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Article

Incorporation of Alternative Ultrasound Biomarkers into Myometrial Invasion-based Model better Predicts Lymph Node Metastasis in Endometrial Cancer: Evidence and Future Prospects

Marcin Liro [1], Marcin Śniadecki [1,*], Ewa Wycinka [2], Szymon Wojtylak [3], Michał Brzeziński [4], Joanna Jastrzębska [1] and Dariusz Wydra [1]

[1] Department of Obstetrics and Gynecology, Gynecologic Oncology and Gynecologic Endocrinology, Medical University of Gdańsk, Poland; mliro@gumed.edu.pl; marcinsniadecki@gumed.edu.pl; dgwydra@gumed.edu.pl; joannajastrzebska@gumed.edu.pl
[2] Department of Statistics, Faculty of Management, Gdańsk University, Gdańsk, Poland; ewa.wycinka@ug.edu.pl
[3] Department of Pathology, Medical University of Gdańsk, Gdańsk, Poland; swojtylak@gumed.edu.pl
[4] Department of Gynecologic Oncology, PCK Marine Hospital in Gdynia, Gdynia, Poland; m.brzezinski@gumed.edu.pl
* Correspondence: marcinsniadecki@gumed.edu.pl; Tel.: +48-501-337-941 (M.Ś.)

Simple Summary: Several ultrasound biomarkers that predict lymph node metastases (LNM) in endometrial cancer (EC) are known. The most common, myometrial invasion (MI) is insufficient without molecular testing, which is not available in many countries. Conversely, the prevalence and accessibility of ultrasound examinations raises the question of whether another biomarker in addition to MI could be used to better describe the progress of EC, thus saving resources for other medical services. This is especially important in the context of the issue of health resources being redistributed because of the COVID-19 epidemic. Our study found that two models, one with, and the other without MI, provided better LNM prediction than MI alone and could be used in preparing EC patients for surgery.

Abstract: Background: Myometrial invasion (MI) is a parameter currently used in transvaginal ultrasound (TVS) in endometrial cancer (EC) to determine local staging, however, without molecular diagnostics, it is insufficient for selection of high-risk cases, i.e., those with a high risk of lymph node metastases (LNM). **Methods:** One hundred sixteen consecutive EC patients, who had received 2D transvaginal ultrasound examinations in their preoperative workup and final histopathology results as a reference standard, were included in this prospective study. Univariate and multivariate logistic models of analyzed TVS biomarkers (tumor [T] size, T area [AREA], T volume [SPE-VOL], MI, T-free distance to serosa [TFD], endo-myometrial irregularity, [EMIR], cervical stromal involvement, CSI) were evaluated to assess the relative accuracy of the possible LNM predictors. To avoid a potential bias in assuming linear relations between LNM and continuous predictors, spline functions were applied. Calculations were made in R with the use of libraries *splines*, *glmulti*, and *pROC*. **Results:** LNM was found in 20 out of 116 (17%) patients. In univariate analysis, only uMI, EMIR, uCSI and uTFD were significant predictors of LNM. Accuracy was 0.707 (AUC 0.684, 95% CI 0.568-0.801) for uMI ($p<0.01$), 0.672 (AUC 0.664, 95% CI 0.547-0.781) for EMIR ($p<0.01$), 0.776 (AUC 0.647, 95% CI 0.529-0.765) for uCSI ($p<0.01$), and 0.638 (AUC 0.683, 95% CI 0.563-0.803) for uTFD ($p<0.05$). The cut-off value for uTFD was 5.2 mm. However, AREA and VOL revealed significant relation by non-linear analysis as well. Among all possible multivariate models, the one comprising interactions of splines of uTFD with uMI and splines of SPE-VOL with uCSI showed most usefulness. Accuracy was 0.802 (AUC 0.791, 95% CI 0.673-0.91) **Conclusions:** A combination of uTFD for patients with uMI>50%, and SPE-VOL for patients with uCSI, allows for the most accurate prediction of LNM in EC, rather than uMI alone.

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Keywords: endometrial cancer; ultrasound; lymph nodes; staging; metastases; biomarkers; model; COVID-19

1. Introduction

Although the histological and molecular features of a tumor facilitate a differential prognosis of endometrial cancer (EC), ultrasonographic (US) examination is a fundamental part of an EC patient's work-up before a proper diagnosis is made [1]. The task of US is to assess the locoregional staging of the disease, which for a gynecological surgeon is a signpost of the scope of therapy, outweighing other, more expensive, and less accessible imaging tests [2]. Ultrasound in EC has value for several reasons, being a diagnostic method that is relatively easy to use and widely available in many centers, and because gynecological ultrasound training begins with the assessment of the uterus and endometrium. So far, most studies have been devoted to analysing the prognostic and predictive utility of US for uterine infiltration, namely, invasion depth in relation to full myometrial thickness, expressed as either greater than or equal to, or less than, 50% [3,4]. However, myometrial invasion measured by ultrasonography (uMI) has its drawbacks, such as the irregularity of the endo-myometrial junction, difficulty in assessing cancer invasion by adenomyosis, or a variety of unusual invasion patterns of EC [5–7]. Therefore, new biomarkers are sought that can either serve as an alternative or as a complement to uMI. The common denominator and reference point for assessing the usefulness of ultrasound biomarkers should be whether they can predict lymph node metastases (LNM), which are a critical characteristic in the high-risk EC group compared to most other EC cases that have a rather good prognosis [8]. So far, several biomarkers have been mentioned in the literature: tumor size [9], tumor surface area [10], tumor volume [11], tumor-free distance (uTFD) [12], myometrial invasion (uMI ≥ 50%) [13], endo-myometrial irregularity (EMIR) [14], and cervical invasion (uCSI) [15]. However, a simultaneous analysis of all these biomarkers has not yet been undertaken with the power to create a model for predicting EC LNM. Our study aims to develop such a model that is as simple to use as uMI, but more effective at predicting the risk of nodal metastases following the diagnosis of EC. The social context of US is also important; as has been demonstrated by the outbreak of the COVID-19 pandemic, which showed that during such a health crisis only the simplest and most effective diagnostic methods are able to be maintained to ensure continuity of quality cancer care. This mainly applies to EC, which is one of the most common cancers in women worldwide [16].

2. Materials and Methods

The authors acknowledge that the portion of the results section, concerning the uTFD parameter and the measurement method discussed as a replacement for the standard uMI parameter, have already been published in "Diagnostics" [17]. The present article, which is a continuation of the previous work, deals with all possible biomarkers more broadly as models, as discussed below, to find which is best in predicting lymph node status. The study was approved by The Research Ethics Committee of the Medical University of Gdansk, and each patient voluntarily gave their written informed consent to participate in the study, on the understanding that therapeutic decisions, except for uMI, were not dependent on the results of these measurements. The study was conducted in accordance with the ethical principles for medical research of the Declaration of Helsinki [18]. The study uses the Standards for Reporting Diagnostic accuracy studies (STARD) 2015 guidelines for reporting results [19].

2.1. Study design and participants

In addition to the data analyses of our previously published work [17], and with the same 116 patients recruited between January 2011 and November 2012 for ultrasound

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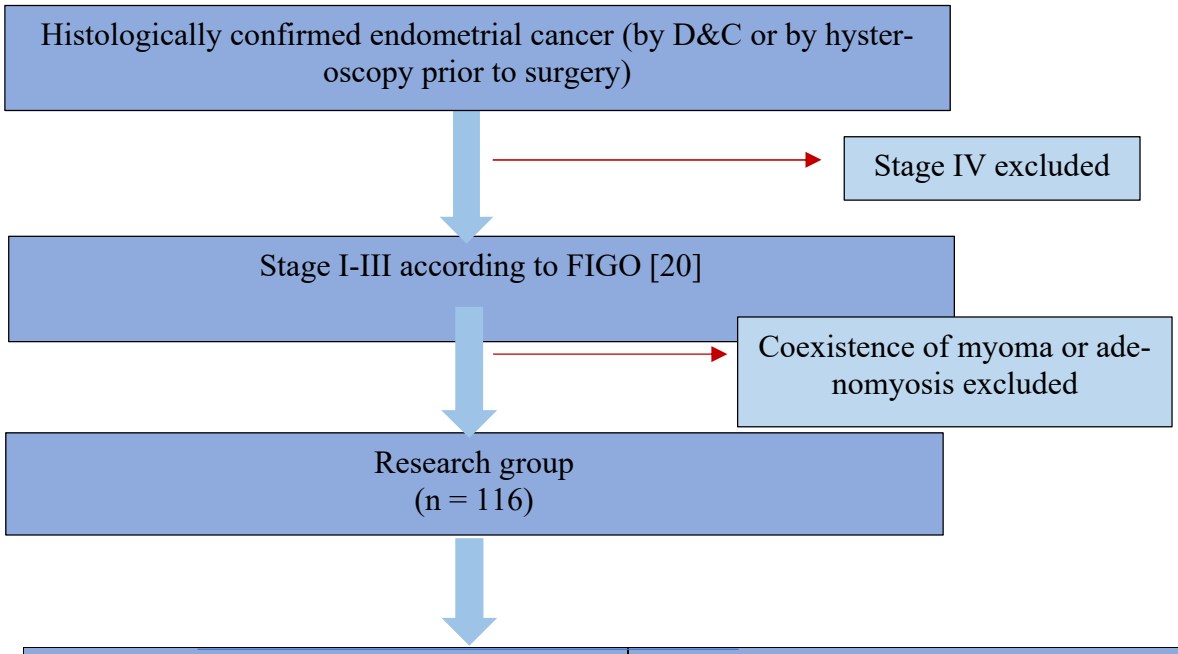
analysis of their endometrial cancer, in the present study we have considered all the parameters that we were collecting at that time, so as to provide more complete data. In the present study, the idea was to use the prospective data on ultrasound biomarkers that we collected to attempt to determine which model consisting of no more than two factors would be better than using uMI alone. In Figure 1, we have presented the study's inclusion and exclusion criteria for patients, which are the same as those used in the previously published study [17].



Ultrasound parameter measurements (u):	Treatment:
○ tumor size (T)	○ Mayo Clinic algorithm for treatment of EC patients [21,22]
○ area of tumor (AREA)	
○ tumor volume (VOL)	○ sentinel lymph node biopsy for low-risk patients
○ myometrial invasion (MI)	○ systematic lymphadenectomy for high-risk patients
○ tumor-free distance (TFD)	
○ endo-myometrial junction irregularity (EMIR)	
○ cervical stromal invasion (CSI)	

Legend: FIGO – International Federation of Gynecology and Obstetrics

Figure 1. Inclusion and exclusion criteria for study patients, and the flow of the study [17].

2.2. Ultrasound examination

2D transvaginal ultrasound (TVS) was performed using the Philips HD7 (Koninklijke Philips N.V.) with vaginal probe (6-10 MHz). The following ultrasound (u) markers were analyzed: tumor size (T), tumor area (AREA), volume (VOL), myometrial invasion (MI), tumor-free distance (TFD), endo-myometrial irregularity (EMIR), and cervical stromal invasion (CSI). T feature was measured as the largest dimension of the tumor at either the

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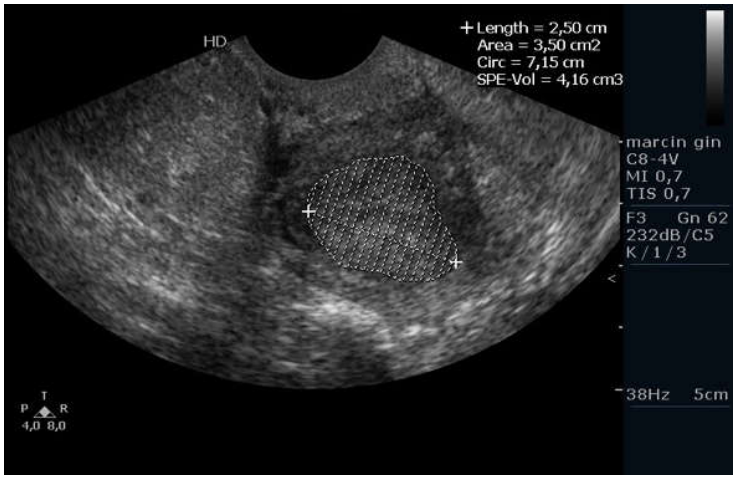
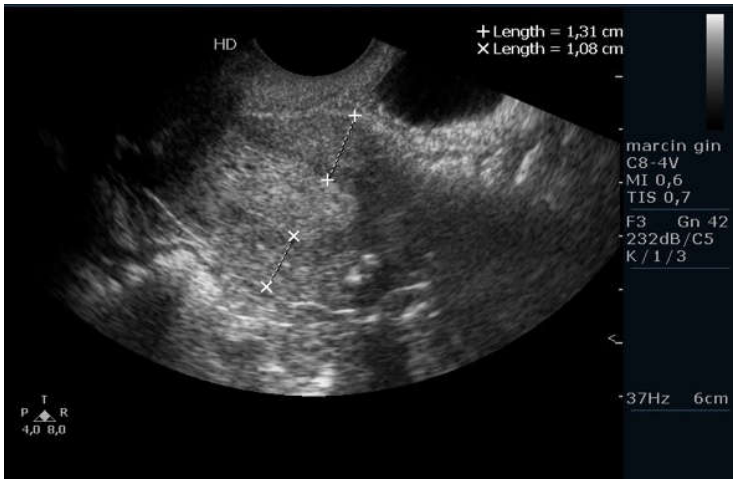
frontal, sagittal, or transverse planes; AREA was assessed at the largest dimension of the tumor (most often in, or near, the sagittal plane). In turn, VOL was measured planimetrically, by calculating the three dimensions of the suspected echogenic structure, according to the formula: $\pi/6$ x d1 x d2 x d3 (where "d" is the dimension). We made three measurements for both AREA and VOL and used the highest value in each parameter to minimise calculation error. In the same three planes, uTFD measurements were made subjectively in the most locally advanced part of the tumor; and the shortest distance from the forehead of the infiltration to the serosa surface was taken into consideration. The ultrasound MI was measured by subtracting the tumor thickness (perpendicular to the long axis) from the distance between endometrium-myometrium interface to the serosa. EMIR was examined by accentuating the endometrial junction - if this border had been breached (it could not be traced), the trait was deemed positive. The CSI measured by ultrasound was defined as the absence of the outline of the tumor, at least in the inner orifice of the cervix. Figure 2 (panel) shows an example of measurement-taking. All measurements were made using a tension-free technique, so as not to compress the tissues, and thus to avoid distortion of the results. The ultrasound researcher was aware of the primary pathological result. The staging was determined preoperatively based on TVUS according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) classification system [20].



(**a**)



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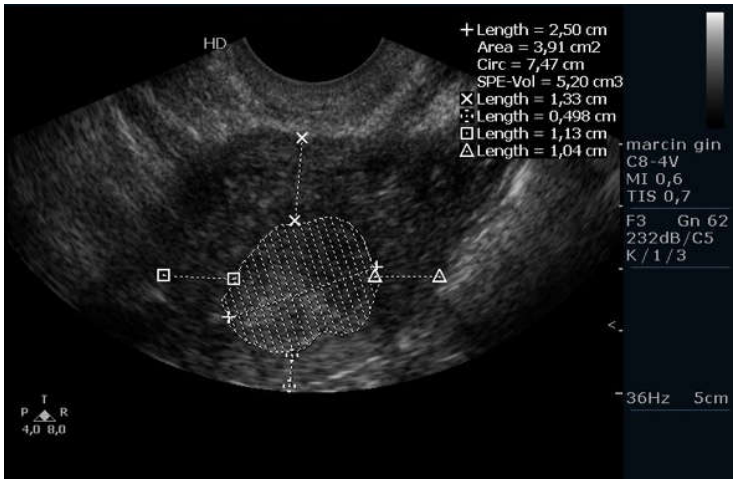

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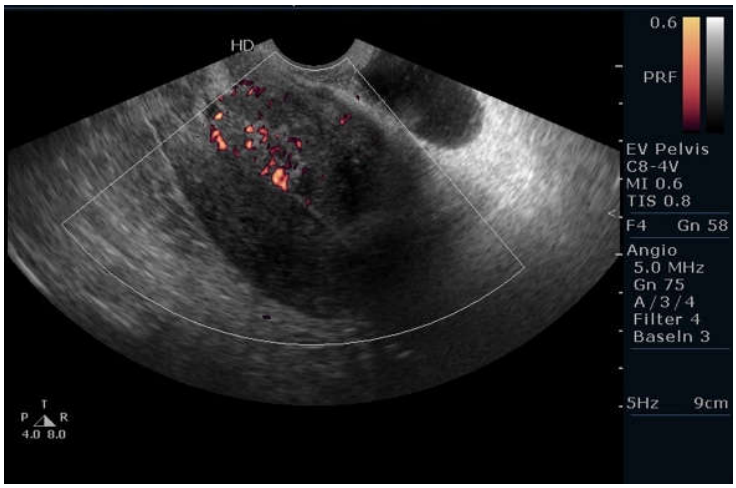

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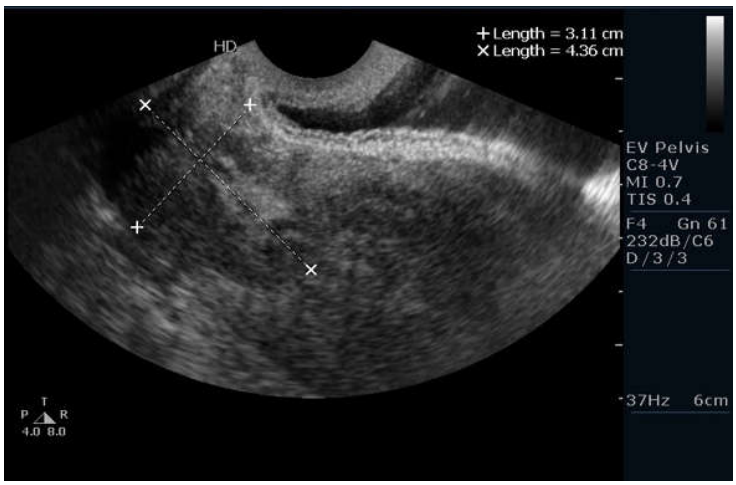

(**e**)

Figure 2. Multiple panels showing how measurements were taken: (**a**) tumor size (T = 2.5 cm), tumor area ("Area" = 3.5 cm2), volume ("SPE-Vol" = 4,16 cm3); (**b**) myometrial invasion (MI = apparently less than 50%); (**c**) tumor-free distance (TFD = 0.498 cm); (**d**) endo-myometrial irregularity (EMIR), here with use of power Doppler; (**e**) cervical stromal invasion (CSI).

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2.3. Surgery including lymph nodes procedure

We described the surgical procedures in our previous publication [17]. The types of surgery included were simple hysterectomy and bilateral salpingo-oophorectomy with sentinel lymph node biopsy (SLNB) or regional lymphadenectomy in endometrioid carcinomas, and total hysterectomy with salpingo-oophorectomy with pelvic and para-aortic lymphadenectomy (in those patients with diagnosed prognostic factors for lymph node recurrence and metastasis). Sentinel lymph node biopsy was performed in patients with contraindications for extensive lymph nodes surgery (e.g., poor general condition or comorbidities).

The SLNB concept that we used was based on combining: Tc99m-nanocolloid applied to the ectocervix mucosa before skin incision, and intraoperative injection of blue dye to the subserosa of the uterine fundus. During the procedure, we assessed node colour and radiotracer uptake - when blue staining occurred, and / or uptake 10 times the background level, they were determined to be SLN positive.

2.4. Histopathology

The pathologist was blinded to the ultrasound results. All the results received from external sites were subject to verification internally. That is, each external institution's blocks (for instance, histological slides) when received, underwent pathologist processing and verification by a specialist of our facility. The excised lymph nodes were subject to routine histopathological treatment (reference standard) [17].

2.5. Statistical analysis

Univariate logit models for continuous predictors were evaluated twice (raw predictors and cubic splines of predictors). Univariate logit models were also evaluated for qualitative predictors (uT, uMI, EMIR, uCSI,). To build multivariate models, all possible combinations of covariates as well as interactions between them were considered. Using the *glmulti* package in R, more than 450 models were estimated. The Akaike Information Criterion (AIC) were used to select the best multivariate model and to omit overestimation [23]. The discrimination ability of models was assessed with the use of receiver operating characteristic curve (ROC) and the area under it (AUC). Accuracies were calculated for points of predictors which maximise Youden's index. The likelihood ratio test (LRT) was used as a global test for models. In logit models to avoid a risk of overfitting, should be used a minimum of 10 outcome events per predictor variable. This rule was established in simulation studies [24]. Thus, having 20 outcome events, the model with up to two predictors can be specified.

3. Results

Table 1 summarises the values of seven ultrasound variables in the study group.

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Table 1. Distribution of ultrasound predictors for lymph nodes metastases in the group of 116 patients with EC.

Ultrasound Variable	Characteristic	Value
T ≤2 cm >2 cm	Number (%)	 76 (66%) 40 (34%)
AREA [cm^2]	Mean±SD (range)	7.49±9.77 (0.161–67)
SPE-VOL [cm^3]	Mean±SD (range)	17.00±26.93 (0.033–127)
TFD [mm]	Mean±SD (range)	7.39±4.83 (0.3–22)
uMI <50% ≥50%	Number (%)	 76 (66%) 40 (34%)
EMIR	Number (%)	44 (38%)
CSI	Number (%)	24 (20.7%)

Legend: AREA – surface area of tumor; CSI – cervical stromal invasion; EMIR – endo-myometrial junction irregularity; MI – myometrial invasion; SPE-VOL – volume of tumor; T – tumor size; TFD – tumor free distance to serosa; Detailed description of the values in the text.

Univariate models show the influence of each of the ultrasound predictors on the risk of neoplastic metastases, namely, the influence of uTFD (C model), SPE-VOL and AREA (nonlinearly, D and E models), uMI (G model), EMIR (H model), uCSI (at the limit of statistical significance), and size (model J). Models A and B were irrelevant, i.e. there is no linear influence of SPEVOL and AREA on LNM. Among univariate models, the highest accuracy was achieved by the uCSI model with a result of 77.6% (Table 2). The multivariate model (K) includes two interactions, the first uMI with bs (uTFD) and the second uCSI with bs (SPE-VOL). According to Akaike information criterion, the K model is the best model (AIC=94.81). For comparison, the best one-way model is uMI (AIC = 101.17). The multivariate (K) model also achieved the highest accuracy (80%) in predicting metastasis. The comparison of the ROC curves of the univariate and multivariate models, proving the superiority of the latter, is presented in the graph in Figure 3.

As SPE-VOL increases to 40 cm-3, the probability of LNM increases and then decreases (Supplementary material 1); in the case of AREA, it is an increase of up to 18 cm-2. As the tumor surface continues to enlarge on ultrasound, the risk of LNM decreases.

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Table 2. Univariate logit models (models A-J) and multivariate logit model (model K).

Model	Covariate	Est.	Std. Error	p-value	AIC	ACC	AUC (95% CI)	p-value (LRT)
A	(Intercept)	-1.79	0.30	0.000				
	SPE-VOL	0.01	0.00	0.143	108.67	0.750	0.652 (0.507–0.796)	0.159
B	(Intercept)	-1.87	0.32	0.000	108.18	0.767	0.646 (0.499–0.794)	0.115
	AREA	0.03	0.02	0.110				
C	(Intercept)	-0.50	0.44	0.254	102.68	0.638	0.683 (0.563–0.803)	0.005
	uTFD	-0.17	0.07	0.012				
D	(Intercept)	-2.68	0.49	0.000				
	bs(SPE-VOL)1	6.81	2.28	0.003	103.86	0.767	0.689 (0.538–0.840)	0.013
	bs(SPE-VOL)2	-3.48	2.94	0.236				
	bs(SPE-VOL)3	2.36	1.78	0.186				
E	(Intercept)	-2.61	0.61	0.000				
	bs(AREA)1	5.18	4.37	0.215	106.23	0.784	0.671 (0.520–0.821)	0.038
	bs(AREA)2	-0.01	13.98	1.000				
	bs(AREA)3	-5.84	38.32	0.878				
F	(Intercept)	-0.62	0.93	0.506				
	bs(uTFD)1	-1.37	4.14	0.740	106.32	0.638	0.683 (0.563–0.803)	0.040
	bs(uTFD)2	-0.58	6.49	0.929				
	bs(uTFD)3	-8.04	11.92	0.500				
G	(Intercept)	-2.29	0.40	0.000	101.17	0.707	0.684 (0.568–0.801)	0.002
	uMI	1.56	0.52	0.003				
H	(Intercept)	-2.23	0.40	0.000	103.34	0.672	0.664 (0.547–0.781)	0.007
	EMIR	1.36	0.52	0.009				
I	(Intercept)	-2.00	0.32	0.000	103.11	0.776	0.647 (0.529–0.765)	0.06
	uCSI	1.49	0.53	0.005				
J	(Intercept)	-3.43	0.82	0.000	104.02	0.690	0.654 (0.535–0.773)	0.010
	Size	1.29	0.51	0.011				
K	(Intercept)	-2.62	0.44	0.000	94.81	0.802	0.791 (0.673–0.91)	
	uMI:bs(uTFD)1	-13.24	4.74	0.005				
	uMI:bs(uTFD)2	-54.62	27.47	0.046				
	uMI:bs(uTFD)3	121.59	60.64	0.044				0.005
	uCSI:bs(SPE-VOL)1	10.09	3.51	0.004				0.006
	uCSI:bs(SPE-VOL)2	-15.04	9.30	0.105				
	uCSI:bs(SPE-VOL)3	9.39	9.07	0.301				

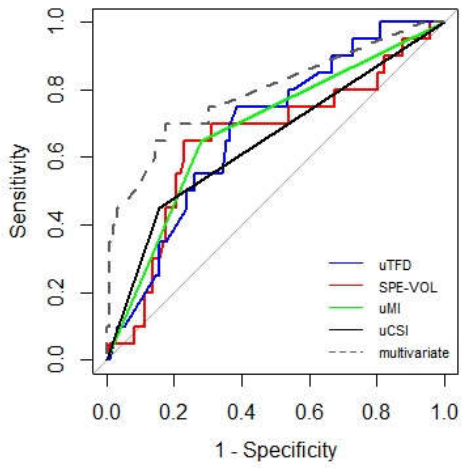

Figure 3. ROC curves showing the most important predictors of lymph node metastasis in a study of 116 women with endometrial cancer.

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For the high-risk group, no single factor model was significant in LNM prediction. Therefore, we tried to find a model where one predictor would be the interaction of the variables. The uCSI:uMI model turned out to be the best (AIC 45.19, ACC 71%) (Table 3).

Table 3. Univariate logit models (models A′-J′) and multivariate logit model (model K′) for high-risk group (n=31).

Model	Covariate	Est.	Std. Error	p-value	AIC	ACC	AUC (95% CI)	p-value (LRT)
A′	(Intercept)	-0.13	0.48	0.786	49.62	0.618	0.479 (0.268–0.689)	0.501
	SPE-VOL	-0.01	0.01	0.509				
B′	(Intercept)	-0.12	0.53	0.827	49.69	0.618	0.475 (0.265–0.685)	0.536
	AREA	-0.01	0.03	0.550				
C′	(Intercept)	0.12	0.58	0.836	49.01	0.588	0.571 (0.375–0.768)	0.304
	uTFD	-0.09	0.08	0.316				
D′	(Intercept)	-1.04	0.77	0.176	50.22	0.765	0.686 (0.483–0.889)	0.278
	bs(SPE.VOL)1	5.11	3.12	0.101				
	bs(SPE.VOL)2	-4.68	3.24	0.148				
	bs(SPE.VOL)3	0.87	1.91	0.650				
E′	(Intercept)	-0.84	0.82	0.307	52.09	0.765	0.643 (0.423–0.863)	0.577
	bs(AREA)1	2.863.28	3.84	0.394				
	bs(AREA)2	-3.11	6.09	0.610				
	bs(AREA)3	-3.58	9.19	0.697				
F′	(Intercept)	-0.14	0.77	0.855	52.57	0.618	0.600 (0.404–0.796)	0.683
	bs(uTFD)1	0.05	3.42	0.989				
	bs(uTFD)2	0.30	3.56	0.933				
	bs(uTFD)3	-2.51	3.49	0.472				
G′	(Intercept)	-0.81	0.60	0.177	49.11	0.559	0.582 (0.416–0.748)	0.328
	uMI	0.72	0.74	0.335				
H′	(Intercept)	-0.69	0.55	0.206	49.38	0.559	0.571 (0.399–0.744)	0.407
	EMIR	0.59	0.71	0.411				
I′	(Intercept)	-0.69	0.55	0.206	49.38	0.559	0.571 (0.399–0.743)	0.407
	uCSI	0.59	0.71	0.411				
J′	(Intercept)	-0.48	1.36	0.726	50.06	0.471	0.507 (0.347–0.667)	0.928
	Size	0.07	0.77	0.928				
K′	(Intercept)	-0.89	0.45	0.048	45.19	0.706	0.675 (0.517–0.833)	.
	uCSI:uMI	1.74	0.82	0.035				0.027

For the low-risk group, the uMI:EMIR interaction is important, but too few metastases were recorded in this large group of patients to build a reliable model (Supplementary material 2).

4. Discussion

Two ultrasound parameters, uMI and uCSI, are the best single predictors of LNM in EC. However, the multivariate model, consisting of uMI:bs(uTFD) and uCSI:bs(uSPE-VOL) pairs, showed higher accuracy than univariate models. The parameters uMI and uCSI are well-known predictors of LNM, while uTFD and uSPE-VOL are new predictors. The latter seem to increase the predictive power of "classical" parameters. In the clinical-anatomical sense, the first, uTFD, shows cancer access to blood vessels with a cut-off value of 5 mm from the serosa, and perhaps this is already evidence of infiltration of the tumor to the lymph vessels. The second ultrasound biomarker, SPE-VOL, shows the tumor-uterus relationship, whereas large tumors, i.e., over 40cm-3, show more mild invasive features. This latter can be explained by the length of their growth until diagnosis, especially since we do not often observe LNM in them. The opposite may be true for rapidly growing, aggressive (possibly metastatic) tumors, which are found to be smaller at diagnosis. The uMI:uCSI model best defined the biological aggressiveness of cancer (high-risk tumors), which can be explained by the fact that both markers in this model are predictors of invasion.

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Our study has several limitations. The first is the low number of cases with positive lymph nodes in our sample (20/116). Therefore, it was impossible to divide the risk groups into more than two categories (high risk / low risk), with the caution that histological and ultrasound features were considered, but not molecular features. In addition, most of the low-risk group did not undergo full lymphadenectomy, which (although consistent with the treatment guidelines) did not allow for a comprehensive comparison with the high-risk group in which full lymphadenectomy had been performed [1,25]. The third limitation was the influence of the diagnostic procedures used prior to the diagnosis of EC (D&C, hysteroscopy) affecting the accuracy of imaging prior to surgery. For example, volumetric-based biomarkers (such as uSPE-VOL) must be taken with caution because during invasive procedures such as D&C and endoscopic techniques, some tissues may be lost before preoperative ultrasound assessment is undertaken. The fourth limitation is that biomarkers indicating continuity disturbance of the boundary between the tumor and healthy tissue (such as uMI and EMIR) can also equally indicate the same critical phenomenon, such as the invasion process, and thus, as one marker overlaps the other the clarity of the modelling is reduced. On the other hand, the difference between uMI and EMIR is that EMIR is a zero-one feature, and uMI is a semi-quantitative biomarker. The next limitation is that we did not include adenomyosis and myoma patients. This is because EMIR cannot be properly assessed in such cases due to the fact that the intraepithelial zone may be poorly reflected in ultrasound if some disease of the uterine muscle is present. Lastly, we did not incorporate grading of cancer in our ultrasound models, to ensure the correct methodology for the ultrasound trial.

It is assumed that ultrasound with an endovaginal probe is a diagnostic tool that does not permit direct visualization of lymph node metastases [26]. More advanced imaging (i.e. computed tomography, magnetic resonance imaging) can detect LNM directly, but it cannot detect micrometastases. This means that at the point of EC diagnosis those tests do not have a significant advantage over TVUS examination [27,28].

Ultrasound-measured MI is a guide for deciding whether to perform lymphadenectomy prior to surgical intervention. This biomarker has proved to be a decisive factor when determining the scope of operation in cases of potentially high-risk tumors. Being imprecise, uMI seems to be a key biomarker for predicting EC cases as high-risk but is not a sufficient parameter for determining LNM risk [29–31]. In general, uMI is associated with other parameters besides uCSI, such as tumor histology. There is little data on the usefulness of ultrasound-only parameter models used for the prediction of metastases [32–34]. Our study is the only one that covers ultrasound-only models, irrespective of grade or tumor type.

The value of ultrasound becomes especially evident during an economic crisis, such as that posed by the COVID-19 pandemic, and when there is a decline in the availability of research.

It is known, however, that histopathological, and nowadays molecular, examination determines further treatment of patients with EC. The question is how widespread these approaches are, and what characterises the "false low risk" group within the "low risk" group. In our study most cases were “low risk”, and we performed a separate analysis for “low risk” ECs. In this group of 6 “false low risk” cases, the risk was higher than it would appear from their classification. There was no stromal infiltration in this subgroup at all, 5/6 had uTFD up to 5.2 mm, 4/6 had EMIR, and 3/6 had uMI >= 50%. This shows that in practice a tumor can be described in several ways and differently (each marker describes a different biological feature).

The best correlation between ultrasound and pathology should be expected in the “expanding type” of tumor growth. This invasion pattern is characterized by a broad front of neoplastic infiltration with a sharp demarcation of tumor tissue from the adjacent healthy tissues. This margin should be clearly identified by ultrasound invasion biomarkers such as MI, TFD, and EMIR. Among these markers, uEMIR seems to be the most subjective and therefore the most difficult to evaluate. This characteristic is reflected by multivariate models in which not only uAREA but also uEMIR is missing. However, the

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latter parameter is promising. In physiology, this structure takes part in facilitating sperm transport through modulation of uterine peristalsis and blastocyst implantation, thus it influences fertility [29]. However, its role in oncology is yet not well elucidated. This intermediate zone between epithelium and muscle layer is lost during EC invasion. Therefore, it can be suggested that EMIR may be a helpful indicator of early invasion. Our observations did not confirm these assumptions. Perhaps the following suggestion is not strongly supported by the current data, but uEMIR may be a marker of late invasion of slow-growing tumors (this biomarker proved to be significant in the low-risk group). EMIR assessment was, for example, included in "REC" (risk of endometrial cancer) scoring system by Dueholm et al., and indicates malignancy in case of postmenopausal bleeding and endometrial thickness ≥5 mm [35]. Molecular studies seem to confirm the potential role of this intermediate zone in the invasion of cancer that may involve HOX genes [36, 37]. Thus far, we have limited knowledge about the role of EMIR assessment in the diagnosis and staging of EC [38].

Tumor volume was included in the scoring systems by Mitamura et al., and Imai et al., who further developed the studies by Todo et al. [39–41], although SPE-VOL was measured by MRI and the scoring systems contain a mix of clinical and pathological features. In all these studies the limit of tumor volume (index) was determined at 36 cm3, and our study produced the similar value of 40 cm3. Active tumors equate with "high risk", and indolent tumors with "low risk". A decrease in LNM risk by tumor volume in a range between 40 and 100 cm3 may reflect the point at which an indolent tumor is recognised or may reflect the substantial number of "low risk" tumors in the study group. A further increase in the risk of metastasis with tumors >100 cm3 can be explained by the size of the tumor, which anatomically infiltrates the internal opening of the cervix thus increasing its access to the lymphatic vessels (Supplementary material 1).

Tumor infiltration is a multidirectional process and what is visible during 2D ultrasound examination may not represent a complete infiltration picture (since ultrasound examination is one dimensional, and histologic sections are multidimensional). In a single TVUS-3D study with the uMI biomarker, not only did Adriano Rodríguez-Trujillo et al., not show the superiority of 3D ultrasound over magnetic resonance imaging in the assessment of infiltration, they also stated that in the case of adenomyosis or uterine fibroids, intraoperative examination of the uterine wall infiltration by cancer was indicated [42]. For these reasons, the uMI, uTFD, uEMIR, and uCSI measurements are observer-dependent and subjective. Therefore, they should be combined in two- or more-factor models. Moreover, the location of tumors within the uterus may cause discrepancies in the proper evaluation of the ultrasound parameters [43,44]. All issues stated above refer mainly to type I EC. In the serous carcinomas the deepest point of neoplastic infiltration is often synonymous with the deepest location of the lymphatic tumor emboli [45]. Many serous ECs present an image of only polypoidal growth, accompanied with broad peritoneal metastases. However, the most controversial group of cases are endometrioid G3 tumors, which belong to type I EC, but may histologically represent a heterogenic group of cancers with frequent multiplication of biological features typical for type II [46]. Taking into account the suggestions stated above, a prospective study comprising analysis of two independent models of uMI:uTFD and uCSI:uSPE-VOL is needed. It would be interesting to know whether the local staging of EC may be enhanced to indicate "high risk" patients that may benefit from limited or no lymphadenectomy. A validation study is necessary.

5. Conclusions

For preoperative ultrasound staging dedicated to LNM risk estimation of endometrial cancer four parameters are essential, grouped in two pairs: uMI:uTFD and uCSI:uSPE-VOL. The two-factor model predicts LNM better than the one-factor model. Discretion should be used in choosing a model pair. In the authors' opinion the first model is easier to use because the component parameters, uMI and uTFD, are now used separately.

There are no perfect methods for assessing invasion of endometrial cancer, and the terminology used is inconsistent. Standardization of the terminology of methods and

measurements would allow for better communication between specialists and perhaps improve the therapeutic qualification for different treatment methods.

Supplementary Materials: Supplementary Material 1 - Figure S1: Tumor volume measured by ultrasound in relation to the probability of metastases to lymph nodes in the study group of 116 women with endometrial cancer. **Supplementary Material 2 - Table S1:** Univariate logit models (models D'-I) and multivariate logit model (model J) for low-risk group (n=82).

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Data Availability Statement: The collected clinical material is in electronic form in the Clinic's repository and may be made available in an anonymised form upon request.

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