

TESE DE DOUTORAMENTO EM MEDICINA

APRESENTADA

À FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

DIAGNOSTIC STUDIES IN ENDOMETRIAL CANCER:

EVALUATING THE IMPACT OF PRE-SURGICAL STAGING

MARIANA ISABEL CRUZ REI

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ESTUDOS DIAGNÓSTICOS NO CARCINOMA DO
ENDOMÉTRIO:
AVALIAÇÃO DO IMPACTO DO ESTADIAMENTO
PRÉ-OPERATÓRIO

MARIANA ISABEL CRUZ REI

PORTO 2024

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apresentada à Faculdade de Medicina da Universidade do Porto.

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assunto

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Presidente: Doutor José Manuel Estevão da Costa, Professor Catedrático da Faculdade de Medicina da Universidade do Porto

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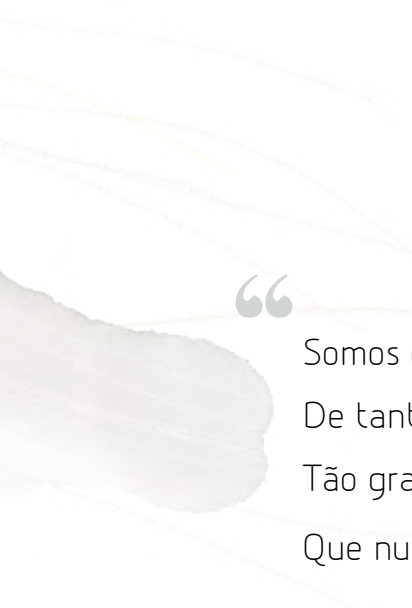
- Doutora Maria Dolores Diestro Tejeda, Professora Associada do Hospital Universitário La Paz, Espanha;
- Doutora Maria João da Silva Fernandes Leal Carvalho, Assistente Convidada da Universidade de Coimbra;
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- Doutora Cristina Maria Nogueira da Costa Santos, Professora Catedrática da Faculdade de Medicina da Universidade do Porto;
- Doutora Maria Antónia Moreira Nunes da Costa, Professora Auxiliar Convidada da Faculdade de Medicina da Universidade do Porto.

Com os melhores cumprimentos,

A Vice-Reitora,



(Prof.ª Doutora Fátima Vieira)



“

Somos o resultado de tanta gente,
De tanta história,
Tão grandes sonhos que vão passando de pessoa em pessoa,
Que nunca estaremos sós.

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ABBREVIATIONS

3D	Three dimensional
ADC	Apparent diffusion coefficient
AUC	Area under the receiver-operating characteristics curve
CI	Confidence interval
CSI	Cervical stromal invasion
DCE	Dynamic contrast enhanced
Dist-OCO	Distance from the lower margin of the tumor to the outer cervical os
DMI	Deep myometrial infiltration
DWI	Diffusion-weighted imaging
EC	Endometrial carcinoma
ES	Endometrial sampling
FIGO	International Federation of Gynecology and Obstetrics
FS	Frozen section
IETA	International Endometrial Tumor Analysis
IGE	Intraoperative gross evaluation
K	Kappa statistic
MI	Myometrial infiltration
MRI	Magnetic resonance imaging
NPV	Negative predictive value
PA	Proportions of agreement
PICOS	Patients, Intervention, Comparator, Outcomes, Study design
PPV	Positive predictive value
SLNB	Sentinel lymph node biopsy
TCGA	The Cancer Genome Atlas
TDS	Tumor distance to serosa
TNM	Tumor-node-metastasis
TVUS [or TVS]	Transvaginal ultrasound
UICC	Union for International Cancer Control
US	Ultrasound

ABSTRACT

Preoperative staging in endometrial cancer has recently been implied as an important contribution to an accurate selection of low-risk cases, ultimately avoiding unnecessary lymph node debulking. Transvaginal ultrasound (TVS) seems promising in clinical staging, as it offers the possibility to assess the depth of myometrial infiltration and cervical stromal invasion. This is a commonly available, non-invasive and low-cost modality, standing as an accurate alternative to Magnetic resonance imaging (MRI), especially in medium and low-income countries, where MRI is not promptly available and costs are an important issue.

Our studies aimed to evaluate the validity of transvaginal ultrasonography in preoperative uterine cancer staging in comparison to pelvic magnetic resonance imaging and to histological exam (permanent section) of the surgical specimen, which stands as the gold standard. We prospectively applied a protocol of subjective and objective ultrasound approaches in order to evaluate myometrial and cervical involvement, which are key preoperative parameters influencing staging and surgical approach. Regarding the evaluation of myometrial invasion, the ultrasound subjective and objective methods of Gordon and Karlsson presented an overall accuracy of 72%, 68% and 73% (95% CI [Confidence intervals] 61-81; 57-78; 63-82), respectively, whereas MRI showed 82% (95% CI 77-98; 52-85; 71-90). Regarding cervical involvement the overall accuracy was 88%, 81% and 94% respectively for subjective, objective TVS and MRI (95% CI 79-94; 68-90; 86-99). Agreement between TVS and MRI was superior in the assessment of cervical invasion, with Proportions of agreement (PA) ranging from 0.82 to 0.93 and Kappa statistic (K) from 0.45 to 0.58, in comparison to myometrial invasion with PA ranging from 0.68 to 0.73 and K from 0.31 to 0.50.

The use of this protocol will potentially impact surgical planning, cost and operating room time management, obviating the need for frozen section exam and allowing an accurate selection of low-risk cases eligible for sentinel lymph node technique.

RESUMO

O estadiamento locorregional pré-operatório do carcinoma do endométrio foi recentemente proposto como uma importante ferramenta na seleção dos casos de baixo risco e de doença confinada ao útero, permitindo dispensar o exame extemporâneo intraoperatório e identificar os casos elegíveis para biópsia de gânglio sentinela, desta forma evitando a indicação de esvaziamento ganglionar e morbilidade associada.

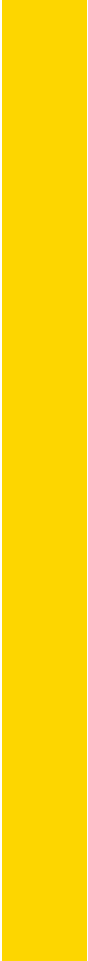
A ecografia via transvaginal realizada por operador experiente surge como uma técnica promissora, uma vez que oferece a possibilidade de determinar a invasão miometrial profunda e o envolvimento estromal cervical. Este é um método de fácil acesso e baixo custo e não invasivo, pelo que se assume como uma alternativa cómoda e fiável à ressonância magnética pélvica, especialmente em contextos hospitalares em que esta última não se encontre disponível ou em que o aspeto financeiro constitua uma limitação.

A presente tese tem como objetivo primário a validação da ecografia transvaginal realizada por operador experiente no estadiamento pré-operatório do carcinoma do endométrio em comparação com a ressonância magnética pélvica e com o exame histopatológico definitivo, estabelecido como o estado da arte no estadiamento da neoplasia endometrial.

Foi prospectivamente aplicado um protocolo de estudo de avaliação ecográfica subjetiva e objetiva para determinação da invasão miometrial e estromal cervical, parâmetros pré-operatórios fulcrais para a decisão de realização de estadiamento cirúrgico ganglionar e que poderão alterar a abordagem cirúrgica e, posteriormente, a necessidade de adjuvância e o prognóstico. Relativamente à avaliação da invasão miometrial, o método ecográfico subjetivo e objetivos de Gordon e Karlsson apresentaram uma acuidade global de 72%, 68% e 73% (95% CI 61-81; 57-78; 63-82), enquanto que a ressonância magnética mostrou 82% (95% CI 77-98; 52-85; 71-90). Relativamente à determinação do envolvimento cervical, a acuidade global foi de 88%, 81% e 94% respetivamente para o método ecográfico subjetivo, objetivo e ressonância magnética (95% CI 79-94; 68-90; 86-99). A concordância entre a ecografia e a ressonância magnética foi superior na avaliação cervical, com proporções de concordância entre 0.82 e 0.93 e coeficiente de K de 0.45 a 0.58, em comparação com a invasão miometrial (proporções de concordância de 0.68 a 0.73 e coeficiente de K de 0.31 a 0.50).

A aplicação por rotina deste protocolo terá um potencial impacto benéfico no planeamento cirúrgico, custos e tempos intra-operatórios, possivelmente obviando a necessidade de realização de exame extemporâneo do útero e permitindo uma seleção fiável dos casos de baixo risco elegíveis para avaliação ganglionar na forma de biópsia de gânglio sentinela.





CHAPTER 1.

INTRODUCTION

An abstract illustration of a mountain range at the bottom of the page. The mountains are rendered in shades of gray, with a prominent dark, jagged peak in the center and lighter, more rounded peaks on either side. The style is soft and painterly, blending into the white background.

1.1. ENDOMETRIAL CARCINOMA

Endometrial carcinoma (EC) is the sixth most common malignancy diagnosed in women and the most common gynaecological cancer in high income countries, with 417.000 new diagnosis globally in 2020 [1]. In the last 30 years overall incidence has risen by 132%, reflecting a progressive upsurge in the risk factors prevalence of: population ageing, reduced benign hysterectomies and growing prevalence of obesity, the latter being the major underlying cause [2]. This tendency is mostly noticed in high-income countries, with a highest rate in North America (86.6/100.000) followed by east and central Europe (52.5-21.9/100.000) [2]. However, a rise in the age-standardized incidence rate has been reported globally, including in sub-Saharan Africa [3], and an inverse correlation between EC incidence to mortality ratio and socioeconomic index is of particular concern.

EC usually presents at an early stage with postmenopausal bleeding and most cases are diagnosed at FIGO stage I, presenting a 5-year survival rate of 90% [4].

The histologic diagnosis is usually based upon the results of an endometrial biopsy or hysterectomy specimens. Pelvic ultrasonography and endometrial sampling have shown efficacy in the initial assessment of postmenopausal women with uterine bleeding [5,6].

The most important prognostic features for EC are FIGO (International Federation of Gynecology and Obstetrics) stage, myometrial infiltration, histological type and differentiation grade [7]. Lately, molecular and genetic findings since the publication of The Cancer Genome Atlas (TCGA) data sheds light on the diverse biological nature of this collection of endometrial cancers and their differing prognostic outcomes [8]. Prognosis is worse in women with high-risk endometrial carcinoma, as defined by the presence of deep myometrial invasion, cervical stromal invasion, grade 3 tumor or non-endometrioid histotype, leading to an increased risk of lymph node metastasis. In contrast, patients with low-risk cancer do not benefit from systematic pelvic and para-aortic lymph node dissection, as it does not improve survival and may lead to increased surgical morbidity [9,10]. Even in low-grade endometrioid tumors, the risk of lymph node metastasis increases from 4% to 15% if the tumor invades the myometrial outer half [11]. Molecular profiling within high-grade endometrioid group can discriminate an excellent prognosis group (POLEmut in early-stage disease) from a poor prognosis group (p53 abnormal [p53abn]) [8].

Classically, EC is a surgically staged disease [8] and therefore routine preoperative evaluation with pelvic imaging to assess myometrial invasion or cervical involvement is often considered unnecessary.

For years, intraoperative frozen section for the evaluation of myometrial and cervical infiltration in clinical stage I endometrial carcinoma has been advocated in order to aid the decision to proceed with lymph node staging, reporting a high accuracy for determining myometrial invasion [12] and cervical stromal invasion [13]. Limitations of this approach includes the fact of being a time-consuming exam and the lack of expertise and reproducibility [14,15].

Therefore, a preoperative evaluation appears to be beneficial, allowing a more carefully planned treatment, an effective selection of high-risk cases elective for more radical surgery with pelvic and paraaortic lymphadenectomy and meanwhile obviating overtreatment in low-risk cases, as well as avoiding longer operative times due to frozen section.

1.2. ONCOLOGICAL STAGING OF ENDOMETRIAL CARCINOMA

1.2.1. SURGERY AND LYMPH NODE STATUS

EC is surgically staged and requires histological confirmation of type, grade and local extent of disease as fundamental criteria for staging [8]. Such criteria are established by the FIGO [8] and tumor-node-metastasis (TNM)-based Union for International Cancer Control (UICC) [16,17].

The surgical mainstay of treatment includes total hysterectomy and salpingo-oophorectomy [15].

Lymph node status as part of surgical staging assumes an important role defining prognosis and therefore regarding decision for adjuvant treatments. Pathologic (histotype and tumor grade) and molecular criteria, lately structured into risk categories, have been used to predict lymph node metastasis and guide surgery [15]. However, significant peri-operative morbidity of pelvic and paraaortic lymph node dissection must be taken into account.

Moreover, the therapeutic role of systematic lymphadenectomy has been challenged in two randomized controlled trials, which did not report survival benefit [9,10,18].

Given the morbidity of systematic lymphadenectomy and balancing the potential risk of lymph node metastasis, it is clear that a subset of patients may not benefit from systematic lymphadenectomy, especially if the risk of lymph node disease is low. While in stage I disease, 3–5% of women with well differentiated tumors and superficial myometrial invasion will have lymph-node involvement, this proportion rises to 20% in poorly differentiated tumors and deep myometrial invasion [10].

Sentinel lymph node biopsy (SLNB) has been recently introduced as a reliable alternative to routine pelvic and paraaortic lymph node dissection and can be considered for staging purposes in patients with low and intermediate risk disease [15]. The emergence of this technique simultaneously allows: 1 - a more intensive pathologic assessment of lymph node status with ultrastaging, which could be missed by standard evaluation [19] and 2 - a substantial lower risk of post-operative morbidity related to extensive lymph node dissection [20].

The NCCN panel recommends that nodal evaluation be performed in patients with EC, including para-aortic lymphadenectomy in high-risk patients. SLNB mapping is the preferred alternative to full lymphadenectomy in the setting of apparent uterine-confined disease [21].

An open-label, non-inferiority, randomized trial (ALICE trial) aiming to confirm that SLNB without systematic node dissection does not negatively impact oncological outcomes is currently completing accrual and will provide more evidence on this subject [22].

1.2.2. PRE-OPERATIVE WORK-UP

Recently, molecular studies have obtained promising results to provide important information for prognosis and for predicting response to novel therapies. The Cancer Genome Atlas Research Network identified four major clinically significant molecular subtypes with differing clinical prognosis: POLE (DNA polymerase epsilon) mutated, microsatellite instability high (MSI-H), copy number low, and copy number high (associated with abnormal p53 expression/TP53mutation) [23]. These studies can be performed either in biopsy or tumor surgical specimens and may impact the need for adjuvant treatments. Additionally, results may indicate the need of germline testing and genetic counseling for inherited cancer risk. Therefore, expert pathology review with additional endometrial biopsy in order to review histology and grade and/or multigene panel testing should be standard of care [21,23].

Concerning preoperative imaging stating, recent international guidelines include expert transvaginal or transrectal ultrasound or pelvic MRI as pre-operative mandatory work-up for the management of patients with EC [15]. These imaging techniques are advocated as a routine procedure in the preoperative assessment of EC in order to estimate tumor local extension and identify patients with endocervical cancer or synchronous ovarian cancer. Developments in this area will be reviewed throughout this chapter.

1.3. METHODOLOGIES FOR PRE AND INTRAOPERATIVE STAGING: TRANSVAGINAL ULTRASOUND, PELVIC MRI AND FROZEN SECTION

1.3.1. TVUS – GENERAL CONCEPT

Ultrasound (US) is usually the first exam performed in women with history of abnormal uterine bleeding. While in premenopausal patients the endometrium demonstrates a wide spectrum of appearances and thickness according to physiologic and hormonal changes, the normal postmenopausal endometrium should appear thin, homogeneous and echogenic [24].

Although there is some controversy regarding the most adequate cut-off value of endometrial thickness in postmenopausal women, most authors consider 5 mm the upper limit of normality. This cut-off value displays a sensitivity of 96% and a specificity of 61% in the diagnosis of EC in postmenopausal women with abnormal uterine bleeding [24].

Studies have largely assessed whether morphological features and vascular pattern on Doppler ultrasound can improve the diagnostic accuracy of EC. The International Endometrial Tumor Analysis (IETA) group in 2010 published a consensus on terminology, definitions and measurements on the endometrium [25]. The most commonly reported features of EC diagnosis, while applying IETA terminology, include heterogeneous echogenicity, irregular or undefined endometrial-myometrial junction and multiple multifocal vessel pattern and a moderate or high color score on Doppler evaluation [26].

1.3.2. TVUS – STAGING ROLE IN ENDOMETRIAL CARCINOMA

Several studies have been evaluating the role of transvaginal ultrasound (TVUS) on clinical staging, as it offers the possibility to assess the depth of myometrial infiltration and cervical stromal invasion [27]. Mascilini *et al.* compared subjective and objective assessment of these features, concluding that subjective evaluation performs at least as well as the objective measurement techniques [28]. Alcázar *et al.* used three dimensional (3D) virtual navigation to determine the myometrial infiltration with high sensitivity [29]. Clinical and ultrasound based models to predict EC have been proposed, presenting high area under the receiver-operating characteristics curve (AUC) and acceptable intra and inter observer agreement [30]. Further studies are needed to evaluate the suggested cut-offs, the predictive and prognostic value of each imaging feature and scoring systems as well as the reproducibility of objective measurement techniques.

1.3.3. ULTRASOUND TECHNIQUES FOR EVALUATION OF MYOMETRIAL INFILTRATION

A systematic review and meta-analysis reviewed the diagnostic accuracy of TVUS in preoperative detection of deep myometrial infiltration in patients with endometrial cancer, comparing subjective and objective methods, based on full text of 24 articles, reporting on 2773 patients between 1994 and 2014 [31]. All studies were prospective and selected according to PICOS (Patients, Intervention, Comparator, Outcomes, Study design) criteria; quality assessment was conducted by the QUADAS-2 tool. Overall pooled sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of TVUS for detecting deep myometrial infiltration were 82% (95% CI, 76-87%), 81% (95% CI, 76-85%), 4.3 (95% CI, 3.6-5.3) and 0.22 (95% CI, 0.16-0.30), respectively. The objective techniques applied for determining myometrial invasion were the Gordon's approach (ratio of the distance between endometrium-myometrium interface and maximum tumor depth to the total myometrial thickness) and Karlsson's approach (endometrial tumor thickness/anteroposterior uterine diameter ratio) [32,33]. Differences among the three methods in terms of diagnostic performance were not observed in this meta-analysis, yet significant heterogeneity between studies was found (I² range, 60.6–95.0). A potentially low reproducibility of the methods is a reasonable explanation for this high heterogeneity. Among test accuracy studies, threshold effect is one of the primary causes of heterogeneity, which occurs when different cut-offs are applied in different studies. Moreover, the main limitation pointed by the authors was that very few studies compared different approaches in the same set of patients [31].

In a prospective multicenter study including 144 women with endometrial cancer, Mascilini *et al.* [28] aimed to compare the diagnostic accuracy of subjective ultrasound assessment with that of objective measurement techniques in the evaluation of myometrial and cervical invasion. Subjective evaluation of myometrial and cervical invasion was performed, as well as the following objective measurements: endometrial thickness, tumor/uterine anteroposterior (AP) diameter ratio, minimal tumor-free margin, minimal tumor-free margin/uterine AP diameter ratio, tumor volume (three-dimensional [3D]) and tumor/uterine volume (3D) ratio. Tumor/uterine AP diameter (at cut-off, 0.53) demonstrated the best performance among all objective measurement techniques, with a sensitivity and specificity of 72% and 76%, respectively. These results did not significantly differ from those of subjective evaluation (sensitivity, 77 [p=0.44]; specificity, 81% [p=0.32]) for the prediction of deep myometrial invasion [28].

A prospective study evaluating 169 consecutive women with well or moderately differentiated endometrioid type endometrial carcinoma aimed to compare the diagnostic performance of six different ultrasound approaches for assessing myometrial infiltration [34]. Approaches for assessing myometrial infiltration included: 1) the subjective impression of examiner; 2) Karlsson's criteria; 3) endometrial thickness, using a cut-off of ≥ 18 mm for predicting $\geq 50\%$ myometrial infiltration as suggested by Mascilini *et al.* [28]; 4) tumor/uterine volume ratio; 5) shortest tumor distance to serosa (TDS), using a cut-off of < 9 mm for predicting $\geq 50\%$ myometrial infiltration; 6) Van Hoslbeke's subjective model, using the formula as proposed $z = -2.6276 + 1.1458 \times (\text{preoperative grading}) + 2.2514 \times (\text{subjective impression})$ and a cut-off of an estimated probability of ≥ 0.50 for predicting $\geq 50\%$ myometrial infiltration [35].

The subjective impression of examiner and subjective model performed similarly, displaying a sensitivity 79.5% and 80.5% and a specificity 89.6% and 90.3%, respectively. Both performed significantly better than Karlsson's criteria (sensitivity 31.8%, $p < 0.05$) and endometrial thickness (sensitivity 47.7%, $p < 0.05$), as well as tumor/uterine volume ratio (specificity 28.3%, $p < 0.05$) and TDS (specificity 41.5%, $p < 0.05$) [34].

Frühauf *et al.* evaluated 210 patients with histological proven endometrial carcinoma in a prospective study, aiming to compare the diagnostic accuracy of subjective *versus* objective techniques, the last one including Karlsson's and Gordon's ratios [36]. Subjective assessment was confirmed to be the most reliable method, showing a sensitivity, specificity and overall accuracy of 79.3%, 73.2% and 75.7%, respectively. Gordon's ratio at a cut-off 0.5 reached 69.6% sensitivity, 65.9% specificity, and 67.3% overall accuracy, while Karlsson's ratio with the same cut-off reached 56.3% sensitivity, 76.4% specificity, and 68.1% overall accuracy [36].

In 2020 Verbakel *et al.* compared the performance of ultrasound measurements and subjective ultrasound assessment in detecting deep myometrial invasion (MI) and cervical stromal invasion (CSI) in women with endometrial cancer, overall and according to whether they had low- or high-grade disease separately, comprising 1538 patients from the IETA-4 prospective multicenter study [37]. The sensitivity and specificity of subjective assessment for detecting deep MI were 70% and 80%, respectively, in patients with a Grade-1 or -2 endometrioid or mucinous tumor *versus* 76% and 64% in patients with a Grade-3 endometrioid or mucinous or a non-endometrioid tumor.

Tumor AP diameter and tumor/uterine AP diameter ratio showed the best performance for predicting deep MI (AUC of 0.76 and 0.77, respectively). However, when fixing sensitivity at the sensitivity level of subjective assessment (72.3%), tumor/uterine AP diameter ratio (at a cut-off ≥ 0.51) had lower specificity (68.9%, i.e. a difference of 7%) and tumor AP diameter (at a cut-off of ≥ 21 mm) had a lower specificity (70.1%, i.e. a difference of 6%) compared to subjective assessment [37].

1.3.4. ULTRASOUND TECHNIQUES FOR ASSESSMENT OF CERVICAL INVOLVEMENT

There are very few publications reporting the use of ultrasound to evaluate cervical stromal invasion. From the available data, the results are generally very good for subjective assessment, with sensitivities ranging from 77 to 93% and specificities ranging from 85 to 99% [38,39].

As previously mentioned, a prospective multicenter study [28] aimed to assess the diagnostic accuracy of subjective and objective ultrasound measurements in the evaluation of cervical invasion. The best objective parameter for the prediction of cervical invasion was the distance from the lower margin of the tumor to the outer cervical os (Dist-OCO), at a cut-off 20.5 mm. Dist-OCO had a non-significantly higher sensitivity compared with subjective evaluation (73% vs. 54%, $p=0.06$), but a significantly lower specificity (63% vs. 93%, $p<0.001$), and the AUC did not differ between these methods [28].

Verbakel *et al.*, in a study comprising 1538 patients for predicting cervical stromal invasion (CSI), subjective assessment had a sensitivity of 50.7% (95% CI, 42.4–59.0%) and a specificity of 93.3% (95% CI, 91.8–94.8%) in patients with a measurable tumor, as compared with 49.3% (95% CI, 41.4–57.3%) and 93.9% (95% CI, 92.6–95.1%) for the whole IETA-4 cohort. Although the Dist-OCO had the best performance for predicting CSI (AUC 0.72), when fixing sensitivity at the same level as that of subjective assessment (50.7%), Dist-OCO (at a cut-off of ≤ 18 mm) had a lower specificity (86.5%, i.e. a difference of –7%) than did subjective assessment [37].

1.3.5. THE ROLE OF THREE-DIMENSIONAL TRANSVAGINAL ULTRASOUND

Costas *et al.* performed a systematic review and meta-analysis in order to assess the diagnostic accuracy of three-dimensional transvaginal ultrasound subjective assessment in the preoperative evaluation of deep myometrial invasion, using definitive histology as the reference standard. Nine studies comprising a total of 581 patients were included, from which five with prospective and four with retrospective design (n varying between 20 and 113), and all studies analyzed myometrial invasion with “subjective impression method” [7]. The pooled estimated sensitivity, specificity, positive likelihood and negative likelihood ratio were 84% (95% CI, 73-90%), 82% (95% CI, 75-88%), 5 (95% CI, 3.1-7.1) and 0.20 (95% CI, 0.11-0.35), respectively [7].

Another recent systematic review and meta-analysis investigated the diagnostic accuracy of three-dimensional transvaginal ultrasound (3D-TVUS) and MRI for deep myometrial infiltration (DMI) and cervical invasion. Five studies comprising a total of 450 women were included, from which two of prospective and three of retrospective design (n varying between 20 and 182). The authors reported a pooled sensitivity, positive likelihood ratio and negative likelihood ratio for detecting DMI using 3D-TVS of 77% (95% CI, 66-85%), 4.57

and 0.31, respectively. The respective values on MRI were 80% (95% CI, 73-86%), 4.22 and 0.24. For detecting cervical invasion the pooled In diagnostic odds ratio was 3.11 (95% CI, 2.09-4.14) for 3D-TVS and 2.36 (95% CI, 0.90-3.83) for MRI. As concluded, 3D-TVUS demonstrated good diagnostic accuracy in terms of sensitivity and specificity for the evaluation of DMI and cervical invasion, with results comparable with those of MRI, thus confirming a potential role of 3D-TVUS in the preoperative staging and surgical planning [40].

1.3.6. INTER-OBSERVER AGREEMENT IN TVUS

A study aimed to assess inter-observer reproducibility among ultrasound experts and gynecologists in the prediction by transvaginal ultrasound of deep myometrial and cervical stromal invasion. Sonographic videoclips of the uterine corpus and cervix of 53 women with endometrial cancer were integrated into a digitalized survey and evaluated by nine ultrasound experts and nine gynecologists. Findings suggest that preoperative ultrasound staging in endometrial cancer is best performed by ultrasound experts: compared with gynecologists, ultrasound experts showed higher sensitivity, specificity and agreement with histopathology in the assessment of cervical stromal invasion (42% [95% CI, 31–53%] vs. 57% [95% CI, 45–68%], $p<0.01$; 83% [95% CI, 78–86%] vs. 87% [95% CI, 83–90%], $p=0.02$; and K 0.45 [95% CI, 0.40–0.49] vs. 0.58 [95% CI, 0.53–0.62], $p<0.001$, respectively) but not of deep myometrial invasion (73% [95% CI, 66–79%] vs. 73% [95% CI, 66–79%], $p=1.0$; 70% [95% CI, 65–75%] vs. 69% [95% CI, 63–74%], $p=0.68$; and K 0.48 [95% CI, 0.44–0.53] vs. 0.52 [95% CI, 0.48–0.57], $p=0.11$, respectively) [41].

1.3.7. TVUS VERSUS RMI PERFORMANCE

MRI is considered the most accurate imaging modality for preoperative assessment of myometrial and cervical invasion, as well as extra-uterine disease, due to its excellent soft-tissue contrast resolution. Sagittal T2-weighted image delineates the uterine anatomy and is a useful tool in the assessment of the depth of myometrial invasion.

However, some pitfalls include the uterine anatomy distorted by leiomyomas and adenomyosis, recent dilation and curettage, tumor haemorrhage and infiltration of the uterine cornu [42]. In these cases, the use of dynamic contrast-enhanced (DCE) images may improve the delineation of the tumor margins and therefore the assessment of the depth of myometrial invasion. Endometrial carcinoma typically presents as isointense to the myometrium on T1-weighted sequences and hypointense relative to the endometrial lining on T2-weighted sequences. On T1 weighted post-contrast images the tumoral lesion usually enhances less than normal myometrial, and more slowly on dynamic contrast [42]. DCE and T2-weighted images combined present an accuracy of 98% for assessing myometrial invasion [24]. Diffusion weighted imaging (DWI) is of particular value in patients who cannot receive intravenous contrast injection of gadolinium-based contrast agent and in the setting of concurrent adenomyosis [24]. On DWI these lesions typically demonstrate restriction diffusion, which translates as an area of high signal intensity on diffusion-weighted images and an area of hypo intensity on apparent diffusion coefficient (ADC) maps [43]. Regarding the assessment of cervical stromal invasion, T2

and dynamic contrast enhanced MR images are important tools, demonstrating a diagnostic accuracy of approximately 90% [24].

In brief, multiparametric MRI using a combination of T2-weighted sequences, DWI and multiphase DCE stands as the mainstay for imaging assessment of EC [44,45]. However, in the last decade a few studies investigated the competing role of TVUS in pre-operative staging of EC, which are mentioned below.

A prospective study with 74 consecutively diagnosed cases of endometrial carcinoma aimed to compare the accuracy of TVUS and magnetic resonance imaging MRI [39]. Both techniques performed equally well, with no statistically significant differences. In the assessment of myometrial infiltration, sensitivity, specificity, positive and negative predictive values, and overall diagnostic accuracy were evaluated, revealing values of 84%, 83%, 79%, 88% and 84%, respectively, for TVUS and 84%, 81%, 77%, 87% and 82% for MRI. Regarding the detection of cervical involvement, values of 93%, 92%, 72%, 98% and 92% were obtained for TVUS; and 79%, 87%, 58%, 95% and 85% for MRI [39].

Taking into account the good diagnostic performance of TVUS by expert regarding local staging of endometrial carcinoma and considering the high costs of MRI, the latter may be offered only to those in whom TVUS produces limited or poor-quality imaging, such as in cases of uterine pathology or obesity.

A recent prospective comparison of the diagnostic accuracies of TVUS and MRI consecutively included 51 women in endometrial cancer [46]. Ultrasound diagnosed more cases of deep myometrial invasion compared to MRI, however, with no statistical significance. The sensitivity and specificity of TVUS and MRI for myometrial assessment were respectively 86% vs. 77% and 66% vs. 76%. For the assessment of cervical involvement, both methods correctly diagnosed the same number of cases. The author then concluded similar diagnostic accuracy between methods [46].

Alcázar *et al.* published a systematic review and meta-analysis including the results of 8 articles, which compared the diagnostic accuracy of TVUS and MRI for detecting myometrial invasion [47]. Pooled estimated sensitivity and specificity for the assessment of deep myometrial infiltration were 75% (95% CI=67%-82%) and 82% (95% CI=75%-93%) for TVUS, and 83% (95% CI=76%-89%) and 82% (95% CI=72%-89%) for MRI, respectively, therefore reporting no statistical differences between methods ($p=0.314$) [47]. A significant heterogeneity for specificity was observed. In addition, some drawbacks of this systematic review must be pointed out, namely the small number of papers reported (eight prospective studies selected according to PICOS criteria, reporting on a total of 560 women, with a number of recruited patients varying from 14 to 177 cases), the timing of those papers varying from 1992 to 2013 and the use of different methodologies, which could explain the reported heterogeneity among the studies.

To our knowledge, there is no published systematic review and meta-analysis evaluating the diagnostic accuracy of two dimensional TVUS and MRI for detecting cervical invasion.

As previously exposed, a systematic review and meta-analysis compared the diagnostic accuracy of 3D-TVUS and MRI for DMI and cervical invasion [40]. In regard to the assessment of DMI, pooled sensitivity, positive likelihood

ratio and negative likelihood ratio were 77% (95% CI, 66–85%), 4.57 and 0.31, respectively using 3D-TVUS and 80% (95% CI, 73–86%), 4.22 and 0.24 for MRI. Bivariate metaregression showed a similar performance of 3D-TVUS and MRI ($p=0.80$) for the correct diagnosis of myometrial invasion. Respecting the detection of cervical invasion, the authors reported pooled diagnostic odds ratio of 3.11 (95% CI, 2.09–4.14) for 3D-TVUS and 2.36 (95% CI, 0.90–3.83) for MRI [40]. This work demonstrates good performance of 3D-TVUS, therefore reinforcing its value for preoperative staging and surgery planning in patients with endometrial cancer.

Regarding inter-observer agreement in MRI, a multicenter retrospective study aimed to perform a multi-reader evaluation using T2-weighted, DWI and DCE sequences to identify the most accurate and its reliability for the best protocol. MRI sequences were independently evaluated by four radiologists (two senior and two novice) to identify deep myometrial invasion, in a total of 92 patients. The performance of the readers did not show significant differences among DWI, DCE and the entire protocol, even though the latter ensures the highest reliability particularly for expert readers (82.6%). The highest inter-observer agreement was obtained with the entire protocol by expert readers (intraclass correlation coefficient = 0.77) [48].

1.3.8. TVUS VERSUS FROZEN SECTION PERFORMANCE

Recent ESGO/ESTRO/ESP guidelines do not recommend the use of intraoperative frozen section for the assessment of myometrial invasion due to its poor reproducibility and interference with adequate pathological processing [15].

A few studies investigated the role of intraoperative frozen section and evaluated inter-observer agreement.

A retrospective study published in 2001 evaluated the records of 460 patients with uterine cancer in order to assess the accuracy of intra-operative frozen section (FS) at identifying the features of high-risk uterine disease. Inter-observer reliability was also determined. Tumor grade and depth of myometrial invasion were accurately reported, respectively, in 88.6% (expected 61.5%, K 0.70) and 94.7% (expected 53.8%, K 0.89), and error resulting in sub-optimal surgical management occurred in 5.3% of cases [49].

Recently, a retrospective study aimed to assess the role of intraoperative FS in guiding decision making for surgical staging of endometrioid endometrial carcinoma, based on the evaluation of 112 patients [50]. The concordance rate of different variables between FS and permanent section was 100%, 89.3% (100/112), 97.3% (109/112), and 95.5% (107/112), regarding to histological subtype, grade, myometrial invasion, and tumor size respectively. Diagnostic accurate rate of combined criteria intraoperatively guiding the decision for surgical staging was 95.5% (107/112); the discordance rate of all was 4.5%, resulting 3 cases (2.7%) of undertreatment and 2 cases (1.8%) of overtreatment [50].

A systematic review and meta-analysis published in 2016 compared diagnostic performance of intraoperative gross evaluation and intraoperative FS for the assessment of DMI, in a total of 35 studies and 6387 patients, from which 17 prospective and 18 retrospective design (n varying between 63 and 816 patients)

[51]. A pooled sensitivity and specificity of 71% and 91% for gross evaluation and of 85% and 97% for FS were found, and both sensitivity ($p=0.0008$) and specificity ($p=0.0021$) were significantly higher for FS [51].

The author of this thesis has produced work regarding this subject, which was presented in the 2017 ESGO Vienna Congress, 20th International Meeting of the European Society of Gynaecological Oncology in Vienna, Austria, November 4-7, 2017, on two abstracts presented as posters, and published as abstract in *Int J Gynecol Cancer*, Vol 27, Suppl 4, Epub 2017 [Supplementary Material – Abstract I and II].

The first, ***Uterine cancer - the importance of histopathological agreement: from endometrial biopsy, through frozen section towards definitive histology***, was a retrospective longitudinal study of all uterine *corpus* malignancies diagnosis/suspicion in a tertiary university centre from January 2007 to March 2017, which aimed to evaluate the histopathological agreement between endometrial sampling (ES), frozen section (FS) and permanent section (PS). A total of 215 cases of uterine *corpus* malignancies were included, from which 104 performed both ES and FS, 171 ES and PS and 113 FS and PS. Among those with final histopathological exam, 84% were epithelial, 9% mesenchymal, 5% miscellaneous and 1% benign and mixed tumours. Concerning the histologic type, the PA between ES and FS was 70% for total agreement and 78% for partial agreement. The majority of discordant cases were due to endometrioid tumours, revealed as complex atypical hyperplasia in ES. A PA of 72% was achieved for total or partial agreement between ES and PS. Regarding agreement between FS and PS a good agreement was found, with a PA of 88% for total or partial concordance. Agreement in tumour grade was moderate between the three groups ($K=0.47, 0.43, 0.43$). In conclusion, a higher agreement was found between FS and PS, compared to ES. Discordance was greater among high risk epithelial, mixed and mesenchymal tumors, suggesting a more challenging diagnosis [Supplementary Material – Abstract I].

The second, entitled ***The role of frozen section in uterine cancer staging - evaluating its accuracy***, was a retrospective chart analysis from January 2007 to March 2017 of all uterine *corpus* malignancies diagnosed in a tertiary university hospital and aimed to determine the accuracy of FS in surgical staging and agreement with permanent section (PS). A total of 205 cases were identified. Regarding myometrial depth invasion, substantial agreement between FS and PS was found ($K=0.63$), which was higher for tumours confined to the inner half of myometrium ($PA=0.92$) and lower for serosa invasion ($PA=0.33$). The sensitivity and specificity for involvement of the outer half was 74% and 99%. For cervical stromal involvement, a sensitivity and specificity of 65% and 99% was found. In conclusion, particularly in low-risk disease and epithelial tumours, comprehensive surgical staging with FS may be an option when other logistical issues are taken into account [Supplementary Material – Abstract II].

A limited body of research has investigated if preoperative methods, namely MRI, can safely replace intraoperative frozen sectioning in the local staging of EC.

Many studies still report slightly better performance of FS for predicting tumor grade (true positive rates of ADC values and FS of 73.3% vs. 66.7%, $p=0.7$; true negative rates of 64.5% vs. 98.7%, $p=0.01$; K of 0.23 and 0.73, respectively) [52] and DMI (diagnostic accuracy of 78.8% for MRI vs. 81.5% for FS, concordance $k=0.54$,

$p < 0.00001$) [53] and with higher agreement (sensitivity, specificity, positive predictive value, and negative predictive value for DMI on MRI were 57.8%, 92.0%, 69.3%, and 87.5%, with a K of 0.53 vs. 66.7%, 97.9%, 90.9%, and 90.4%, with a K of 0.71 on FS) [54, 55].

However, some studies consider that both methods are highly accurate (accuracy rates of 88.7% for MRI versus 94.4% in FS, with no statistical differences, $p = 0.057$) [56] and that frozen-section analysis can be avoided if the preoperative MRI study includes DWI sequences and ADC maps [57].

There is scarce evidence comparing TVUS with FS. A comparative prospective study including 64 women evaluated DMI by TVUS, MRI, and FS, with no statistically significant differences in overall diagnostic performance for the preoperative and intraoperative assessment of myometrial invasion, although with a slightly better performance for FS (overall accuracy of 78%, 81% and 91%, and Cohen Kappa of 0.534, 0.597 and 0.776, respectively for TVUS, MRI and FS) [58]. This research group has also published on the evaluation of cervical involvement in the same cohort [59], with similar conclusions: no statistically significant differences in overall diagnostic performance for the preoperative and intraoperative assessment of cervical involvement (accuracy rates of respectively 90.6%, 92.2% and 95.5% for TVS, MRI and FS).

Additionally, there is insufficient evidence and lack of prospective cost-analysis studies clarifying if one of the methods (MRI or frozen section) could be avoided or safely replaced by TVUS with equivalent diagnostic accuracy and cost-benefit.

Regarding this subject, a decision analysis model aiming to determine cost-effectiveness compared two surgical strategies: 1) routine lymphadenectomy independent of intraoperative risk factors or 2) selective lymphadenectomy for women with high or intermediate risk tumors based on intraoperative assessment on FS. The authors concluded that a strategy of selective lymphadenectomy based on intraoperative risk factors was less cost-effective than systematic lymphadenectomy even considering the impact of post-operative complications as lymphedema [60].

As a final remark, recent studies looking into precision pathology state that patient specimens processed in clinical routine may be subjected to factors that may alter their molecular quality and composition and skew data from molecular analyses, render analysis data uninterpretable, or even preclude analysis altogether if the integrity of a specimen is severely compromised [61]. Endometrial specimens are known to be particularly vulnerable to pre-analytical issues as hot and cold ischemia time and time to first processing step, which could impair the intraoperative and definite histopathological evaluation. A careful specimen handling and documentation will improve the accuracy of biomarker test results, which is crucial for the advancement of precision medicine.

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1.5. SUPPLEMENTARY MATERIAL – ABSTRACT I

ENDOMETRIAL CANCER

ESGO7-0927

UTERINE CANCER - THE IMPORTANCE OF HISTOPATHOLOGICAL AGREEMENT: FROM ENDOMETRIAL BIOPSY, THROUGH FROZEN SECTION TOWARDS DEFINITIVE HISTOLOGY

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Aims

Preoperative oncological uterine stratification based on endometrial sampling (ES) influences surgical planning and radically. The aim was to evaluate the histopathological agreement between ES, frozen section (FS) and permanent section (PS).

Method

Retrospective longitudinal study of all uterine corpus malignancies diagnosis/suspicion in a tertiary university centre from January 2007 to March 2017. Kappa (K) statistics and proportions of agreement (PA) with 95% confidence intervals (CI) were applied to evaluate agreement between pre, intra and postoperative histology, regarding histologic type, subtype and grade.

Results

A total of 215 cases of uterine corpus malignancies were included, from which 104 performed both ES and FS, 171 ES and PS and 113 FS and PS. Among those with final histopathological exam, 84% were epithelial, 9% mesenchymal, 5% miscellaneous and 1% benign and mixed tumours. Concerning the histologic type, the PA between ES and FS was 70% for total agreement and 8% for partial agreement. The majority of discordant cases were due to endometrioid tumours, revealed as complex atypical hyperplasia in ES. A PA of 72% was achieved for total or partial agreement between ES and PS. Regarding agreement between FS and PS a good agreement was found, with a PA of 88% for total or partial concordance. Agreement in tumour grade was moderate between the three groups (K= 0.47, 0.43, 0.43).

Conclusion

A higher agreement was found between FS and PS, compared to ES. Discordance was greater among high risk epithelial, mixed and mesenchymal tumors, suggesting a more challenging diagnosis.

1.6. SUPPLEMENTARY MATERIAL – ABSTRACT II

ENDOMETRIAL CANCER II**ESGO7-0912****THE ROLE OF FROZEN SECTION IN UTERINE CANCER STAGING - EVALUATING ITS ACCURACY***M. Reij¹, R. Portugal², C. Costa-Santos³, J. Bernardes⁴, A. Costa⁵**¹Faculdade de Medicina da Universidade do Porto- i3S Instituto de Inovação e Investigação em Saúde- University of Porto, Serviço de Ginecologia e Obstetria- Centro Hospitalar de São João- EPE Porto, Porto, Portugal**²Serviço de Anatomia Patológica- Centro Hospitalar de São João- EPE Porto,**Serviço de Anatomia Patológica- Centro Hospitalar de São João- EPE Porto, Porto, Portugal**³Department of Health Informatics and Decision Sciences- Medical School- University of Porto- Centre for Research in Health Information Systems and Technologies CINTESIS,**Department of Health Informatics and Decision Sciences- Medical School- University of Porto- Centre for Research in Health Information Systems and Technologies CINTESIS, Porto, Portugal**⁴Faculdade de Medicina da Universidade do Porto- i3S Instituto de Inovação e Investigação em Saúde- University of Porto- Department of Health Informatics and Decision Sciences- Medical School- University of Porto,**Faculdade de Medicina da Universidade do Porto- i3S Instituto de Inovação e Investigação em Saúde- University of Porto- Department of Health Informatics and Decision Sciences- Medical School- University of Porto, Porto, Portugal**⁵Serviço de Ginecologia e Obstetria- Centro Hospitalar de São João- EPE Porto Faculdade de Medicina da Universidade do Porto i3S Instituto de Inovação e Investigação em Saúde- University of Porto,**Serviço de Ginecologia e Obstetria- Centro Hospitalar de São João- EPE Porto Faculdade de Medicina da Universidade do Porto- i3S Instituto de Inovação e Investigação em Saúde- University of Porto, Porto, Portugal***Aims**

Surgical staging, treatment and prognosis of uterine corpus malignancies are highly dependent on disease severity and spread, factors that can be intraoperatively assessed by frozen section (FS). We aimed to determine the accuracy of FS in surgical staging and agreement with permanent section (PS); to evaluate the association between adverse prognostic intraoperative findings with final histopathology.

Method

Retrospective chart analysis from January 2007 to March 2017 of all uterine corpus malignancies diagnosed in a tertiary university hospital. Kappa (K) statistics and proportions of agreement (PA) with 95% confidence intervals were applied. Exclusion criteria were absence of FS, clinically advanced disease, coexisting second malignancy.

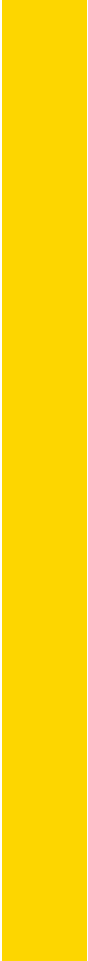
Results

A total of 205 cases were identified. Regarding myometrial depth invasion, substantial agreement between FS and PS was found (K= 0.63), which was higher for tumours confined to the inner half of myometrium (PA= 0.92) and lower for serosa invasion (PA= 0.33). The sensitivity and specificity for involvement of the outer half was 74% and 99%. For cervical stromal involvement, a sensitivity and specificity of 65% and 99% was found. Regarding adverse prognostic oncological factors: the rate of adjacent organ invasion was higher in mesenchymal tumours (50%); lymph node involvement was more frequent in serous (45%) and mixed cell adenocarcinoma (40%), followed by mullerian mixed tumours (33%). Positive ascitic fluid was more frequent in mixed (50%) and miscellaneous (33%) categories.

Conclusion

Particularly in low risk disease and epithelial tumours, comprehensive surgical staging with FS seems beneficial. For locally aggressive tumours, preoperative imagiological staging might have a promising role.

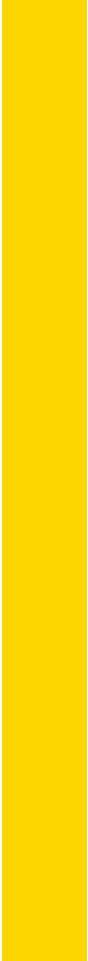




CHAPTER 2.

OBJECTIVES

1. To assess the role of clinical implementation of endovaginal/transrectal ultrasonography in the locoregional staging of endometrial carcinoma.
2. To define the accuracy of pelvic endovaginal/transrectal ultrasonography in the evaluation of myometrial invasion and cervical involvement, considering the final histology as the gold standard.
3. To compare the accuracy of different ultrasonographic approaches regarding the evaluation of myometrial invasion.
4. To compare the accuracy of different ultrasonographic approaches regarding the evaluation of cervical invasion.
5. To compare the accuracy of endovaginal/transrectal ultrasonography regarding the evaluation of myometrial and cervical invasion with other pre or intraoperative methods of locoregional staging (pelvic MRI and frozen section).
6. To establish agreement between preoperative methods of locoregional staging of endometrial carcinoma.



CHAPTER 3.

OUTLINE

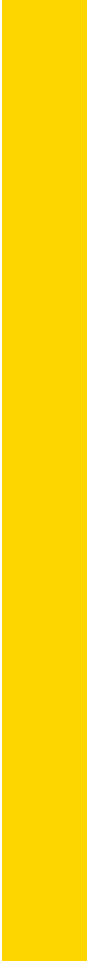
Following the **Introduction** and **Objectives** presented above respectively in **Chapters 1** and **2**, the main body of the thesis is organized in three chapters that describe the work performed for the dissertation.

A description of these chapters follows.

Chapter 4 is embodied by the paper entitled “*Endometrial cancer: Preoperative versus intraoperative staging*”, published in 2019 in the Journal of Gynecology Obstetrics and Human Reproduction. This is a retrospective longitudinal study, which aims to determine the accuracy of transvaginal ultrasonography (TVUS) and pelvic magnetic resonance imaging (MRI) compared to frozen section (FS) in the assessment of myometrial invasion, in a cohort of 187 cases of endometrial cancer.

Chapter 5 incorporates the article entitled “*Preoperative staging of uterine cancer: can transvaginal ultrasonography play a role?*”, published in 2023 in the journal Frontiers in Oncology. This was a prospective longitudinal multicentre trial between 2017 and 2018, which included the recruitment of eighty-two eligible patients. The main purpose of this study was to evaluate the validity of TVUS in preoperative staging of endometrial cancer compared to pelvic MRI and permanent section.

Finally, **Chapter 6** provides an overall discussion on the obtained results set by side with the state of the art and current literature. Each of the six objectives defined on chapter 2 will be fully explored and replied. These considerations will shed light into a conclusion. **Chapter 7** presents the author’s final remarks.



CHAPTER 4.

PAPER 1

Rei M, Rodrigues I, Condeço P, Igreja F, Veríssimo C, Mendinhos G. ***Endometrial cancer: Preoperative versus intraoperative staging*** [published online ahead of print, 2019 Nov 21]. J Gynecol Obstet Hum Reprod. 2019;101647. doi:10.1016/j.jogoh.2019.101647

Endometrial cancer: Preoperative versus intraoperative staging

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Keywords: endometrial cancer, staging, frozen section, ultrasonography, magnetic resonance

Reference:

Rei M, Rodrigues I, Condeço P, Igreja F, Veríssimo C, Mendinhos G. *Endometrial cancer: Preoperative versus intraoperative staging* [published online ahead of print, 2019 Nov 21]. J Gynecol Obstet Hum Reprod. 2019;101647. doi:10.1016/j.jogoh.2019.101647

4.1. ABSTRACT

Purpose: To determine the accuracy of transvaginal ultrasonography (TVS) and pelvic magnetic resonance imaging (MRI) in preoperative staging of endometrial cancer in comparison to frozen section (FS) for the assessment of myometrial invasion, considering permanent section as the gold standard.

Methods: A retrospective longitudinal study of all endometrial carcinomas diagnosed in our institution between March 2012 and October 2018 was conducted. Women with histologically confirmed endometrial malignancy, planned for surgery as primary treatment and submitted either to TVS, MRI and/or intraoperative FS followed by comprehensive surgical staging were eligible.

Results: From a total of 187 endometrial carcinomas, 156 were eligible for the study. The most frequent histology was endometrioid carcinoma (n=115), followed by serous carcinoma (n=25); the majority presented a FIGO stage IA (n=85) or IB (n=21). TVS, MRI and FS presented a sensitivity 56%, 71% and 67% (95% CI 0.35-0.75; 0.49-0.87; 0.45-0.84) and a specificity of 90%, 78% and 94% (95% CI 0.79-0.97; 0.58-0.91; 0.84-0.98), respectively. FS was the method with the lowest overestimation rate (6.5%, 95% CI 0.02-0.16), whereas MRI showed the lowest underestimation rate (29.2%, 95% CI 0.13-0.51). Agreement was superior between MRI and FS (Pa=0.79, K=0.56) and secondly between MRI and TVS (Pa=0.78, K=0.47).

Conclusions: Intraoperative FS presents the higher specificity and the lowest overestimation rate, while MRI seems to be the exam with the highest sensitivity in the evaluation of myometrial invasion. Agreement between the different methods is reasonable, suggesting that the best alternative will be highly dependent on the availability and experience of each institution.

4.2. INTRODUCTION

Endometrial carcinoma is the most common gynecologic malignancy diagnosed in developed countries, with an increasing incidence of 14.7 per 100.000 given to higher rates of metabolic syndrome and associated risk factors in the population ^[1]. Over 70% of cases of endometrial cancer are diagnosed at an early stage, when the reported 5-year survival rate is 90% ^[2]. Major prognostic features for endometrial carcinoma include FIGO (International Federation of Gynecology and Obstetrics) staging, myometrial infiltration, cervical involvement, histological type and differentiation grade ^[2]. Particularly, myometrial infiltration is associated with pelvic lymph-node dissemination and parametrium extension, which may alter both staging and therapeutic approach. The assessment of the depth of myometrial invasion will allow an adequate selection of low risk cases and avoid unnecessary lymphadenectomy and its associated morbidity.

Although classically endometrial cancer is a surgically staged disease, recent studies have recognized the role of preoperative imaging techniques as a reasonable alternative to frozen section (FS), with the potential

benefit of surgical planning. Pelvic magnetic resonance imaging (MRI) and expert transvaginal ultrasonography (TVS) have been recently adopted by international guidelines as an option for assessment of myometrial invasion, presenting an overall accuracy of respectively 82% and 74% [3-7]. The aim of this study is to evaluate the accuracy of TVS in the assessment of myometrial invasion in endometrial cancer, in comparison to pelvic MRI and FS, using final histology as the gold standard.

4.3. METHODS

A retrospective longitudinal study was conducted between March 2012 and October 2018 at our institution. Our sample was selected from women referred to the Gynecological Oncology consultation due to endometrial cancer suspicion. Women with histologically confirmed endometrial malignancy (by dilation and curettage, hysteroscopy or endometrial biopsy) or strong radiological suspicion, planned for surgery as primary treatment and undergoing either pelvic TVS, MRI and/or FS for staging purposes were eligible to participate in the study. Contraindication to surgery as primary treatment, emergency life-saving surgery not allowing pre-surgical diagnosis and staging were considered exclusion criteria. Myometrial invasion (<50% or ≥50%) was evaluated either by pelvic US (based on subjective and/or objective measurements), MRI and/or intraoperative FS by clinicians with substantial expertise in the gynecologic oncology field. Final histology of the specimen obtained by hysterectomy was considered the gold standard. Tumor classification, pathological and clinical staging were based on the recommendations of World Health Organization (WHO), International Union Against Cancer (TNM Classification of malignant tumors) and FIGO 2009 criteria respectively [8,9]. The protocol from the current study was approved by the institutional review board (N/Ref.a 2426/2018_MJHNO).

Statistical analysis

A descriptive analysis of the population was performed (**Table 1**). Sensitivity, specificity, positive and negative predictive values (PPV and NPV) as well as ROC (Receiver Operating Characteristics) curves assessing myometrial invasion were calculated for each staging method (TVS, MRI and FS) in comparison to the final histology. Agreement was assessed using proportions of agreement (Pa), and reliability with the Kappa statistic (K), the latter measuring agreement beyond what is expected by chance. If the lower limit of the 95% confidence intervals (CI) for the Pa is under 0.50, agreement will be considered poor. K statistic will be categorized in slight, fair, moderate agreement, substantial or perfect according to the value [6]. All calculations were performed using STATA version 14 (STATA Corp., Texas, USA) and p value below 0.05 were considered statistically significant.

4.4. RESULTS

From a total of 187 endometrial carcinomas, 156 were eligible for the study. The thirty-one excluded cases were either advanced stages not eligible for surgery as primary treatment, contraindication or refusal for surgery, lost to follow-up before surgical treatment, other tumors in final histology rather than endometrial cancer and absence of pre or intraoperative staging. The study population presented a mean age of 69 years (± 10). More than a half were obese (87/156) with a mean BMI of 31 kg/m² and 33% presented with diabetes *mellitus* (51/156) (**Table 1**). The most frequent histology was endometrioid carcinoma (n=115), followed by serous carcinoma (n=25); the majority presented in FIGO a stage IA (n=85) or IB (n=21).

Table 1. Population characteristics.

	N	mean (std) or n (%)
Age (mean $\pm$ standard deviation)	156	69 $\pm$ 10
BMI (mean $\pm$ std)	156	31 (7)
Histology n (%)	156	-
Endometrioid	-	115 (74)
Serous	-	25 (16)
Carcinossarcoma	-	8 (5)
Undifferentiated carcinoma	-	4 (3)
Mixed	-	4 (3)
FIGO Stage n (%)	156	-
IA	-	85 (55)
IB	-	21 (13)
II	-	15 (10)
III	-	30 (19)
IV	-	5 (3)

The sensitivity, specificity, PPV and NPV of each method in the evaluation of myometrial invasion are presented in **Table 2**. TVS presented the lowest sensitivity (56%, 95% CI 0.35-0.75) and the second highest specificity (90%, 95% CI 0.79-0.97), whereas MRI showed the highest sensibility (71%, 95% CI 0.49-0.87) and the lowest specificity (78%, 95% CI 0.58-0.91).

Table 2. Accuracy of TVS, MRI and FS in the evaluation of myometrial invasion.

	n	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]	AUC
TVS	78	0.56 [0.35-0.75]	0.90 [0.79-0.97]	0.75 [0.51-0.91]	0.79 [0.67-0.89]	0.73 [0.62-0.83]
MRI	51	0.71 [0.49-0.87]	0.78 [0.58-0.91]	0.74 [0.51-0.90]	0.75 [0.55-0.89]	0.74 [0.62-0.87]
FS	86	0.67 [0.45-0.84]	0.94 [0.84-0.98]	0.80 [0.56-0.94]	0.88 [0.78-0.95]	0.80 [0.70-0.90]

PPV: Positive predictive value; NPV: Negative Predictive Value; AUC: area under receiver–operating characteristics curve.

FS was the most specific method (94%, 95% CI 0.84-0.98) and presented the highest PPV, NPV and overall accuracy of 80%, 88% and 80%, respectively. In addition, it was the method with the lowest overestimation rate (6.5%, 95% CI 0.02-0.16); MRI showed the lowest underestimation rate (29.2%, 95% CI 0.13-0.51) but the highest false positive rate (22.2%, 95% CI 0.09-0.42) (Table 3).

Table 3. Over and underestimation rates in the assessment of myometrial invasion by method.

	n	Overestimation rate [95% CI]	Underestimation rate [95% CI]
TVS	78	9.8% [0.03-0.21]	44.4% [0.25-0.65]
MRI	51	22.2% [0.09-0.42]	29.2% [0.13-0.51]
FS	86	6.5% [0.02-0.16]	33.3% [0.16-0.55]

The highest agreement was found between MRI and FS (Pa=0.79, K=0.56). Regarding both preoperative methods, a moderate agreement was found (Pa=0.78, K=0.47), with the highest agreement verified between cases with no invasion of the outer half of myometrium. A fair agreement was found between TVS and frozen section (Pa=0.74, K=0.35) (Table 4).

Table 4. Agreement in myometrial invasion between TVS, MRI and FS measured by PA and Kappa statistics.

	n	PA	K	Std error
TVS vs. MRI	27	0.78	0.47	0,189
TVS vs. FS	42	0.74	0.35	0,152
MRI vs. FS	24	0.79	0.56	0,184

4.5. DISCUSSION

In our study, TVS, MRI and FS presented a sensitivity of 56%, 71% and 67% and a specificity of 90%, 78% and 94%, respectively. FS was the method with the lowest overestimation rate (6.5%), whereas MRI revealed the lowest underestimation rate (29.2%). We found a moderate agreement between MRI and FS and between both preoperative methods, and fair values between US and FS.

Currently, the role of MRI as a non-invasive technique for preoperative local staging of corpus uteri malignancies is well established. Previous studies found an overall accuracy in the assessment of myometrial invasion with MRI of 66–82% [10,11] and recent meta-analysis showed pooled sensitivities and specificities ranging from 81 to 90% and from 82 to 89%, respectively [12,13].

Regarding the assessment of myometrial infiltration by TVS, a recent meta-analysis reported an overall pooled sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of 82% (95% CI 76–87%), 81% (95% CI, 76–85%), 4.3 (95% CI 3.6– 5.3) and 0.22 (95% CI 0.16–0.30), respectively [5]. Similarly, a prospective study with a cohort of 210 patients evaluated the diagnostic accuracy of subjective and objective ultrasound evaluation of myometrial invasion, reporting a sensitivity of 56.3–79.3% and a specificity of 65.9–76.4%, with higher values for subjective assessment [6]. In our series, TVS was the method with the lowest sensitivity and the highest underestimation rate (44%). We should take into account that a large amount of TVS was performed before a strong malignancy suspicion or histologic confirmation by endometrial biopsy. Other identified pitfalls include the retrospective nature of the study design; the lack of protocol for preoperative assessment of endometrial carcinoma, with large heterogeneity in the methodologies to assess depth of myometrial invasion (subjective *versus* objective Gordon or Karlsson approaches) and no technique standardization.

In the current study, FS showed the highest overall accuracy and the lowest false positive rate. Other reports have established intraoperative FS as a reliable diagnostic method to assess myometrial invasion. Stephan *et al.* evaluated the agreement rates between the intra-operative FS and the final paraffin sections in a cohort of 116 cases on endometrioid adenocarcinoma, reporting a 93% sensitivity, 99% specificity, 93% PPV, and 99% NPV, with a K of 0.918, showing an almost perfect agreement [14]. Another report retrospectively assessing 164 cases of endometrial cancer revealed a 93% overall accuracy of FS in predicting myometrial invasion with similar results for early stages [15]. Comparing the performance of the three methods, a prospective study found no statistically significant differences in overall diagnostic, although FS seemed to be slightly superior to the imaging techniques [16]. A recent cross-sectional study concluded that MRI and intraoperative FS have adequate diagnostic yields for determining myometrial invasion with a moderate degree of agreement, though intraoperative FS seems slightly better, with 81.5% diagnostic accuracy, 9.24% underestimation and 9.24% overestimation (*versus* 78.8%, 13.12% and 8.13% in the MRI respectively) [17]. The slightly better performance of the intraoperative FS might be related to a longer and higher level of expertise in our institution and worldwide. However, our data reveals similar AUC curves and high agreement between methods, suggesting a growing pathway of expertise of the preoperative counterparts.

Preoperative methods provide an opportunity for surgical planning, cost and time management in the operating department, since we could obviate the need for FS exam and preoperatively define the surgical approach and the need of a multidisciplinary team. Ultrasonography is a commonly available, safe, non-invasive and low-cost modality that stands as a reliable alternative to MRI, especially in medium and low-income countries where MRI is not promptly available and costs are an important issue. Although MRI is costly and time-consuming, it may have a role whenever highquality ultrasonography is not possible [18].

Therefore, TVS seems a promising preoperative method for the myometrial assessment in endometrial cancer. However, multicentric studies with prospective designs and standardized protocols are needed. While different study groups have been developing protocols for endometrial cancer staging with encouraging results, including subjective and objective techniques to determine myometrial invasion and cervical involvement, there is still a large heterogeneity among the published data. In addition, studies evaluating inter and intra-observer agreement as well as the impact of US training would be of great interest in order to better define the future role of TVS in endometrial cancer preoperative staging.

In conclusion, both preoperative and intraoperative methods seem to have adequate diagnostic yields for determining myometrial invasion in endometrial cancer, although intraoperative FS showed a slightly better performance. However, agreement between methods is reasonable, suggesting that the best alternative will be highly dependent on the availability and expertise of each institution.

4.6. AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mariana Rei and Inês Rodrigues. The first draft of the manuscript was written by Mariana Rei and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Gustavo Mendinhos supervised the project.

4.7. DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest. No funding sources supported this investigation.

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4.9. SUPPLEMENTARY MATERIALS

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Original Article

Endometrial cancer: Preoperative versus intraoperative staging[☆]

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ABSTRACT

Purpose: To determine the accuracy of transvaginal ultrasonography (TVS) and pelvic magnetic resonance imaging (MRI) in preoperative staging of endometrial cancer in comparison to frozen section (FS) for the assessment of myometrial invasion, considering permanent section as the gold standard.

Methods: A retrospective longitudinal study of all endometrial carcinomas diagnosed in our institution between March 2012 and October 2018 was conducted. Women with histologically confirmed endometrial malignancy, planned for surgery as primary treatment and submitted either to TVS, MRI and/or intraoperative FS followed by comprehensive surgical staging were eligible.

Results: From a total of 187 endometrial carcinomas, 156 were eligible for the study. The most frequent histology was endometrioid carcinoma (n = 115), followed by serous carcinoma (n = 25); the majority presented a FIGO stage IA (n = 85) or IB (n = 21). TVS, MRI and FS presented a sensitivity 56 %, 71 % and 67 % [95 %CI 0.35-0.75; 0.49-0.87; 0.45-0.84] and a specificity of 90 %, 78 % and 94 % [95 %CI 0.79-0.97; 0.58-0.91; 0.84-0.98], respectively. FS was the method with the lowest overestimation rate (6.5 %, 95 %CI 0.02-0.16), whereas MRI showed the lowest underestimation rate (29.2 %, 95 %CI 0.13-0.51). Agreement was superior between MRI and FS (Pa = 0.79, K = 0.56) and secondly between MRI and TVS (Pa = 0.78, K = 0.47).

Conclusions: Intraoperative FS presents the higher specificity and the lowest overestimation rate, while MRI seems to be the exam with the highest sensitivity in the evaluation of myometrial invasion. Agreement between the different methods is reasonable, suggesting that the best alternative will be highly dependent on the availability and experience of each institution.

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Introduction

Endometrial carcinoma is the most common gynecologic malignancy diagnosed in developed countries, with an increasing incidence of 14.7 per 100,000 given to higher rates of metabolic syndrome and associated risk factors in the population [1]. Over 70 % of cases of endometrial cancer are diagnosed at an early stage, when the reported 5-year survival rate is 90 % [2].

Major prognostic features for endometrial carcinoma include FIGO (International Federation of Gynecology and Obstetrics) staging, myometrial infiltration, cervical involvement, histological

type and differentiation grade [2]. Particularly, myometrial infiltration is associated with pelvic lymph-node dissemination and parametrium extension, which may alter both staging and therapeutic approach. The assessment of the depth of myometrial invasion will allow an adequate selection of low risk cases and avoid unnecessary lymphadenectomy and its associated morbidity.

Although classically endometrial cancer is a surgically staged disease, recent studies have recognized the role of preoperative imaging techniques as a reasonable alternative to frozen section (FS), with the potential benefit of surgical planning. Pelvic magnetic resonance imaging (MRI) and expert transvaginal ultrasonography (TVS) have been recently adopted by international guidelines as an option for assessment of myometrial invasion, presenting an overall accuracy of respectively 82 % and 74 % [3-7].

The aim of this study is to evaluate the accuracy of TVS in the assessment of myometrial invasion in endometrial cancer, in comparison to pelvic MRI and FS, using final histology as the gold standard.

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Methods

A retrospective longitudinal study was conducted between March 2012 and October 2018 at our institution. Our sample was selected from women referred to the Gynecological Oncology consultation due to endometrial cancer suspicion. Women with histologically confirmed endometrial malignancy (by dilation and curettage, hysteroscopy or endometrial biopsy) or strong radiological suspicion, planned for surgery as primary treatment and undergoing either pelvic TVS, MRI and/or FS for staging purposes were eligible to participate in the study. Contraindication to surgery as primary treatment, emergency life-saving surgery not allowing pre-surgical diagnosis and staging were considered exclusion criteria.

Myometrial invasion (<50 % or ≥50 %) was evaluated either by pelvic US (based on subjective and/or objective measurements), MRI and/or intraoperative FS by clinicians with substantial expertise in the gynecologic oncology field. Final histology of the specimen obtained by hysterectomy was considered the gold standard. Tumor classification, pathological and clinical staging were based on the recommendations of World Health Organization (WHO), International Union Against Cancer (TNM Classification of malignant tumors) and FIGO 2009 criteria respectively [8,9]. The protocol from the current study was approved by the institutional review board (N/Ref.ª 2426/2018_MJHNO).

Statistical analysis

A descriptive analysis of the population was performed (Table 1). Sensitivity, specificity, positive and negative predictive values (PPV and NPV) as well as ROC (Receiver Operating Characteristics) curves assessing myometrial invasion were calculated for each staging method (TVS, MRI and FS) in comparison to the final histology.

Agreement was assessed using proportions of agreement (Pa), and reliability with the kappa statistic (K), the latter measuring agreement beyond what is expected by chance. If the lower limit of the 95 % confidence intervals (CI) for the Pa is under 0.50, agreement will be considered poor. K statistic will be categorized in slight, fair, moderate agreement, substantial or perfect according to the value [6]. All calculations were performed using STATA version 14 (STATA Corp., Texas, USA) and p value below 0.05 were considered statistically significant.

Results

From a total of 187 endometrial carcinomas, 156 were eligible for the study. The thirty-one excluded cases were either advanced

stages not eligible for surgery as primary treatment, contraindication or refusal for surgery, lost to follow-up before surgical treatment, other tumors in final histology rather than endometrial cancer and absence of pre or intraoperative staging. The study population presented a mean age of 69 years (± 10). More than a half were obese (87/156) with a mean BMI of 31 kg/m² and 33 % presented with diabetes mellitus (51/156) (Table 1). The most frequent histology was endometrioid carcinoma (n = 115), followed by serous carcinoma (n = 25); the majority presented in FIGO a stage IA (n = 85) or IB (n = 21).

The sensitivity, specificity, PPV and NPV of each method in the evaluation of myometrial invasion are presented in Table 2. TVS presented the lowest sensitivity (56 %, 95 % CI 0.35-0.75) and the second highest specificity (90 %, 95 % CI 0.79-0.97), whereas MRI showed the highest sensibility (71 %, 95 % CI 0.49-0.87) and the lowest specificity (78 %, 95 % CI 0.58-0.91).

FS was the most specific method (94 %, 95 % CI 0.84-0.98) and presented the highest PPV, NPV and overall accuracy of 80 %, 88 % and 80 %, respectively. In addition, it was the method with the lowest overestimation rate (6.5 %, 95 % CI 0.02-0.16); MRI showed the lowest underestimation rate (29.2 %, 95 % CI 0.13-0.51) but the highest false positive rate (22.2 %, 95 % CI 0.09-0.42) (Table 3).

The highest agreement was found between MRI and FS (Pa = 0.79, K = 0.56). Regarding both preoperative methods, a moderate agreement was found (Pa = 0.78, K = 0.47), with the highest agreement verified between cases with no invasion of the outer half of myometrium. A fair agreement was found between TVS and frozen section (Pa = 0.74, K = 0.35) (Table 4).

Discussion

In our study, TVS, MRI and FS presented a sensitivity of 56 %, 71 % and 67 % and a specificity of 90 %, 78 % and 94 %, respectively. FS was the method with the lowest overestimation rate (6.5 %), whereas MRI revealed the lowest underestimation rate (29.2 %). We found a moderate agreement between MRI and FS and between both preoperative methods, and fair values between US and FS.

Currently, the role of MRI as a non-invasive technique for preoperative local staging of corpus uteri malignancies is well established. Previous studies found an overall accuracy in the assessment of myometrial invasion with MRI of 66–82 % [10,11] and recent meta-analysis showed pooled sensitivities and specificities ranging from 81 to 90 % and from 82 to 89 %, respectively [12,13].

Regarding the assessment of myometrial infiltration by TVS, a recent meta-analysis reported an overall pooled sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of 82 % (95 % CI 76–87 %), 81 % (95 % CI, 76–85 %), 4.3 (95 % CI 3.6–5.3) and 0.22 (95 % CI 0.16–0.30), respectively [5]. Similarly, a prospective study with a cohort of 210 patients evaluated the diagnostic accuracy of subjective and objective ultrasound evaluation of myometrial invasion, reporting a sensitivity of

Table 1
Population characteristics.

	N	mean (std) or n (%)
Age (mean ± standard deviation)	156	69 ± 10
BMI (mean ± std)	156	31 (7)
Histology n (%)	156	
Endometrioid		115 (74)
Serous		25 (16)
Carcinosarcoma		8 (5)
Undifferentiated carcinoma		4 (3)
Mixed		4 (3)
FIGO Stage n (%)	156	
IA		85 (55)
IB		21 (13)
II		15 (10)
III		30 (19)
IV		5 (3)

Table 2
Accuracy of TVS, MRI and FS in the evaluation of myometrial invasion.

	n	Sensitivity [95 % CI]	Specificity [95 % CI]	PPV [95 % CI]	NPV [95 % CI]	AUC
TVS	78	0,56 [0,35-0,75]	0,90 [0,79-0,97]	0,75 [0,51-0,91]	0,79 [0,67-0,89]	0,73 [0,62-0,83]
MRI	51	0,71 [0,49-0,87]	0,78 [0,58-0,91]	0,74 [0,51-0,90]	0,75 [0,55-0,89]	0,74 [0,62-0,87]
FS	86	0,67 [0,45-0,84]	0,94 [0,84-0,98]	0,80 [0,56-0,94]	0,88 [0,78-0,95]	0,80 [0,70-0,90]

PPV: Positive predictive value; NPV: Negative Predictive Value; AUC: area under receiver-operating characteristics curve.

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Table 3
Over and underestimation rates in the assessment of myometrial invasion by method.

	n	Overestimation rate [95 % CI]	Underestimation rate [95 % CI]
TVS	78	9.8 % [0.03-0.21]	44.4 % [0.25-0.65]
MRI	51	22.2 % [0.09-0.42]	29.2 % [0.13-0.51]
FS	86	6.5 % [0.02-0, [15]	33.3 % [0.16-0.55]

Table 4
Agreement in myometrial invasion between TVS, MRI and FS measured by PA and kappa statistics.

	n	PA	K	Std error
TVS vs MRI	27	0.78	0.47	0.189
TVS vs FS	42	0.74	0.35	0.152
MRI vs FS	24	0.79	0.56	0.184

PA: proportions of agreement; K: kappa statistics.

56.3–79.3% and a specificity of 65.9–76.4 %, with higher values for subjective assessment [6]. In our series, TVS was the method with the lowest sensitivity and the highest underestimation rate (44 %). We should take into account that a large amount of TVS was performed before a strong malignancy suspicion or histologic confirmation by endometrial biopsy. Other identified pitfalls include the retrospective nature of the study design; the lack of protocol for preoperative assessment of endometrial carcinoma, with large heterogeneity in the methodologies to assess depth of myometrial invasion (subjective versus objective Gordon or Karlsson approaches) and no technique standardization.

In the current study, FS showed the highest overall accuracy and the lowest false positive rate. Other reports have established intraoperative FS as a reliable diagnostic method to assess myometrial invasion. Stephan et al evaluated the agreement rates between the intra-operative FS and the final paraffin sections in a cohort of 116 cases on endometrioid adenocarcinoma, reporting a 93 % sensitivity, 99 % specificity, 93 % PPV, and 99 % NPV, with a kappa statistic of 0.918, showing an almost perfect agreement [14]. Another report retrospectively assessing 164 cases of endometrial cancer revealed a 93 % overall accuracy of FS in predicting myometrial invasion with similar results for early stages [15]. Comparing the performance of the three methods, a prospective study found no statistically significant differences in overall diagnostic, although FS seemed to be slightly superior to the imaging techniques [16]. A recent cross-sectional study concluded that MRI and intraoperative FS have adequate diagnostic yields for determining myometrial invasion with a moderate degree of agreement, though intraoperative FS seems slightly better, with 81.5 % diagnostic accuracy, 9.24 % underestimation and 9.24 % overestimation (versus 78.8 %, 13.12 % and 8.13 % in the MRI respectively) [17]. The slightly better performance of the intraoperative FS might be related to a longer and higher level of expertise in our institution and worldwide. However, our data reveals similar AUC curves and high agreement between methods, suggesting a growing pathway of expertise of the preoperative counterparts.

Preoperative methods provide an opportunity for surgical planning, cost and time management in the operating department, since we could obviate the need for FS exam and preoperatively define the surgical approach and the need of a multidisciplinary team. Ultrasonography is a commonly available, safe, non-invasive and low-cost modality that stands as a reliable alternative to MRI, especially in medium and low-income countries where MRI is not

promptly available and costs are an important issue. Although MRI is costly and time-consuming, it may have a role whenever high-quality ultrasonography is not possible [18].

Therefore, TVS seems a promising preoperative method for the myometrial assessment in endometrial cancer. However, multicentric studies with prospective designs and standardized protocols are needed. While different study groups have been developing protocols for endometrial cancer staging with encouraging results, including subjective and objective techniques to determine myometrial invasion and cervical involvement, there is still a large heterogeneity among the published data. In addition, studies evaluating inter and intra-observer agreement as well as the impact of US training would be of great interest in order to better define the future role of TVS in endometrial cancer preoperative staging.

In conclusion, both preoperative and intraoperative methods seem to have adequate diagnostic yields for determining myometrial invasion in endometrial cancer, although intraoperative FS showed a slightly better performance. However, agreement between methods is reasonable, suggesting that the best alternative will be highly dependent on the availability and expertise of each institution.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mariana Rei and Inês Rodrigues. The first draft of the manuscript was written by Mariana Rei and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Gustavo Mendinhos supervised the project.

Declaration of Competing Interest

The authors declare no conflicts of interest. No funding sources supported this investigation.

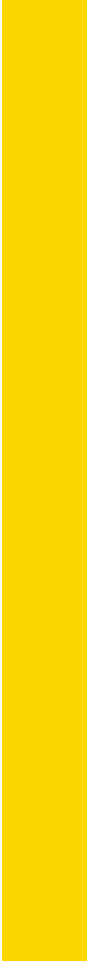
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CHAPTER 5.

PAPER 2

Rei M, Costa-Santos C, Bernardes J, Costa A.
***Preoperative staging of uterine cancer: can
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Preoperative staging of uterine cancer: can transvaginal ultrasonography play a role?

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5.1. ABSTRACT

Introduction: Preoperative staging of uterine cancer has recently been implied as an important contribution to an accurate selection of low-risk cases, ultimately avoiding unnecessary lymph node debulking. The aim of this study was to evaluate the validity of transvaginal ultrasonography (TVS) in preoperative staging of uterine cancer in comparison to pelvic magnetic resonance imaging (MRI) and permanent section.

Methods: We conducted a prospective longitudinal multicentre trial between 2017 and 2018. Inclusion criteria comprised cases of endometrial neoplasia histologically confirmed or strong imaging suspicion, candidates for elective surgery as primary treatment. Proportions of Agreement (PA), Kappa statistic (K), sensitivity, specificity and accuracy were calculated with 95% confidence intervals (95% CI).

Results: Eighty-two patients were eligible for the study, presenting a mean age of 68 years (standard deviation 11). In what concerns the TVS evaluation of myometrial invasion, the subjective and objective methods of Gordon and Karlsson presented a sensitivity of 79%, 79% and 67% (95% CI 63-91; 63-91; 50-81), a specificity of 65%, 58% and 79% (95% CI 49-79; 42-73; 64-89) and an overall accuracy of 72%, 68% and 73% (95% CI 61-81; 57-78; 63-82). MRI presented respectively a sensitivity, specificity and overall accuracy of 92%, 70% and 82% (95% CI 77-98; 52-85; 71-90). Regarding cervical involvement, the sensitivity was respectively 31%, 50% and 67% (95% CI 9-61; 21-79; 35-90) for the subjective method, objective TVS and MRI, and the specificity was 98%, 90% and 100% (95% CI 92-100; 77-97; 94; 100). Agreement between TVS and MRI was superior in the assessment of cervical invasion, with PA ranging from 0.82 to 0.93 and K from 0.45 to 0.58, in comparison to myometrial invasion with PA ranging from 0.68 to 0.73 and K from 0.31 to 0.50.

Considering the assessment of cervical involvement, as MRI showed a specificity of 100% it is not possible to increase the specificity. However, it was possible to increase the sensitivity, considering the combination of TVS with objective approach and MRI.

Conclusion: TVS may have a promising role as a tool for preoperative staging of endometrial carcinoma, presenting a performance that approximates to MRI, with a higher agreement in the assessment of cervical invasion.

5.2. INTRODUCTION

Endometrial carcinoma is the sixth most common malignancy diagnosed in women and the most common gynaecological cancer in high income countries, with 417.000 new diagnosis globally in 2020 ^[1] and a 5-year relative survival of 76% according to the EURO CARE-5 study ^[2].

Classically, endometrial cancer was a surgically staged disease, and therefore routine preoperative work-up to assess myometrial invasion or cervical involvement was often considered unnecessary. With the advent of

the sentinel node technique, lymph node biopsy is an acceptable alternative to systematic lymphadenectomy in stage I/II, therefore avoiding lymphadenectomy-related morbidity [3, 4].

Major prognostic features for endometrial carcinoma include grading, histotype, nodal metastasis and deep myometrial infiltration [5]. Particularly, myometrial infiltration and cervical involvement, which can be assessed by ultrasound, are key preoperative parameters, that might alter staging and surgical approach [6, 7].

As the frozen section is now discouraged for myometrial invasion assessment due to poor reproducibility, and sentinel node biopsy is increasingly used for lymph node staging in stage I/II endometrial cancer [4], the use of the most suitable preoperative work-up is a fundamental feature for surgical planning and correct inform consent.

Our study aimed to evaluate the validity of transvaginal ultrasonography (TVS) in preoperative staging of endometrial cancer, especially regarding myometrial and cervical stromal invasion, in comparison to the gold-standard of tumor staging which is final histology. We also aimed to determine the agreement between TVS and pelvic magnetic resonance imaging (MRI), the established standard of care in pre-surgical imaging staging.

5.3. MATERIALS AND METHODS

This is a prospective longitudinal multicentre trial conducted between October 2017 to December 2018 in three portuguese hospitals: Centro Hospitalar de São João, in Oporto, Instituto Português de Oncologia in Coimbra and Hospital Beatriz Ângelo, in Loures. The patient enrollment comprised women referred to gynecological oncology consultation in one of these three centers due to uterine cancer suspicion. Women with histologically confirmed endometrial malignancy (by dilation and curettage, hysteroscopy or endometrial biopsy) or strong imaging suspicion and planned surgery as primary treatment were eligible to participate in the study. Suspicion of uterine malignancy without histological confirmation, contraindication to undergo pelvic MRI, technical issues not allowing transvaginal or transrectal ultrasonography, contraindication to surgery as primary treatment, urgent or emergency life-saving surgery not allowing pre-surgical diagnosis and staging were considered exclusion criteria. The protocol and consent forms were approved by the institutional review board of each center, and all participants or their legally authorized representative provided written informed consent before enrollment.

Recruitment protocol

Recruitment for the trial and data collection was delivered as one-to-one consultation by the principal investigator.

The diagnostic features collected, while performing TVS, followed a designated protocol based on previous studies [6,8,9] which included: uterine and tumor dimensions and volume; endometrial thickness;

intracavitary fluid; tumor vascularization; regularity of the endometrial contour and junctional zone; myometrial invasion using the subjective approach (based on evaluation of disrupted endometrial/myometrial border and a subjective correlation of the width of myometrium along with the depth of tumour invasion) and the objective assessment by Gordon (the distance between the maximum tumour depth and the total myometrial thickness) and Karlsson methods (the maximum anteroposterior thickness of the endometrial lesion measured in the sagittal plane divided by the anteroposterior uterine diameter) [10,11]; cervical involvement (subjective assessment and objective method using a cut-off less than 20mm between the outer cervical orifice to the lower margin of the tumor) [6,12]; invasion of adjacent organs; ascites; other gynecological findings prone for altering the staging. MRI criteria were defined by the radiologist expert based on international consensus in endometrial cancer imaging staging [13].

TVS was either performed by a resident fellow (5 years of training) or a specialist (more than 5 years of expertise in gynecologic imaging). The equipment to employ was a GE Voluson E8 (GE Medical systems Austria) US system, equipped with RICS5-9 transducer, or equivalent device. Pelvic MRI was interpreted by a radiology specialist with more than 5 years of expertise in gynecologic imaging (one per center). All imaging exams were blinded for the remaining clinical and imaging data and systematically followed a predefined protocol, filled out by the investigator after each examination.

Comprehensive surgical staging comprises abdominopelvic washings, total hysterectomy, bilateral salpingo-oophorectomy and pelvic and para-aortic lymph node dissection according to intra-operative frozen section staging or histologic criteria in preoperative biopsy [14]. Histologic assessment following surgery was considered the gold standard. Post-operative staging was performed by a dedicated pathologist with expertise in gynecologic oncology, based on the most recent FIGO 2009 staging guidelines [15].

Statistical analysis

Sensitivity, specificity and overall accuracy were calculated, with ninety-five percent confidence intervals (95% CI) for each staging method (TVS or MRI), in comparison to the final histopathological exam, that was considered the gold standard. These calculations evaluated myometrium and cervical invasion once these are key features that might impact staging and different surgical approaches.

Agreement was assessed by overall and specific Proportions of Agreement (PA) from each category and reliability with the Kappa statistic (K). If the lower limit of the 95% CI for the PA is under 0.50, agreement will be considered poor [16]. PA for a specific category estimates the conditional probability, given that one of the methods makes a rating in that category, the other method will also do so. A K value lower than 0.20 was considered poor reliability; it was categorized by fair between 0.21 and 0.40, moderate between 0.41 and 0.60, substantial between 0.61 and 0.80 and excellent if higher than 0.80 [17].

5.4. RESULTS

Eighty-two patients were eligible for the study, presenting a mean age of 68 years (standard deviation [sd]=11): 88% postmenopausal (72/82), 66% with arterial hypertension (54/81), 57% obese (47/82), 26% diabetic (21/82) and 11% with high risk endometrial cancer mutations (9/82). The majority of endometrial cancers included in our study were of endometrioid histological type (79%, 65/82) of which 78% (51/65) were well to moderately differentiated (grade 1 or 2). Most malignancies were diagnosed at FIGO stage 1 or 2 (66/82, 80%) (Table 1).

Table 1. Population characteristics.

	N	mean (sd) or n (%)
Age mean (sd)	82	68 (11)
Age of menopause mean (sd)	72	50 (6)
Body mass index (kg/m ²) mean (sd)	79	32 (7)
Type 2 Diabetes <i>mellitus</i> n (%)	82	21 (26)
Arterial hypertension n (%)	81	54 (66)
BRCA or Lynch mutation n (%)	82	9 (11)
Histology n (%)	82	-
Endometrioid	-	65 (79.3)
Serous	-	9 (11.0)
Clear cells	-	1 (1.2)
Mixed	-	2 (2.4)
Undifferentiated	-	2 (2.4)
Carcinossarcoma	-	3 (3.7)
FIGO Stage n (%)	82	-
IA	-	35 (43)
IB	-	23 (28)
II	-	8 (10)
III	-	16 (19)

sd: standard deviation.

Regarding myometrial invasion, we found a sensitivity of 79%, 79% and 67% and a specificity of 65%, 58% and 79% for the subjective, Gordon and Karlsson approaches respectively. An overall accuracy of 72%, 68% and 73% was found respectively for the subjective, Gordon and Karlsson methods. Contrast-enhanced MRI provided a sensitivity, specificity and overall accuracy of 92%, 70% and 82% for myometrial invasion (**Table 2**).

Table 2. Sensitivity, specificity and accuracy of TVS and MRI in the evaluation of myometrial invasion.

Myometrial invasion	N	Sensitivity [95% CI]	Specificity [95% CI]	Accuracy [95% CI]
TVS Subjective method	82	0.79 [0.63; 0.91]	0.65 [0.49; 0.79]	0.72 [0.61; 0.81]
TVS Gordon method	82	0.79 [0.63; 0.91]	0.58 [0.42; 0.73]	0.68 [0.57; 0.78]
TVS Karlsson method	82	0.67 [0.50; 0.81]	0.79 [0.64; 0.89]	0.73 [0.63; 0.82]
MRI	70	0.92 [0.77; 0.98]	0.70 [0.52; 0.85]	0.82 [0.71; 0.90]

Considering the evaluation of cervical stromal invasion, we found a sensitivity and specificity of 31% and 98% for the ultrasound subjective approach and 50% and 90% for the objective method; overall accuracy reached respectively 88% and 81% for both methods. Contrast-enhanced MRI provided a sensitivity, specificity and overall accuracy of 67%, 100% and 94% for cervical involvement (**Table 3**).

Table 3. Sensitivity, specificity and accuracy of TVS and MRI in the evaluation of cervical invasion.

Cervical invasion	N	Sensitivity [95% CI]	Specificity [95% CI]	Accuracy [95% CI]
TVS Subjective method	82	0.31 [0.09; 0.61]	0.98 [0.92; 1.00]	0.88 [0.79; 0.94]
TVS Objective method	53	0.50 [0.21; 0.79]	0.90 [0.77; 0.97]	0.81 [0.68; 0.90]
MRI	70	0.67 [0.35; 0.90]	1.00 [0.94; 1.00]	0.94 [0.86; 0.98]

The reliability between MRI and TVS for the three ultrasound approaches was fair to moderate (K=0.31 to 0.50) in the evaluation of myometrial deep invasion and moderate (K=0.45 to 0.58) regarding cervical involvement. Agreement in the assessment of cervical invasion was better for “no” category than for “yes”. (**Table 4**).

Table 4. Reliability and agreement between TVS and MRI regarding myometrial deep invasion and cervical involvement.

Myometrial invasion	K [95% CI]	PA [95% CI]
TVS Karlsson method vs. MRI	0.50 [0.30; 0.70]	0.74 [0.63; 0.84]
Superficial	-	0.57 [0.41; 0.72]
Deep	-	0.61 [0.45; 0.74]
TVS Subjective method vs. MRI	0.43 [0.21; 0.65]	0.73 [0.61; 0.82]
Superficial	-	0.49 [0.32; 0.65]
Deep	-	0.63 [0.49; 0.76]
TVS Gordon method vs. MRI	0.31 [0.07; 0.54]	0.68 [0.55; 0.78]
Superficial	-	0.39 [0.24; 0.56]
Deep	-	0.59 [0.45; 0.72]
Cervical invasion		
TVS Subjective method vs. MRI	0.58 [0.22; 0.93]	0.93 [0.83; 0.97]
No	-	0.92 [0.82; 0.97]
Yes	-	0.44 [0.15; 0.77]
TVS Objective method vs. MRI	0.45 [0.10; 0.79]	0.82 [0.67; 0.91]
No	-	0.80 [0.64; 0.90]
Yes	-	0.38 [0.15; 0.68]
TVS Subjective vs. Objective method	0.62 [0.30; 0.94]	0.90 [0.78; 0.96]
No	-	0.89 [0.76; 0.96]
Yes	-	0.50 [0.20; 0.96]

While combining techniques, we found a sensitivity of 75%, 72% and 67% and a specificity of 82%, 82% and 88% for the subjective, Gordon and Karlsson approaches respectively for the evaluation of myometrial invasion, when these approaches were combined with MRI for a positive deep myometrial invasion. If at least one approach classifies as positive deep myometrial invasion, we found a sensitivity of 97%, 100% and 94% and a specificity of 50%, 44% and 65% for the subjective, Gordon and Karlsson approaches respectively when combined with MRI (Table 5).

Table 5. Sensitivity, specificity and accuracy of combination of TVS and MRI (“yes” if both approaches classify “yes” and “yes” if at least one approach classifies “yes”) in the evaluation of myometrial invasion.

Myometrial invasion	N	Sensitivity [95% CI]	Specificity [95% CI]	Accuracy [95% CI]
“yes” if both approaches classify “yes”				
TVS Subjective method and MRI	70	0.75 [0.58; 0.88]	0.82 [0.65; 0.93]	0.78 [0.67; 0.87]
TVS Gordon method and MRI	70	0.72 [0.55; 0.86]	0.82 [0.65; 0.93]	0.77 [0.66; 0.86]
TVS Karlsson method and MRI	70	0.67 [0.49; 0.81]	0.88 [0.72; 0.97]	0.77 [0.66; 0.86]
“yes” if at least one approach classifies “yes”				
TVS Subjective method and MRI	70	0.97 [0.85; 0.99]	0.50 [0.32; 0.67]	0.74 [0.62; 0.84]
TVS Gordon method and MRI	70	1.00 [0.90; 1.00]	0.44 [0.27; 0.62]	0.73 [0.61; 0.83]
TVS Karlsson method and MRI	70	0.94 [0.81; 0.99]	0.65 [0.46; 0.80]	0.80 [0.60; 0.89]

5.5. DISCUSSION

Imaging has been advocated in the preoperative management of patients with uterine corpus cancer [6]. The recently reviewed European guidelines for the management of patients with endometrial carcinoma include expert transvaginal or transrectal ultrasound or pelvic MRI in the pre-operative mandatory work-up [4].

Regarding myometrial invasion, in our study MRI presented the highest sensitivity for myometrial invasion assessment (92%). We found an overlapping sensitivity for the subjective and Gordon approach (79%); Karlsson approach displayed the lowest sensitivity (67%) but the highest specificity (79%). If both TVS and MRI preoperative exams classify as positive to deep myometrial invasion, we report a combined sensitivity of 75%, 72% and 67% and a specificity of 82%, 82% and 88% for the subjective, Gordon and Karlsson approaches respectively. If at least one approach classifies as positive deep invasion, we found a higher sensitivity (97%, 100% and 94%) and lower specificity (50%, 44% and 65%) for the subjective, Gordon and Karlsson approaches respectively when combined with MRI. Of notice, MRI displayed a higher overall accuracy compared to the ultrasonographic methods (82% vs. 68-73%).

A systematic review and meta-analysis of 24 studies reviewing the diagnostic accuracy of TVS in the preoperative detection of deep myometrial infiltration [18] revealed an overall pooled sensitivity and specificity of respectively 82% and 81%, with no statistical differences between subjective and objective methods.

Alcázar *et al.* conducted a trial comparing the diagnostic performance of six different approaches for assessing myometrial infiltration using transvaginal or transrectal ultrasound in women with grade 1 or 2 endometrioid carcinoma, including the impression of examiner, Karlsson’s criteria, endometrial thickness, tumor/uterine 3D volume ratio, tumor distance to myometrial serosa and van Holsbeke’s subjective

model. The impression of examiner and subjective model seemed to be the best approaches for assessing myometrial infiltration (sensitivity of 79.5% and 80.5% and specificity of 89.6% and 90.3% respectively), both with significantly better sensitivity than Karlsson's criteria (sensitivity 31.8%) (19). Frühauf *et al.* highlighted similar results, reporting a sensitivity, specificity and overall accuracy of respectively 79.3%, 73.2 and 75.7% for subjective assessment, 69.6%, 65.9% and 67.3% for Gordon's ratio and 56.3%, 76.4% and 68.1% for Karlsson's approach [20]. Our results reinforce these studies: the subjective approach yielded a high diagnostic overall accuracy (72%) and Karlsson's criteria performed poorer than subjective evaluation in terms of sensitivity (67% vs. 79%).

In a recent systematic review and meta-analysis, Alcázar *et al.* compared MRI and TVS for detecting myometrial infiltration in endometrial carcinoma, reporting a pooled estimated sensitivity and specificity of 75% and 82% for TVS, and 83% and 82% for MRI, respectively [21]. Our study found comparable accuracies for both preoperative methods.

Agreement regarding deep myometrial invasion between preoperative transvaginal ultrasound and intraoperative macroscopic examination in low-risk endometrioid carcinoma was evaluated in an observational study comprising 152 women; although the agreement between the two approaches was only moderate, both methods had similar accuracy when compared with frozen section histology, reinforcing the use of preoperative ultrasound by expert [22].

Concerning other factors affecting the preoperative staging, Fischerova *et al.* did not confirm the expected correlation between ultrasound failure and obesity, uterus position or the quality of ultrasound imaging [23]. A recent retrospective analysis indicates FIGO stage IB as the main significant confounding factor, with worse diagnostic accuracy in patients with concomitant benign uterine pathologies as diffuse fibromatosis and adenomyosis [24]. In regard to the use of 3D transvaginal ultrasound, it did not show improved diagnostic accuracy of myometrial infiltration in comparison to 2D ultrasound in a large multicentre study [25]. However, a recent systematic review reported that 3D-TVUS is at least comparable, in fact superior in one study, to MRI and mostly equivalent to 2D-TVUS, thus underlining a promising role [26].

A total of four examiners participated in the study, but due to a low number of exams performed by each physician it was not possible to understand slight differences in the overall diagnostic accuracy rate per physician and potential differences between experts and residents. Concerns regarding the reproducibility of the different measure techniques in real-time ultrasonography examinations remain. Ericsson *et al.* demonstrated a higher degree of agreement with histopathology and greater interobserver reproducibility in the assessment of cervical stromal invasion among ultrasonography experts, but not of deep myometrial invasion [27].

Regarding cervical stromal invasion, our data shows that MRI provided the highest specificity for cervical involvement (67%). The ultrasound subjective approach displayed a low sensitivity, but a high specificity (98%), slightly overcome by MRI; the objective methodology reached similar results for specificity, but with higher

sensitivity compared to the subjective approach. Overall accuracy was higher for MRI (94%) but reasonably similar to the subjective method (88%). Agreement between subjective and objective TVS methodologies was fair to moderate. As regard the agreement between MRI and TVS, subjective measurement performed slightly better comparing to the objective method. A previous study found that a cut-off less than 20mm from the outer cervical ostium to the lower margin of the tumor was correlated to the probability of cervical stromal invasion; however, subjective assessment seemed to perform significantly better [12].

The main drawback of our study is a small number of referred patients meeting the inclusion criteria (n=82), even though the recruitment has taken place in three centres with oncology care. The small sample size may jeopardize generalization of results and analysis of reproducibility. Additionally, the study may be underpowered to determine the accuracy of pre-surgical staging regarding cervical invasion, due to lack of patients with advanced disease and unsuitable for surgery. The number of enrolled patients, which did not perform MRI (12/82, 15%) is not negligible and must also be taken into account. Another identified pitfall was the impossibility of performing an inter-observer agreement study of ultrasound and MRI measurement techniques. Regarding the shortcomings of TVUS, the evaluation is examiner dependent and might be influenced by the equipment and patient profile. An additional drawback is the tumor profile itself: a large polypoid bulky endometrial cancer may lead to an overestimation on myometrial invasion due to the stretching effect on the surrounding myometrium, while a small uterus with a supposedly thin, hypoechoic or non defined endometrial stripe may be deeply infiltrative [6].

Strengths of our study include the prospective multicentre design and the fact that both gynecology and radiology experts performing imaging staging were blinded to the remaining results. Although the sample size remains below the expected for extracting robust evidence for clinical applicability and decision-making, still it is comparable to the current scientific papers published regarding this issue. The authors believe that a large multicentre trial with more controlled variables (namely technique expertise and patient confounders) will be largely beneficial, since it will allow an increase in the sample size as well as a better representation of the Portuguese population. Although this was not a trial conducted to evaluate reproducibility, it was interesting to understand the differences in applying the same protocol in different hospital settings around the country and performed by professionals with different levels of expertise. Moreover, a classical reproducibility study, implying the performance of TVS by different experts could raise issues of compliance and enrollment due to discomfort of a long examination, possibly compromising the final sample size. Still, the authors believe it would be important to address this matter in further studies.

Ultrasonography is a commonly available, non-invasive and low-cost modality, standing as a reliable alternative to MRI, especially in medium and low income countries where MRI is not promptly available and costs are an important issue. Additionally, its use will potentially impact surgical planning, cost and time management in the operating room, obviating the need for frozen section exam and allowing an accurate selection of low and intermediate risk cases eligible for sentinel lymph node technique. According to recent international guidelines, sentinel lymph node biopsy is an acceptable alternative to systematic

lymphadenectomy for lymph node staging even in stage I/II high-intermediate and high-risk disease [4].

In conclusion, pelvic ultrasound can play an important role in the preoperative staging of endometrial cancer, presenting a high sensitivity for the myometrial invasion assessment with a fair to moderate agreement with MRI and a better, although still moderate, agreement with MRI in the assessment of cervical invasion. However, further studies with larger sample sizes including several referral centers and assessing reproducibility are needed in order to reinforce our conclusions.

5.6. CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.7. AUTHOR CONTRIBUTIONS

Author 1 conceived and designed the analysis, collected the data and wrote the paper. Author 2 conceived and designed the analysis, contributed to data and analysis tools and performed the analysis. Author 3 and 4 performed a critical revision of the article for important intellectual content and approved the version to be published.

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5.11. SUPPLEMENTARY MATERIALS



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Preoperative staging of uterine cancer: can transvaginal ultrasonography play a role?

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Introduction: Preoperative staging of uterine cancer has recently been implied as an important contribution to an accurate selection of low-risk cases, ultimately avoiding unnecessary lymph node debulking. The aim of this study was to evaluate the validity of transvaginal ultrasonography (TVS) in preoperative staging of uterine cancer in comparison to pelvic magnetic resonance imaging (MRI) and permanent section.

Methods: We conducted a prospective longitudinal multicenter trial between 2017 and 2018. Inclusion criteria comprised cases of endometrial neoplasia histologically confirmed or strong imaging suspicion, candidates for elective surgery as primary treatment. Proportions of Agreement (PA), kappa statistic (K), sensitivity, specificity and accuracy were calculated with 95% confidence intervals (95%CI).

Results: Eighty-two patients were eligible for the study, presenting a mean age of 68 years (standard deviation 11). In what concerns the TVS evaluation of myometrial invasion, the subjective and objective methods of Gordon and Karlsson presented a sensitivity of 79%, 79% and 67% [95%CI 63-91; 63-91; 50-81], a specificity of 65%, 58% and 79% [95%CI 49-79; 42-73; 64-89] and an overall accuracy of 72%, 68% and 73% [95%CI 61-81; 57-78; 63-82]. MRI presented respectively a sensitivity, specificity and overall accuracy of 92%, 70% and 82% [95%CI 77-98; 52-85; 71-90]. Regarding cervical involvement, the sensitivity was respectively 31%, 50% and 67% [95%CI 9-61; 21-79; 35-90] for the subjective method, objective TVS and MRI, and the specificity was 98%, 90% and 100% [95%CI 92-100; 77-97; 94-100]. Agreement between TVS and MRI was superior in the assessment of cervical invasion, with PA ranging from 0.82 to 0.93 and K from 0.45 to 0.58, in comparison to myometrial invasion with PA ranging from 0.68 to 0.73 and K from 0.31 to 0.50. Considering the assessment of cervical involvement, as MRI showed a specificity of 100% it is not possible to increase

the specificity. However, it was possible to increase the sensitivity, considering the combination of TVS with objective approach and MRI.

Conclusion: TVS may have a promising role as a tool for preoperative staging of endometrial carcinoma, presenting a performance that approximates to MRI, with a higher agreement in the assessment of cervical invasion.

KEYWORDS

uterine cancer, endometrial neoplasia, pelvic ultrasound, myometrial invasion, cervical invasion, staging

1 Introduction

Endometrial carcinoma is the sixth most common malignancy diagnosed in women and the most common gynecological cancer in high income countries, with 417,000 new diagnosis globally in 2020 (1) and a 5-year relative survival of 76% according to the EUROCare-5 study (2).

Classically, endometrial cancer was a surgically staged disease, and therefore routine preoperative work-up to assess myometrial invasion or cervical involvement was often considered unnecessary. With the advent of the sentinel node technique, lymph node biopsy is an acceptable alternative to systematic lymphadenectomy in stage I/II, therefore avoiding lymphadenectomy-related morbidity (3, 4).

Major prognostic features for endometrial carcinoma include grading, histotype, nodal metastasis and deep myometrial infiltration (5). Particularly, myometrial infiltration and cervical involvement, which can be assessed by ultrasound, are key preoperative parameters, that might alter staging and surgical approach (6, 7).

As the frozen section is now discouraged for myometrial invasion assessment due to poor reproducibility, and sentinel node biopsy is increasingly used for lymph node staging in stage I/II endometrial cancer (4), the use of the most suitable preoperative work-up is a fundamental feature for surgical planning and correct inform consent.

Our study aimed to evaluate the validity of transvaginal ultrasonography (TVS) in preoperative staging of endometrial cancer, especially regarding myometrial and cervical stromal invasion, in comparison to the gold-standard of tumor staging which is final histology. We also aimed to determine the agreement between TVS and pelvic magnetic resonance imaging (MRI), the established standard of care in pre-surgical imaging staging.

2 Materials and methods

This is a prospective longitudinal multicenter trial conducted between October 2017 to December 2018 in three portuguese hospitals: Centro Hospitalar de São João, in Oporto, Instituto Português de Oncologia in Coimbra and Hospital Beatriz Ângelo, in Loures. The patient enrollment comprised women referred to

gynecological oncology consultation in one of these three centers due to uterine cancer suspicion. Women with histologically confirmed endometrial malignancy (by dilation and curettage, hysteroscopy or endometrial biopsy) or strong imaging suspicion and planned surgery as primary treatment were eligible to participate in the study. Suspicion of uterine malignancy without histological confirmation, contraindication to undergo pelvic MRI, technical issues not allowing transvaginal or transrectal ultrasonography, contraindication to surgery as primary treatment, urgent or emergency life-saving surgery not allowing pre-surgical diagnosis and staging were considered exclusion criteria. The protocol and consent forms were approved by the institutional review board of each center, and all participants or their legally authorized representative provided written informed consent before enrollment.

2.1 Recruitment protocol

Recruitment for the trial and data collection was delivered as one-to-one consultation by the principal investigator.

The diagnostic features collected, while performing TVS, followed a designated protocol based on previous studies (6, 8, 9) which included: uterine and tumor dimensions and volume; endometrial thickness; intracavitary fluid; tumor vascularization; regularity of the endometrial contour and junctional zone; myometrial invasion using the subjective approach (based on evaluation of disrupted endometrial/myometrial border and a subjective correlation of the width of myometrium along with the depth of tumour invasion) and the objective assessment by Gordon ((the distance between the maximum tumour depth and the total myometrial thickness) and Karlsson methods (the maximum anteroposterior thickness of the endometrial lesion measured in the sagittal plane divided by the anteroposterior uterine diameter) (10, 11); cervical involvement (subjective assessment and objective method using a cut-off less than 20mm between the outer cervical orifice to the lower margin of the tumor) (6, 12); invasion of adjacent organs; ascites; other gynecological findings prone for altering the staging. MRI criteria were defined by the radiologist expert based on international consensus in endometrial cancer imaging staging (13).

TVS was either performed by a resident fellow (5 years of training) or a specialist (more than 5 years of expertise in gynecologic imaging). The equipment to employ was a GE Voluson E8 (GE Medical systems Austria) US system, equipped with RICS5-9 transducer, or equivalent device. Pelvic MRI was interpreted by a radiology specialist with more than 5 years of expertise in gynecologic imaging (one per center). All imaging exams were blinded for the remaining clinical and imaging data and systematically followed a predefined protocol, filled out by the investigator after each examination.

Comprehensive surgical staging comprise abdominopelvic washings, total hysterectomy, bilateral salpingo-oophorectomy and pelvic and para-aortic lymph node dissection according to intra-operative frozen section staging or histologic criteria in preoperative biopsy (14). Histologic assessment following surgery was considered the gold standard. Post-operative staging was performed by a dedicated pathologist with expertise in gynecologic oncology, based on the most recent FIGO 2009 staging guidelines (15).

2.2 Statistical analysis

Sensitivity, specificity and overall accuracy were calculated, with ninety-five percent confidence intervals (95% CI) for each staging method (TVS or MRI), in comparison to the final histopathological exam, that was considered the gold standard. These calculations evaluated myometrium and cervical invasion once these are key features that might impact staging and different surgical approaches.

Agreement was assessed by overall and specific Proportions of Agreement (PA) from each category and reliability with the kappa statistic (K). If the lower limit of the 95% CI for the PA is under 0.50, agreement will be considered poor (16). PA for a specific category estimates the conditional probability, given that one of the methods makes a rating in that category, the other method will also do so. A K value lower than 0.20 was considered poor reliability; it was categorized by fair between 0.21 and 0.40, moderate between 0.41 and 0.60, substantial between 0.61 and 0.80 and excellent if higher than 0.80 (17).

3 Results

Eighty-two patients were eligible for the study, presenting a mean age of 68 years (standard deviation [sd] =11): 88% postmenopausal (72/82), 66% with arterial hypertension (54/81), 57% obese (47/82), 26% diabetic (21/82) and 11% with high risk endometrial cancer mutations (9/82). The majority of endometrial cancers included in our study were of endometrioid histological type (79%, 65/82) of which 78% (51/65) were well to moderately differentiated (grade 1 or 2). Most malignancies were diagnosed at FIGO stage 1 or 2 (66/82, 80%) (Table 1).

Regarding myometrial invasion, we found a sensitivity of 79%, 79% and 67% and a specificity of 65%, 58% and 79% for the subjective, Gordon and Karlsson approaches respectively. An

overall accuracy of 72%, 68% and 73% was found respectively for the subjective, Gordon and Karlsson methods. Contrast-enhanced MRI provided a sensitivity, specificity and overall accuracy of 92%, 70% and 82% for myometrial invasion (Table 2).

Considering the evaluation of cervical stromal invasion, we found a sensitivity and specificity of 31% and 98% for the ultrasound subjective approach and 50% and 90% for the objective method; overall accuracy reached respectively 88% and 81% for both methods. Contrast-enhanced MRI provided a sensitivity, specificity and overall accuracy of 67%, 100% and 94% for cervical involvement (Table 3).

The reliability between MRI and TVS for the three ultrasound approaches was fair to moderate (K= 0.31 to 0.50) in the evaluation of myometrial deep invasion and moderate (K= 0.45 to 0.58) regarding cervical involvement. Agreement in the assessment of cervical invasion was better for “no” category than for “yes” (Table 4).

While combining techniques, we found a sensitivity of 75%, 72% and 67% and a specificity of 82%, 82% and 88% for the subjective, Gordon and Karlsson approaches respectively for the evaluation of myometrial invasion, when these approaches were combined with MRI for a positive deep myometrial invasion. If at least one approach classifies as positive deep myometrial invasion, we found a sensitivity of 97%, 100% and 94% and a specificity of 50%, 44% and 65% for the subjective, Gordon and Karlsson approaches respectively when combined with MRI (Table 5).

TABLE 1 Population characteristics. sd: standard deviation.

	n	mean (sd) or n (%)
Age mean (sd)	82	68 (11)
Age of menopause mean (sd)	72	50 (6)
Body mass index (kg/m ²) mean (sd)	79	32 (7)
Type 2 Diabetes mellitus n (%)	82	21 (26)
Arterial hypertension n (%)	81	54 (66)
BRCA or Lynch mutation n (%)	82	9 (11)
Histology n (%)	82	
Endometrioid		65 (79.3)
Serous		9 (11.0)
Clear cells		1 (1.2)
Mixed		2 (2.4)
Undifferentiated		2 (2.4)
Carcinosarcoma		3 (3.7)
FIGO Stage n (%)	82	
IA		35 (43)
IB		23 (28)
II		8 (10)
III		16 (19)

TABLE 2 Sensitivity, specificity and accuracy of TVS and MRI in the evaluation of myometrial invasion.

Myometrial invasion	N	Sensitivity [95%CI]	Specificity [95%CI]	Accuracy [95%CI]
TVS Subjective method	82	0.79 [0.63; 0.91]	0.65 [0.49;0.79]	0.72 [0.61; 0.81]
TVS Gordon method	82	0.79 [0.63; 0.91]	0.58 [0.42; 0.73]	0.68 [0.57; 0.78]
TVS Karlsson method	82	0.67 [0.50; 0.81]	0.79 [0.64; 0.89]	0.73 [0.63; 0.82]
MRI	70	0.92 [0.77; 0.98]	0.70 [0.52; 0.85]	0.82 [0.71; 0.90]

4 Discussion

Imaging has been advocated in the preoperative management of patients with uterine corpus cancer (6). The recently reviewed European guidelines for the management of patients with endometrial carcinoma include expert transvaginal or transrectal ultrasound or pelvic MRI in the pre-operative mandatory work-up (4).

Regarding myometrial invasion, in our study MRI presented the highest sensitivity for myometrial invasion assessment (92%). We found an overlapping sensitivity for the subjective and Gordon approach (79%); Karlsson approach displayed the lowest sensitivity (67%) but the highest specificity (79%). If both TVS and MRI preoperative exams classify as positive to deep myometrial invasion, we report a combined sensitivity of 75%, 72% and 67% and a specificity of 82%, 82% and 88% for the subjective, Gordon and Karlsson approaches respectively. If at least one approach classifies as positive deep invasion, we found a higher sensitivity (97%, 100% and 94%) and lower specificity (50%, 44% and 65%) for the subjective, Gordon and Karlsson approaches respectively when combined with MRI. Of notice, MRI displayed a higher overall accuracy compared to the ultrasonographic methods (82% versus 68 – 73%).

A systematic review and meta-analysis of 24 studies reviewing the diagnostic accuracy of TVS in the preoperative detection of deep myometrial infiltration (18) revealed an overall pooled sensitivity and specificity of respectively 82% and 81%, with no statistical differences between subjective and objective methods.

Alcazar et al. conducted a trial comparing the diagnostic performance of six different approaches for assessing myometrial infiltration using transvaginal or transrectal ultrasound in women with grade 1 or 2 endometrioid carcinoma, including the impression of examiner, Karlsson's criteria, endometrial thickness, tumor/uterine 3D volume ratio, tumor distance to myometrial serosa and van Holsbeke's subjective model. The impression of examiner and subjective model seemed to be the best approaches

for assessing myometrial infiltration (sensitivity of 79.5% and 80.5% and specificity of 89.6% and 90.3% respectively), both with significantly better sensitivity than Karlsson's criteria (sensitivity 31.8%) (19). Frühauf et al. highlighted similar results, reporting a sensitivity, specificity and overall accuracy of respectively 79.3%, 73.2 and 75.7% for subjective assessment, 69.6%, 65.9% and 67.3% for Gordon's criterion and 56.3%, 76.4% and 68.1% for Karlsson's approach (20). Our results reinforce these studies: the subjective approach yielded a high diagnostic overall accuracy (72%) and Karlsson's criteria performed poorer than subjective evaluation in terms of sensitivity (67% vs 79%).

In a recent systematic review and meta-analysis, Alcazar et al. compared MRI and TVS for detecting myometrial infiltration in endometrial carcinoma, reporting a pooled estimated sensitivity and specificity of 75% and 82% for TVS, and 83% and 82% for MRI, respectively (21). Our study found comparable accuracies for both preoperative methods.

Agreement regarding deep myometrial invasion between preoperative transvaginal ultrasound and intraoperative macroscopic examination in low-risk endometrioid carcinoma was evaluated in an observational study comprising 152 women; although the agreement between the two approaches was only moderate, both methods had similar accuracy when compared with frozen section histology, reinforcing the use of preoperative ultrasound by expert (22).

Concerning other factors affecting the preoperative staging, Fischerova et al. did not confirm the expected correlation between ultrasound failure and obesity, uterus position or the quality of ultrasound imaging (23). A recent retrospective analysis indicates FIGO stage IB as the main significant confounding factor, with worse diagnostic accuracy in patients with concomitant benign uterine pathologies as diffuse fibromatosis and adenomyosis (24). In regard to the use of 3D transvaginal ultrasound, it did not show improved diagnostic accuracy of myometrial infiltration in comparison to 2D ultrasound in a large multicentre study (25).

TABLE 3 Sensitivity, specificity and accuracy of TVS and MRI in the evaluation of cervical invasion.

Cervical invasion	N	Sensitivity [95%CI]	Specificity [95%CI]	Accuracy [95%CI]
TVS Subjective method	82	0.31 [0.09; 0.61]	0.98 [0.92; 1.00]	0.88 [0.79; 0.94]
TVS Objective method	53	0.50 [0.21; 0.79]	0.90 [0.77; 0.97]	0.81 [0.68; 0.90]
MRI	70	0.67 [0.35;0.90]	1.00 [0.94; 1.00]	0.94 [0.86; 0.98]

TABLE 4 Reliability and agreement between TVS and MRI regarding myometrial deep invasion and cervical involvement.

Myometrial invasion	kappa [95%CI]	PA [95%CI]
TVS Karlsson method vs MRI	0.50 [0.30; 0.70]	0.74 [0.63; 0.84]
Superficial		0.57 [0.41; 0.72]
Deep		0.61 [0.45; 0.74]
TVS Subjective method vs MRI	0.43 [0.21; 0.65]	0.73 [0.61; 0.82]
Superficial		0.49 [0.32; 0.65]
Deep		0.63 [0.49; 0.76]
TVS Gordon method vs MRI	0.31 [0.07; 0.54]	0.68 [0.55; 0.78]
Superficial		0.39 [0.24; 0.56]
Deep		0.59 [0.45; 0.72]
Cervical invasion		
TVS Subjective method vs MRI	0.58 [0.22; 0.93]	0.93 [0.83; 0.97]
No		0.92 [0.82; 0.97]
Yes		0.44 [0.15; 0.77]
TVS Objective method vs MRI	0.45 [0.10; 0.79]	0.82 [0.67; 0.91]
No		0.80 [0.64; 0.90]
Yes		0.38 [0.15; 0.68]
TVS Subjective vs Objective method	0.62 [0.30; 0.94]	0.90 [0.78; 0.96]
No		0.89 [0.76; 0.96]
Yes		0.50 [0.20; 0.96]

However, a recent systematic review reported that 3D-TVUS is at least comparable, in fact superior in one study, to MRI and mostly equivalent to 2D-TVUS, thus underlining a promising role (26).

A total of four examiners participated in the study, but due to a low number of exams performed by each physician it was not possible to understand slight differences in the overall diagnostic accuracy rate per physician and potential differences between experts and residents. Concerns regarding the reproducibility

of the different measure techniques in real-time ultrasonography examinations remain. Ericsson et al. demonstrated a higher degree of agreement with histopathology and greater interobserver reproducibility in the assessment of cervical stromal invasion among ultrasonography experts, but not of deep myometrial invasion (27).

Regarding cervical stromal invasion, our data shows that MRI provided the highest specificity for cervical involvement (67%). The ultrasound subjective approach displayed a low sensitivity, but a high specificity (98%), slightly overcome by MRI; the objective methodology reached similar results for specificity, but with higher sensitivity compared to the subjective approach. Overall accuracy was higher for MRI (94%) but reasonably similar to the subjective method (88%). Agreement between subjective and objective TVS methodologies was fair to moderate. As regard the agreement between MRI and TVS, subjective measurement performed slightly better comparing to the objective method. A previous study found that a cut-off less than 20 mm from the outer cervical ostium to the lower margin of the tumor was correlated to the probability of cervical stromal invasion; however, subjective assessment seemed to perform significantly better (12).

The main drawback of our study is a small number of referred patients meeting the inclusion criteria (n=82), even though the recruitment has taken place in three centres with oncology care. The small sample size may jeopardize generalization of results and analysis of reproducibility. Additionally, the study may be underpowered to determine the accuracy of pre-surgical staging regarding cervical invasion, due to lack of patients with advanced diseased and unsuitable for surgery. The number of enrolled patients, which did not perform MRI (12/82, 15%) is not negligible and must also be taken into account. Another identified pitfall was the impossibility of performing an inter-observer agreement study of ultrasound and MRI measurement techniques. Regarding to the shortcomings of TVUS, the evaluation is examiner dependent and might be influenced by the equipment and patient profile. An additional drawback is the tumor profile itself: a large polypoid bulky endometrial cancer may lead to an overestimation on myometrial invasion due to the stretching effect on the surrounding myometrium, while a small uterus with a

TABLE 5 Sensitivity, specificity and accuracy of combination of TVS and MRI ("yes" if both approaches classify "yes" and "yes" if at least one approach classifies "yes") in the evaluation of myometrial invasion.

Myometrial invasion	N	Sensitivity [95%CI]	Specificity [95%CI]	Accuracy [95%CI]
"yes" if both approaches classify "yes"				
TVS Subjective method and MRI	70	0.75 [0.58; 0.88]	0.82 [0.65; 0.93]	0.78 [0.67; 0.87]
TVS Gordon method and MRI	70	0.72 [0.55; 0.86]	0.82 [0.65; 0.93]	0.77 [0.66; 0.86]
TVS Karlsson method and MRI	70	0.67 [0.49; 0.81]	0.88 [0.72; 0.97]	0.77 [0.66; 0.86]
"yes" if at least one approach classifies "yes"				
TVS Subjective method and MRI	70	0.97 [0.85; 0.99]	0.50 [0.32; 0.67]	0.74 [0.62; 0.84]
TVS Gordon method and MRI	70	1.00 [0.90; 1.00]	0.44 [0.27; 0.62]	0.73 [0.61; 0.83]
TVS Karlsson method and MRI	70	0.94 [0.81; 0.99]	0.65 [0.46; 0.80]	0.80 [0.60; 0.89]

supposedly thin, hypoechoic or non-defined endometrial stripe may be deeply infiltrative (6).

Strengths of our study include the prospective multicentre design and the fact that both gynecology and radiology experts performing imaging staging were blinded to the remaining results. Although the sample size remains below the expected for extracting robust evidence for clinical applicability and decision-making, still it is comparable to the current scientific papers published regarding this issue. The authors believe that a large multicentre trial with more controlled variables (namely technique expertise and patient confounders) will be largely beneficial, since it will allow an increase in the sample size as well as a better representation of the Portuguese population. Although this was not a trial conducted to evaluate reproducibility, it was interesting to understand the differences in applying the same protocol in different hospital settings around the country and performed by professionals with different levels of expertise. Moreover, a classical reproducibility study, implying the performance of TVS by different experts could raise issues of compliance and enrollment due to discomfort of a long examination, possibly compromising the final sample size. Still, the authors believe it would be important to address this matter in further studies.

Ultrasonography is a commonly available, non-invasive and low-cost modality, standing as a reliable alternative to MRI, especially in medium and low income countries where MRI is not promptly available and costs are an important issue. Additionally, its use will potentially impact surgical planning, cost and time management in the operating room, obviating the need for frozen section exam and allowing an accurate selection of low and intermediate risk cases eligible for sentinel lymph node technique. According to recent international guidelines, sentinel lymph node biopsy is an acceptable alternative to systematic lymphadenectomy for lymph node staging even in stage I/II high-intermediate and high-risk disease (4).

In conclusion, pelvic ultrasound can play an important role in the preoperative staging of endometrial cancer, presenting a high sensitivity for the myometrial invasion assessment with a fair to moderate agreement with MRI and a better, although still moderate, agreement with MRI in the assessment of cervical invasion. However, further studies with larger sample sizes including several referral centers and assessing reproducibility are needed in order to reinforce our conclusions.

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Data availability statement

The raw data supporting the conclusions of this article will be made available without undue reservation. Further inquiries can be directed to the corresponding author.

Author contributions

MR conceived and designed the analysis, collected the data and wrote the paper. CC-S conceived and designed the analysis, contributed to data and analysis tools and performed the analysis. JB and AC performed a critical revision of the article for important intellectual content and approved the version to be published. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5.12. SUPPLEMENTARY MATERIAL II – DATA COLLECTION TABLES

5.12.1. ULTRASOUND DATA



Protocolo de Ecografia Endovaginal: Neoplasias do Endométrio

Dimensão do útero em 3 planos (mm)	
Posição	antevertido / retrovertido / intermédio
Longitudinal x AP x Transverso	_____ x _____ x _____ mm
Volume	_____ mm ³
Comprimento colo	_____ mm
Dimensão da lesão em 3 planos (mm)	
Longitudinal x AP x Transverso	_____ x _____ x _____ mm
Volume	_____ mm ³
Localização da lesão	istmo, corpo, fundo parede anterior /posterior parede lateral direita / esquerda
Caracterização do endométrio	
Espessamento endometrial (mm)	_____ mm
Tipo de espessamento	local vs difuso
Ecogenecidade	• Homogéneo: hiperecogénico / isoecogénico / hipoeecogénico • Heterogéneo: hiperecogénica / isoecogénica / hipoeecogénica (porção dominante)
Líquido intracavitário	sim / não
Diâmetro AP (mm)	_____ mm
Ecogenecidade	anecóide / baixa ecogenecidade
Vascularização tumoral	
Score de fluxo (1 a 4)	1 - ausente 2 - mínimo 3 - moderado 4 - abundante
Padrão	• Origem: focal / multifocal • Vascularização dominante: única / múltipla • Tipo: Simples / ramificada / dispersa

Linha endometrial externa	linear / não linear/ irregular / não definida	
Junção endométrio-miometrial	regular / irregular / interrompida / não definida	
Avaliação <u>subjectiva</u> de invasão miometrial	superficial (< 50% parede miometrial) profunda (> 50% parede miometrial)	
Localização da invasão miometrial mais profunda	istmo, corpo, fundo parede anterior /posterior parede lateral direita / esquerda	
Avaliação <u>objectiva</u> de invasão miometrial		
Rácio diâmetro AP (mm) tumor / útero	_____, superficial (rácio > 0.5) / profunda (rácio < 0.5)	
Parede miometrial íntegra (mm)	_____ mm	
Margem mínima livre de lesão (mm)	_____ mm	
Avaliação <u>subjectiva</u> de invasão cervical	sem invasão vs. suspeita de invasão	
Distância ao OCE (mm)	_____ mm	
Avaliação da invasão local:		
• vagina	sim / não	
• anexos	sim / não	
• vesical	sim / não	
• rectal	sim / não	
Presença de Ascite	sim / não	
Volume	pequeno / moderado / grande	
Outros achados concomitantes:		
• Neoformações anexiais	sim / não, se sim _____	
• Leiomiomas uterinos	sim / não, se sim _____	
• Adenomiose	sim / não, se sim _____	
• Endometriose pélvica profunda	sim / não, se sim _____	
Implantes peritoneais pélvicos	sim / não	
Avaliação ganglionar pélvica	Direitos I Esquerdos	
Visualização	sim / não	sim / não
Caracterização	normais / reactivos	normais / reactivos
Dimensões	_____ x _____ mm	_____ x _____ mm

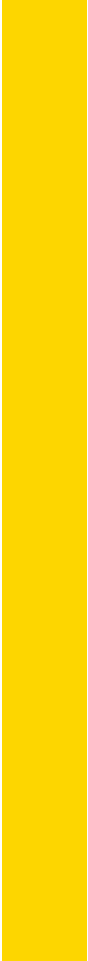
5.12.2. MRI DATA

Checklist para RMN pélvica

Participante: _____ ND: _____

Dimensão corpo uterino em 3 planos (mm)	
Longitudinal Antero-posterior Transverso Volume	
Dimensão da lesão em 3 planos (mm)	
Longitudinal Antero-posterior Transverso Volume	
Rácio do diâmetro anteroposterior tumoral/uterino	
Rácio do volume tumoral/uterino	
Espessamento endometrial (mm)	
Líquido intracavitário (sim/não)	
Invasão miometrial	
Superficial (<50% da espessura miometrial) Profunda (≥50% da espessura miometrial) Disrupção da serosa uterina	
Margem mínima livre de lesão (mm)	
Invasão do estroma do colo uterino	
Invasão de (sim/não) • vagina • anexos • paramétrios • ligamentos úterossagrados • vesical • rectal	
Presença de Ascite (sim/não) Volume (pequeno / moderado / grande)	
Evidência de doença peritoneal (sim/não)	
Metástases ganglionares (número): - Gânglios pélvicos ou de morfologia nodular ≥8mm de menor eixo - Gânglios inguinais > 15mm de menor eixo axial	





CHAPTER 6.

DISCUSSION AND CONCLUSIONS

6.1. GENERAL DISCUSSION

The **first objective** of this thesis was to assess the clinical benefit of the implementation of pelvic endovaginal/transrectal ultrasonography in the locoregional staging of endometrial carcinoma. This topic was widely address in the introduction, chapter one.

As we perceive new knowledge about the lack of therapeutic role of routine lymphadenectomy in endometrial cancer, which has been pointed by two randomized trials, the sentinel-node-biopsy (SLBN) emerges as an adequate approach to nodal assessment [1,2,3]. As preoperative work-up, pelvic endovaginal/transrectal ultrasonography by expert arises as a competitive tool in the locoregional staging of endometrial carcinoma, namely the assessment of myometrial and cervical invasion. These two essential criteria, in the past recognized as prognostic features, may impact surgical procedures (the need to perform systemic pelvic and paraaortic lymphadenectomy), maintain its key importance in the era of SLBN. As frozen section becomes obsolete and pelvic MRI is often inaccessible and cost and time consuming, pelvic ultrasonography as preoperative staging confirms disease confined to the uterus and allows to sort the low-risk cases from those requiring lymph node staging after SLBN procedures.

Although the ultrasound by expert has been acknowledged as preoperative staging tool in international guidelines [4], there is still a long way until oncologic units manage the learning curve, validate their preoperative staging protocol and become certified, ensuring at least a comparable accuracy to other available pre or intraoperative tools.

In the **second objective**, we aimed to define the accuracy of pelvic endovaginal/transrectal ultrasonography in the evaluation of myometrial invasion and cervical involvement, considering the final histology as the gold standard.

In **paper 1**, a retrospective longitudinal study evaluating 156 eligible cases of endometrial carcinoma, the aim of this study is to establish TVUS accuracy of at the assessment of myometrial invasion in endometrial cancer in comparison to pelvic MRI and FS, using final histology as the gold standard. TVUS demonstrated a sensitivity of 56% (0.35-0.75) and a specificity of 90% (0.79-0.97); the positive and negative predictive values were respectively 75% (0.51-0.91) and 79% (0.67-0.89); an area under receiver–operating characteristics curve of 0.73 (0.62-0.83) was reported.

Paper 2 was a prospective longitudinal multicentre trial aiming to evaluate the validity of transvaginal ultrasonography (TVUS) in preoperative staging of endometrial cancer in comparison to pelvic magnetic resonance imaging (MRI) and permanent section. In what concerns the TVUS evaluation of myometrial invasion, subjective and objective methods were evaluated individually. An overall accuracy of 72%, 68% and 73% was found respectively for the subjective, Gordon and Karlsson methods. The comparison between ultrasound methodologies will be further clarified.

A systematic review and meta-analysis of 24 studies aimed to determine the accuracy of TVUS in the assessment of myometrial infiltration and reported an overall pooled sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of 82% (95% CI 76–87%), 81% (95% CI, 76–85%), 4.3 (95% CI 3.6– 5.3) and 0.22 (95% CI 0.16–0.30), respectively [5]. Another systematic review and meta-analysis from Alcázar *et al.* outlined a pooled estimated sensitivity and specificity for diagnosing deep MI of 75% (95% CI=67%-82%) and 82% (95% CI=75%-93%) for TVUS [6].

The reported accuracies for the assessment of myometrial infiltration by TVUS in recent meta-analysis are slightly higher compared to the ones reported in paper 1 and 2. However, it must be highlighted that those meta-analysis included mostly studies performed in centers with great expertise in ultrasound protocols in gynecologic oncology. On the other hand, paper 1 was of retrospective nature and ultrasound was not carried out with the aim of performing a preoperative loco-regional staging; moreover, paper 2 was the first national multicentre study attempting the implementation of a preoperative protocol in endometrial cancer. Before this trial no Portuguese centre was performing ultrasound with this aim and there was no expertise in the field; the applied protocol was adapted from international publications. Therefore, it was mostly expected to perform least successfully in comparison to other centres.

The accuracy of ultrasound on the evaluation of stromal cervical involvement was only addressed in paper 2. A sensitivity of 31% and 50% was found respectively for the TVUS subjective and objective approaches; the specificity was respectively 98% and 90% and the overall accuracy reached respectively 88% and 81% for both methods.

There are very few studies evaluating the TVUS performance in cervical involvement. Likewise, there are less described methodologies in the literature. Mascillini *et al.* delivered that goal in a prospective multicenter study including 144 women with endometrial cancer [7]. In this study the authors report a sensitivity of 54% and 63% for the subjective and objective approaches; a specificity of 93% and 63% and an AUC of 74% and 75% respectively. The sensitivity values are low but comparable to those reported in paper 2, whereas our study provides better results for specificity and better performance expressed by a higher AUC.

In none of the papers it was possible to evaluate performance according to histology. As expected, in both retrospective and prospective studies the majority of cases were endometrioid histologies (paper 1 - 74%, n= 115/156; paper 2 - 79%, n= 65/82), precluding a feasible statistical analysis between histologic types.

The **third aim** was to compare the accuracy of different ultrasonographic approaches regarding the evaluation of myometrial invasion.

Our prospective multicentre study evaluated different ultrasound methodologies to assess deep myometrial tumoral invasion.

The subjective approach presented a sensitivity 79% (95% CI 0.63-0.91) and a specificity of 65% (95% CI 0.49-0.79) with an overall accuracy of 72% (95% CI 0.61-0.81). Our study protocol included two objective methodologies: Gordon and Karlsson approaches. These methods presented respectively a sensitivity 79% and 67% (95% CI 0.63-0.91; 0.50-0.81), a specificity of 58% and 79% (95% CI 0.42-0.73; 0.64-0.89) with an overall accuracy of 68% and 73% (95% CI 0.57-0.78; 0.63-0.82).

Alcázar *et al.* compared the diagnostic performance of six different approaches for assessing myometrial infiltration, including the impression of examiner, Karlsson's criteria, endometrial thickness, tumor/uterine 3D volume ratio, tumor distance to myometrial serosa (TDS) and van Holsbeke's subjective model in a cohort of 169 consecutive women with low grade endometrioid type endometrial carcinoma [8]. The results have shown similar performance between the impression of examiner and subjective model (sensitivity 79.5% and 80.5%; specificity 89.6% and 90.3%, respectively). The previous methods had significantly better sensitivity than Karlsson's criteria (31.8%, $p < 0.05$) and endometrial thickness (47.7%, $p < 0.05$), and higher specificity than tumor/uterine volume ratio (28.3%, $p < 0.05$) and TDS (41.5%, $p < 0.05$).

Likewise, in another study prospectively including 210 patients, subjective assessment was confirmed as the most reliable method to assess myometrial invasion (79.3% sensitivity, 73.2% specificity, 75.7% overall accuracy) [9]. Gordon's ratio reached 69.6% sensitivity, 65.9% specificity, and 67.3% overall accuracy, whereas Karlsson's ratio with the same 50% cut-off reached 56.3% sensitivity, 76.4% specificity, and 68.1% overall accuracy [9].

There are several considerations to take into account, while discussing these results.

Subjective ultrasound evaluation correlates the width of myometrium with the depth of tumor invasion, as well as doppler color score and vascular pattern.

Among the objective approaches, the most frequently reported in literature and therefore the ones selected for our prospective study were the Karlsson and Gordon methods.

The Karlsson approach is based on a ratio between anteroposterior endometrial tumor thickness and anteroposterior uterine diameter measured in the sagittal plane, with $\geq 50\%$ indicating the deep myometrial invasion. Gordon's approach is a ratio of the distance between endometrium myometrium interface and maximum tumor depth to the total myometrial thickness, with $\geq 50\%$ indicating the deep myometrial invasion.

The finding of absence of statistical differences in terms of diagnostic performance between subjective impression and both objective measurements is quite foreseeable: before taking measurements for objective assessment, subjective impression is firstly used to distinguish between normal healthy tissue and tumoral lesion.

According to Fisherova *et al.*, the accuracy of subjective assessment of myometrial and cervical invasion by ultrasound is significantly influenced by tumor size, density of tumor vascularization, tumor vessel architecture and histological grading, but not by body mass index, uterine position and image quality [10].

Tumor growth characteristics may hamper the subjective and objective evaluation of myometrial invasion. For instance, a polipoip exophytic tumor protruding into the cavity may lead to a high Karlsson ratio due to the distend of the cavity, but still there may be no infiltration of the endometrial myometrial interface, and thus this ratio may be deceitful. In opposition, the extension of infiltrative tumors with an isoechoic or hypoechoic echogenicity may be difficult to define. Other morphological patterns, as the microcystic, elongated, fragmented (MELF) pattern can be found in 7–48% of endometrioid histologies and may lead to tumor understaging, since the epithelial formations in the MELF pattern are often separated from the superficial, more easily identified invasive tumor from which they originated, thereby inducing an underestimation of the deepest extent of myometrial invasion [11].

Similarly, the presence of uterine pathology namely diffuse adenomyosis or leiomyomatosis and assymetries between myometrial walls may affect the aforementioned ratios, thus inducing an erroneous calculation of the myometrial infiltration.

Noneless, not only Karlsson method may be prone to deceivable results. Gordon's ratio presupposes the measurement of maximum tumour depth to the total myometrial thickness. The accurate identification of the endometrial-myometrial interface and the area of the deepest infiltration, as the correct measurement of the intact myometrial wall has to be accomplished in order to obtain a good performance.

In our prospective study, the overall accuracy between subjective approach and objective Karlsson method were appreciably similar (72% CI 95% 0.61-0.81 vs. 73% CI 95% 0.63-0.82). While the sensitivity of Gordon method overlaps the subjective evaluation (79%, CI 95% 0.63-0.91 for both methods) and exceeds the Karlsson assessment (67%, CI 95% 0.50-81), the specificity of the first is considerably lower (58%, CI 95% 0.42 - 0.63), jeopardizing overall accuracy.

As previously defined, the **fourth objective** was to compare the accuracy of different ultrasonographic approaches regarding the evaluation of cervical invasion. This analysis was exclusively carried out in the prospective study.

The retrospective nature of **paper 1** precluded the evaluation of this feature, given that only protocols of loco-regional staging include subjective evaluation and objective measurements of stromal cervical involvement.

In **paper 2**, considering the evaluation of cervical stromal invasion, overall accuracy reached respectively 88% and 81% for both subjective and objective TVUS methods. The TVUS subjective measurement showed a higher specificity (98%, 95% CI 0.92-1,00 vs. 90%, 95% CI 0.77-0.97) and better overall accuracy (88% [0.79-0.94] vs. 81% [0.68-0.90]). These findings are consistent with the results previously reported in the literature.

As previously reported by Mascilini *et al.*, the best objective parameter for the prediction of cervical invasion was the distance from outer cervical os to lower margin of tumor (Dist-OCO), showing a non-significantly higher

sensitivity compared with subjective evaluation (73% vs. 54%, $p=0.06$), but a significantly lower specificity (63% vs. 93%, $P<0.001$) [7].

This interesting finding is likely to be related to the dynamic nature of pelvic ultrasound. The TVUS subjective approach to evaluate stromal cervical invasion takes into account factors as doppler evaluation and bulging *versus* infiltration of the tumor, features that are highly dependent on a dynamic evaluation rather than a simple measurement. The movement of the endovaginal probe, described as uterine sliding sign, frequently used in the evaluation of deep endometriosis, may be a simple sonographic predictor for presence of deep infiltrating endometriosis of the rectovaginal septum [12]. A similar rationale in the evaluation of cervical tumoral infiltration may apply. The sliding movement of the endovaginal probe over the cervix will allow the protruding tumor to slide within the canal, but not the infiltrative. Conversely, the cervical stromal infiltration will display different features: absence of mobility within the canal, loss of clear demarcation of the endometrial lesion toward the cervical stroma and enhanced tumor perfusion.

In the **fifth objective**, we aimed to compare the accuracy of endovaginal/transrectal ultrasonography regarding the evaluation of myometrial and cervical invasion with other pre or intraoperative methods of locoregional staging (pelvic MRI and frozen section).

The author's retrospective longitudinal study included a total of 187 endometrial carcinomas in a Portuguese referral center, in order to determine the accuracy of TVUS and MRI in the diagnosis of deep myometrial invasion in comparison to frozen section FS. In this paper, TVUS, MRI and FS presented respectively a sensitivity of 56%, 71% and 67% (95% CI 0.35-0.75; 0.49-0.87; 0.45-0.84) and a specificity of 90%, 78% and 94% (95% CI 0.79-0.97; 0.58-0.91; 0.84-0.98), respectively.

FS was the most specific method and presented the highest PPV, NPV and overall accuracy (80%, 88% and 80%, respectively), thus yielding the lowest overestimation rate (6.5%, 95% CI 0.02-0.16). MRI showed the highest sensitivity and the lowest specificity, which warrants the lowest underestimation rate (29.2%, 95% CI 0.13-0.51), but the highest false positive rate (22.2%, 95% CI 0.09-0.42).

TVUS presented the lowest sensitivity. The limitations in the diagnostic accuracy of TVUS are warranted by the retrospective nature of the study. TVUS was often performed without a suspicion of endometrial neoplasia and protocols of local staging were only occasionally applied.

As previously reported, our prospective study showed an overall accuracy for deep myometrial invasion of 72%, 68% and 73% respectively for the subjective, Gordon and Karlsson methods (95% CI 0.61-0.81; 0.57-0.78; 0.63-0.82). In this study, contrast-enhanced MRI provided a sensitivity, specificity and overall accuracy of 92%, 70% and 82% for myometrial invasion (95% CI 0.77-0.98; 0.52-0.85; 0.71-0.90). MRI yielded a higher sensitivity and overall accuracy compared to any ultrasound methodologies.

Similarly, MRI revealed better performance in the assessment of cervical stromal invasion. A sensitivity, specificity and overall accuracy of 67%, 100% and 94% were reported (95% CI 0.35-0.90; 0.94-0.100; 0.86-0.98), consistently superior to the results obtained for either subjective and objective TVUS methods (respectively an overall accuracy of 88%, 95% CI 0.79-0.94, and 81%, 95% CI 0.68-0.90). Still, the limited number of cases with cervical involvement, FIGO stage II (n=8/82), must be outlined as an important drawback in the analysis.

There is a reasonable body of evidence in the literature clarifying the role of TVUS in local staging and reporting different ranges of accuracy in the assessment of deep myometrial and cervical invasion. The reported sensitivities (specificities) [accuracies] of TVUS for the detection of deep myometrial invasion range from 71 to 85% (72–90%) [72–84%] and cervical stromal invasion from 29–93% (92–94%) [78–92%] [5,8,10,13,14].

A systematic review and meta-analysis comparing TVUS *versus* MRI for preoperative assessment of myometrial infiltration in patients with endometrial cancer confirms this data [6]. A total of 8 articles and 560 patients were included in the final analyses. Pooled estimated sensitivity and specificity for deep myometrial invasion were respectively 75% (95% CI=67%-82%) and 82% (95% CI=75%-93%) for TVUS, and 83% (95% CI=76%-89%) and 82% (95% CI=72%-89%) for MRI. No statistical differences were found between methods (p=0.314) [6].

Likewise, the most recent systematic review and head-to-head meta-analysis on preoperative assessment of myometrial infiltration in patients with low-grade endometrioid endometrial cancer, comprising four articles and 577 patients, reports no statistical differences between both imaging techniques (p>0.05; pooled sensitivity and specificity of 65% and 85% for MRI and 71% and 76% for TVS, respectively), stating that the diagnostic performance of TVS and MRI for the evaluation of deep MI in women with low-grade endometrioid endometrial cancer is similar [15].

Despite of the lack of previous experience in local staging by ultrasound and the prospective implementation of a brand-new protocol in three different hospital settings, we can assume comparable accuracies for both methods and a successful accomplishment.

Similar diagnostic inaccuracy reflects the same limitations in both methods, revealing a comparable tendency to overestimate myometrial invasion and underestimate cervical stromal invasion.

Regarding intraoperative methods, Alcázar *et al.* published a meta-analysis comparing diagnostic performance of intraoperative gross evaluation (IGE) and intraoperative FS for the assessment of myometrial invasion in patients with endometrial cancer [16]. A total of 35 studies were included, and 6387 women were evaluated intraoperatively with any of the methods. Pooled sensitivity and specificity for IGE were 71% (95% CI 63%-77%) and 91% (95% CI 89%-93%), whereas it was respectively 85% (95% CI, 81%-88%) and 97% (95% CI, 96%-98%) for FS. The authors concluded that FS has better diagnostic performance than IGE for the intraoperative assessment of myometrial invasion [16].

Comparing the data from this metanalysis with the results obtained in paper 1, the lower pooled sensitivity of F (67% [95% CI 0.45-0.84]) can be easily elucidated. In the institution where the recruitment took place, in case

of low macroscopic suspicion of deep myometrial invasion the pathology team would occasionally perform only intraoperative gross evaluation rather than intraoperative frozen section. These results can be framed as a consequence of this clinical practice. The aim of our prospective study was to validate pre-operative but not intraoperative staging tools, thus the analysis did not include data on the performance of FS.

As the **final goal**, our objective was to establish agreement between preoperative methods of loco-regional staging of endometrial carcinoma.

Agreement analysis was performed in both publications and was calculated using proportions of agreement (Pa), and reliability with the K, the latter measuring agreement beyond what is expected by chance. If the lower limit of the 95% CI for the PA is under 0.50, agreement would be considered poor. The K values were categorized by poor if lower than 0.20, fair between 0.21 and 0.40, moderate between 0.41 and 0.60, substantial between 0.61 and 0.80 and excellent if higher than 0.80.

In **paper 1**, agreement in the assessment of deep myometrial invasion was higher between MRI and FS (Pa=0.79, K=0.56) and secondly between MRI and TVUS (Pa=0.78, K=0.47).

In **paper 2**, reliability between MRI and TVUS for the three ultrasound approaches was fair to moderate (K values ranging from 0.31 to 0.50) in the evaluation of deep myometrial invasion and moderate (K values ranging from 0.45 to 0.58) regarding cervical involvement.

In what concerns to deep myometrial invasion, the highest agreement was obtained between Karlsson method and MRI (PA 0.74, 95% CI 0.63-0.84) followed by subjective method and MRI (PA 0.73, 95% CI 0.61-0.82).

While exploring agreement by staging (IA *versus* IB FIGO staging or superficial *versus* deep myometrial invasion), agreement is consistently higher for higher stages (TVS Karlsson method *vs.* MRI: PA 0.61 [95% CI 0.45-0.749] in deep invasion *vs.* 0.57 [95% CI 0.41-0.72] in superficial; TVS Subjective method *vs.* MRI: PA 0.63 [95% CI 0.49-0.76] in deep invasion *vs.* 0.49 [95% CI 0.32-0.65] in superficial; TVS Gordon method *vs.* MRI: PA 0.59 [95% CI 0.45-0.72] in deep invasion *vs.* 0.39 [95% CI 0.24-0.56] in superficial). By way of explanation, it seems that reliability may be better in cases with positive deep myometrial invasion.

A recently published retrospectively analysis of 290 patients stated that FIGO stage IB is the main factor worsening the diagnostic accuracy of transvaginal ultrasound in endometrial cancer patients ($p=.004$). Among the 82 of 290 patients with histologically proven FIGO stage IB, 27 (32.9%) had a wrong ultrasound prediction of myometrial infiltration [17]. Our results do not confirm these findings.

In what regards to cervical stromal invasion, the highest agreement was obtained between subjective ultrasound method and MRI (PA 0.93, 95% CI 0.83-0.97). For all of the comparisons, agreement is consistently higher in the absence of cervical invasion, with worst reliability in case of positive tumoral infiltration (TVS subjective method *versus* MRI: PA 0.44 [95% CI 0.15-0.77] if tumoral invasion *versus* 0.92 [95% CI 0.82-0.97] if absence; TVS objective method *versus* MRI: PA 0.38 [95% CI 0.15-0.68] if tumoral invasion *versus* 0.80 [95%

CI 0.64-0.90] if absence). Less expertise in evaluating positive tumoral infiltration of the cervix due to a low number of FIGO stage II cases may provide support for this finding.

Very little was found in the literature on the question of agreement between preoperative methods. A recent prospective study with 51 women aimed to compare the diagnostic accuracies of ultrasound and MRI for deep ($\geq 50\%$) myometrial invasion and cervical stromal invasion in women with endometrial cancer. Ultrasound and MRI had a moderate agreement for deep myometrial invasion ($k=0.49$; 95% CI=0.26-0.73), whereas the agreement for cervical stromal invasion was substantial ($k=0.69$; 95% CI=0.36-1.00) [18]. Our prospective study obtained slightly better results for myometrial invasion but worst reliability for cervical assessment.

To the best of our knowledge, there are no earlier studies addressing the accuracy of combined preoperative techniques to determine deep myometrial invasion. In paper 2, if both ultrasound and MRI classifies the examination as positive deep myometrial invasion, the overall accuracy will be inarguably higher than using exclusively ultrasound staging, with an overall accuracy of 77%-78% (95% CI 0.66-0.86; 0.67-0.87) and higher specificity values (0.82-0.88; 95% CI 0.65-0.93; 0.72-0.97). If we consider a positive result the positivity of at least one approach (either ultrasound or MRI), we will clearly obtain higher sensitivity results but worst specificity. At a time were frozen section is becoming obsolete and sentinel lymph node protocol requires the preoperative knowledge of local staging (disease confined to the uterus), a protocol combining both techniques may be advisable until the learning curve on ultrasound preoperative staging is concluded.

6.2. STRENGTHS

This thesis includes the first multicentre prospective national study evaluating the role of ultrasound in the preoperative staging of endometrial carcinoma.

National and international guidelines latterly acknowledge the role of ultrasound preoperative in order to evaluate and establish disease confined to the uterus, in some international centers even replacing the use of pelvic RMI and intraoperative frozen section. However, to our knowledge, none oncologic center in Portugal has validated a protocol of ultrasound by expert as preoperative work up in endometrial cancer. Therefore, this thesis shows a unique and significant effort in the direction of a validated protocol in endometrial carcinoma.

Patients from north, middle and south areas of the country were recruited, which allowed the authors to portray the Portuguese population.

The **paper 2** is the first to our knowledge to analyze and compare the accuracy of combined preoperative techniques, including the conjunction of different ultrasound methods (subjective or objective methodologies) and pelvic MRI evaluation.

6.3. DRAWBACKS

Transvaginal ultrasound plays a pivotal role in the management of women with suspicion of endometrial malignancy and is usually performed by the treating gynecologist while guiding diagnosis. However, performing an office ultrasound is not equivalent to a specialized ultrasonography. A reliable ultrasound diagnostic is highly dependent on the skill of the examiners and their ability to obtain an accurate image plane, and therefore TVUS can be affected by subjective interpretations and may be particularly prone to inter and intraobserver variability issues. Moreover, the ultrasound scanner's control settings are likely to affect image quality (depth of field, compound imaging and tissue harmonic imaging).

The sample size is small and remains below the expected for extracting robust evidence for clinical applicability and decision-making. However, it still matches the current evidence in the field, even when confronted with some of the studies included in the most recent systematic reviews and meta-analysis.

Due to limitations of the sample size in both retrospective and prospective studies, it remains to be evaluated the accuracy of different ultrasound methodologies according to sonographic morphology and histological type. Moreover, in the multicenter prospective study it was not possible to assess differences between recruitment centers due to unbalanced numbers recruited from each center. The authors believe that a large multicenter trial would allow to compare important variables as the technique expertise (by professional and years/level of expertise) and to control to patient confounders.

The multicenter study design in paper 2, which aimed to recruit the possible largest sample size at the shortest time, imposes the need of different gynecology and radiology experts. The authors attempted to minimize the variability in these experts by defining one gynecology and radiology expert by center.

Due to limitations in equipment issues, in neither of the three ultrasound devices it was possible to collect videos of the examinations. In one of the centers there was no centralized equipment to collect images. Considering that ultrasound technique is highly operator dependent, this issue represented a major drawback: it was almost impracticable to verify the quality of ultrasound planes obtained to perform the assessment of myometrial and cervical invasion, therefore precluding the chance of recalculating the objective measurements. In addition, it importantly limited educational purposes, the opportunity to teach younger practitioners, to revise the work and to have feedback on personal performance. It also makes it unfeasible to perform reproducibility studies. In the absence of collected imaging, a classical reproducibility study, implying the performance of TVUS by different experts in different time frames could raise issues of compliance and enrollment due to discomfort of a long or repeated examination.

6.4. CONTRIBUTION TO THE FUTURE

The pelvic ultrasound stands as promising tool in the preoperative work up of endometrial carcinoma. It is a low cost and promptly accessible method, with no harm or discomfort to the patient and allowing a dynamic evaluation of the pelvic structures and the anatomical relation to surrounding viscera, which presents a clear advantage over the remaining pre-operative techniques, namely RMI. Additionally, it is performed preoperatively, allowing a tailored surgical procedure and obviating the increase of operative times and unnecessary costs inherent to intraoperative frozen section.

Therefore, this modality emerges as a reliable alternative to RMI and frozen section.

As previously seen with the role of ultrasound in obstetrics and benign gynecology, also in the field of gynecologic oncology we will notice a shift in clinical practice: the ultrasound by specialists in gynecologic oncology will take place in certified centers.

6.5. CONCLUSIONS

Preoperative imaging is crucial to enable a tailored surgical procedure in endometrial cancer, and TVUS and pelvic MRI are the preferred methods for the local staging. However, all imaging methods are restrained by non-perfect performances and limitations in reproducibility.

Preoperative pelvic ultrasound performed by expert seems to have adequate diagnostic yields for determining myometrial invasion in endometrial cancer comparing to other preoperative or intraoperative tools. Still, pelvic MRI seems to display a slightly better performance. Regarding the evaluation of cervical stromal involvement, the overall accuracy was higher for MRI but reasonably similar to the ultrasound subjective method. Therefore, comparable diagnostic inaccuracy reflects the same drawbacks in both methods, revealing a similar tendency to overestimate myometrial invasion and underestimate cervical stromal invasion.

Agreement between methods is reasonable, suggesting that the best alternative will be highly dependent on the availability and expertise of each institution. Because of imaging availability, ultrasound by expert will become the preferred option, whereas MRI will remain relevant in cases of reduced acoustic visibility due to limiting factors as uterine pathology, acoustic shadows or uterine position.

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CHAPTER 7.

PERSONAL REMARKS AND FUTURE DIRECTIONS

As a specialist dedicated to gynecologic oncology, I have a view of paramount investigation topics and the means of continuing clinical research in this field. There are a few research gaps I will endeavor to fulfill in the future, which are highlighted below:

- To establish objective differences between ultrasound patterns according to histologic type of endometrial cancer and the potential impact on the accuracy of ultrasound techniques in preoperative staging;
- To evaluate the reproducibility of different ultrasound methods in the assessment of myometrial and cervical invasion, according to the level of expertise (between residents, fellows and gynecologic oncology specialists) and in different hospital settings around the country;
- A cost-analysis study including pre-operative (TVUS and MRI) and intra-operative methods (FS);
- To create an image and video library of clinical cases with educational, training and research purposes.

I have always felt that a good balance between clinical practice and research is crucial to improve the provided healthcare. As a clinician, I recognize the challenges and obstacles that exist to conciliate these two worlds, but I do believe that they grow better together: clinical practice raises the relevant questions, while research helps to find answers and promotes progress. Therefore, I aim to encourage fellows to engage in clinical research and I look forward to supervising future PhD students to go further on this field.



Ama como a estrada começa.

Mário Cezariny

Mariana Isabel Cruz Rei

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