

REVIEW

Transvaginal ultrasound versus magnetic resonance imaging for preoperative assessment of myometrial infiltration in patients with low-grade endometrioid endometrial cancer: A systematic review and head-to-head meta-analysis

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Abstract

Purpose: We aimed to compare the diagnostic performance of magnetic resonance imaging (MRI) and transvaginal ultrasound (TVS) for detecting myometrial invasion (MI) in patients with low-grade endometrioid endometrial carcinoma.

Methods: A comprehensive search of MEDLINE (Pubmed), Web of Science, Embase and Scopus (from January 1990 to December 2022) was performed for articles comparing TVS and MRI in the evaluation of myometrial infiltration in low-grade (grade 1 or 2) endometrioid endometrial carcinoma in the same group of patients. We used QUADAS-2 tool for assessing the risk of bias of studies.

Results: We found 104 citations in our extensive research. Four articles were ultimately included in the meta-analysis, after excluding 100 reports. All articles were considered low risk of bias in most of the domains assessed in QUADAS-2. We observed that pooled sensitivity and specificity for detecting deep MI were 65% (95% confidence interval [CI] = 54%–75%) and 85% (95% CI = 79%–89%) for MRI, and 71% (95% CI = 63%–78%) and 76% (95% CI = 67%–83%) for TVS, respectively. No statistical differences were found between both imaging techniques ($p > 0.05$). We observed low heterogeneity for sensitivity and high for specificity regarding TVS; and moderate for both sensitivity and specificity in case of MRI.

Conclusions: The diagnostic performance of TVS and MRI for the evaluation of deep MI in women with low-grade endometrioid endometrial cancer is similar. However, further research is needed as the number of studies is scanty.

KEYWORDS

endometrial neoplasms, low-grade endometrioid, magnetic resonance imaging, myometrium infiltration, transvaginal, ultrasound

1 | INTRODUCTION

Cancer of the endometrium is the commonest gynecological malign tumor in developed countries and second in mortality, being surpassed only by ovarian cancer. In Europe, its 5-year prevalence rises up to 34.7% causing nearly 30 000 deaths per year.¹ Its incidence is 20.3 cases/100 000 women/year and it has been rising in recent years, due to obesity and an aging population among other factors.² However, mortality rates have decreased over the same period despite the bigger amount of deaths related to endometrial cancer.³

The diagnosis and staging are fundamental for the prognosis and for designing the optimal therapy for each patient.⁴ Surgery is needed for performing the staging as well as for definitive diagnosis of the histological type and grade. Therefore, when diagnosing a patient with endometrial cancer, before the surgery a preoperative and clinical staging with imaging test is highly recommended.⁵

The preoperative work-up allows to determine whether the patient is a candidate for surgical treatment and to assess the radicality of surgery knowing the disease extension.

ESGO-ESP-ESTRO have defined these tumors in different groups according to the prognosis related to the risk of relapse. The treatment differs according to these groups allowing us to be more conservative in the low-risk group than in intermediate or high-risk. Thus, it is important to define clearly which group each patient belongs to.⁵

Low-risk group includes all those patients who have an endometrioid, low-grade tumor with negative lymphovascular space invasion and confined to endometrium or infiltrating <50% of the myometrial wall. The management of these cases consists of total hysterectomy, bilateral salpingo-oophorectomy and sentinel-lymph-node biopsy, the latter if possible. Any adjuvant treatment will not be necessary. Therefore, to determine whether an endometrial cancer is a low-risk case, the assessment of tumor type and grade as well as the myometrial infiltration is essential. Tumor type and grade can be accurately assessed by endometrial biopsy.⁵ However, myometrial infiltration is assessed by intraoperative gross or frozen section analysis or by preoperative imaging. Alcazar et al. demonstrated in a meta-analysis that intraoperative gross assessment has a limited diagnostic performance.⁶ Furthermore, the most recent ESGO guideline states that myometrial infiltration should not be assessed by frozen section because of poor reproducibility and agreement with definitive paraffin sections.⁵

Therefore, preoperative imaging assessment of myometrial infiltration becomes clinically relevant. magnetic resonance imaging (MRI) traditionally assesses image assessment of myometrial invasion (MI). However, there is evidence that transvaginal ultrasound (TVS) may offer similar diagnostic performance than MRI.^{7,8} TVS has the advantages over MRI that it is less expensive, the use of contrast is not necessary, it is more readily available, and it requires less time to complete the diagnostic exam.⁹ In addition, international guidelines of the management of endometrial cancer as ESGO or the Spanish Society of Gynecology and Obstetrics guideline consider the use of TVS as an alternative for the assessment of uterine invasion.^{5,10}

Different meta-analyses have analyzed the specificity and sensitivity of MRI versus TVS in endometrial tumors. However, they do not specify its degree, but rather include all of them.^{11,12} Alcazar et al. noted that the clinical application of studies that include both high-grade and low-grade tumors for the comparison between the diagnostic ability of MRI versus TVS might be affected by the overestimation caused by high-grade tumors, since they carry a higher risk probability of myometrial infiltration.¹³ Therefore, a reevaluation would be convenient to assess and compare the performance of both methods in a priori low-risk cases.

Hence, we performed a head-to-head meta-analysis with the aim to compare the diagnostic performance of transvaginal sonography versus MRI for preoperative assessment of MI in patients with low-grade (G1–G2) endometrioid endometrial cancer.

2 | MATERIALS AND METHODS

This meta-analysis was performed according to the PRISMA recommendations (<http://www.prisma-statement.org/>) and the Synthesizing Evidence from Diagnostic Accuracy Tests (SEDATe) guidelines.

We defined the exclusion and inclusion criteria for selecting articles prior to start the meta-analysis. We did not register the protocol in PROSPERO. Given the nature and design of this study, ethics committee approval was not required.

Three authors (H.C., N.F., and J.V.) used four electronic databases to identify potentially eligible articles that were published between January 1990 and December 2022: Web of Science, SCOPUS, Embase and MEDLINE (PubMed). The search terms included the following keywords: “Transvaginal ultrasound,” “Magnetic resonance,” “Endometrial cancer,” “Myometrial infiltration,” “Myometrial invasion,” and “Myometrial staging.” Searching was limited to English language papers.

Furthermore, two authors (H.C., N.F.) combined the searches from the above-mentioned databases. Duplicated articles and non-English articles were excluded. Subsequently, citations were screened first by the titles, then by abstracts for identifying irrelevant articles to exclude, as those not related to the topic or were not primary studies (i.e., reviews, case reports, letters to Editor, systematic reviews). Full-texts articles of the remaining citations were read for the identification of potentially eligible papers. Two reviewers (H.C. and N.F.) used the following criteria for selecting the articles:

1. Prospective and retrospective cohort studies which include a set of patients who underwent both techniques, TVS and MRI, in order to evaluate myometrial infiltration in low-grade (G1–G2) endometrioid endometrial cancer as an index test.
2. Surgical evaluation of the myometrial infiltration regarding the permanent frozen histopathological diagnosis as reference standard.
3. Reporting of data allowing building 2 × 2 contingency table of diagnostic performance to be constructed as the minimum data required.

We excluded those articles which were not specifically related to the issue under review, that did not report data about myometrial infiltration in low-grade endometrioid cancer or those articles in which TVS and MRI were not performed in the same set of patients. Any other study not containing the necessary data to build a contingency table were also excluded.

Three of the authors (S.T., J.R.P.V., and J.V.) gathered blinded each other, data about true positives, true negatives, false positives and false negatives of each imaging method as well as any further relevant information from selected primary studies. Any disagreement during this process was solved by consensus discussed among the three authors (S.T., J.R.P.V., and J.V.).

The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) was used to evaluate the risk of bias as well as concerns of applicability of all studies included in this meta-analysis.¹⁴ This tool comprises four areas, namely "patient selection," "index test," "reference standard," and "flow and timing." Risk of bias and concerns about applicability were analyzed and rated as low, high, or unclear risk for each domain except for the domain of flow and timing. The results of quality assessment had a descriptive purpose in order to assess the global quality of the articles analyzed and to identify any potential factors of heterogeneity. The methodological quality was assessed independently by three authors (S.T., J.R.P.V., and J.L.A.) using a standard form with quality assessment criteria and a flow chart. Disagreements were solved by reaching a consensus by all three reviewers (S.T., J.R.P.V., and J.L.A.).

The evaluation of the study's quality was based on some information such as study's design, description of exclusion and inclusion criteria, description about how the index test (TVS/MRI) was performed and interpreted, description of the reference standard performed and whether or not the pathologists were blinded to index test. Histopathological diagnosis of the presence of myometrial infiltration was defined as the reference standard. To assess the flow-and-timing domain, we evaluated the descriptions of the time elapsed between TVS/MRI examination and surgery.

Information on diagnostic performance of MRI and TVS was gathered. Pooled specificity, sensitivity, positive (+LR) and negative likelihood ratio (−LR) was determined using a random-effects model. We used bivariate method to compare the diagnostic performance between MRI and TVS for detecting deep MI.¹⁵ To calculate post-test probabilities, we used the mean prevalence of deep myometrial infiltration depending on the index test evaluated and plotted on Fagan nomograms.¹⁵

We assessed the presence of heterogeneity for sensitivity and specificity using the I^2 index.¹⁶ I^2 values above 75% would be considered to indicate high heterogeneity, respectively. Forest plots of sensitivity and specificity of all studies were calculated and added to this document. When high heterogeneity was observed, we used meta-regression to evaluate covariates. These covariates were prevalence, sample size, number of observers (single/multiple) and mean patient age. Summary receiver-operating characteristics (sROC) curves were plotted to illustrate the relationship between sensitivity and specificity. Publication bias was assessed according to Deek's method.¹⁷

All analyses were performed using MIDAS command in STATA (Stata Corporation, College Station, TX, United States) version 12.0 for Windows. Statistical significance was defined as a p value <0.05.

3 | RESULTS

Our research yielded 1004 citations. Eight hundred and three citations remained after excluding eight articles published in non-English languages and 193 duplicate records. After reading titles, 755 citations were further discarded. Abstracts of the remaining 48 papers were read by two authors (H.C. and N.F.), who ruled out and 21 more citations. Then, we reviewed the full length of the remaining 27 articles and 23 papers were excluded because they did not meet the inclusion criteria (articles that evaluated MRI and TVS in different groups of patients, studies that not analyze only low-grade (G1–G2) endometrioid endometrial cancer, absence of data required to make the 2×2 table). Thus, four articles were ultimately included in this systematic review and meta-analysis. All authors also reviewed all references cited in the articles included looking for additional relevant papers, although, no relevant studies were found. A flow diagram summarizing the literature search performed is shown in Figure 1.

Four articles reporting on 577 patients and published between January 2021 and December 2022 were included in the ultimate analysis.^{18–21} Among 577 patients, 141 cases had histopathological diagnosis of deep myometrial infiltration confirmed after surgery. Mean prevalence of deep myometrial infiltration was 25.4%, ranging from 12.8% to 34.3%. All papers described some clinical features of the cohort of patients included in the respective studies. Mean age of the patients was provided in three out of four studies and ranged from 31 to 93 years.^{18–20} In one article,²¹ the mean age of the patients was not provided specifically for the target group (subgroup women with low risk).

According to the four articles, the ultrasound equipment used was medium or high-brand machines in all studies. Three studies reported that the MRI magnetic field was 1.5 or 3 T, but one study did not report on this information. All articles indicate the number of observers who performed either TVS or MRI. The pathologist was blind to the index test results in the four articles. All these features are summarized in Table 1.

Table 2 shows the assessment of the risk of bias and concerns according to applicability of the selected articles. All data concerning patient selection, index test and reference standard domains shown were available for all articles. Information regarding flow and timing domain was only available in two articles.^{18,21} The study design was clearly defined as prospective in all the studies. Two papers were considered as high risk for *patient selection* because of patients unsatisfactory evaluated in the TVS exam were excluded.^{18,21} Three studies^{18,19,21} adequately described the method of *index text* (concerning TVS) as well as its implementation and interpretation. One was considered as high risk since TVS examiners had no previous experience in preoperative assessment of myometrial infiltration in endometrial cancer, even though they received a theoretical-practical

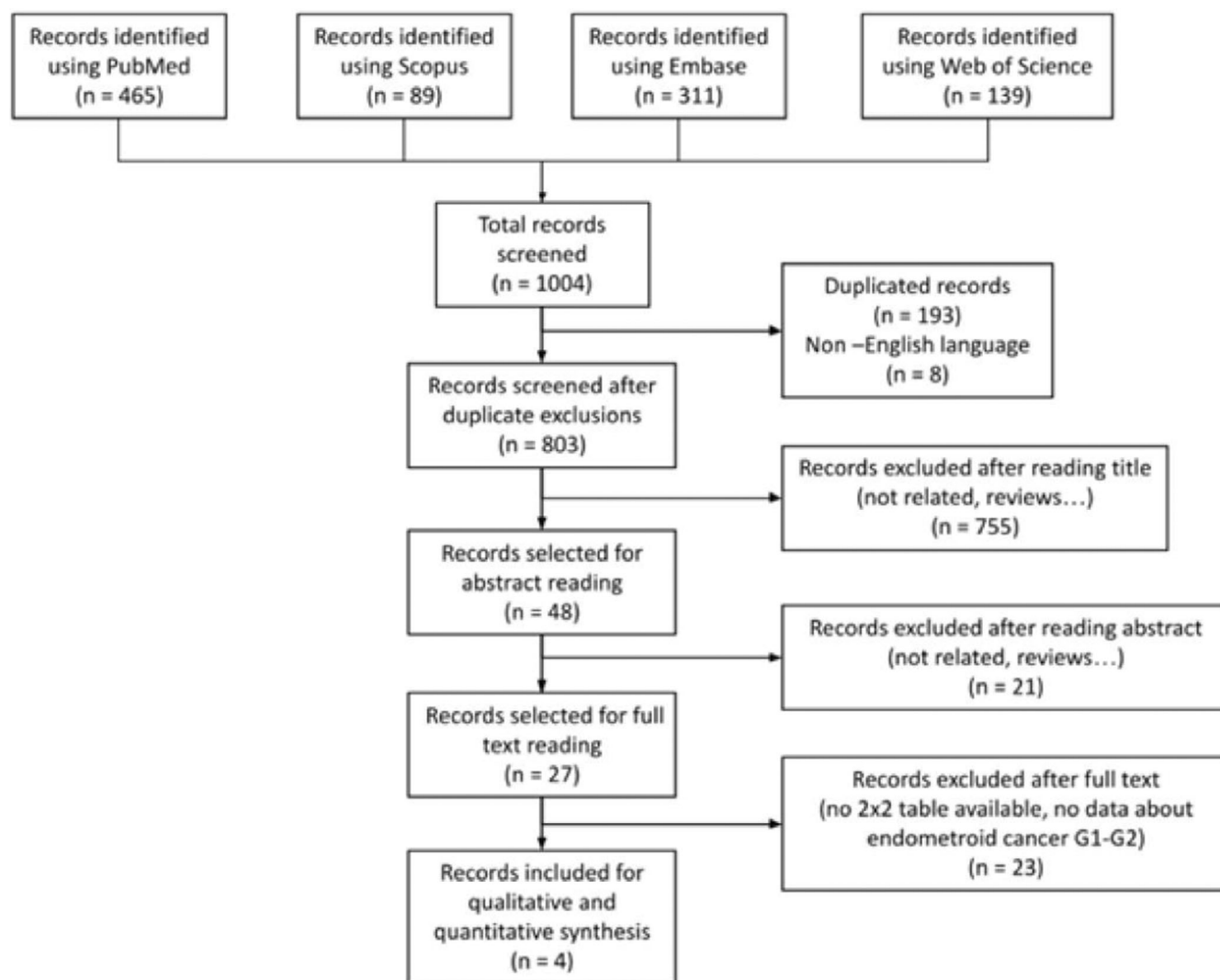


FIGURE 1 Flowchart showing the study selection process, indicating the titles found in each database, the exclusion process, and the final number of articles included in the meta-analysis.

TABLE 1 Main characteristics of the studies included in the meta-analysis.

Author	Year	Study design	Consecutive series	N	N > 50% MI	TVS scan	MRI scan	Observers TVS	Observers MRI	Blind pathologist
Cubo ¹⁸	2021	Prospective	No	131	45	Voluson E6, GE	1.5 T and T3	Single	Multiple (3)	Yes
Gastón ¹⁹	2022	Prospective	Yes	156	20	Voluson E6, GE	1.5 T	Single	Single	Yes
Palmer ²⁰	2022	Prospective	Yes	TVS: 257, MRI: 259	TVS: 66 MRI: 67	Voluson E10, Voluson S6/E6	NA	Multiple (32)	Multiple (2)	Yes
Wong ²¹	2022	Prospective	No	31	9	Voluson E8, GE	NA	Single	Multiple	Yes

training.²⁰ With regard to MRI, all four studies adequately described the method of index test as well as how it was performed and interpreted. Regarding the domain *flow and timing*, the time elapsed between the index test and reference standard was unclear in two studies.^{19,20} For the domain *reference standard*, all studies were likely to correctly classify the target condition by the reference standard. However, in only one study it was clearly specified if the results of

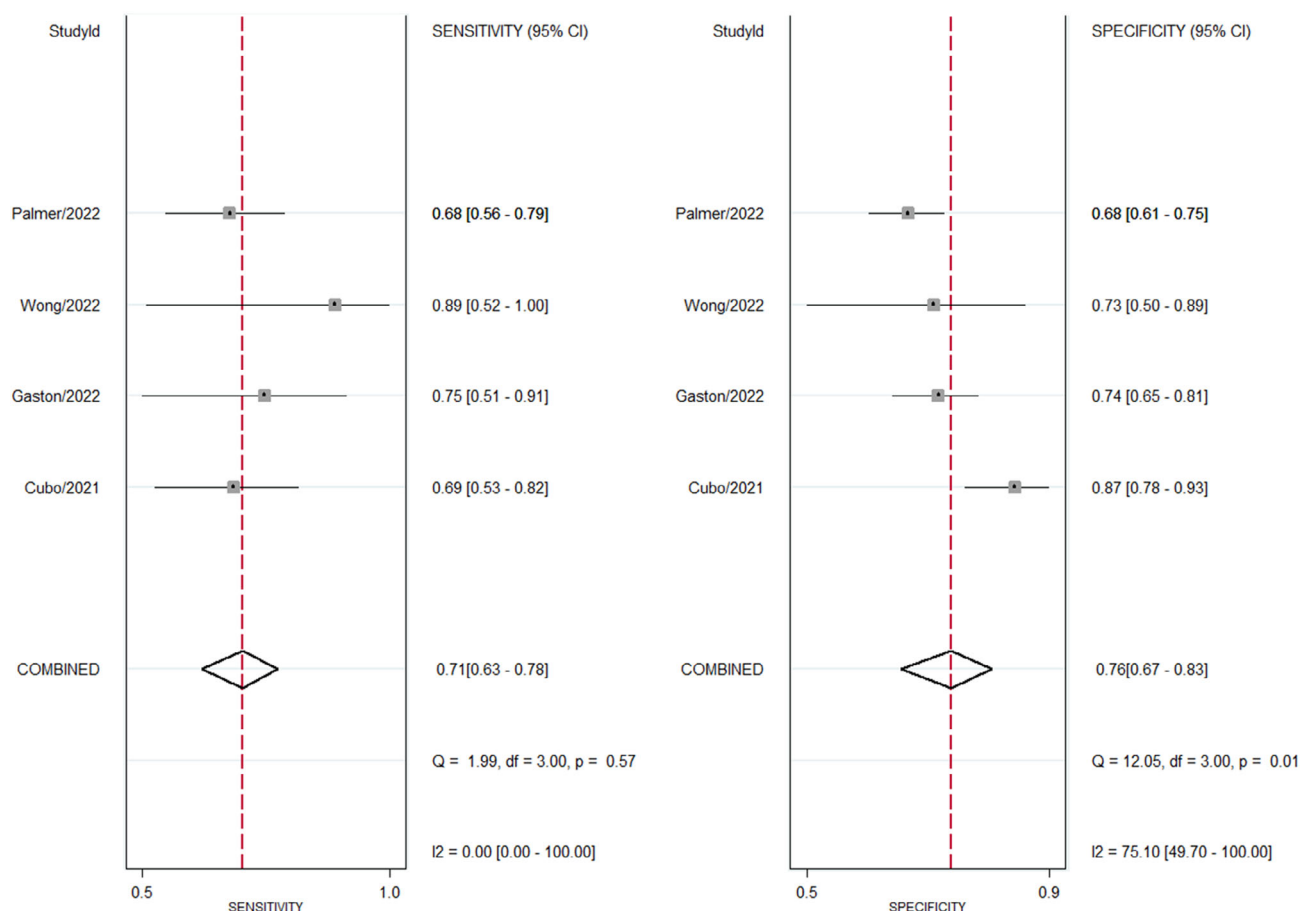
the reference standard were interpreted using permanent frozen section.¹⁹ In addition, all studies reported specifically that pathologists were blinded to imaging results.

Regarding concerns of applicability, all studies were considered as low risk for all three domains.

Pooled sensitivity, specificity, LR+, LR– and diagnostic odd ratio (DOR) of TVS for detecting deep myometrial infiltration were

TABLE 2 QUADAS-2 analysis of the studies included in this meta-analysis.

Risk of bias						Concerns of applicability		
Author	Patient selection	Index test (TVS)	Index test (MRI)	Reference test	Flow and timing	Patient selection	Index tests	Reference test
Cubo ¹⁷	High	Low	Low	Low	Low	Low	Low	Low
Gaston ¹⁸	Low	Low	Low	Low	Unclear	Low	Low	Low
Palmer ¹⁹	Low	High	Low	Low	Unclear	Low	Low	Low
Wong ²⁰	High	Low	Low	Low	Low	Low	Low	Low

**FIGURE 2** Forest plot for sensitivity and specificity for all studies concerning the diagnostic performance transvaginal ultrasound (TVS) for deep myometrial infiltration.

71% (95% confidence interval [CI] = 63%–78%), 76% (95% CI = 67%–83%), 2.9 (95% CI = 2.1–4.2), and 0.39 (95% CI = 0.29–0.51) and 8 (95% CI = 4–13), respectively. As Figure 2 shown, high heterogeneity was found for specificity ($I^2 = 75.10$) but no heterogeneity was found for sensitivity ($I^2 = 0.00$). Meta-regression showed that prevalence, sample size, number of observers and mean patient age did not explain heterogeneity observed for specificity.

On the other hand, pooled sensitivity, specificity, LR+, LR–, and DOR of MRI for detecting deep myometrial infiltration were 65% (95%

CI = 54%–75%), 85% (95% CI = 79%–89%), 4.3 (95% CI = 3.2–5.9), 0.41 (95% CI = 0.31–0.45), and 11 (95% CI = 7–17), respectively. Moderate heterogeneity was found for sensitivity ($I^2 = 54.57$) and specificity ($I^2 = 57.27\%$), respectively. This is shown in Figure 3. When comparing both methods we did not find any statistical differences ($p = 0.314$).

sROC curves for TVS and MRI are shown in Figures 4 and 5, respectively. It can be observed that both techniques had similar areas under the curve, AUC for TVS was 0.73 (95% CI: 0.69–0.76) and AUC for MRI was 0.83 (95% CI: 0.80–0.86).

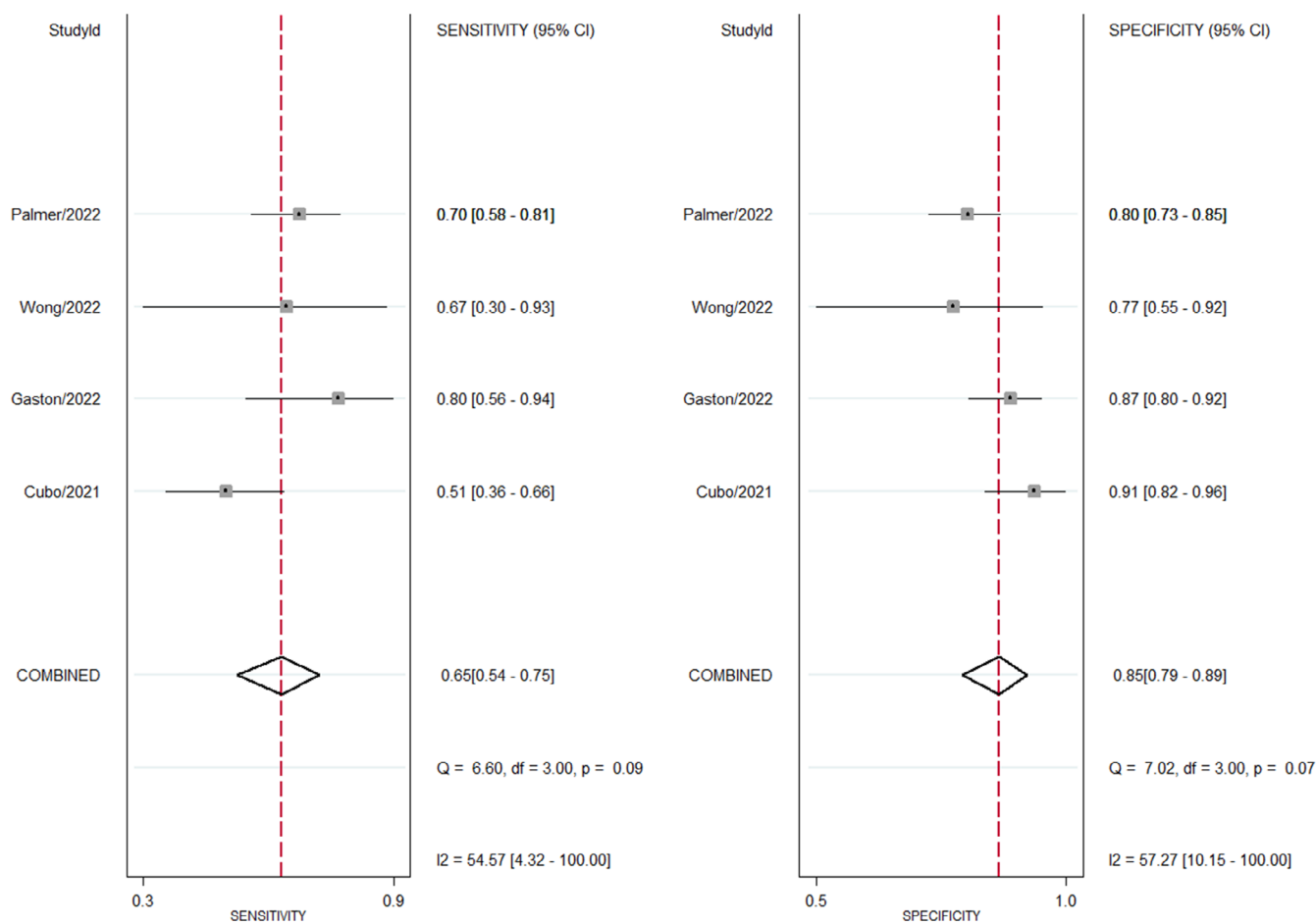


FIGURE 3 Forest plot for sensitivity and specificity for all studies concerning the diagnostic performance of magnetic resonance imaging (MRI) for detecting deep myometrial infiltration.

Fagan nomograms show that a positive test for TVS and MRI increases the pretest probability of deep myometrial infiltration, from 25% to 49% in case of TVS and from 25% to 59% in case of MRI. While a negative test decreases the pretest probability, from 25% to 11% in case of TVS and from 25% to 12% in case of MRI (Figures 6 and 7).

No publication bias was found, neither for TVS ($p = 0.33$) nor for MRI ($p = 0.79$).

4 | DISCUSSION

4.1 | Summary of findings

In the present meta-analysis, we observed that TVS had a higher pooled sensitivity as compared to MRI (71% vs. 65%). On the contrary, pooled specificity was higher for MRI as compared to TVS (85% vs. 76%). However, these differences did not reach statistical significance. Heterogeneity was low for sensitivity and high for specificity for TVS; and moderate for both sensitivity and specificity in MRI. The likelihood ratios of these methods were also limited for predicting or ruling out deep myometrial infiltration. It is interesting to note that

positive likelihood ratio was low and negative likelihood ratio was high for both techniques. This could be explained for the small number of patients included in the meta-analysis.

We found that the quality of the analyzed studies was rather limited.

4.2 | Interpretation of findings in clinical context

Patients with low-grade endometrioid EC are at priori a low-risk group where systematic lymphadenectomy is not recommended in all patients.⁵ In these patients, assessment of MI is essential in order to determine the need of lymphadenectomy and plan optimal surgery. Current guidelines do not recommend performing histological intraoperative assessment of myometrial infiltration due to its low reproducibility.⁵ A false negative assessment of deep myometrial infiltration can lead to the omission of lymphadenectomy with the consecutive oncologic implications. Preoperative imaging assessment for determining myometrial infiltration can be performed with MRI or TVS. Some guidelines current recommend MRI for the preoperative assessment in the management of these patients.^{22,23}

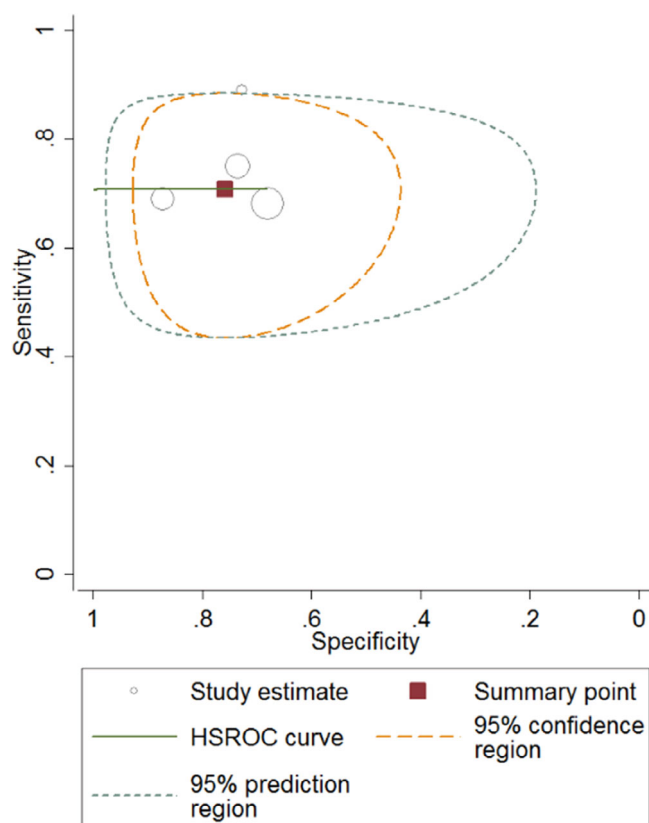


FIGURE 4 Summary receiver-operating characteristics (ROC) curve for the diagnostic performance of transvaginal ultrasound (TVS) to detect deep myometrial invasion (MI).

The evaluation the diagnostic performance of MRI in detecting deep myometrial infiltration in endometrial cancer has been already published in several meta-analyses.^{11,24–26} One of the most recent meta-analysis from Bi et al.,²⁶ which included 14 studies, concluded that MRI has a good diagnostic performance for assessing myometrial infiltration in patients with endometrial cancer. Their analysis showed a pooled sensitivity and specificity of 0.79 (95% CI: 0.75, 0.83) and 0.81 (95% CI: 0.78, 0.83), respectively. In patients younger than 60 years they observed a higher sensitivity and specificity of 0.84 (95% CI: 0.78, 0.89) and 0.90 (95% CI: 0.88, 0.93) respectively. However, in all these meta-analyses, no specific assessment of low-grade endometrioid cancers was done, as we have recently highlighted.²⁷

TVS is a cheaper and more accessible imaging test than MRI. As our results show that the diagnostic performance of assessing myometrial infiltration in low-grade endometrioid endometrial cancer is similar, we believe that TVS is a good option for first-line imaging technique for saving costs, especially in low-income countries, where the possibility to perform MRI is much more limited.

It is important to underline that there is a higher variability in the interpretation of ultrasound scans rather than MRI. This is probably due to the fact that MRI has a more standardized technique than TVS.²⁷ Green et al.²⁸ studied this variability in detail. Their aim was to estimate the agreement between two-dimensional TVS and three-dimensional volume contrast imaging in the diagnosis of deep

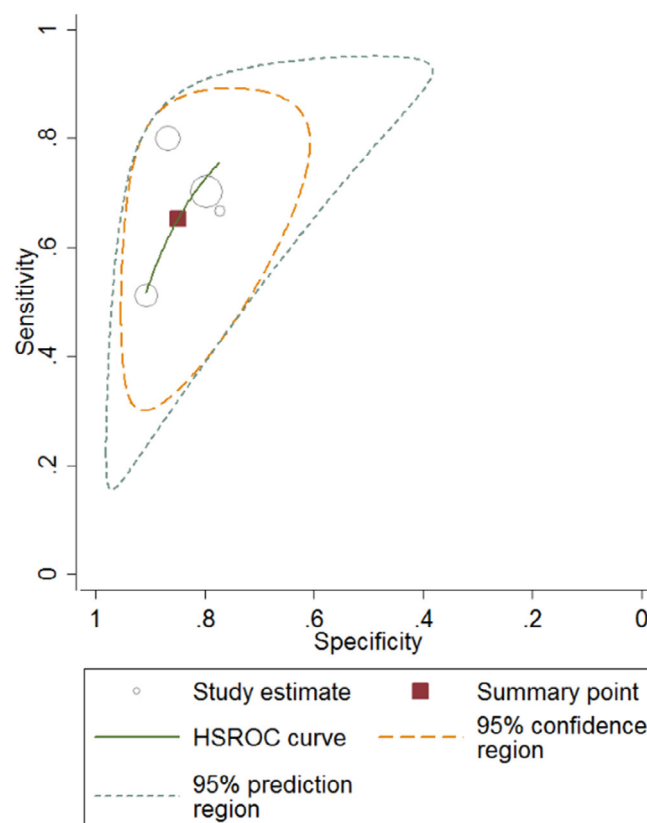


FIGURE 5 Summary receiver-operating characteristics (ROC) curve for the diagnostic performance of magnetic resonance imaging (MRI) to detect deep myometrial invasion (MI).

myometrial infiltration of endometrial cancer as well as to compare the two techniques according to inter-rater reliability and diagnostic accuracy. They showed 58 3D volumes and 2D TVS video clips from patients with biopsy-confirmed endometrial cancer to 15 ultrasound experts. The gold standard was the histological diagnosis after hysterectomy. Interrater reliability was measured using kappa. Spearman's rank correlation coefficient was used to measure accuracy. The agreement between both techniques was 76% (median), with a range 64%–93%. 2D TVS achieved better interrater reliability (Fleiss' kappa 0.41) and better accuracy for diagnosis of deep myometrial infiltration rather than volume contrast 3D. Furthermore, they found that the number of cases performed annually had a positive impact in the diagnostic accuracy of deep myometrial infiltration of endometrial cancer, though they recommended centralizing these examinations to high volume centers.²⁸

In another study, Eriksson et al.²⁹ aimed to assess interobserver reproducibility in the prediction of deep myometrial infiltration of endometrial cancer among ultrasound experts and non-experts. Nine ultrasound experts and 9 non-experts assessed 53 sonographic video clips of patients with endometrial cancer. Their study included endometrioid adenocarcinoma G1, G2, and G3 and non-endometrioid endometrial cancers. They used histopathology after hysterectomy as the gold standard. They concluded that ultrasound non-experts were equally good at predicting deep myometrial infiltration as experts but

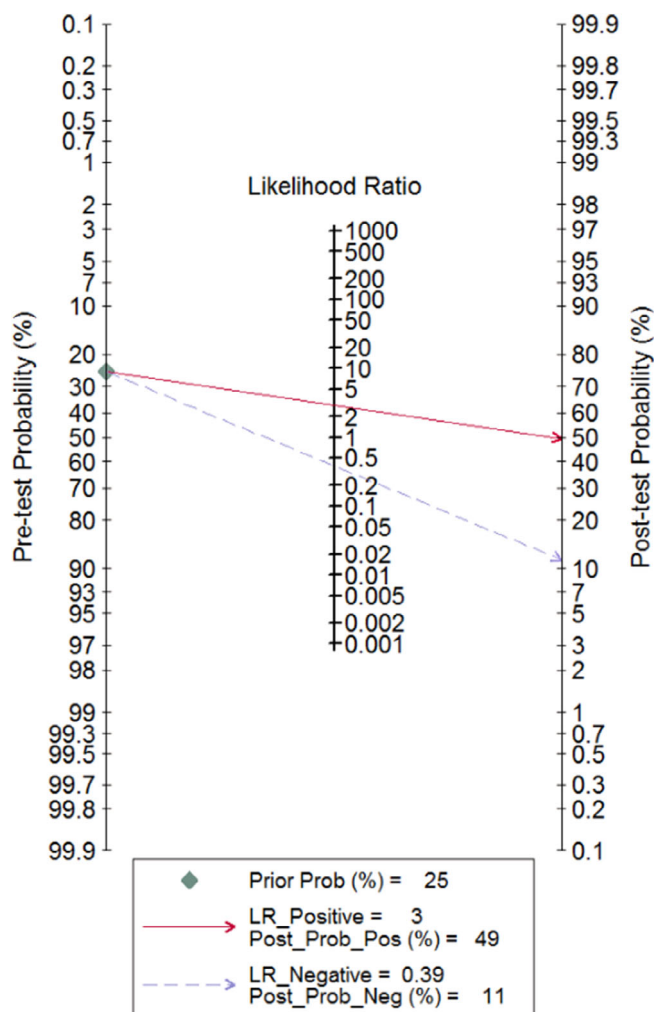


FIGURE 6 Fagan's nomogram for transvaginal ultrasound (TVS).

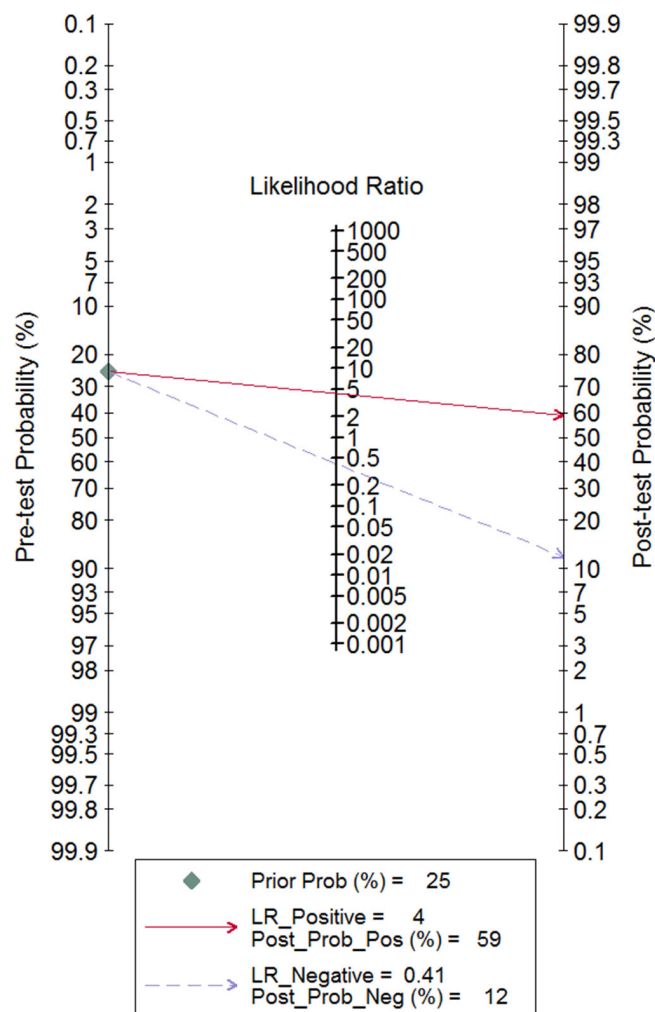


FIGURE 7 Fagan's nomogram for magnetic resonance imaging (MRI).

those showed higher agreement with histopathology, sensitivity, and specificity regarding the evaluation of cervical stromal invasion. Inter-observer reproducibility regarding the prediction of deep myometrial infiltration was similar in both groups (experts, 34% and non-experts, 22%, $p = 0.13$). Interobserver agreement according to kappa was 0.52 (95% CI, 0.48–0.57) and 0.48 (95% CI, 0.44–0.53), $p = 0.11$, for deep myometrial infiltration for ultrasound experts and non-experts respectively.

Recently, there are numerous publications studying the importance of training programs and the implementation of standardized techniques to evaluate MI in EC via TVS in order to achieve an improvement in diagnostic accuracy.^{30–32} Xholi et al.³¹ conducted a retrospective study in order to evaluate the learning curve for TVS assessment of MI in women with EC. Sixty-seven real-time ultrasound scanning examinations performed by two expert examiners were assessed using the learning curve cumulative summation (LC-CUSUM) test. After the learning phase, the cumulative summation test was applied to assess performance maintenance. The pathologic result was the reference standard. They used Karlsson's method for assessing MI and established a failure rate at 25%. They observed that one

observer performed 42 examinations. It was not until the 29th examination when he reached competence and kept it under control afterwards. The second observer performed 25 examinations but did not achieve competence.

Chacón et al.³² studied this learning curve for assessing MI in cases of endometrial cancer using TVS. Five performers (four fourth year obstetrics and gynecology residents and one radiologist) underwent a specific training on the evaluation of MI in EC. They had no previous experience in the evaluation of such pathology. The training consisted of one specific lecture about this topic and the visualization of a video explaining 10 cases by the trainer. After this, all of them visualized 45 clips of uterine ultrasound scans of endometrial cancer cases. The evaluation of the endometrial infiltration was based on the subjective impression and the histology was used as a reference standard. The learning curve cumulative summation was used to evaluate each trainee's learning curve. All trainees completed the study. One trainee did not reach competence and another one reached competence but could not maintain it. The others trainees reached competence at the 33rd, 35th, and 36th case, respectively. Also, they kept

the process under control after reaching this competence. Therefore, they conclude that 30–40 cases would be needed to obtain optimal skills in determining myometrial infiltration.

As far as we know, there is no previous head-to-head meta-analysis on this topic and we think this is the main strength of our study. Furthermore, we have only included papers in which patients underwent both MRI and TVS techniques. As a result, this makes the comparison between both techniques better and more reliable.

Additionally, the timing of papers included in our study varies from 2021 to 2022. This implies that technological advances in both TVS and MRI and current state of knowledge should be homogeneous among the four included studies.

However, caution should be taken when interpreting these results because this article is not exempt from some limitations. The main limitation is the small number of included papers. Therefore, results derived from the analysis of these studies are based on data from a total of 577 patients, which is certainly a small sample size.

Taking into account the results of our meta-analysis, we do think that there is a need for prospective randomized trials and cost-effective studies comparing the diagnostic accuracy of MRI and TVS assessing myometrial infiltration in patients with low-grade endometrioid endometrial cancer before surgery. This research would be particularly relevant for low-income countries, where the availability of high technology, such as MRI, is much more limited than for TVS.

5 | CONCLUSIONS

For detection of deep myometrial infiltration in women with low-grade endometrioid endometrial cancer, TVS showed similar diagnostic performance to MRI. Therefore, TVS should be considered as an alternative for being used with proper training. However, further research on this topic is needed, as the number of studies is scanty.

AUTHOR CONTRIBUTIONS

Conceptualization: Juan Luis Alcázar. **Methodology:** Sara Tameish, Juan Ramón Pérez Vidal, Natalia Florez, Hui Chen, Julio Vara, and Juan Luis Alcázar. **Formal analysis:** Juan Luis Alcázar. **Investigation:** Sara Tameish, Juan Ramón Pérez Vidal, Natalia Florez, Hui Chen, and Juan Luis Alcázar. **Data curation:** Juan Luis Alcázar. **Writing—original draft preparation:** Juan Luis Alcázar, Sara Tameish, and Juan Ramón Pérez Vidal. **Writing—review and editing:** Sara Tameish, Natalia Florez, Juan Ramón Pérez Vidal, Hui Chen, Julio Vara, and Juan Luis Alcázar. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

ETHICS STATEMENT

Due to the nature of the study, ethical approval was waived.

INSTITUTIONAL REVIEW BOARD STATEMENT

Institutional Review Board (IRB) was waived due to study's design.

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