Testing Transvaginal Ultrasound as a Non-Invasive Diagnostic Tool for Endometriosis

Testing Transvaginal Ultrasound as a Non-Invasive Diagnostic Tool for Endometriosis

By Shay M. Freger (MPH, HBSC)

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Master of Science

Descriptive Note

McMaster University MASTER OF SCIENCE (2024) Hamilton, Ontario (Medical Science)

TITLE: Enhancing Transvaginal Ultrasound as a Non-Invasive Diagnostic Tool for Endometriosis AUTHOR: Shay M. Freger (MPH, HBSc). SUPERVISOR: Dr. Mathew Leonardi

NUMBER OF PAGES: 81

Lay Abstract

Endometriosis is a common gynecological disease involving the abnormal growth of uterine-like cells outside the uterus, causing significant negative impacts on quality of life and diagnostic delays. Deep endometriosis (DE) is the most aggressive form, infiltrating surrounding tissues and leading to complex disease states. The uterosacral ligaments (USLs; connective structures between the lower spine and uterus) are the most common site for DE, but diagnosing them non-invasively remains challenging, aiding the diagnostic delay. Following updated classification guidelines, the overarching aim of this thesis is to enhance our understanding of transvaginal ultrasound (TVS) as a safe and rapid diagnostic for DE of the USLs and TU. In doing so, this thesis aims to assess the accuracy of a new TVS technique for DE of the USLs and determine how other related health conditions might affect the accuracy of this diagnostic approach. The findings from this study indicate that using TVS could greatly assist in diagnosing DE in the USLs, potentially leading to more personalized treatment approaches by healthcare providers and better outcomes for individuals with endometriosis. In summary, this research contributes significantly to our understanding and management of this complex condition.

Abstract

Endometriosis is a heterogeneous chronic pain and inflammatory disease associated with negative impacts on quality of life. Among the phenotypes of endometriosis, deep endometriosis (DE) is the most aggressive form of the disease, associated with complex disease states, such as adhesions within the pouch of Douglas (POD) and bowel DE. The most common site of DE is the uterosacral ligaments (USLs), which are bilateral structures between the uterus and sacrum conjoined by the torus uterinus (TU), with a prevalence of 20 to 70%. The USLs have historically been the hardest to visualize using non-invasive modalities, such as transvaginal ultrasound (TVS), resulting in poor identification of endometriosis when present on/within the USLs, contributing to the significant diagnostic delay associated with the disease.

This thesis details a novel diagnostic approach, utilizing TVS within the posterior vaginal fornix as the index test and laparoscopic visualization as the reference standard, aiming to evaluate the diagnostic accuracy of TVS for DE of the USLs and TU. Additionally, the USLs and TU are commonly associated with complex disease presentations, including POD obliteration and bowel DE, though the impact on diagnostic accuracy remains unknown. We theorize that these concurrent complex disease states will lead to the distortion of the anatomical environment and, in turn, negatively alter the diagnostic performance of the novel posterior approach. This thesis further aimed to determine the impact of concurrent complex disease states on diagnostic performance.

We found enhanced diagnostic accuracy in the detection of endometriosis in the left USL, right USL, and TU compared to previous studies, with our sensitivity ranging from 75.0-100%, specificity of 100%, positive predictive values of 100%, and negative predictive value ranging from 88.6-100%. Furthermore, contrary to our hypothesis, diagnostic performance appeared unaffected by the presence of complex disease states. The ability to diagnose USL DE non-invasively can have profound implications for introducing personalized treatment plans in a timely manner, which should improve patient outcomes. With this enhanced diagnostic performance, fewer people will require a surgical diagnosis, which reduces the burden on the health system and decreases surgical complications associated with diagnostic surgery.

Acknowledgments

I am profoundly grateful to my supervisor, Dr. Mathew Leonardi, whose mentorship has been the cornerstone of my academic and professional journey. Dr. Leonardi's unwavering support, guidance, and belief in my potential have been instrumental in shaping me into a leader within the field. Throughout this dynamic period, Dr. Leonardi has fostered an environment where curiosity thrives and innovation flourishes. His encouragement to think creatively and explore outside the confines of conventional wisdom has been invaluable, allowing me to push boundaries and discover new perspectives. I am deeply appreciative of the countless opportunities he has provided and his steadfast support through both triumphs and challenges. Without Dr. Leonardi's mentorship, my success and completion of this period would not have been possible.

I extend my heartfelt appreciation to my committee members, Dr. Julia Muraca and Rohan D'Souza, for their guidance, wisdom, and encouragement. Dr. Muraca's insights bridging public health and clinical work have greatly influenced my academic growth, while Dr. D'Souza's encouragement to explore beyond disciplinary boundaries has broadened my perspective. Special thanks to Kyle McGowan for his invaluable assistance in navigating the complexities of research, a skill that will continue to shape my academic career.

Dr. Warren (Lauren) Foster holds a special place in my academic journey as the one who introduced me to the field of endometriosis. Dr. Foster's mentorship has been foundational,

instilling in me independence, critical thinking, and a deep appreciation for the intricacies of academia. I am also grateful for the camaraderie and support of my past lab mates, including Anna Leonova, Emerson Brazil, and Victoria Turnip, whose friendship and collaboration have enriched my research experience during the highs and lows of my academic journey. Special thanks to current colleagues, including Ido Mick, Mahsa Gholiof, and Melissa Marien, who ensured my academic journey was never a dull moment and fostered countless laughs. Additionally, I extend my gratitude to past and present members of the Holloway lab, particularly Dr. Alison Holloway, Genevieve Perono, and Laiba Jamshed, for their support.

To my family, I owe an immeasurable debt of gratitude for their unwavering support and encouragement throughout my entire life. Your belief in my potential and encouragement to pursue my dreams, no matter how audacious, has been the driving force behind my achievements. I am deeply thankful for their sacrifices and unwavering commitment to my dreams. I also extend my appreciation to my closest friends back home, as well as Amer and Tabitha, for their support and encouragement. Special thanks to Murphy, Mackenzie, and, most importantly, Remi for being my pillars of strength throughout my journey.

Finally, I dedicate this work to my late grandfather, Semyon Freger, whose boundless love and support have left an indelible mark on my life. Though he is no longer with us, his legacy of kindness, resilience, and unwavering support will continue to inspire me as I navigate the path ahead.

Table of Contents

Lists of Figures and Tables

Chapter I.

Chapter II.

Chapter III.

List of Abbreviations

AFAB – Assigned Female Sex at Birth

DE – Deep Endometriosis

ESHRE – European Society of Human Reproduction and Embryology

FIGO - Federation of Gynecology and Obstetrics

IDEA - International Deep Endometriosis Analysis

OE – Ovarian Endometriosis

POD – Pouch of Douglas

QUADAS-2 – Quality Assessment of the Diagnostic Accuracy of Studies

SE – Superficial Endometriosis

STARD – Standards for Reporting Diagnostic Accuracy

TU – Torus Uterinus

TVS – Transvaginal Ultrasound

USL – Uterosacral Ligament

Declaration of Academic Achievement

The study design, measurements, respective analysis, and manuscript submission were completed by SMF under the supervision of ML. However, transvaginal ultrasound and all procedures, including laparoscopic surgery, were performed by ML. Measurements were obtained, recorded, and analyzed by SMF. VT assisted in cross-validation of data collection to ensure unbiased collection, and KM assisted as the departmental research coordinator.

Chapter I. Introduction

I.I Endometriosis Overview

Endometriosis is a whole-body, heterogeneous disease affecting approximately 1 in 9 people assigned female sex at birth (AFAB) [1]. The disease is characterized by the abnormal growth of endometrial-like stroma and glandular epithelial cells outside of the uterus, leading to chronic pain-like symptoms, inflammation, and infertility [2]. Beyond the clinical experiences, endometriosis generally precipitates significant impairment on quality of life, with negative consequences on employment, education, relationships, social life, finances, and physical and mental health [3]. While endometriosis often presents with hallmark characteristics, it may also exhibit unique presentations with or without the hallmark characteristics [4].

I.II Endometriosis Phenotypes

Although endometriosis may be defined histologically by the ectopic growth of endometrial-like cells, it may be further divided into three phenotypes, including ovarian endometriosis (OE; 'endometrioma' or cystic-like growth along or within either or bilateral ovaries), superficial endometriosis (SE; the most prevalent phenotype, present as non-infiltrative lesions along the surface of the peritoneum or surrounding anatomy), and deep endometriosis (DE; the infiltrative type, leading to significant distortion of anatomy) [5].

I.II.I Ovarian Endometriosis

Endometriomas (OE), cystic growths within the ovary or ovaries, comprise up to 25% of endometriosis cases [6]. Although endometriosis is generally considered benign, the possibility of OE undergoing malignant transformation has been reported, focusing on histological hyperplasia, atypia, and molecular changes [2].

I.II.II Superficial Endometriosis

Superficial endometriosis, also known as peritoneal endometriosis, has a reported prevalence of 80% among cases of endometriosis [6]. Though the phenotype has historically been described as subtle or a non-severe stage of disease, SE may drive similar symptoms and systemic changes. SE rarely appears alone; on the other hand, it is usually diffusely present in multiple regions of the pelvis, such as the pelvic sidewalls, ovaries, ligaments, and uterine surface [7–9]. As its nomenclature suggests, SE does not infiltrate beneath the surface (i.e. peritoneum) [5]. SE may vary significantly in its appearance, including size and colour (red, black, or white/clear) [10], which has been previously suspected to indicate lesion age or inflammation activity level, though this remains to be elucidated.

I.II.III Deep Endometriosis

Of the three phenotypes, DE is the most invasive form of the disease, with infiltration into anatomic locations [11]. The current estimated prevalence of DE is 20% in general gynecological clinics [8,12], though notably higher, estimated at 57% among those with chronic pelvic pain (CPP)

[13]. Though DE may be present anywhere within the pelvis, locales include the bowel, pouch of Douglas (POD; space between the rectum and uterus), posterior vaginal fornix, and most commonly, within the uterosacral ligaments (USLs; bilateral connective structures between the uterus and sacrum) [8,12]. Historically, DE was defined by >5mm infiltration within surrounding tissue, while SE was characterized by <5mm. In 2021, these classification guidelines underwent a revision [14]. As per the updated criteria, DE is now defined by any level of infiltration within the surrounding tissue, whereas the absence of infiltration in the surrounding tissue describes SE [14].

Anatomical distortion is a cornerstone of DE. In particular, bowel DE, generally within the anterior wall of the rectum or sigmoid, usually yields a severe form of anatomical distortion called POD obliteration (adhesions between the bowel and uterus and/or vagina and uterus) [15–17]. This is broadly believed to be one of the highest levels of endometriosis complexity. In this complex disease state of bowel DE and/or POD obliteration, other DE disease sites are usually present [17,18]. The distortion of the anatomical environment may lead to difficulties in diagnosing, characterizing, and treating endometriosis in specific disease locations [19], including the USLs.

I.III Diagnostic Limitations and Diagnostic Delay

The endometriosis landscape has undergone extensive shifts in recent years, yet it remains highly underrepresented and poorly understood [4]. In a qualitative assessment of patient experiences, the largest limitation within the field remains the extensive diagnostic delay from the onset of symptoms to the time of diagnosis [20,21]. The delay is estimated to be 5.8 years globally [22] and 5.4 years within Canada [23]. This delay limits patients from initiating appropriate management of the

disease, subjecting patients to a prolonged negative quality of life [21]. Although the pathophysiological and symptomatologic evolution of endometriosis has been heavily debated, this limited access to care may lead to worsening symptoms and exacerbate negative impacts on quality of life throughout a patient's life course [24].

The origin of the diagnostic delay associated with endometriosis is multifaceted and composed of social phenomena such as stigmatization, poor education and awareness of the disease, and systemic issues within the medical milieu [23,25,26]. One of the largest contributors to the delay throughout the clinical care pathway is inadequate recognition of symptoms and poor adoption of non-invasive methods as front-line diagnostics [27,28]. In the absence of first-line diagnostics and their ubiquitous adoption, patients are subjected to inadequate care, prolonged negative impact on quality of life, and significant economic burdens nationally and globally [3].

I.IV Diagnostics and Endometriosis

I.IV.I Laparoscopy Visualization and Histological Confirmation

Historically, laparoscopy followed by histological confirmation was considered the gold standard for diagnosing endometriosis [29]. A thorough examination of the pelvis and abdomen is performed during laparoscopy to identify all phenotypic presentations of endometriosis [30]. Laparoscopy may allow for a simultaneous diagnosis and treatment of disease, whereby suspected endometriosis is excised and confirmed histologically [31]. However, the technique does have several notable limitations, including resource implications and exacerbating diagnostic delay

through prolonged surgical wait times [32]. Patients may encounter wait periods for surgery ranging from 6 months to 3 years following their initial visit [33]. Furthermore, recent studies have highlighted the discrepancies between laparoscopy and histology, stemming from the dependence on the surgeon to completely remove all affected tissue and the meticulous examination demanded by intact histological specimens [32]. Additionally, the challenge posed by the simultaneous identification and treatment of complex disease states demands specialized expertise, considerably lengthier procedural time, coordination with other surgical services, patient preparation (including bowel preparation), and explicit patient consent [34]. Although laparoscopy is an invasive procedure associated with several risks, it is the most widely used for diagnosing endometriosis, especially in cases where non-invasive approaches have not provided conclusive results, such as limited detection of SE [35].

I.IV.II Transvaginal Ultrasound

Non-invasive imaging techniques are crucial in diagnosing endometriosis and providing valuable information about the disease, including presence/absence, locale, size, and extent. A rapid, non-invasive imaging-based diagnosis of endometriosis in an outpatient setting enables timely management. Furthermore, non-invasive modalities may be further used to guide treatment approaches, including laparoscopy, whereby surgical planning is facilitated. Guided treatment may also uphold patient autonomy, allowing individuals to select treatment options based on informed education.

The European Society of Human Reproduction and Embryology (ESHRE) guidelines have recommended that transvaginal ultrasound (TVS) should be employed as a first-line imaging modality due to its accessibility, cost-effectiveness, and ability to assess pelvic structures dynamically [36]. This development has been primarily instigated by improved diagnostic performance and classifications of TVS, summarized by the International Deep Endometriosis Analysis (IDEA) consensus statement, curated in response to inconsistencies describing anatomical structures and disease locale [37]. The IDEA consensus proved pivotal in promoting standardization in reporting the location and extent of endometriosis while simultaneously allowing for meaningful comparisons from scan to scan, locally and across the globe [37]. Considering this, recent publications using the IDEA consensus have suggested that TVS may reliably diagnose OE with improved accuracy in identifying and diagnosing DE [38–41], though SE remains elusive. Despite some improvement, anatomical site-specific variation in accuracy exists, with some disease locales, including USLs, still exhibiting poor diagnostic test accuracy with TVS [39].

It's important to highlight that the current assessment of diagnostic accuracy for TVS in diagnosing DE relies on previous classification guidelines, limiting our comprehension of accuracy when applied to newer guidelines. Considering this, there is a need for further investigation into the diagnostic efficacy of TVS for detecting DE, aligning with the IDEA consensus and the revised classification guidelines.

I.V Diagnostic Test Accuracy

I.V.II Diagnostic Accuracy Methodology

The most common method of determining a diagnostic test's ability to discriminate between those with endometriosis and those without includes traditional diagnostic accuracy methodology [40]. In a recent review evaluating the effectiveness of diagnostic modalities in diagnosing endometriosis, methodology mainly included sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), and overall accuracy [40]. This approach compares an index test (new diagnostic of interest) to a reference test (typically a gold standard), whereby parameters are calculated to determine diagnostic performance [42] *(Table 1*).

Table 1. Diagnostic accuracy performance parameters and their respective formulas

Diagnostic Accuracy Parameters	**Formula**
Sensitivity	$\frac{True\ Positives}{True\ Positives + False\ Negatives}$
Specificity	$\frac{True\ Negatives}{True\ Negatives + False\ Positives}$
Positive Predictive Value	$\frac{True\ Positives}{True\ Positives + False\ Positives}$
Negative Predictive Value	$\frac{True\ Negatives}{True\ Negatives + False\ Negatives}$

When assessing the index test, the test's sensitivity (true positive rate) refers to the probability of the test positively diagnosing the disease under the condition that they have the disease [43]. In comparison, specificity (true negative rate) is the probability of a negative test result among those who are truly negative [43]. Sensitivity and specificity are inversely proportional; when one

increases, the other decreases [42]. A highly sensitive test will have fewer false negatives or missed diagnoses, whereas a test with high specificity will have fewer false positives. In the context of disease screening, tests with low specificities are ineffective, as those without endometriosis will be diagnosed with a positive test result, leading to inappropriate management [44–46].

A predictive value of a test refers to the probability of a positive or negative test among those with and without the disease, respectively [43]. In endometriosis, if the disease was identified or absent in an individual, predictive values elucidate the likelihood that the patient is truly positive or negative. It should be noted that PPV and NPV depend on disease prevalence, sensitivity, and specificity [46]. To ensure a clinically relevant conclusion is made, it is critical to ensure an appropriate sample size when determining diagnostic accuracy parameters, as when the prevalence increases, PPV will increase, NPV will decrease, and vice versa when sample size decreases.

Although diagnostic test accuracy parameters are the most common method of determining the effectiveness of new diagnostic tests, particularly in binary classification problems posed by imaging modalities, other methods may be used. For example, it is common to see receiver operator curves (ROC) and area under the curve (AUC) metrics for biomarkers [47–49], where a single summary measure or a global metric is provided, plotted between sensitivity and specificity. Although these methods may be used, they do not provide predictive capabilities of a test nor elucidate the impact of individual parameters in the model [50]. For example, one test may have a high sensitivity and a low specificity or vice versa yet yield the same AUC. Global

measures, such as ROCs/AUCs and diagnostic odds ratios, may be helpful when comparing two or more diagnostic tests, though they have limited utility in their clinical significance [50].

I.V.II Diagnostic Accuracy of TVS in Endometriosis

In developing novel non-invasive methods for diagnosing endometriosis, diagnostic accuracies must be determined among phenotypes and disease locales to ensure clinical utility. The most adopted reference standards for comparing novel index tests include laparoscopy and/or histological confirmation when tissue is available [39]. Considering this, given the development of guidelines and increased standardized reporting, studies have elucidated the diagnostic potential of various non-invasive modalities, particularly TVS. However, given the potential inconsistencies between laparoscopy and histology and the lack of a definitive negative diagnosis, as healthy tissue is seldom removed, comparisons and definitive conclusions should be made cautiously.

Alongside developing the IDEA consensus, the International Ovarian Tumour Analysis (IOTA) criteria have enabled TVS to diagnose OE reliably [51]. In a Cochrane Review evaluating the efficacy of TVS in diagnosing OE, the pooled sensitivity was 93% and a specificity of 96% [52], though a more recent multisite study by Leonardi et al., adhering to the IDEA guidelines suggests a sensitivity of 92.1 – 92.2 % and a specificity 90.8 – 92.4% [39]. Although there is widespread trust in TVS reliably diagnosing OE, the accuracy of TVS diagnosing DE remains variable depending on the locale [40]. The bowel, including the rectum and rectosigmoid, is the site with the highest diagnostic accuracy for DE diagnosed by TVS, with a recent meta-analysis

suggesting a pooled sensitivity of 91% (95% CI 88.1–93.5) and specificity of 98% (95% CI 96.7–99.0) [53]. Given these findings, TVS has been accepted as a reliable diagnostic for OE and certain sites of DE, though other locales, including the USLs and TU, remain limited in their accuracy [37,39].

I.V.III Diagnostic Accuracy of the Uterosacral Ligaments

The USLs are the most common site of DE, with a prevalence ranging from 20% to 70% [54,55], depending on the population and setting. The USLs are connective structures between the sacral spine and posterior uterus, conjoined by the TU, maintaining normal anatomical positioning of the pelvic environment [56]. These structures also relay crucial vessels, lymphatics, and nerves, which provide sensory and autonomic innervation throughout the pelvic cavity [57,58]. In the context of endometriosis, infiltration of DE within the USLs has been associated with chronic pelvic pain-like symptoms and dyspareunia, with symptoms improving upon excision of the disease [59,60]. It has been recently appreciated that DE nodules may affect individual USLs as discrete nodules or affect them bilaterally with or without TU involvement. Complex disease states, including POD obliteration and bowel DE, are commonly associated with USL and TU disease, requiring a more meticulous diagnostic and surgical approach [19].

The most utilized TVS technique to diagnose USL DE includes placement of the TVS probe in the anterior vaginal fornix. Historically, TVS for DE of the USLs and TU has had the lowest diagnostic accuracy, [38,39,41,55] with the most recent prospective multisite study adhering to the IDEA consensus, suggesting a sensitivity of 44.4 – 58.7%, specificity of 77.8 – 88.2%, PPV of

63.3 – 75.5% and NPV of 66.1 – 77.5% [39]. As such, there has been particular interest in improving the diagnostic capacity of TVS in diagnosing and characterizing DE among these structures.

Although the exact deficiencies in diagnosing USL DE are unclear, we have hypothesized that it is likely two-fold. Firstly, there is universal unfamiliarity with how to visualize these structures non-invasively as they have never been included in the structures intending to be assessed on pelvic ultrasound prior to the IDEA consensus statement. Secondly, there may be a negative influence of the concurrent complex disease states on diagnostic test accuracy for DE in the adjacent USL/TU [19,27]. In complex disease states, anatomical landmarks are largely distorted, potentially impacting the ability to identify the structures and diagnose the disease [15,19]. Improving the ability of TVS to diagnose DE of the USLs/TU and understanding the impact of concurrent complex disease states on diagnostic accuracy is pivotal in addressing the significant diagnostic delay associated with the disease and improving guided treatment.

I.VI Project Rationale, Hypothesis, and Thesis Objectives

The significance of USLs and TU in endometriosis is evident, as they represent pivotal structures and are the primary site of DE. Historical diagnostic imaging practices have consistently yielded the lowest accuracy in diagnosing DE of the USLs and TU. This diagnostic challenge contributes substantially to the pronounced delay in endometriosis diagnoses, perpetuating the existing obstacles in timely intervention and tailored treatment planning, particularly concerning surgical

interventions. The lack of precision in diagnosing endometriosis within the USLs not only prolongs the suffering of affected individuals but also complicates surgical planning and fosters an economic burden on the health system.

The inefficiencies in site-specific diagnosis of endometriosis within the USLs underscore the critical need to enhance diagnostic methodologies, particularly TVS, which has been accepted as a reliable first-line imaging modality. A comprehensive understanding of how concurrent complex disease states influence the diagnostic accuracy of TVS is paramount for advancing the overall diagnosis and mapping of endometriosis. By unravelling the intricacies of endometriosis within the USLs, clinicians can gain valuable insights that will guide surgical management. This, in turn, holds the promise of optimizing patient care and outcomes, breaking the cycle of delayed diagnoses, and ensuring that individuals with deep endometriosis receive timely and effective interventions.

To address this critical gap in knowledge, the present work aimed not only to reevaluate the diagnostic accuracy of TVS in identifying DE within the USLs but also to introduce a novel approach to enhance the precision of TVS. Using a novel approach – called the posterior TVS approach - we hypothesize that there will be an improved diagnostic accuracy for DE of the USL and TU relative to what has been described in the literature. Furthermore, the investigations sought to elucidate how complex disease states impact the accuracy of TVS in detecting DE of the USLs and TU. We hypothesized that TVS would have reduced diagnostic performance in diagnosing DE of USLs and TU in the presence of concurrent complex disease states. Through

these endeavours, the overarching aim of this thesis is to enhance TVS methodology and our understanding of the modality as a non-invasive diagnostic for DE of the USLs and TU to combat diagnostic delay and improve patient care.

II. Chapter II

Published Manuscript.

II.I. Preface and Significance to Thesis

Endometriosis, characterized by endometrial-like tissue outside the uterine cavity, poses a substantial diagnostic challenge. Timely and accurately identifying endometriotic lesions is crucial for effective management and improved patient outcomes. The USLs, the most common sites of DE, and their neighbouring TU have emerged as focal points for diagnostic investigation.

This study aims to target the first objective of the thesis, whereby a novel posterior TVS approach was adopted to prospectively evaluate the diagnostic accuracy of TVS in assessing DE within the USLs and TU. We hope that the findings presented herein will not only contribute to the expanding body of knowledge surrounding endometriosis diagnostics but will also pave the way for more effective and accessible diagnostic modalities. The findings of this study suggest that TVS may be a reliable diagnostic modality for the USLs and TU using this novel posterior approach. May this contribution serve as a foundation for future advancements in endometriosis research and diagnostics, fostering a deeper understanding of this complex condition and, ultimately, improving the lives of those affected.

II.II Authors' Contribution

The following study was spearheaded by SMF, under the supervision of ML, and included study design, ethics, data collection, analysis, tables, and manuscript development and submission. ML performed all imaging procedures, including advanced TVS and laparoscopy. KM and VT coordinated the study. The final manuscript was completed by SMF and edited by ML prior to submission.

II.III Prospective diagnostic test accuracy of uterosacral ligament and torus uterinus endometriosis using transvaginal ultrasound posterior approach (Freger et al., 2024; https://doi.org/10.1002/uog.27492).

Prospective diagnostic test accuracy of uterosacral ligament and torus uterinus endometriosis using transvaginal ultrasound posterior approach

Shay M Freger [1], Victoria Turnbull [1], Kyle McGowan [1], Mathew Leonardi [1,2]

1. *Department of Obstetrics & Gynecology, McMaster University, Hamilton, Ontario, Canada.*
2. *Robinson Research Institute, University of Adelaide, Adelaide, Australia*

Corresponding Author:

Mr. Shay M. Freger

Department of Obstetrics and Gynecology. Faculty of Medical Sciences.

McMaster University

1280 Main Street West. Hamilton, Ontario, Canada. L8S 4K1

Phone: (416) 938 - 5926

E-mail: smfreger@gmail.com

Running Head: Endometriosis, Ultrasound, and USLs

Keywords: Endometriosis, Transvaginal Ultrasound, Diagnostics Test Accuracy, Uterosacral Ligaments

ABSTRACT

Objective: To determine the diagnostic test accuracy of transvaginal ultrasound (TVS) using a standardized technique for the diagnosis of deep endometriosis (DE) of the uterosacral ligaments (USLs) and adjacent torus uterinus (TU).

Methods: This was a prospective diagnostic test accuracy study conducted at the McMaster University Medical Center Tertiary Endometriosis Clinic, Hamilton, ON, Canada. Consecutive participants were enrolled if they successfully underwent TVS and surgery by our team from 10 August 2020 to 31 October 2021. The index test was TVS using a standardized posterior approach performed and interpreted by an expert sonologist. The reference standard included direct surgical visualization on laparoscopy by the same person who performed and interpreted the ultrasound scans. Accuracy, sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative likelihood ratios were calculated for the TVS posterior approach for each location using the reference standard.

Results: There were 54 consecutive participants included upon completion of laparoscopy and histological assessment. The prevalence of DE for the left USL, right USL, and TU was 42.6%, 22.2%, and 14.8%, respectively. Based on surgical visualization as the reference standard, TVS demonstrated an accuracy of 92.6% (95%CI, 82.1–97.9%), sensitivity of 82.6% (95%CI, 61.2–95.1%), specificity of 100% (95%CI, 88.8–100%), PPV of 100% and NPV of 88.6% (95%CI, 76.1–95.0%) for diagnosing DE in the left USL. For DE of the right USL, TVS demonstrated an accuracy of 94.4% (95%CI, 84.6–98.8%), sensitivity of 75.0% (95%CI, 42.8–94.5%), specificity

of 100% (95%CI, 91.6–100%), PPV of 100% and NPV of 93.3% (95%CI, 84.0–97.4%). For DE of the TU, TVS demonstrated an accuracy of 100% (95%CI, 93.4–100%), sensitivity of 100% (95%CI, 63.1–100%), specificity of 100% (95%CI, 92.3–100%), PPV of 100% and NPV of 100%.

Conclusions: We observed high diagnostic test accuracy of the evaluated standardized TVS technique for assessing DE of the USLs and TU. Further studies evaluating this technique should be performed, particularly with less experienced observers, before considering this technique as the standard approach.

Introduction

Endometriosis is a highly prevalent gynecological disease affecting approximately 10% of women and individuals assigned female at birth. It is characterized by the presence of endometrial-like tissue in areas outside the uterus, resulting in a chronic state of inflammation [1–3], extensive pelvic adhesions, and severe morbidity in the form of pelvic pain and/or infertility [3]. Endometriosis lesions are divided into one of three phenotypes: superficial (most common, but non-infiltrative), ovarian (cysts within the ovaries), and deep endometriosis (DE; infiltrative and most aggressive phenotype, leading to significant distortion of the surrounding anatomical and physiological milieu) [2,4,5].

Historically, to achieve a diagnosis of endometriosis, a combination of direct visualization of endometriosis lesions at surgery combined with histopathological analysis was required [6]. However, due to extensive surgical wait times, invasiveness, diagnostic delay and inconsistencies in diagnostic accuracies [7], the European Society of Human Reproduction and Embryology (2022) guidelines [6] now recommend the use of non-invasive imaging modalities such as transvaginal ultrasound (TVS) [6]. In 2016, the International Deep Endometriosis Analysis group (IDEA) consensus was developed to improve and describe the sonographic features of endometriosis [8], improving standardization and characterization of disease phenotypes, including anatomical structures in the posterior compartment such as the uterosacral ligament (USL) and the torus uterinus (TU; junction of the two USLs at the retrocervix). With this development, TVS has become a reliable, non-invasive, and rapid diagnostic modality for diagnosing DE, with a

sensitivity ranging from 88 to 90% and specificities of 76 to 79% [9], depending on the location of endometriosis.

Despite these recent developments, the USLs remain the most difficult to diagnose with moderate accuracy [10], including a sensitivity and specificity of 60-67% and 86-95% [11,12], respectively, despite being the most common location of DE [10,13]. Several authors have argued that the TVS methodology to visualize normal and abnormal USLs and the TU is limited, yielding these poor diagnostic test accuracy scores [11,14].

Our objective was to assess the diagnostic accuracy of TVS through a standardized posterior approach [14,15], with the probe in the posterior vaginal fornix for diagnosing DE of the USLs and TU. We hypothesized that this TVS technique would have an improved diagnostic accuracy for the USL and TU DE relative to what was previously described in the literature.

Methods

The study is reported according to Standards for Reporting Diagnostic Accuracy (STARD; 2015) guidelines [16] to assist in standardization and transparency of reporting diagnostic accuracy with consideration for the Quality Assessment of the Diagnostic Accuracy of Studies (QUADAS-2) checklist [17].

Study Design

This is a prospective diagnostic test accuracy study of patients who underwent TVS and laparoscopy (with surgical treatment of endometriosis when it was identified) at McMaster University Medical Center, Hamilton Health Sciences. Participant recruitment took place from August 10, 2020 – October 31, 2021. A single sonologist interpreter (ML), considered an expert based on the study coordinator and European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) (level 3) [18], was responsible for the final review and reporting of ultrasound findings. All ultrasound scans were performed within one year of surgery. Similarly, the reference standard surgical procedures were all performed by a single surgeon (ML) with minimally invasive gynecologic surgery training and working in a center of surgical excellence with available colorectal and urologic surgery.

Participants

Consecutive participants were recruited if they met the inclusion criteria. Inclusion criteria included: age between 18 and 50 years, assigned female sex at birth, pre-menopausal and post-menarchal, history of chronic pelvic pain and/or endometriosis, able to undergo TVS and consented to laparoscopic surgery for endometriosis. Exclusion criteria included previous diagnoses or current active gynecologic malignancy or if they ultimately underwent laparoscopy at a different center. Patients on various medical therapies were not excluded.

Ethical approval

This study was approved by the Hamilton Integrative Research Ethics Board (HiREB: 12617).

Study Procedure & Test Methods

All data, including TVS, surgical, and histological findings, were collected prospectively in real-time. The data was reviewed and entered by two investigators independently and confirmed and audited for accuracy by the supervising investigator (ML).

Index test

The index test, TVS, was performed and reported in accordance with the IDEA consensus with the additional posterior approach technique, hypothesized to improve the diagnostic accuracy described by Leonardi and colleagues [14,15] for the evaluation of DE of the USLs and TU (Appendix A). Mindray Zonare machines were used with an E9-4 (4-9 MHz) transducer.

Transvaginal Ultrasound Posterior Approach

The technique for TVS assessment of the USL and TU involves the insertion of the TVS probe into the posterior vaginal fornix, angled towards the rectum in the midsagittal position. Once the hypoechoic posterior vaginal fornix and the overlying hyperechoic rectouterine peritoneum (thin white line) are visualized, the probe is lateralized and rotated clockwise (right USL) or counter-clockwise (left USL), usually no more than 45°. Upon lateralization and rotation, there is a progressive thickening of the white line of the peritoneum, revealing the USL at the thickest point. If pelvic vessels are noted in concentration, the probe tip has been lateralized too far. Suspected DE presents as a hypoechoic nodule within the surrounding hyperechoic USL and

should be measured in three orthogonal planes. Characterization of DE of the USLs and TU using the standardized posterior approach is illustrated in *Figures 1 and 2*. DE nodules in this space can be regular or irregular and can be confluent with other nodules nearby in the posterior vaginal fornix, bowel, TU, or parametrium. Sonographically, the TU is defined as the thin hyperechoic layer of tissue immediately posterior to the cervix. The TU tissue simply continues into the USLs without a clear anatomical border, but the edge of the cervix can be used as an estimate of a border.

Reference Standards

Surgical Visualization

The index test was compared primarily to direct visualization through laparoscopy as the reference standard. When tissue was available, the index test was compared to histological confirmation as a secondary reference standard. Direct visualization involved a systematic evaluation of the abdomen and pelvis of all surfaces as per the local standardized protocol (Appendix B). The reporting of the surgical findings mirrored the systematic ultrasound evaluation approach. The surgeon, ML, was not blinded to the ultrasound findings, which reflects the real clinical experience of surgeons who utilize preoperative information to appropriately plan and perform surgery. Complete excision of endometriosis was performed unless the risk of excision outweighed the benefit as per the patient's preferences and informed consent, in which case occasional intentional incomplete excision was performed. Direct visualization yielded the presence of DE when irregular nodules of varying pigmentations with a fibrotic (or hard) tactile feedback on palpation were noted [19]. An endometrioma was noted when

an ovarian cyst containing dark brown material ("chocolate cyst") was present. Obliteration of the rectouterine pouch was noted when the peritoneum between and inferior to the USLs was not visible or present due to the presence of adhesions. Normal peritoneum and other pelvic surfaces were noted when no evidence of endometriosis (of any subtype) or adhesions were present. If in doubt about the appearance of an area as normal versus abnormal, it was considered suspicious for endometriosis based on direct visualization and excision was performed to achieve a histological assessment.

Histology

Microscopically, DE is characterized by endometrial stroma and/or glands with fibrosis, along with hyperplastic and hypertrophic smooth-muscle fibres [20]. The pathologists performing the histological evaluation were not blinded to the clinical history, which similarly reflects the real clinical experience of pathologists. However, they were blinded to the index test results. Histology was only evaluated when samples were available and provided as supplementary data.

Other Variables

Variables collected for univariate analysis include age, endometriosis phenotypes and distribution present at laparoscopy and confirmed histologically, presence of adenomyosis described by the presence of Morphological Uterus Sonographic Assessment (MUSA) features, presence of fibroids described by the International Federation of Gynecology and Obstetrics (FIGO) classification features, symptoms, (dysmenorrhea, dyspareunia, dyschezia, dysuria, and abnormal uterine bleeding), and previous diagnosis and surgery for endometriosis.

Sample Size

The sample size for the TVS technique was determined based on Buderer's formula [21]. Using the expected prevalence of surgically confirmed USL DE within our clinical population of 35% and an expected sensitivity and specificity from previously reported diagnostic accuracy studies of 90% and 85%, respectively (confidence level of 95%; 0.85 power), a total of n = 49 participants was required.

Analysis

Data was collected using the REDCap electronic data capture tool (Vanderbilt University, Nashville, Tennessee, USA) and imported into Microsoft Excel for Windows 10 (Microsoft Corporation, Santa Rosa, CA, USA). Cleaned data was transferred and analyzed using IBM SPSS statistics V29 software (SPSS Inc., Chicago, IL, USA).

Descriptive statistics were used to summarize all variables. Continuous variables were described with means and respective standard deviations. Categorical variables were described as frequencies and percentages. Additional pertinent variables identified during TVS and/or laparoscopy with histological confirmation, including endometriosis phenotypes and co-presentation of benign gynecological diseases, were reported as frequencies and percentages relative to the total population.

Accuracy, sensitivity, specificity, negative and positive predictive value, negative (LR-) and positive (LR+) likelihood ratios with 95 % confidence interval (CI) were calculated for the index test relative to laparoscopy as the primary reference standard among all participants. The diagnostic parameters for the index test were then calculated relative to histology as a secondary reference standard. All accuracy parameters were determined using the cross-tabulation function in SPSS.

Results

Participant Characteristics

Of the 160 consecutive patients identified in the clinic setting, 54 met the inclusion criteria and were included in the study (Figure 3). All participants underwent the index test and surgical visualization. Only one participant underwent diagnostic laparoscopy without surgical excision of endometriosis despite its presence; the surgery was abandoned due to anesthesiology concerns.

Participant characteristics are summarised in Table 1. Of those who were included, the mean age at the time of evaluation was 35.2±7.2 years, with a mean duration from the index test to the reference standard of 177.4±100.5 days. The most common symptoms among the patients included 94.4% (51/54) dysmenorrhea, followed by 72.2% (39/54) abnormal uterine bleeding, 66.7% (36/54) dyspareunia, 59.3% (32/54) dyschezia, and 25.9% (14/54) dysuria. Approximately 70.4% (38/54) of the patients had previously been diagnosed with endometriosis before assessment at McMaster University Medical Center, which included a presumptive clinical diagnosis, imaging-based diagnoses, or surgical diagnosis. Additional medical history further indicated a rate of anxiety and/or depression at 40.7% (22/54). Past surgical history was reported at 88.9% (48/54), of which 44.4% (24/54) included past surgery for endometriosis (laparoscopic ablation or excision). In addition to endometriosis features, 24.1% (13/54) and 37.0% (20/54) of patients exhibited fibroids and adenomyosis features on TVS, respectively.

Surgical and Histological Findings

The left USL had the highest prevalence of DE, seen in 42.6% (23/54) of patients laparoscopically, with a prevalence of 33.3% (18/54) upon histological assessment. Right USL DE was present in 22.2% (12/54) of patients laparoscopically and 18.5% (10/54) through histological assessment. TU DE was the least prevalent, seen in 14.8% (8/54) laparoscopically and 13.0% (7/54) through histological assessment. The left USL, right USL, and TU nodules were solitary in 25.9% (14/54), 5.5% (3/54), and 7.4% (4/54) of cases, respectively, with 16.7% (9/54) of nodules existing within multiple sites.

When assessing lesion dimensions in three orthogonal planes sonographically, the mean length, width, and height of the left USL with the respective standard deviations, were 9.3mm (± 4.5mm), 5.0mm (± 3.0mm), and 10.0mm (± 3.9mm), respectively. For the right USL, 10.4mm (± 7.0mm), 6.8mm (± 4.8mm), and 8.5mm (± 3.8mm), respectively. For the TU, 7.8mm (± 3.6mm), 5.6mm (± 2.7mm), and 10.0mm (± 4.2mm), respectively.

Beyond the USL and TU nodules of interest, laparoscopically visualized and histologically confirmed superficial endometriosis was present in 70.4% (38/54) of all patients. Laparoscopic and histologically confirmed OE was present in 29.6% (16/54) of patients, with 11.1% (6/54) having confirmed non-OE benign cysts. Beyond the USLs, the most common location for DE was the bowel, with a prevalence of 18.5% (10/54) and a prevalence of laparoscopically confirmed rectouterine pouch obliteration among 24.1% (13/54) of patients.

Diagnostic Performance

The diagnostic test performance for the index test relative to the reference standard for three anatomical structures in the posterior compartment is presented in Tables 1-3. Diagnostic test performance of TVS in the location of the left USL, with surgical visualization as the reference standard, respectively, was as follows: accuracy 92.6% (95% CI 82.1 – 97.9%), sensitivity 82.6% (95% CI 61.2 – 95.1%), specificity 100% (95% CI 88.8 – 100%), PPV 100%, NPV 88.6% (95% CI 76.1 – 95.0%), LR+ 33.2 (95% CI 4.8 – 229.5%) and LR- 0.2 (95% CI 0.2 – 0.4), respectively.

Diagnostic test performance of TVS in the location of the right USL, with surgical visualization as the reference standard, respectively, was as follows: Accuracy 94.4% (95% CI 84.6 – 98.9%), sensitivity 75.0% (95% CI 42.8 – 94.5%), specificity 100% (95% CI 91.6 – 100%), PPV 100%, NPV 93.3% (95% CI 84.0 – 97.4%), and LR- 0.3 (95% CI 0.1 – 0.7) respectively. The TU was the location with the overall highest diagnostic test performance relative to the reference standard, including an accuracy 100% (95% CI 93.4 -100%), sensitivity 100% (95% CI 63.1 – 100%), specificity 100% (95% CI 92.3-100%), PPV 100%, and NPV 100%, respectively. Diagnostic test performance of TVS with histology as a reference standard, under the assumption that the surgeon was correct in the absence of disease, is provided as *Supplementary Table 1*.

Discussion

Main Findings

In this diagnostic accuracy study, we evaluated the accuracy of a standardized TVS posterior approach for identifying and characterizing DE of the USL and TU, proposed by Leonardi and colleagues in 2020 [14]. Our findings suggest that identifying DE in its most common location, the USLs, with TVS placed within the posterior vaginal fornix may yield improved diagnostic accuracy scores relative to those reported in the literature [11,12]. When assessing diagnostic performance, our results suggest anatomical site and reference test-specific variations, though subtle, as suggested by the large overlap in 95% CI's.

Interpretation and Significance

There is a ubiquitous acceptance of the need for USL evaluation as part of the TVS examination of endometriosis [11]. Although the USLs are the most common location of DE, they remain the most difficult to diagnose due to the small size of nodules and a pervasive unfamiliarity in evaluating these structures using imaging [10–12]. Despite recent advancements made through the development of the IDEA consensus in aiding the diagnosis of endometriosis, the diagnostic test performance of TVS of these anatomical areas and the presence of DE have not improved [9,11]. A recent study evaluating the diagnostic performance of TVS using the IDEA consensus among all anatomical sites suggested the USLs and TU had the lowest accuracy, with overall accuracies ranging between 65.2% to 74.4%, sensitives 44.2 to 58.7%, and specificities 77.8% to 88.2% among the three sites [9]. Our findings suggest that implementing using the standardized posterior approach proposed by Leonardi and colleagues (i.e., approaching the posterior compartment

through the posterior vaginal fornix) may improve the visualization of the USLs and TU – normal and abnormal and the diagnostic test performance of TVS. Because the USLs are the most common location for DE and the only site of disease in some individuals, the *overall* diagnostic accuracy of TVS for endometriosis and lengthy diagnostic delay in those suffering from endometriosis can be improved by optimizing the imaging diagnostic performance of the USLs. Beyond diagnostic improvements, understanding USL DE may have clinical utility; the USLs are highly innervated supporting structures, carrying crucial neurological components from the spinal cord, including the inferior hypogastric plexus, supplying pelvic and perineal organs with parasympathetic and sympathetic innervation for normal physiological phenomena to occur [22]. Endometriosis of the USL may be clinically linked to symptoms such as dyspareunia and chronic pelvic pain [23]. Identifying endometriosis of the USLs and respective TU is essential in advancing our understanding of this enigmatic disease with varying presentations of symptoms. Lastly, it should be noted that scanning the USLs and TU is possible through the anterior vaginal fornix, providing a different perspective than the posterior approach, which on occasion, may provide added value.

Strengths and Limitations

All efforts were made to ensure this study's robustness through adherence to the STARD guidelines [16]. The prospective nature of this study involved utilizing standardized reporting forms to ensure consistency among consecutive patients. Furthermore, the current study was performed in a specialized center with high-quality ultrasound and surgical equipment, allowing for detailed characterization of the posterior compartment. To ensure generalizability, the technique adheres primarily to the methodology described by Leonardi and colleagues [14,15], on

the background of the IDEA approach, which has previously shown an improvement in diagnostic accuracy using a generalizable, multisite approach [8,9]. Lastly, the strength of the study lays in the innovation of evaluating the diagnostic accuracy of TVS imaging using a standardized posterior approach.

Several limitations should be noted, particularly in the interpretation of the results. First, our study's reported accuracy and generalizability may be impacted by the nature of the TVS, and surgery being performed by the same highly trained surgeon sonologist and the use of high-quality equipment. Given the current study design, the same person who developed the technique was the same person conducting all TVS scans and surgeries, making double-blind evaluation unfeasible. Though our overall design is common in endometriosis diagnostic accuracy studies, future studies could consider a design which involves blinding the surgeon to ultrasound findings. In addition, one could consider a design comparing non-experienced ultrasound operators (index test) against experienced operators (reference standard), or vice versa, to potentially enhance the generalizability of the study's technique. Furthermore, a large portion of the patient population did not undergo histological evaluation due to the absence of endometriosis seen during laparoscopically – biopsies of entirely normal tissue, which were not acquired for ethical and resource-limitation reasons. In such cases, it is uncertain whether these patients were truly absent of disease, as samples were not obtained. The study relies on the accuracy of surgical visualization, which has limitations [7]. Specifically, deep endometriosis lesions could hypothetically be missed by a surgeon's eye [24]. Although the diagnosis of endometriosis typically relies on the final histological confirmation, histological assessment does have limitations, including in such cases where stroma and epithelial cells are altered/damaged [25]

or the presence of fibrous obliteration [26]. Similar limitations arise upon using laparoscopy, where studies have suggested 50% of surgical biopsies suspected of being endometriosis were proven histologically [27], and 25% of atypical appearing tissue not suspected of being endometriosis were proven to be endometriosis [28]. The studies elucidate the reliance on surgical experience and expertise. Due to the imperfect relationship between what is seen laparoscopically and histologically, the assumption that the surgeon was correct in their diagnosis was adopted [7]. Additionally, this study had a relatively small sample size, with 44.4% of the participants previously having endometriosis surgery, which may create bias, potentially artificially increasing the diagnostic performance of TVS. Lastly, we did not include a comparison with an anterior TVS approach to truly suggest whether a posterior approach is superior in imaging the USLs.

Conclusion

DE of the USLs and TU is highly prevalent, though poorly recognized and characterized using TVS in previous studies. We have tested a previously proposed technique predicated on the TVS probe evaluating the posterior compartment from the posterior vaginal fornix, which we call the posterior approach. Our findings suggest that the posterior approach may yield improved accuracy for the USLs and TU compared to previous studies. External validation and larger-scale studies would be valuable in strengthening or refuting these findings.

References

1. Zondervan KT, Becker CM, Missmer SA. Endometriosis. Longo DL, ed. *N Engl J Med*. 2020;382(13):1244-1256. doi:10.1056/NEJMra1810764

2. Scioscia M, Bruni F, Ceccaroni M, Steinkasserer M, Stepniewska A, Minelli L. Distribution of endometriotic lesions in endometriosis stage IV supports the menstrual reflux theory and requires specific preoperative assessment and therapy. *Acta Obs Gynecol Scand*. 2011;90(2):136-139. doi:10.1111/J.1600-0412.2010.01008.X

3. Giudice LC, Kao LC. Endometriosis. *Lancet*. 2004;364(9447):1789-1799. doi:10.1016/S0140-6736(04)17403-5

4. Ianieri MM, Mautone D, Ceccaroni M. Recurrence in deep infiltrating endometriosis: A systematic review of the literature. *J Minim Invasive Gynecol*. 2018;25(5):786-793. doi:10.1016/J.JMIG.2017.12.025

5. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril*. 1997;68(4):585-596. doi:10.1016/S0015-0282(97)00191-X

6. Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, King K, Kvaskoff M, Nap A, Petersen K, Saridogan E, Tomassetti C, van Hanegem N, Vulliemoz N, Vermeulen N, Group EEG, Altmäe S, Ata B, Ball E, Barra F, Bastu E, Bianco-Anil A, Knudsen UB, Brubel R, Cambitzi J, Cantineau A, Cheong Y, Daniilidis A, Bie B De, Exacoustos C, Ferrero S, Gelbaya T, Goetz-Collinet J, Hudelist G, Hussain M, Indrielle-Kelly T, Khazali S, Kumar SL, Maggiore ULR, Maas JWM, McLaughlin H, Metello J, Mijatovic V, Miremadi Y, Muteshi C, Nisolle M, Oral E, Pados G, Parades D, Pluchino N, Supramaniam PR, Schick M, Seeber B, Seracchioli R, Laganà AS, Stavroulis A, Tebache L, Uncu G, Van den Broeck U, van Peperstraten A, Vereczkey A, Wolthuis A, Bahat PY, Yazbeck C. ESHRE guideline: endometriosis. *Hum Reprod Open*. 2022;2022(2):1-26. doi:10.1093/HROPEN/HOAC009

7. Pascoal E, Wessels JM, Aas-Eng MK, Abrao MS, Condous G, Jurkovic D, Espada M, Exacoustos C, Ferrero S, Guerriero S, Hudelist G, Malzoni M, Reid S, Tang S, Tomassetti C, Singh SS, Van den Bosch T, Leonardi M. Strengths and limitations of diagnostic tools for endometriosis and relevance in diagnostic test accuracy research. *Ultrasound Obs Gynecol*. 2022;60(3):309-327. doi:10.1002/UOG.24892

8. Guerriero S, Condous G, Bosch T van den, Valentin L, Leone FPG, Schoubroeck D Van, Exacoustos C, Installé AJF, Martins WP, Abrao MS, Hudelist G, Bazot M, Alcazar JL, Gonçalves MO, Pascual MA, Ajossa S, Savelli L, Dunham R, Reid S, Menakaya U, Bourne T, Ferrero S, Leon M, Bignardi T, Holland T, Jurkovic D, Benacerraf B, Osuga Y, Somigliana E, Timmerman D. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. *Ultrasound Obs Gynecol*. 2016;48(3):318-332. doi:10.1002/UOG.15955

9. Leonardi M, Uzuner C, Mestdagh W, Lu C, Guerriero S, Zajicek M, Dueckelmann A, Filippi F, Buonomo F, Pascual MA, Stepniewska A, Ceccaroni M, Van den Bosch T, Timmerman D, Hudelist G, Condous G. Diagnostic accuracy of transvaginal ultrasound for detection of

endometriosis using International Deep Endometriosis Analysis (IDEA) approach: prospective international pilot study. *Ultrasound Obs Gynecol*. 2022;60(3):404-413. doi:10.1002/UOG.24936

10. Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V, Vacher-Lavenu MC, Dubuisson JB. Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. *Hum Reprod*. 2003;18(1):157-161. doi:10.1093/HUMREP/DEG009

11. Maple S, Chalmers KJ, Bezak E, Henry K, Parange N. Ultrasound characteristics and scanning techniques of uterosacral ligaments for the diagnosis of endometriosis. *J Ultrasound Med*. 2022;42(6):1193-1209. doi:10.1002/JUM.16129

12. Guerriero S, Ajossa S, Minguez JA, Jurado M, Mais V, Melis GB, Alcazar JL. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in uterosacral ligaments, rectovaginal septum, vagina and bladder: systematic review and meta-analysis. *Ultrasound Obs Gynecol*. 2015;46(5):534-545. doi:10.1002/UOG.15667

13. Manieri Rocha R, Leonardi M, Eathorne A, Armour M, Condous G. Anatomical distribution of endometriosis: A cross-sectional analysis of transvaginal ultrasound in symptomatic patients. *Australas J Ultrasound Med*. Published online 2023:https://doi.org/10.1002/ajum.12327. doi:10.1002/AJUM.12327

14. Leonardi M, Martins WP, Espada M, Arianayagam M, Condous G. Proposed technique to visualize and classify uterosacral ligament deep endometriosis with and without infiltration into parametrium or torus uterinus. *Ultrasound Obs Gynecol*. 2020;55(1):137-139. doi:10.1002/UOG.20300

15. Leonardi M, Condous G. A pictorial guide to the ultrasound identification and assessment of uterosacral ligaments in women with potential endometriosis. *Australas J Ultrasound Med*. 2019;22(3):157-164. doi:10.1002/AJUM.12178

16. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, De Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF, Alonzo T, Azuara-Blanco A, Bachmann L, Blume J, Boutron I, Büller H, Buntinx F, Byron S, Chang S, Cooper R, De Groot J, Deeks J, Dendukuri N, Dinnes J, Fleming K, Guyatt G, Heneghan C, Hilden J, Horvath R, Hunink M, Hyde C, Ioannidis J, Janes H, Kleijnen J, Knottnerus A, Lange S, Leeflang M, Lord S, Lumbreras B, Macaskill P, Magid E, Mallett S, McInnes M, Mc-Neil B, McQueen M, Moons K, Morris K, Mustafa R, Obuchowski N, Ochodo E, Onderdonk A, Overbeke J, Pai N, Peeling R, Pepe M, Petersen S, Price C, Ravaud P, Rutjes A, Schunemann H, Simel D, Simera I, Smidt N, Steyerberg E, Straus S, Summerskill W, Takwoingi Y, Thompson M, Van De Bruel A, Van Maanen H, Vickers A, Virgili G, Walter S, Weber W, Westwood M, Whiting P, Wilczynski N, Ziegler A. STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies. *Clin Chem*. 2015;61(12):1446-1452. doi:10.1373/CLINCHEM.2015.246280

17. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MMG, Sterne JAC, Bossuyt PMM. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536. doi:10.7326/0003-4819-155-8-201110180-00009/SUPPL_FILE/155-8-529-SUPPLEMENT.PDF

18. European Federation of Societies for ultrasound in medicine and biology. Minimum training requirements for the practice of Medical Ultrasound in Europe. *Ultraschall Med*. 2010;31(4):426-427. doi:10.1055/s-0030-1263214

19. Stegmann BJ, Sinaii N, Liu S, Segars J, Merino M, Nieman LK, Stratton P. Using location, color, size, and depth to characterize and identify endometriosis lesions in a cohort of 133 women. *Fertil Steril*. 2008;89(6):1632-1636. doi:10.1016/j.fertnstert.2007.05.042

20. Benagiano G, Brosens I. Review: The history of endometriosis: identifying the disease. *Hum Reprod*. 1991;6(7):963-968. doi:10.1093/OXFORDJOURNALS.HUMREP.A137470

21. Fenn Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Acad Emerg Med*. 1996;3(9):895-900. doi:10.1111/j.1553-2712.1996.tb03538.x

22. Ramanah R, Berger MB, Parratte BM, De Lancey JOL. Anatomy and histology of apical support: A literature review concerning cardinal and uterosacral ligaments. *Int Urogynecol J*. 2012;23(11):1483-1494. doi:10.1007/S00192-012-1819-7/METRICS

23. Hummelshoj L, De Graaff A, Dunselman G, Vercellini P. Let's talk about sex and endometriosis. *J Fam Plann Reprod Heal Care*. 2014;40(1):8-10. doi:10.1136/JFPRHC-2012-100530

24. Dinh T, Leonardi M, Espada M, Vanza K, Condous G. The Use of Ultrasound in Detecting Endometriosis: Endometriotic Nodule Detected on Ultrasound but not Visualized on Laparoscopy. *J Obstet Gynaecol Canada*. 2020;42(8):1016. doi:10.1016/J.JOGC.2019.12.013

25. Clement PB. The pathology of endometriosis: a survey of the many faces of a common disease emphasizing diagnostic pitfalls and unusual and newly appreciated aspects. *Adv Anat Pathol*. 2007;14(4):241-260. doi:10.1097/PAP.0B013E3180CA7D7B

26. Agarwal N, Subramanian A. Endometriosis - morphology, clinical presentations and molecular pathology. *J Lab Physicians*. 2010;2(1):001-009. doi:10.4103/0974-2727.66699

27. Walter AJ, Hentz JG, Magtibay PM, Cornella JL, Magrina JF. Endometriosis: correlation between histologic and visual findings at laparoscopy. *Am J Obs Gynecol*. 2001;184(7):1407-1413. doi:10.1067/MOB.2001.115747

28. Albee RB, Sinervo K, Fisher DT. Laparoscopic excision of lesions suggestive of endometriosis or otherwise atypical in appearance: relationship between visual findings and final histologic diagnosis. *J Minim Invasive Gynecol*. 2008;15(1):32-37. doi:10.1016/J.JMIG.2007.08.619

Table and Figure Captions

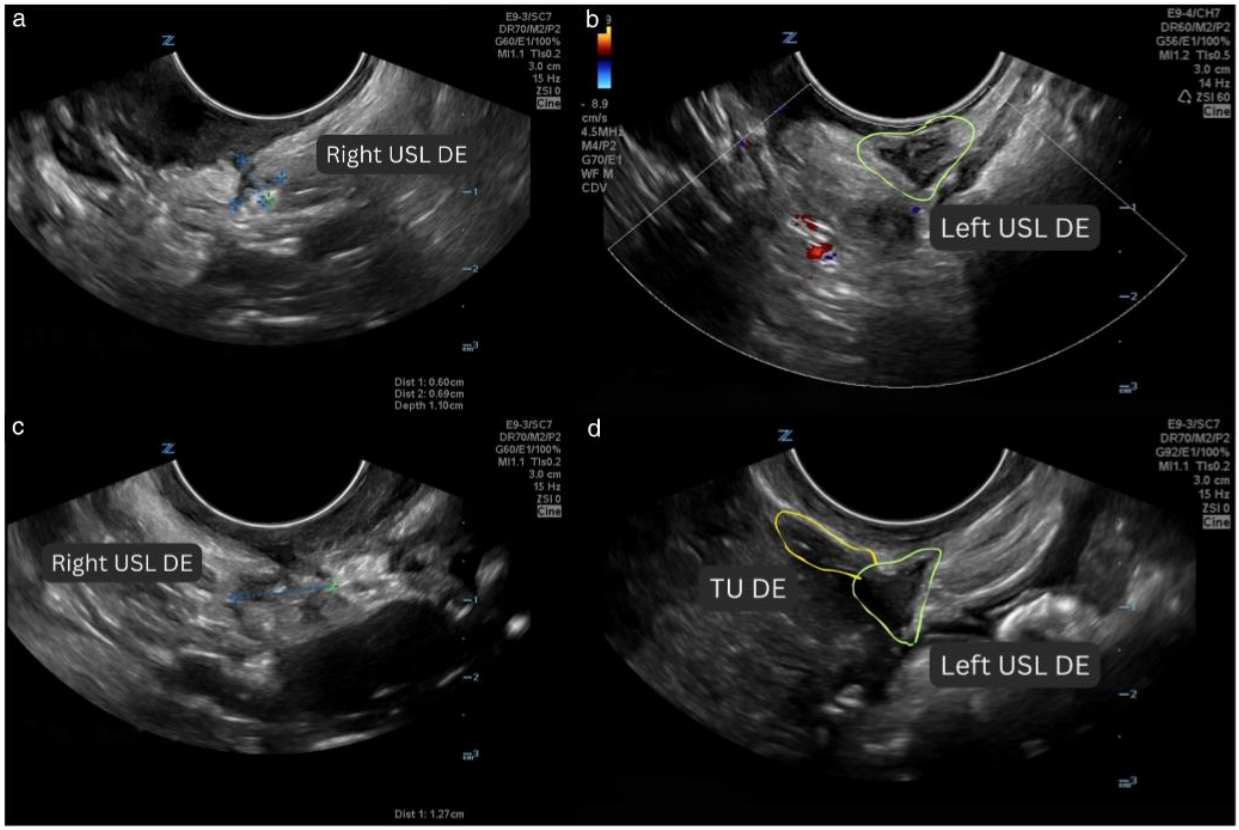


Figure 3. Deep endometriosis (DE) of the right uterosacral ligament (USL) (a,c), left USL and torus uterinus (TU) (b,d) in oblique longitudinal(a,b) and transverse (c,d) views.

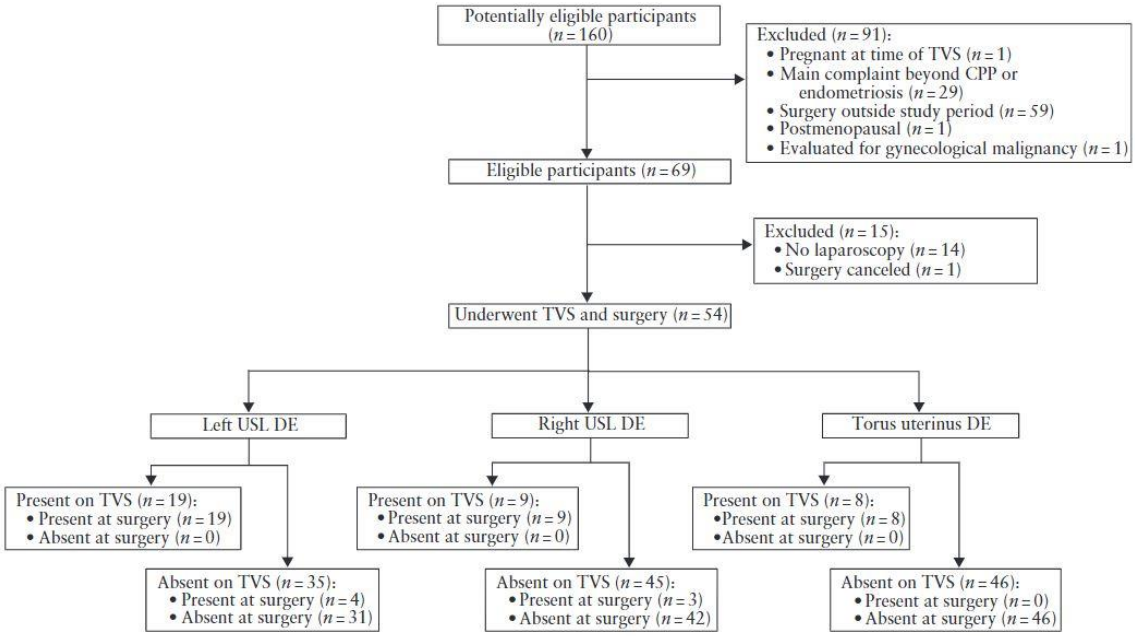


Figure 4. Flowchart summarizing inclusion of participants in the study and diagnostic performance of transvaginal ultrasound (TVS) posterior approach in detecting deep endometriosis (DE) in uterosacral ligaments (USLs) and torus uterinus. CPP, chronic pelvic pain.

Table 1. Characteristics of study population (n=54)

Characteristic	*Value*
Age (years)	35.2 ± 7.2
Time from index test to reference standard (days)	177.4 ± 100.5
Symptoms	
Dysmenorrhea	51 (94.4)
Abnormal uterine bleeding	39 (72.2)
Dyspareunia	36 (66.7)
Dyschezia	32 (59.3)
Dysuria	14 (25.9)
Anxiety/depression	22 (40.7)
Previous endometriosis diagnosis	38 (70.4)
Surgical history	48 (88.9)
Endometriosis-specific (excision, ablation)	24 (44.4)
Comorbidity	
Adenomyosis	20 (37.0)
Fibroids	13 (24.1)

Data are given as mean ± SD or *n* (%).

Table 2. Diagnostic test accuracy of transvaginal ultrasound posterior approach for left uterosacral ligament (USL), right USL and torus uterinus deep endometriosis relative to laparoscopy as the reference standard in 54 patients.

	Left USL *n = 54* *%* *(95% CI)*	**Right USL** *n = 54* *%* *(95% CI)*	**TU** *n = 54* *%* *(95% CI)*
Accuracy	92.6% (82.1-97.9)	94.4% (84.6-98.8)	100% (93.4-100)
Sensitivity	82.6% (61.2-95.1)	75.0% (42.8-94.5)	100% (63.1-100)
Specificity	100% (88.8-100)	100% (91.6-100)	100% (92.3-100)
PPV	100% (-)	100% (-)	100% (-)
NPV	88.6% (76.1-95.0)	93.3% (84.0-97.4)	100% (-)
LR+	-	-	-
LR -	0.2 (0.1-0.4)	0.3 (0.1 to 0.7)	-

Values in parentheses are 95% CI. The positive likelihood ratio was not calculable for any location due to a specificity of 100%. — , incalculable value; LR−, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

III. Chapter III

Submitted for publication.

III.I. Preface and Significance to Thesis

This portion of the thesis represents a sequential inquiry following the prior study examining the prospective diagnostic accuracy of a novel posterior TVS approach in diagnosing DE of the USLs and TU. The current investigation delves into the nuanced dynamics surrounding complex disease states associated with endometriosis, addressing its influence on the accuracy of TVS diagnoses pertaining to DE of the USLs and TU. The previous study laid the groundwork for comprehending the diagnostic potential of TVS using novel guidelines and the classification of DE. Nevertheless, the intricate interplay between concurrent complex disease states and diagnostic accuracy remains a critical aspect necessitating further exploration to enhance TVS as a diagnostic modality. This thesis project serves as an extension, honing in on the distinctive challenges of concurrent complex disease states on the diagnostic accuracy of TVS.

Severe endometriosis presents a range of complexities that can mask subtle manifestations and hinder accurate identification through TVS. By concentrating on DE of the USLs, we can conduct a thorough examination of how disease severity might affect the sensitivity and specificity of TVS as a diagnostic modality. This endeavour aligns with the broader goal of improving diagnostic methodologies to cater to the diverse spectrum of endometriosis presentations, enhancing patient care and outcomes.

III.II Authors' Contribution

The following study was spearheaded by SMF, under the supervision of ML, and included study design, ethics, data collection, analysis, tables, and manuscript development and submission. ML performed all imaging procedures, including advanced TVS and laparoscopy. The final manuscript was completed by SMF and edited by ML prior to submission.

III.III The influence of severe endometriosis on the accuracy of transvaginal ultrasound diagnosis of uterosacral ligament endometriosis

The influence of severe endometriosis on the accuracy of transvaginal ultrasound diagnosis of uterosacral ligament endometriosis

Shay M Freger [1] and Mathew Leonardi [1,2]

1. Department of Obstetrics & Gynecology, McMaster University, Hamilton, Ontario, Canada.
1. Robinson Research Institute, University of Adelaide, Adelaide, Australia

Corresponding Author:
Department of Obstetrics and Gynecology. Faculty of Medical Sciences.

McMaster University

1280 Main Street West. Hamilton, Ontario, Canada. L8S 4K1

Phone: (416) 938 - 5926

E-mail: smfreger@gmail.com

Running Head: Endometriosis, Ultrasound, and Complex Disease

Keywords: Endometriosis, Transvaginal Ultrasound, Diagnostics Test Accuracy, pouch of Douglas obliteration, Uterosacral Ligaments

Abstract

Objective: We aimed to determine the impact of anatomical distortion caused by severe endometriosis on transvaginal ultrasound (TVS) diagnosis of deep endometriosis (DE) when using the posterior approach in the uterosacral ligaments (USLs) and torus uterinus (TU).

Methods: This was a secondary analysis of data from a prospective diagnostic test accuracy study conducted at the McMaster University Medical Center Tertiary Endometriosis Clinic, using consecutively recruited participants. We included data from all 54 participants, where the index test was TVS using a standardized posterior approach, and the reference standard was laparoscopy. Two forms of complex disease states were considered for this study due to their potential influence and co-occurrence on highly prevalent USL and TU disease, including surgically confirmed pouch of Douglas (POD) obliteration and DE of the bowel. Diagnostic accuracy parameters were calculated for each site, 1) excluding those with POD obliteration and 2) excluding those with DE of the bowel, where they were compared to the previously identified baseline performance.

Results: The diagnostic performance of TVS based on surgical visualization as a reference for the left USL, right USL, and TU excluding those with POD obliteration were as follows: Accuracy 90.2%, 97.6%, and 100%; Sensitivity 73.3%, 85.7%, and 100%; Specificity 100%, 100%, 100%; Positive Predictive Value (PPV) 100%, 100%, 100%; Negative Predictive Value (NPV) 86.7%, 97.1%, and 100%; respectively. Upon excluding those with DE of the bowel, the diagnostic performance was as follows: Accuracy 93.2%, 95.5%, and 100%; Sensitivity 82.4%, 71.4%,

100%; Specificity 100%, 100%, 100%; PPV 100%, 100%, 100%; NPV 90.0%, 94.9%, and 100%; respectively.

Conclusion: Severe endometriosis substantially disturbs the anatomical environment, potentially making it difficult to identify prominent anatomical landmarks and map normal and diseased tissue using non-invasive diagnostics. Our results suggest a strong diagnostic performance regardless of disease complexity, alluding to the potential robustness in diagnosing and detecting DE through TVS.

Introduction

Endometriosis is a chronic pain and inflammatory disease characterized by abnormal growth of endometrial-like cells within pelvic structures, including stroma and glandular epithelial cells [1]. Deep endometriosis (DE) is the most aggressive form, composing approximately 20% of all endometriosis diagnoses [2–5]. DE is characterized by its infiltrative nature within surrounding structures, leading to significant distortion of the surrounding anatomical milieu through adhesions and displacement of intrapelvic structures, depicted in *Figure 1*. [6,7].

The most common location for DE remains the uterosacral ligaments (USLs), bilateral connective structures between the uterus and sacrum, conjoined by the torus uterinus (TU) [7–9]. The USLs are pivotal structures within the posterior aspect of the pelvis, creating the lateral boundaries of the POD, the space between the bowel and uterus [10,11]. The structures maintain normal pelvic anatomy and relay crucial nerves and vessels throughout the pelvis, orchestrating normal physiological activity [10–12].

Beyond clinical manifestation, DE of the USLs is highly associated with hallmark symptoms associated with endometriosis, including dyspareunia and pelvic pain [13,14]. USLs may be affected by discrete nodules or contiguously, whereby bilateral structures may be affected with TU involvement [15,16]. In severe cases, USL nodules may co-occur with highly prevalent pelvic adhesions [5], or be involved with additional disease locales within surrounding pelvic structures, including the parametrium, posterior vaginal fornix, ovaries, ureters, and most commonly, the bowel. Alongside aggressive forms of endometriosis, the POD may be “obliterated,” whereby

dense adhesions are present between the uterus/retrocervix and rectum, leading to the absence of mobility between the posterior aspect of the uterus and bowel and an inability to surgically visualize the space between and inferior to the USLs [17,18]. Without accurate pre-operative mapping of DE lesions in these anatomically distorted states, there is a possibility that surgeons may be performing incomplete excision, unbeknownst to them or their patients.

Although the USLs remain invaluable structures and the most common locale for DE, they have been historically associated with low diagnostic accuracy sonographically, with marginal improvement with the development of the pivotal IDEA (International Deep Endometriosis Analysis group) consensus [8,9,19]. Recently, our previous findings evaluating the diagnostic accuracy of a standardized TVS *posterior approach* in diagnosing DE of the USLs suggest an overall improved diagnostic performance relative to those previously reported [15]. Whilst several studies have elucidated the diagnostic accuracy of DE when multiple sites are impacted, including the USLs, the impact of complex disease states and anatomical distortion on diagnostic accuracy remains unexplored. As such, we hypothesize that TVS has a reduced diagnostic performance in the presence of complex disease states when diagnosing DE of USLs and TU. The objective of this study is to provide an explorative analysis to evaluate the impact of these complex disease states on the diagnostic accuracy of DE of the USLs and TU.

Methods

The study is a secondary analysis of prospective data initially used to investigate the diagnostic accuracy of TVS using a standardized posterior approach in diagnosing DE of the USLs and TU [15]. The diagnostic test accuracy scores between the two cohorts of interest are compared. This study was done and reported in accordance with Standards for Reporting Diagnostic Accuracy

(STARD; 2015) [20] guidelines, with consideration for the Quality Assessment of the Diagnostic Accuracy of Studies (QUADAS-2) checklist [21].

Participants

The "complex disease" state was defined surgically. This specifically involved complete POD obliteration and/or bowel DE. The other cohort, the "non-complex disease" state, included those without complete POD obliteration or bowel DE. All 54 participants in the initial study were included in the secondary analysis. In brief, all participant data was collected consecutively, where the reference standard was performed within one year of the index test. Participant inclusion criteria included 1) age between 18 and 50 years, 2) assigned female sex at birth, 3) pre-menopausal and post-menarchal, 4) history of chronic pelvic pain and/or endometriosis, 5) able to undergo TVS and consented to laparoscopic surgery for endometriosis. Participants were excluded if they had a current or previous gynecologic malignancy or if they underwent laparoscopy at a different center.

Study Design

The initial study was a prospective diagnostic accuracy study of DE of the USLs and TU, conducted at McMaster University Medical Center, Hamilton Health Sciences. Data was prospectively collected in real-time between August 10, 2020 – October 31, 2021.

Ethics Approval

The study was conducted in accordance with the Hamilton Integrative Research Ethics Board (HiREB: 12617).

Index Test, Reference Standard, and the Identification of Complex Disease States

The index test was TVS using a standardized posterior approach and reported in accordance with the IDEA consensus, and the reference standard was laparoscopic visualization and histology when tissue was available, which was performed by a single expert surgeon-sonologist. Characterization of anatomical landmarks and DE of the USLs/TU using the posterior approach were as previously described [15]. DE nodules are described sonographically as hypoechoic with regular or irregular outer borders, generally breaking the smooth peritoneal surface echogenicity. Surgically, DE was characterized by a nodule portraying diverse pigmentation and a firm tactile response upon palpation. POD obliteration was assessed on the index test, TVS, using the 'sliding sign' method in assessing mobility between the posterior aspect of the uterus and the anterior side of the bowel [22]. The presence of DE of the bowel was characterized by any level of infiltration of a nodule into the muscularis of the bowel, including the lower rectum, upper rectum and rectosigmoid, in accordance with the IDEA consensus statement.

All patients required the reference standard, laparoscopy. To be included in this "complex disease" state, patients needed surgically confirmed complete POD obliteration and/or laparoscopically visualized/palpated bowel DE (including histologically confirmed DE of the bowel when tissue was available following colorectal surgical excision). Bowel DE was appreciable at surgery in the location of most dense adhesions and palpable with the bowel graspers at the site of fibrotic (i.e. hard) nodularity within the anterior bowel wall. [22] Obliteration was confirmed surgically by the inability to visualize the peritoneum of the POD, between and inferior to the USLs, due to the presence of adhesions between the uterus and bowel. Partial

obliteration, defined as a partial visualization of the peritoneum of the POD, was considered non-complex disease and grouped within the non-complex cohort.

Analysis

The prevalence of POD obliteration and DE of the bowel was previously collected and reported in the initial analysis [15]. Baseline diagnostic accuracy parameters for bilateral USLs and TU, including accuracy, sensitivity, specificity, negative and positive predictive value, negative (LR-) and positive (LR+) likelihood ratios with 95% confidence interval (CI), were previously described. To assess the impact of complex disease on diagnostic accuracy of DE of the USLs and TU, diagnostic accuracy parameters were calculated: 1) excluding those with complete POD obliteration, and 2) excluding those with DE of the bowel. Both complex disease states were descriptively compared individually to the previously reported baseline parameters using overall accuracies and CIs.

Data was collected using the REDCap electronic data capture tool (Vanderbilt University, Nashville, Tennessee, USA) and imported into Microsoft Excel for Windows 10 (Microsoft Corporation, Santa Rosa, CA, USA). Cleaned data was transferred and analyzed using IBM SPSS statistics V29 software (SPSS Inc., Chicago, IL, USA). All accuracy parameters were determined using the cross-tabulation function in SPSS.

Results

Surgical and Histological Findings

Patient characteristics, lesion dimensions, and baseline diagnostic accuracy results of the left USL, right USL, and TU were as previously reported [15].

Prevalence of POD obliteration was similarly reported, with 24% (13/54) having complete obliteration, which was assessed sonographically and confirmed laparoscopically. Two patients had partial obliteration of the POD and were included in the non-complex cohort. Laparoscopically confirmed DE of the bowel was present in 19% (10/54) of participants, with 70% (7/10) being histologically confirmed and 30% (3/10) not histologically confirmed as colorectal surgery was not performed. Though the complex disease states were evaluated individually, 17% (9/54) had both POD obliteration and DE of the bowel. Regarding location, 80% (8/10) of nodules were within the upper rectum, and 20% (2/10) had nodules within the rectosigmoid junction.

Diagnostic Performance

Diagnostic parameters for each complex disease state and locale relative to the baseline parameters are reported in *Table 1*. The diagnostic test performance of TVS of the left USL, right USL, and TU, excluding those with POD obliteration ((-) POD obliteration/ (+) Bowel DE) with surgical visualization as the reference standard was as follows: Accuracy of 90.2% (95% CI 76.9 – 97.3%), 97.6% (95% CI 87.1 – 99.9%), and 100% (95% CI 91.4 – 100%); Sensitivity 73.3% (95% CI 44.9 – 92.2%) 85.7% (95% CI 42.1 – 99.6%), and 100% (95% CI 29.2 – 100%); Specificity 100% (95% CI 86.8 – 100%), 100% (95% CI 89.7 – 100%), and 100% (95% CI 90.8 – 100%); PPV 100%, 100%, and 100%; NPV 86.7% (95% CI 73.7 – 93.8%), 97.1% (95% CI 84.7 – 99.5%), and 100%, respectively.

Upon excluding those with DE of the bowel ((+) POD obliteration/ (-) Bowel DE) with surgical visualization as the reference standard for the left USL, right USL, and TU, was as follows: Accuracy 93.2% (95% CI 81.3 – 98.6%), 95.5% (95% CI 84.5 – 99.4%), and 100% (95% CI 92.0 – 100%); Sensitivity 82.4% (95% CI 56.5 – 96.2%), 71.4% (95% CI 29.0 – 96.3%), and 100% (95% CI 47.8 – 100%); Specificity 100% (95% CI 87.2 – 100%), 100% (95% CI 90.5 – 100%), and 100% (95% CI 91.0 – 100%); PPV 100%, 100%, and 100%; NPV 90.0% (95% CI 76.3 – 96.2%), 94.9% (95% CI 85.2 – 98.4%), and 100%, respectively.

Compared to all participants (n = 54), the diagnostic test accuracy scores for all three sites (left USL, right USL, and TU) amongst those without POD obliteration yielded similar 95% CIs for accuracies (95% CI 82.1 - 100% vs 95% CI 76.9 - 100%), specificities (95% CI 88.8 - 100% vs 95% CI 86.8 - 100%), PPVs (95% CI 100% vs 100%) and NPVs (95% CI 76.1 - 100% vs 95% CI 73.7 - 100%), respectively. Similarly, relative to all participants, the diagnostic test accuracy scores for all three sites (LUSL, RUSL, TU) amongst those without bowel DE yielded similar 95% CIs for accuracies (95% CI 82.1 - 100% vs 95% CI 81.3 - 100%), specificities (95% CI 88.8 - 100% vs 95% CI 87.2 - 100%), PPV (95% CI 100% vs 95% CI 100%), and NPV (95% CI 76.1 - 100% vs 95% CI 76.3 - 100%), respectively. Notably, the lower limit in 95% CIs for sensitivities widens for the left USL, right USL, and TU upon the exclusion of POD obliteration (95% CIs 42.8 - 100% vs 95% CIs 29.2 - 100%) and exclusion of bowel DE (95% CIs 42.8 - 100% vs 95% CIs 29.0 - 100%).

Discussion

Main Findings

In this secondary analysis, utilizing data collected prospectively, we assessed the influence of commonly co-occurring complex disease states, such as POD obliteration and DE of the bowel, on the accuracy of TVS diagnoses of DE within the USLs and TU. Given the 95% CI overlap among sites and complex disease states relative to baseline parameters, the findings of this study suggest that the presence of complex disease states, including POD obliteration and DE of the bowel, did not directly impact the diagnostic accuracy in our centre.

All diagnostic parameters for TU remained unchanged, irrespective of complex disease. Among all three sites relative to baseline, accuracy, specificity, PPV, and LR+ values remain largely unchanged. Reassuringly, when the disease was present on TVS, it was always detected surgically with no false positive results, irrespective of complex disease states. Interestingly, relative to baseline, there was a slight reduction in sensitivity for the left USLs upon excluding those with POD obliteration and DE of the bowel, from 82.6% (95% CI 61.2 – 95.1%) to 73.3% (95% CI 44.9 – 92.2%) and 82.4% (95% CI 56.6 – 96.2%), respectively. In comparison, the right USL showed an improvement in sensitivity upon excluding those with POD obliteration and reduction upon excluding DE of the bowel, from 75.0% (95% CI 42.8 – 94.5%) to 85.7% (95% CI 42.1 – 99.6%) and 71.4% (95% CI 29.0 – 96.3%), respectively. However, there was substantial overlap among the 95% CIs, with the lower limit of the sensitivity intervals widening, likely due to the reduction in sample size or exclusion of true positives among the sites, given the common occurrence of these complex states with DE nodules. Given the substantial CI overlap, it is unlikely there is a true clinically significant difference upon excluding those with complex disease states.

Interpretation and Significance

The USLs remain the most common location of DE, with historically the lowest diagnostic accuracy among all anatomical sites described through TVS [7,9,23]. Despite recent advancements, including our primary study suggesting an improved accuracy using a standardized posterior approach [15,16], no studies have reported the influence of complex disease states on the diagnostic accuracy of TVS for DE. Understanding the impact of these highly prevalent and co-occurring disease states is pivotal in surgical planning and gauging disease severity sonographically. In the case of severe endometriosis, the anatomical environment is typically distorted due to extensive adhesions [5,24]. This hypothetically leads to difficulties in mapping and characterizing normal and diseased tissue, reducing diagnostic performance. Although we expected an improved accuracy upon excluding those with complex disease, this was not the case. Despite the increased challenge in scanning these patients and mapping all disease sites, we suspect the limited change of accuracy is a product of the enhanced critical investigative nature of the sonologist, instigated by the presence of obliteration and/or DE of the bowel. In other words, a red flag is raised when these severe states are recognized, and the sonologist makes concerted efforts to map other adjacent pathology. Moreover, the maintenance of strong diagnostic performance may also be secondary to the posterior approach technique with the positioning of the probe in the posterior vaginal fornix, which allows for closer proximity to disease sites.

Reflecting on deficiencies in diagnostic test accuracy in this study and others, we may consider how the distorted anatomical environment and landmarks are heavily distorted in severe endometriosis, potentially yielding a discrepancy in where a nodule is labelled at ultrasound

versus surgery. We must consider that the left USL may not always feel tactile on sonographic assessment or even physical examination to be “left”. Normalizing anatomy through adhesiolysis and excision of other pathology (e.g. large endometriomas) throughout the surgery may result in a more accurate description of the location of the disease. However, it does not mean the ultrasound test did not correctly identify the presence of a specific site of endometriosis; instead, it only incorrectly labelled the location. It is most likely that this type of confusion will exist with the retrocervical structures, the USLs and TU, and potentially the peritoneum of the POD, which is in continuity with these structures. For example, it is possible that a nodule may be sonographically labelled as TU but surgically labelled as left USL or as TU *and* left USL. A potential solution to this dilemma is considering the entire USL/TU complex as one retrocervical complex (or a “horseshoe region”) from a diagnostic test accuracy perspective, particularly in POD obliteration. This may be particularly important in settings where the surgeon is not the sonologist, and discrepancies in the localization of a deposit could hypothetically result in false negative classifications of disease locations at surgery or unnecessary excision of regions believed to be impacted by DE. The unification of this as a singular location when both USLs are impacted would not diminish our ability to study concepts such as disease location and symptom correlation or etiology/pathophysiology. It is most likely appropriate to distinguish the horseshoe region from the POD peritoneum. Similarly, the bowel and vagina have distinctive anatomical landmarks, even in the context of severe disease, so it is our belief that these regions should be distinctly assessed and characterized, producing more clarity on disease localization.

Strengths and Limitations

This secondary analysis benefits from parallel strengths previously reported in the primary study, including adherence to the STARD guidelines, prospective recruitment, and use of high-quality ultrasound and surgical equipment. The study also benefits from the novel exploration of the impact of complex disease states on the diagnostic accuracy of TVS diagnosis of DE of the USLs and TU.

Although our recent study suggested improved accuracy using a standardized posterior approach, several limitations were noted, which remain in this concurrent secondary study. Specifically, the generalizability may remain impacted given that the same highly trained surgeon-sonologist performed both TVS and surgeries, where future studies should incorporate an aspect of blinding and/or use experienced and inexperienced sonographers and surgeons. Similarly, this type of study should simultaneously be performed when the surgeon and the diagnostic imaging teams are entirely different. This would reveal gaps in our current technical approach and our language across the sonography and surgery spaces. Future studies should also consider including a comparison to a standardized anterior TVS approach in the presence of complex disease states, which may provide a less sonographically distorted view since the posterior approach yields a further stretch on the posterior compartment tissues. Lastly, it is likely that the origin of the extensive 95% CI overlap is due to the limited sample size of this study. It remains possible that even within our population, there may indeed be a difference in the diagnostic performance of TVS between the groups that are not identified due to methodologic limitations. However, the findings of this study initiate an important dialogue and can assist future studies with sample size calculations in evaluating complex disease states on diagnostic accuracy.

Conclusion

The USLs and TU are common locations for DE and commonly exist in complex disease states, including POD obliteration and DE of the bowel. Despite the challenges posed by severe endometriosis, which distorts the anatomical environment with extensive adhesions, our results demonstrate strong diagnostic performance regardless of disease complexity. The findings of this secondary analysis elucidate the robustness of diagnosing and detecting DE through TVS using the posterior approach.

References

1. Zondervan KT, Becker CM, Missmer SA. Endometriosis. Longo DL, ed. *N Engl J Med*. 2020;382(13):1244-1256. doi:10.1056/NEJMra1810764
2. Scioscia M, Bruni F, Ceccaroni M, Steinkasserer M, Stepniewska A, Minelli L. Distribution of endometriotic lesions in endometriosis stage IV supports the menstrual reflux theory and requires specific preoperative assessment and therapy. *Acta Obs Gynecol Scand*. 2011;90(2):136-139. doi:10.1111/J.1600-0412.2010.01008.X
3. Koninckx PR, Ussia A, Adamyan L, Wattiez A, Donnez J. Deep endometriosis: definition, diagnosis, and treatment. *Fertil Steril*. 2012;98(3):564-571. doi:10.1016/J.FERTNSTERT.2012.07.1061
4. Alson S, Jokubkiene L, Henic E, Sladkevicius P. Prevalence of endometrioma and deep infiltrating endometriosis at transvaginal ultrasound examination of subfertile women undergoing assisted reproductive treatment. *Fertil Steril*. 2022;118(5):915-923. doi:10.1016/j.fertnstert.2022.07.024

5. Chaggar P, Tellum T, Thanatsis N, De Braud L V., Setty T, Jurkovic D. Prevalence of deep and ovarian endometriosis in women attending a general gynecology clinic: prospective cohort study. *Ultrasound Obstet Gynecol*. 2023;61(5):632-641. doi:10.1002/UOG.26175

6. International Working Group of AAGL EE and W, Tomassetti C, Johnson NP, Petrozza J, Abrao MS, Einarsson JI, Horne AW, Lee TTM, Missmer S, Vermeulen N, Zondervan KT, Grimbizis G, De Wilde RL. An international terminology for endometriosis, 2021,. *Hum Reprod Open*. 2021;2021(4):16. doi:10.1093/HROPEN/HOAB029

7. Guerriero S, Condous G, Bosch T van den, Valentin L, Leone FPG, Schoubroeck D Van, Exacoustos C, Installé AJF, Martins WP, Abrao MS, Hudelist G, Bazot M, Alcazar JL, Gonçalves MO, Pascual MA, Ajossa S, Savelli L, Dunham R, Reid S, Menakaya U, Bourne T, Ferrero S, Leon M, Bignardi T, Holland T, Jurkovic D, Benacerraf B, Osuga Y, Somigliana E, Timmerman D. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. *Ultrasound Obs Gynecol*. 2016;48(3):318-332. doi:10.1002/UOG.15955

8. Guerriero S, Ajossa S, Minguez JA, Jurado M, Mais V, Melis GB, Alcazar JL. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in uterosacral ligaments, rectovaginal septum, vagina and bladder: systematic review and meta-analysis. *Ultrasound Obs Gynecol*. 2015;46(5):534-545. doi:10.1002/UOG.15667

9. Leonardi M, Uzuner C, Mestdagh W, Lu C, Guerriero S, Zajicek M, Dueckelmann A, Filippi F, Buonomo F, Pascual MA, Stepniewska A, Ceccaroni M, Van den Bosch T, Timmerman D, Hudelist G, Condous G. Diagnostic accuracy of transvaginal ultrasound

for detection of endometriosis using International Deep Endometriosis Analysis (IDEA) approach: prospective international pilot study. *Ultrasound Obs Gynecol*. 2022;60(3):404-413. doi:10.1002/UOG.24936

10. Scioscia M, Scardapane A, Virgilio BA, Libera M, Lorusso F, Noventa M. Ultrasound of the Uterosacral Ligament, Parametrium, and Paracervix: Disagreement in Terminology between Imaging Anatomy and Modern Gynecologic Surgery. *J Clin Med 2021, Vol 10, Page 437*. 2021;10(3):437. doi:10.3390/JCM10030437
11. Vu D, Haylen BT, Tse K, Farnsworth A. Surgical anatomy of the uterosacral ligament. *Int Urogynecol J*. 2010;21(9):1123-1128. doi:10.1007/S00192-010-1147-8/FIGURES/6
12. Fujii M, Sagae S, Sato T, Tsugane M, Murakami G, Kudo R. Investigation of the Localization of Nerves in the Uterosacral Ligament: Determination of the Optimal Site for Uterosacral Nerve Ablation. *Gynecol Obstet Invest*. 2002;54(Suppl. 1):11-17. doi:10.1159/000066289
13. Chapron C, Dubuisson JB. Laparoscopic treatment of deep endometriosis located on the uterosacral ligaments. *Hum Reprod*. 1996;11(4):868-873. doi:10.1093/OXFORDJOURNALS.HUMREP.A019268
14. Vercellini P, Aimi G, Busacca M, Apolone G, Uglietti A, Crosignani PG. Laparoscopic uterosacral ligament resection for dysmenorrhea associated with endometriosis: Results of a randomized, controlled trial. *Fertil Steril*. 2003;80(2):310-319. doi:10.1016/S0015-0282(03)00613-7
15. Freger SM, Mathew L, Freger SM, Turnbull V, Mcgowan K, Leonardi M, Shay M, Freger M. Prospective diagnostic test accuracy of uterosacral ligament and torus uterinus

endometriosis using transvaginal ultrasound posterior approach. *Ultrasound Obstet Gynecol*. Published online September 19, 2023. doi:10.1002/UOG.27492

16. Zhang Y, Xiao X, Xu F, Lin Q, Xu J, Du B. Evaluation of uterosacral ligament involvement in deep endometriosis by transvaginal ultrasonography. *Front Pharmacol*. 2019;10(APR):430427. doi:10.3389/FPHAR.2019.00374/BIBTEX
17. Reid S, Lu C, Casikar I, Reid G, Abbott J, Cario G, Chou D, Kowalski D, Cooper M, Condous G. Prediction of pouch of Douglas obliteration in women with suspected endometriosis using a new real-time dynamic transvaginal ultrasound technique: the sliding sign. *Ultrasound Obstet Gynecol*. 2013;41(6):685-691. doi:10.1002/UOG.12305
18. Leonardi M, Martins WP, Espada M, Georgousopoulou E, Condous G. Prevalence of negative sliding sign representing pouch of Douglas obliteration during pelvic transvaginal ultrasound for any indication. *Ultrasound Obstet Gynecol*. 2020;56(6):928-933. doi:10.1002/UOG.22023
19. Guerriero S, Ajossa S, Orozco R, Perniciano M, Jurado M, Melis G, Alcazar J. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in the rectosigmoid: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2016;47(3):281-289. doi:10.1002/UOG.15662
20. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, De Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF, Alonzo T, Azuara-Blanco A, Bachmann L, Blume J, Boutron I, Büller H, Buntinx F, Byron S, Chang S, Cooper R, De Groot J, Deeks J, Dendukuri N, Dinnes J, Fleming K, Guyatt G, Heneghan C, Hilden J, Horvath R, Hunink M, Hyde C, Ioannidis J, Janes H, Kleijnen J, Knottnerus A, Lange S, Leeflang M, Lord S, Lumbreras

B, Macaskill P, Magid E, Mallett S, McInnes M, Mc-Neil B, McQueen M, Moons K, Morris K, Mustafa R, Obuchowski N, Ochodo E, Onderdonk A, Overbeke J, Pai N, Peeling R, Pepe M, Petersen S, Price C, Ravaud P, Rutjes A, Schunemann H, Simel D, Simera I, Smidt N, Steyerberg E, Straus S, Summerskill W, Takwoingi Y, Thompson M, Van De Bruel A, Van Maanen H, Vickers A, Virgili G, Walter S, Weber W, Westwood M, Whiting P, Wilczynski N, Ziegler A. STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies. *Clin Chem*. 2015;61(12):1446-1452. doi:10.1373/CLINCHEM.2015.246280

21. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MMG, Sterne JAC, Bossuyt PMM. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536. doi:10.7326/0003-4819-155-8-201110180-00009/SUPPL_FILE/155-8-529-SUPPLEMENT.PDF

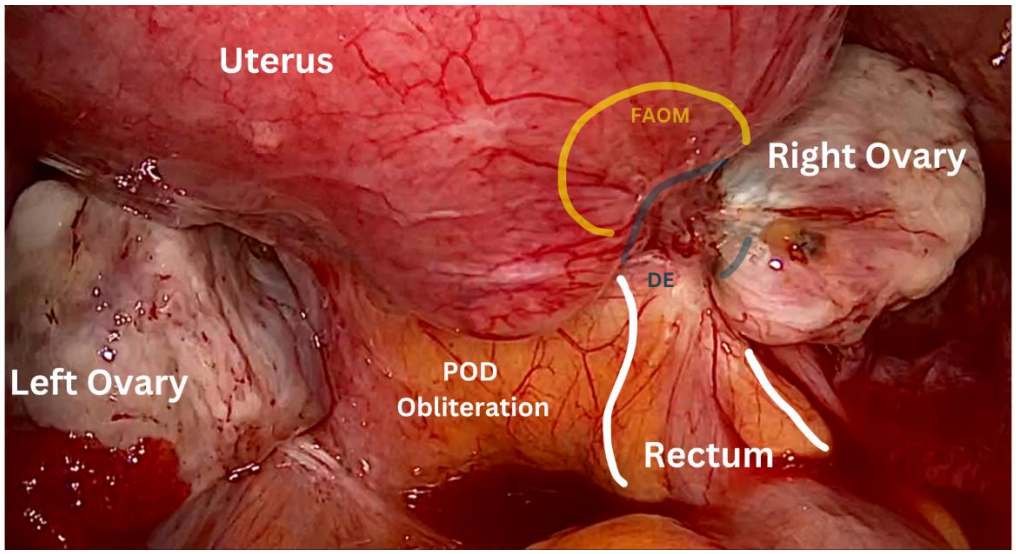
22. Leonardi M, Martins WP, Espada M, Georgousopoulou E, Condous G. Prevalence of negative sliding sign representing pouch of Douglas obliteration during pelvic transvaginal ultrasound for any indication. *Ultrasound Obstet Gynecol*. 2020;56(6):928-933. doi:10.1002/UOG.22023

23. Guerriero S, Ajossa S, Minguez JA, Jurado M, Mais V, Melis GB, Alcazar JL. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in uterosacral ligaments, rectovaginal septum, vagina and bladder: systematic review and meta-analysis. *Ultrasound Obs Gynecol*. 2015;46(5):534-545. doi:10.1002/UOG.15667

24. Porpora MG, Koninckx PR, Piazze J, Natili M, Colagrande S, Cosmi E V. Correlation between endometriosis and pelvic pain. *J Am Assoc Gynecol Laparosc*. 1999;6(4):429-434. doi:10.1016/S1074-3804(99)80006-1

Table and Figure Captions

Figure 1. Laparoscopic view of a distorted pelvic environment due to the presence of DE and POD obliteration.



Legend: DE = Deep Endometriosis, FOAM = Focal Adenomyosis of the Outer Myometrium, POD = Pouch of Douglas

Table 1. The impact of surgically confirmed POD obliteration and DE of the bowel on the diagnostic accuracy of TVS diagnosis of DE of bilateral USLs and TU relative to baseline performance.

	Left USL			**Right USL**			**TU**		
	Whole* *n=54* *% (95% CI)*	***(X) POD*** *n= 41* *% (95% CI)*	***(X) Bowel*** *n=44* *% (95% CI)*	***Whole**** *n=54* *% (95% CI)*	***(X) POD*** *n=41* *% (95% CI)*	***(X) Bowel*** *n=44* *% (95% CI)*	***Whole**** *n=54* *% (95% CI)*	***(X) POD*** *n=41* *% (95% CI)*	***(X) Bowel*** *n=44* *% (95% CI)*
Accuracy	92.6% (82.1-97.9)	90.2% (76.9-97.3)	93.2% (81.3-98.6)	94.4% (84.6-98.8)	97.6% (87.1-99.9)	95.5% (84.5-99.4)	100% (93.4-100)	100% (91.4-100)	100% (92.0-100)
Sensitivity	82.6% (61.2-95.1)	73.3% (44.9-92.2)	82.4% (56.6-96.2)	75.0% (42.8-94.5)	85.7% (42.1-99.6)	71.4% (29.0-96.3)	100% (63.1-100)	100% (29.2-100)	100% (47.8-100)
Specificity	100% (88.8-100)	100% (86.8-100)	100% (87.2-100)	100% (91.6-100)	100% (89.7-100)	100% (90.5-100)	100% (92.3-100)	100% (90.8-100)	100% (91.0-100)
PPV	100% (-)	100% (-)	100% (-)	100% (-)	100% (-)	100% (-)	100% (-)	100% (-)	100% (-)
NPV	88.6% (76.1-95.0)	86.7% (73.7-93.8)	90.0% (76.3-96.2%)	93.3% (84.0-97.4)	97.1% (84.7-99.5)	94.9% (85.2-98.4)	100% (-)	100% (-)	100% (-)
LR+	-	-	-	-	-	-	-	-	-
LR -	0.2 (0.1-0.4)	0.3 (0.1-0.6)	0.2 (0.06-0.5)	0.3 (0.1-0.7)	0.1 (0.02-0.9)	0.3 (0.09-0.9)	-	-	-

**Baseline findings from an initial study conducted by Freger et al. (2023).*

Legend: CI = Confidence Interval, LR- = Negative Likelihood Ratio, LR+ = Positive Likelihood Ratio, n = number, NPV - = Negative Predictive Value, POD = Pouch of Douglas, PPV- = Positive Predictive Value, TU = Torus Uterinus, USL = Uterosacral Ligaments, X = Excluded from analysis

Chapter IV. Discussion

Endometriosis is a challenging disease, burdened by a significant diagnostic delay, leading to short- and long-term consequences on the quality of life among those affected. One of the most significant contributors to this delay includes inadequate adoption and understanding of non-invasive imaging modalities, notably TVS, with ongoing diagnostic limitations despite recent developments [27]. Though OE is reliably diagnosable, and there have been notable improvements in several locales of DE [40], the most common locale, the USLs and TU, have maintained the lowest diagnostic accuracy using non-invasive imaging tests [38,39,55], exacerbating the diagnostic delay and limiting guided treatment.

The prevailing method for diagnosing and characterizing endometriosis sonographically involves TVS probe placement within the anterior vaginal fornix. The performance and reporting of TVS are increasingly being done according to standards outlined in the landmark 2016 IDEA consensus. While effective for assessing the anterior and adnexal compartments, this standard "anterior" technique yields challenges in characterizing the posterior aspect, where the USLs and TU exist. The lower diagnostic accuracy associated with the USLs and TU may be attributed, in part, to methodological limitations inherent in an anterior approach, given the further proximity of the probe to posterior anatomical structures.

In addition to methodological considerations, the evolution of DE classification guidelines [14] introduces a degree of variability despite the standardization achieved through the IDEA consensus. Our current understanding of TVS diagnostic accuracy relies on literature that

adheres to the previous guidelines, defining DE as infiltration exceeding 5mm. The new classification, characterizing DE based on any level of infiltration, raises concerns about the alignment of previously reported accuracies with the current paradigm. Smaller nodules with limited infiltration, such as those measuring 1-2mm, traditionally pose more significant diagnostic challenges than extensively infiltrating nodules [39]. While the size of nodules may not significantly impact our ability to diagnose sites with high accuracy, such as the bowel, it could markedly affect challenging locations like the USLs and TU, which have historically exhibited low accuracy. Given the methodological and standardization limitations, a critical reassessment of diagnostic performance is warranted, urging a nuanced approach to enhance the visualization of DE within the USLs and TU using TVS.

In the work presented in this thesis, we introduce a novel posterior TVS approach for the diagnosis of DE of the USLs and TU, involving placement of the probe within the posterior vaginal fornix. Additionally, the index test evaluated in this study was performed and reported in adherence with the IDEA consensus and the novel classification guidelines for DE. The most recent study by Leonardi et al., involving a multisite comparison of TVS to both laparoscopy and histology for DE of the USLs and TU, showed a diagnostic performance of sensitivity of 44.4 – 58.7% and specificity of 77.8 – 88.2% [39]. In our current study, using the posterior TVS approach with the novel classification guidelines, the sensitivity was 75.0 – 100% and a specificity of 100% among the three sites. Similar improvements were seen in predictive values. The multisite study suggested a PPV of 63.3 – 75.5% and NPV of 66.1 – 77.5%, whereas our study suggested a PPV of 100% and NPV of 88.6 – 100%.

The observed increase in accuracy is likely attributed to the new technique, placing the probe within the posterior vaginal fornix, positioning it a few millimetres from the USLs and TU. However, it is crucial to acknowledge that various factors, such as using high-quality instrumentation, may also contribute to this improvement. Furthermore, while the TU exhibited the highest accuracy, followed by the right and left USL, these differences were subtle and inversely proportional to disease prevalence. Although it is plausible that the accuracy of TVS in diagnosing TU disease may be influenced by its low prevalence within our population, it is equally likely that it is, in fact, the most accurate site. Notably, the presence of 'horseshoe' nodules affecting both left and right USLs naturally directs a sonologist to investigate the TU critically.

Lesion dimensions are seldom reported in literature solely evaluating diagnostic accuracy; however, the findings of our study using the novel DE classification suggest a mean level of infiltration among the left USL, right USL, and TU of 5.0mm (±3.0), 6.8mm (±4.8), and 5.6mm (±2.7), respectively. With lower infiltration limits reaching as low as 2mm, a large proportion of these nodules would have historically been considered SE and would have contributed to lower diagnostic accuracy. Interestingly, a prospective study by Koninckx et al. (1991), which involved laparoscopically evaluating the depth of nodule infiltration among patients with infertility, identified a similar depth of 2mm [81]. Given that the infertility population is generally more representative than patients seen in advanced endometriosis centers [82], the similarities in nodule depth may suggest that our findings may have broader applicability compared to studies conducted solely in advanced endometriosis centers.

The work described in this thesis is the first to evaluate the impact of complex disease states on the diagnostic accuracy of TVS in diagnosing DE. The findings presented in this work suggest that the accuracy of the novel TVS approach remains unaffected by complex disease states in our center. The parameters, upon exclusion of these states, demonstrate a range of diagnostic performance: sensitivity from 71.4 – 100%, specificity at 100%, PPV at 100%, NPV 86.7 – 100%, and an overall accuracy spanning from 90.2 – 100%, across all three locales. Although only subtle differences in accuracy parameters were noted, they are likely not clinically relevant.

These results not only underscore the robustness of the novel posterior TVS approach in diagnosing DE of the USLs and TU but also highlight its resilience in the presence of co-occurring complex disease states. The resilience may be similarly attributed to the new technique, given the proximity of the probe to the locales of interest. However, complex disease states typically raise a ’red flag’ to a sonologist, warranting further sonographic investigation for DE. Similarly, the findings of the present work set the foundation for a nuanced approach, where diagnostic accuracy studies and diagnosis within clinical settings should consider the entirety of the pelvis rather than siloed investigation of structures. The findings enhance our understanding of the dynamic nature of TVS and curate a crucial dialogue within the field, warranting similar studies among other sites and disease locales.

IV.I Strengths and Limitations

The studies throughout the thesis are strengthened by robust methodological design for diagnostic test accuracy, adhering meticulously to the STARD guidelines [69] to enhance standardization in reporting. Simultaneously, adopting the QUADAS-2 [70] checklist helped mitigate biases in patient selection, encompassing both index and reference tests, as well as flow and timing. Furthermore, the studies potentially benefited from a generalizable population to other tertiary centres, given that DE sizes reported in our study reflected others. Noteworthy is the dual benefit derived from the novelty of the approach and the investigative methods employed, contributing to the thesis's overall comprehensiveness and impact.

While rigorous efforts were undertaken to ensure a robust study design, it is crucial to acknowledge limitations arising from inherent constraints within the field and the chosen study design. The primary limitation is that the same individual who conducted the index test also performed the reference standard. This introduces the possibility of observation and interpretation bias, where preconceptions formed during the index test may have influenced how the reference standard was executed or interpreted. That said, while surgical findings were unblinded, pathologists performing histological analysis were not subject to knowledge of the index test. Future studies would benefit from incorporating an element of blinding, ensuring that the index and reference tests are conducted by separate individuals blinded to the results. The generalizability of the methodology may also face limitations, considering that the surgeon-sonologist possesses advanced training in diagnosing endometriosis. While this study may offer repetitive insights for experts in the field or endometriosis specialists sonologists, its findings

may not readily apply to generalist obstetrician-gynecologists or generalist radiologists. Subsequent studies should explore a combination of trained and untrained operators, including of different specializations, to enhance the generalizability of the findings. Additionally, this study did not account for intra- and inter-operator variability, a factor that should be considered in future investigations to enhance the generalizability of the results.

The study faced limitations in its sample size, which was initially calculated based on the prevalence of left USL disease (as the most common site of DE in the entire body). Given the lower prevalence of both right USL and TU disease, it is conceivable that the study might not have been adequately powered equally for all sites. Considering this, the varying prevalences of disease in our study and others may affect our confidence in distinguishing differences among sites. Similar limitations persist in evaluating complex disease states. Though the aim of the study was largely explorative and hypothesis-driving for future studies, the secondary analysis was performed using the previous sample size without considering multiple comparisons or posthoc analysis. Future studies should prioritize a larger, adequately powered sample size to effectively determine diagnostic accuracy differences among less prevalent sites.

An additional notable limitation arises from the absence of histology as a reference standard in certain circumstances. The challenge lies in the fact that healthy tissue is seldom removed, making it impossible to definitively diagnose true negatives histologically. In the cases of normal-appearing pelvic anatomy, the surgeon’s eyes alone acted as the reference standard, unlike the scenario in which abnormal findings were identified surgically, providing a

histological reference standard. Despite a strong alignment between the surgeon's positively identified specimens and histological confirmation, notable discrepancies between surgeons and pathologists have been observed in literature [32]. Consequently, a significant limitation within the field, evident in this study, is the potential discordance between surgeons and pathologists, relying on the assumption that the surgeon's diagnosis is correct. Lastly, in this study, our objective was to identify areas where the posterior TVS approach surpasses the diagnostic accuracy previously reported using an anterior approach. While our findings suggested improvement, a direct comparison between the posterior and anterior TVS approaches is necessary, which we did not complete.

Conclusion

Endometriosis, a prevalent chronic pain and inflammatory disease, imposes a substantial burden on the quality of life and often entails prolonged diagnostic delays among those affected. Despite advancements in diagnostic techniques, historically challenging sites, including the USLs and TU, continue to pose diagnostic challenges, with guideline changes further complicating our understanding of diagnostic accuracy among these locales. These challenges contribute to diagnostic delays, complicate surgical planning, and impose a considerable economic burden on healthcare systems. The findings presented in this thesis underscore the efficacy of a novel posterior approach in TVS, surpassing previous diagnostic accuracies. Secondary analyses corroborate the efficacy of the approach, demonstrating sustained accuracy in the presence of complex disease states. By highlighting advancements in diagnostic accuracy for endometriosis,

this thesis contributes valuable insights into improving patient care and mitigating the burdens of prolonged diagnostic delays.

References

1. Zondervan KT, Becker CM, Missmer SA. Endometriosis. Longo DL, ed. *N Engl J Med*. 2020;382(13):1244-1256. doi:10.1056/NEJMRA1810764

2. Burney RO, Giudice LC. Pathogenesis and Pathophysiology of Endometriosis. *Fertil Steril*. 2012;98(3):511-519. doi:10.1016/J.FERTNSTERT.2012.06.029

3. Moradi M, Parker M, Sneddon A, Lopez V, Ellwood D. Impact of endometriosis on women's lives: A qualitative study. *BMC Womens Health*. 2014;14(1). doi:10.1186/1472-6874-14-123

4. Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, Singh SS, Taylor HS. Clinical diagnosis of endometriosis: a call to action. *Am J Obstet Gynecol*. 2019;220(4):354.e1-354.e12. doi:10.1016/J.AJOG.2018.12.039

5. Johnson NP, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS, Abrao MS, Bush D, Kiesel L, Tamimi R, Sharpe-Timms KL, Rombauts L, Giudice LC, Consortium WESSP, Abrao M, Adamson GD, Advincula A, Allaire C, Andersson E, Arche JC, Becker C, Bush D, Chwalisz K, Condous G, Critchley H, de Bie B, D'Hooghe T, Dunselman G, Evers J, Farquhar C, Faustmann T, Ferriani R, Flores I, Forman A, Fourquet J, Fraser I, Giudice L, Guidone H, Guo SW, Hull L, Hummelshoj L, Johnson N, Keckstein J, Kiesel L, Koninckx P, Lam A, Lessey B, Maher P, Marcellin L, Marriott J, Menakaya U, Missmer S, Mol B, Nisenblat V, Paredes D, Petta C, Reis F, Rolla E, Rombauts L, Seckin T, Sharpe Timms K, Soriano D, Stratton P, Tamimi R, Taylor H, Taylor R, Tsaltas J, Verhagen-Kamberbeek W, Zondervan K. World Endometriosis Society consensus on the classification of endometriosis. *Hum Reprod*. 2017;32(2):315-324. doi:10.1093/HUMREP/DEW293

6. Piessens S, Edwards A. Sonographic Evaluation for Endometriosis in Routine Pelvic Ultrasound. *J Minim Invasive Gynecol*. 2020;27(2):265-266. doi:10.1016/j.jmig.2019.08.027

7. Fernando R, P S, L M, B B, MC LP, C C. Superficial Peritoneal Endometriosis: Clinical Characteristics of 203 Confirmed Cases and 1292 Endometriosis-Free Controls. *Reprod Sci*. 2020;27(1):309-315. doi:10.1007/S43032-019-00028-1

8. Manieri Rocha R, Leonardi M, Eathorne A, Armour M, Condous G. Anatomical distribution of endometriosis: A cross-sectional analysis of transvaginal ultrasound in symptomatic patients. *Australas J Ultrasound Med*. Published online 2023:https://doi.org/10.1002/ajum.12327. doi:10.1002/AJUM.12327

9. Mackenzie SC, Stephen J, Williams L, Daniels J, Norrie J, Becker CM, Byrne D, Cheong Y, Clark TJ, Cooper KG, Cox E, Doust AM, Fernandez P, Hawe J, Holland T, Hummelshoj L, Jackson LJ, King K, Maheshwari A, Martin DC, Sutherland L, Thornton J, Vincent K, Vyas S, Horne AW, Whitaker LHR. Effectiveness of laparoscopic removal of isolated superficial peritoneal endometriosis for the management of chronic pelvic pain in women (ESPriT2): protocol for a multi-centre randomised controlled trial. *Trials*.

2023;24(1):1-15. doi:10.1186/S13063-023-07386-X/TABLES/3

10. Mettler L, Schollmeyer T, Lehmann-Willenbrock E, Schüppler U, Schmutzler A, Shukla D, Zavala A, Lewin A. Accuracy of Laparoscopic Diagnosis of Endometriosis. *JSLS J Soc Laparoendosc Surg*. 2003;7(1):15. Accessed September 9, 2021. /pmc/articles/PMC3015470/

11. Abrao MS, Andres MP, Miller CE, Gingold JA, Rius M, Neto JS, Carmona F. AAGL 2021 Endometriosis Classification: An Anatomy-based Surgical Complexity Score. *J Minim Invasive Gynecol*. 2021;28(11):1941-1950.e1. doi:10.1016/J.JMIG.2021.09.709

12. Audebert A, Petousis S, Margioula-Siarkou C, Ravanos K, Prapas N, Prapas Y. Anatomic distribution of endometriosis: A reappraisal based on series of 1101 patients. *Eur J Obstet Gynecol Reprod Biol*. 2018;230:36-40. doi:10.1016/J.EJOGRB.2018.09.001

13. Janssen EB, Rijkers ACM, Hoppenbrouwers K, Meuleman C, D'Hooghe TM. Prevalence of endometriosis diagnosed by laparoscopy in adolescents with dysmenorrhea or chronic pelvic pain: a systematic review. *Hum Reprod Update*. 2013;19(5):570-582. doi:10.1093/HUMUPD/DMT016

14. International Working Group of AAGL EE and W, Tomassetti C, Johnson NP, Petrozza J, Abrao MS, Einarsson JI, Horne AW, Lee TTM, Missmer S, Vermeulen N, Zondervan KT, Grimbizis G, De Wilde RL. An international terminology for endometriosis, 2021,. *Hum Reprod Open*. 2021;2021(4):16. doi:10.1093/HROPEN/HOAB029

15. Leonardi M, Martins WP, Espada M, Georgousopoulou E, Condous G. Prevalence of negative sliding sign representing pouch of Douglas obliteration during pelvic transvaginal ultrasound for any indication. *Ultrasound Obstet Gynecol*. 2020;56(6):928-933. doi:10.1002/UOG.22023

16. D'alterio MN, D'ancona G, Raslan M, Tinelli R, Daniilidis A, Angioni S. Management Challenges of Deep Infiltrating Endometriosis. *Int J Fertil Steril*. 2021;15(2):88-94. doi:10.22074/IJFS.2020.134689

17. Rocha TP, Andres MP, Carmona F, Baracat EC, Abrão MS. Deep Endometriosis: the Involvement of Multiple Pelvic Compartments Is Associated with More Severe Pain Symptoms and Infertility. *Reprod Sci*. 2023;30(5). doi:10.1007/s43032-022-01104-9

18. Kuznetsov L, Dworzynski K, Davies M, Overton C. Diagnosis and management of endometriosis: summary of NICE guidance. *BMJ*. 2017;358. doi:10.1136/BMJ.J3935

19. Leonardi M, Singh SS, Murji A, Satkunaratnam A, Atri M, Reid S, Condous G. Deep Endometriosis: A Diagnostic Dilemma With Significant Surgical Consequences. *J Obstet Gynaecol Canada*. 2018;40(9):1198-1203. doi:10.1016/j.jogc.2018.05.041

20. Surrey E, Soliman AM, Trenz H, Blauer-Peterson C, Sluis A. Impact of Endometriosis Diagnostic Delays on Healthcare Resource Utilization and Costs. *Adv Ther*. 2020;37(3):1087. doi:10.1007/S12325-019-01215-X

21. Hudelist G, Fritzer N, Thomas A, Niehues C, Oppelt P, Haas D, Tammaa A, Salzer H. Diagnostic delay for endometriosis in Austria and Germany: Causes and possible consequences. *Hum Reprod*. 2012;27(12):3412-3416. doi:10.1093/HUMREP/DES316

22. Requadt E, Nahlik AJ, Jacobsen A, Ross WT. Patient experiences of endometriosis diagnosis: A mixed methods approach. *BJOG An Int J Obstet Gynaecol*. Published online 2023. doi:10.1111/1471-0528.17719

23. Singh S, Soliman AM, Rahal Y, Robert C, Defoy I, Nisbet P, Leyland N. Prevalence, Symptomatic Burden, and Diagnosis of Endometriosis in Canada: Cross-Sectional Survey of 30 000 Women. *J Obstet Gynaecol Canada*. 2020;42(7):829-838. doi:10.1016/J.JOGC.2019.10.038

24. Missmer SA, Tu FF, Agarwal SK, Chapron C, Soliman AM, Chiuve S, Eichner S, Flores-Caldera I, Horne AW, Kimball AB, Laufer MR, Leyland N, Singh SS, Taylor HS, As-Sanie S. Impact of Endometriosis on Life-Course Potential: A Narrative Review . *Int J Gen Med*. 2021;14:9-25. doi:10.2147/IJGM.S261139

25. Ballard K, Lowton K, Wright J. What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. *Fertil Steril*. 2006;86(5):1296-1301. doi:10.1016/J.FERTNSTERT.2006.04.054

26. Van Der Zanden M, Teunissen DAM, Van Der Woord IW, Braat DiDM, Nelen WLDM, Nap AW. Barriers and facilitators to the timely diagnosis of endometriosis in primary care in the Netherlands. *Fam Pract*. 2020;37(1):131-136. doi:10.1093/FAMPRA/CMZ041

27. Leonardi M, Robledo K, Goldstein S, Benacerraf B, Condous G. International survey finds majority of gynecologists are not aware of and do not utilize ultrasound techniques to diagnose and map endometriosis. *Ultrasound Obstet Gynecol*. 2020;56(3):324-328. doi:10.1002/UOG.21996

28. Leonardi M, Rocha R, Tun-Ismail A, Robledo K, Armour M, Condous G. Assessing the knowledge of endometriosis diagnostic tools in a large, international lay population: an online survey. *BJOG An Int J Obstet Gynaecol*. Published online 2021. doi:10.1111/1471-0528.16865

29. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. *Nat Rev Dis Prim 2018 41*. 2018;4(1):1-25. doi:10.1038/s41572-018-0008-5

30. Hudelist G, Valentin L, Saridogan E, Condous G, Malzoni M, Roman H, Jurkovic D, Keckstein J. What to choose and why to use – a critical review on the clinical relevance of rASRM, EFI and Enzian classifications of endometriosis. *Facts, Views Vis ObGyn*. 2021;13(4):331. doi:10.52054/FVVO.13.4.041

31. Leonardi M, Gibbons T, Armour M, Wang R, Glanville E, Hodgson R, Cave AE, Ong J, Tong YYF, Jacobson TZ, Mol BW, Johnson NP, Condous G. When to Do Surgery and When Not to Do Surgery for Endometriosis: A Systematic Review and Meta-analysis. *J Minim Invasive Gynecol*. 2020;27(2):390-407.e3. doi:10.1016/j.jmig.2019.10.014

32. Pascoal E, Wessels JM, Aas-Eng MK, Abrao MS, Condous G, Jurkovic D, Espada M, Exacoustos C, Ferrero S, Guerriero S, Hudelist G, Malzoni M, Reid S, Tang S, Tomassetti C, Singh SS, Van den Bosch T, Leonardi M. Strengths and limitations of diagnostic tools for endometriosis and relevance in diagnostic test accuracy research. *Ultrasound Obs Gynecol*. 2022;60(3):309-327. doi:10.1002/UOG.24892

33. Shapiro J, Karol D, Bridge-Cook P, McCaffrey C, Murji A, Kroft J. A Team-Based, Single-Entry Model for Managing Endometriosis Referrals: An Innovative and Equitable Approach. *J Obstet Gynaecol Canada*. 2023;45(6):393-394. doi:10.1016/j.jogc.2023.03.014

34. Leonardi M, Gibbons T, Armour M, Wang R, Glanville E, Hodgson R, Cave AE, Ong J, Tong YYF, Jacobson TZ, Mol BW, Johnson NP, Condous G. When to Do Surgery and When Not to Do Surgery for Endometriosis: A Systematic Review and Meta-analysis. *J Minim Invasive Gynecol*. 2020;27(2):390-407.e3. doi:10.1016/j.jmig.2019.10.014

35. Leonardi M, Robledo K, Espada M, Vanza K, Condous G. SonoPODography: A new diagnostic technique for visualizing superficial endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2020;254:124-131. doi:10.1016/J.EJOGRB.2020.08.051

36. Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, King K, Kvaskoff M, Nap A, Petersen K, Saridogan E, Tomassetti C, van Hanegem N, Vulliemoz N, Vermeulen N, Group EEG, Altmäe S, Ata B, Ball E, Barra F, Bastu E, Bianco-Anil A, Knudsen UB, Brubel R, Cambitzi J, Cantineau A, Cheong Y, Daniilidis A, Bie B De, Exacoustos C, Ferrero S, Gelbaya T, Goetz-Collinet J, Hudelist G, Hussain M, Indrielle-Kelly T, Khazali S, Kumar SL, Maggiore ULR, Maas JWM, McLaughlin H, Metello J, Mijatovic V, Miremadi Y, Muteshi C, Nisolle M, Oral E, Pados G, Parades D, Pluchino N, Supramaniam PR, Schick M, Seeber B, Seracchioli R, Laganà AS, Stavroulis A, Tebache L, Uncu G, Van den Broeck U, van Peperstraten A, Vereczkey A, Wolthuis A, Bahat PY, Yazbeck C. ESHRE guideline: endometriosis. *Hum Reprod Open*. 2022;2022(2):1-26. doi:10.1093/HROPEN/HOAC009

37. Guerriero S, Condous G, Bosch T van den, Valentin L, Leone FPG, Schoubroeck D Van, Exacoustos C, Installé AJF, Martins WP, Abrao MS, Hudelist G, Bazot M, Alcazar JL, Gonçalves MO, Pascual MA, Ajossa S, Savelli L, Dunham R, Reid S, Menakaya U, Bourne T, Ferrero S, Leon M, Bignardi T, Holland T, Jurkovic D, Benacerraf B, Osuga Y, Somigliana E, Timmerman D. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. *Ultrasound Obs Gynecol*. 2016;48(3):318-332. doi:10.1002/UOG.15955

38. Indrielle-Kelly T, Frühauf F, Fanta M, Burgetova A, Lavu D, Dundr P, Cibula D, Fischerova D. Diagnostic Accuracy of Ultrasound and MRI in the Mapping of Deep Pelvic Endometriosis Using the International Deep Endometriosis Analysis (IDEA) Consensus. *Biomed Res Int*. 2020;2020. doi:10.1155/2020/3583989

39. Leonardi M, Uzuner C, Mestdagh W, Lu C, Guerriero S, Zajicek M, Dueckelmann A, Filippi F, Buonomo F, Pascual MA, Stepniewska A, Ceccaroni M, Van den Bosch T, Timmerman D, Hudelist G, Condous G. Diagnostic accuracy of transvaginal ultrasound for detection of endometriosis using International Deep Endometriosis Analysis (IDEA) approach: prospective international pilot study. *Ultrasound Obs Gynecol*. 2022;60(3):404-413. doi:10.1002/UOG.24936

40. Avery JC, Deslandes A, Freger SM, Leonardi M, Lo G, Carneiro G, Condous G, Hull ML, Hull L, Carneiro G, Avery J, O'Hara R, Condous G, Knox S, Leonardi M, Panuccio C,

Sirop A, Abbott J, Gonzalez-Chica D, Wang H, Lo G, Chen T, Deslandes A, To MS, Zhang Y, Yang N, Uzuner C, Holdsworth-Carson S, Nguyen T, Freger S, Abeygunasekara N, Richards M, Simpson A, Voyvodic F, Jenkins M. Non-invasive Diagnostic Imaging for Endometriosis Part 1: A Systematic review of recent developments in Ultrasound, Combination Imaging and Artificial Intelligence. *Fertil Steril*. 2023;0(0). doi:10.1016/j.fertnstert.2023.12.008

41. Piessens S, Edwards A. Sonographic Evaluation for Endometriosis in Routine Pelvic Ultrasound. *J Minim Invasive Gynecol*. 2020;27(2):265-266. doi:10.1016/J.JMIG.2019.08.027

42. Wong HB, Lim GH. Measures of diagnostic accuracy: Sensitivity, specificity, PPV and NPV. *Proc Singapore Healthc*. 2011;20(4). doi:10.1177/201010581102000411

43. Akobeng AK. Understanding diagnostic tests 1: Sensitivity, specificity and predictive values. *Acta Paediatr Int J Paediatr*. 2007;96(3). doi:10.1111/j.1651-2227.2006.00180.x

44. Swift A, Heale R, Twycross A. What are sensitivity and specificity? *Evid Based Nurs*. 2020;23(1). doi:10.1136/ebnurs-2019-103225

45. Alberg AJ, Park JW, Hager BW, Brock M V., Diener-West M. The use of "overall accuracy" to evaluate the validity of screening or diagnostic tests. *J Gen Intern Med*. 2004;19(5 PART 1):460-465. doi:10.1111/J.1525-1497.2004.30091.X/METRICS

46. Grimes DA, Schulz KF. Uses and abuses of screening tests. *Lancet*. 2002;359(9309). doi:10.1016/S0140-6736(02)07948-5

47. Vodolazkaia A, El-Aalamat Y, Popovic D, Mihalyi A, Bossuyt X, Kyama CM, Fassbender A, Bokor A, Schols D, Huskens D, Meuleman C, Peeraer K, Tomassetti C, Gevaert O, Waelkens E, Kasran A, De Moor B, D'Hooghe TM. Evaluation of a panel of 28 biomarkers for the non-invasive diagnosis of endometriosis. *Hum Reprod*. 2012;27(9). doi:10.1093/humrep/des234

48. Mihalyi A, Gevaert O, Kyama CM, Simsa P, Pochet N, De Smet F, De Moor B, Meuleman C, Billen J, Blanckaert N, Vodolazkaia A, Fulop V, D'Hooghe TM. Non-invasive diagnosis of endometriosis based on a combined analysis of six plasma biomarkers. *Hum Reprod*. 2010;25(3). doi:10.1093/humrep/dep425

49. Othman EEDR, Hornung D, Salem HT, Khalifa EA, El-Metwally TH, Al-Hendy A. Serum cytokines as biomarkers for nonsurgical prediction of endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2008;137(2). doi:10.1016/j.ejogrb.2007.05.001

50. Šimundić AM. Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC*. 2009;19(4).

51. Kaijser J, Bourne T, Valentin L, Sayasneh A, Van Holsbeke C, Vergote I, Testa AC, Franchi D, Van Calster B, Timmerman D. Improving strategies for diagnosing ovarian cancer: a summary of the International Ovarian Tumor Analysis (IOTA) studies. *Ultrasound Obstet Gynecol*. 2013;41(1):9-20. doi:10.1002/UOG.12323

52. Nisenblat V, Bossuyt PMM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev*. 2016;2016(2). doi:10.1002/14651858.CD009591.PUB2/MEDIA/CDSR/CD009591/URN:X-

WILEY:14651858:MEDIA:CD009591:CD009591-AFIG-FIG05

53. Hudelist G, English J, Thomas AE, Tinelli A, Singer CF, Keckstein J. Diagnostic accuracy of transvaginal ultrasound for non-invasive diagnosis of bowel endometriosis: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2011;37(3):257-263. doi:10.1002/UOG.8858

54. Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V, Vacher-Lavenu MC, Dubuisson JB. Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. *Hum Reprod*. 2003;18(1):157-161. doi:10.1093/HUMREP/DEG009

55. Maple S, Chalmers KJ, Bezak E, Henry K, Parange N. Ultrasound characteristics and scanning techniques of uterosacral ligaments for the diagnosis of endometriosis. *J Ultrasound Med*. 2022;42(6):1193-1209. doi:10.1002/JUM.16129

56. Vu D, Haylen BT, Tse K, Farnsworth A. Surgical anatomy of the uterosacral ligament. *Int Urogynecol J*. 2010;21(9):1123-1128. doi:10.1007/S00192-010-1147-8/FIGURES/6

57. Ramanah R, Berger MB, Parratte BM, De Lancey JOL. Anatomy and histology of apical support: A literature review concerning cardinal and uterosacral ligaments. *Int Urogynecol J*. 2012;23(11):1483-1494. doi:10.1007/S00192-012-1819-7/METRICS

58. Azaïs H, Collinet P, Delmas V, Rubod C. Uterosacral ligament and hypogastric nerve anatomical relationship. Application to deep endometriotic nodules surgery. *Gynécologie Obs Fertil*. 2013;41(3):179-183. doi:10.1016/J.GYOBFE.2013.01.004

59. Chopin N, Vieira M, Borghese B, Foulot H, Dousset B, Coste J, Mignon A, Fauconnier A, Chapron C. Operative management of deeply infiltrating endometriosis: Results on pelvic pain symptoms according to a surgical classification. *J Minim Invasive Gynecol*. 2005;12(2):106-112. doi:10.1016/j.jmig.2005.01.015

60. Chapron C, Dubuisson JB. Laparoscopic treatment of deep endometriosis located on the uterosacral ligaments. *Hum Reprod*. 1996;11(4):868-873. doi:10.1093/OXFORDJOURNALS.HUMREP.A019268

61. Zondervan KT, Becker CM, Missmer SA. Endometriosis. Longo DL, ed. *N Engl J Med*. 2020;382(13):1244-1256. doi:10.1056/NEJMra1810764

62. Scioscia M, Bruni F, Ceccaroni M, Steinkasserer M, Stepniewska A, Minelli L. Distribution of endometriotic lesions in endometriosis stage IV supports the menstrual reflux theory and requires specific preoperative assessment and therapy. *Acta Obs Gynecol Scand*. 2011;90(2):136-139. doi:10.1111/J.1600-0412.2010.01008.X

63. Giudice LC, Kao LC. Endometriosis. *Lancet*. 2004;364(9447):1789-1799. doi:10.1016/S0140-6736(04)17403-5

64. Ianieri MM, Mautone D, Ceccaroni M. Recurrence in deep infiltrating endometriosis: A systematic review of the literature. *J Minim Invasive Gynecol*. 2018;25(5):786-793. doi:10.1016/J.JMIG.2017.12.025

65. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic

nodules of the rectovaginal septum are three different entities. *Fertil Steril*. 1997;68(4):585-596. doi:10.1016/S0015-0282(97)00191-X

66. Guerriero S, Ajossa S, Minguez JA, Jurado M, Mais V, Melis GB, Alcazar JL. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in uterosacral ligaments, rectovaginal septum, vagina and bladder: systematic review and meta-analysis. *Ultrasound Obs Gynecol*. 2015;46(5):534-545. doi:10.1002/UOG.15667

67. Leonardi M, Martins WP, Espada M, Arianayagam M, Condous G. Proposed technique to visualize and classify uterosacral ligament deep endometriosis with and without infiltration into parametrium or torus uterinus. *Ultrasound Obs Gynecol*. 2020;55(1):137-139. doi:10.1002/UOG.20300

68. Leonardi M, Condous G. A pictorial guide to the ultrasound identification and assessment of uterosacral ligaments in women with potential endometriosis. *Australas J Ultrasound Med*. 2019;22(3):157-164. doi:10.1002/AJUM.12178

69. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, De Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF, Alonzo T, Azuara-Blanco A, Bachmann L, Blume J, Boutron I, Büller H, Buntinx F, Byron S, Chang S, Cooper R, De Groot J, Deeks J, Dendukuri N, Dinnes J, Fleming K, Guyatt G, Heneghan C, Hilden J, Horvath R, Hunink M, Hyde C, Ioannidis J, Janes H, Kleijnen J, Knottnerus A, Lange S, Leeflang M, Lord S, Lumbreras B, Macaskill P, Magid E, Mallett S, McInnes M, Mc-Neil B, McQueen M, Moons K, Morris K, Mustafa R, Obuchowski N, Ochodo E, Onderdonk A, Overbeke J, Pai N, Peeling R, Pepe M, Petersen S, Price C, Ravaud P, Rutjes A, Schunemann H, Simel D, Simera I, Smidt N, Steyerberg E, Straus S, Summerskill W, Takwoingi Y, Thompson M, Van De Bruel A, Van Maanen H, Vickers A, Virgili G, Walter S, Weber W, Westwood M, Whiting P, Wilczynski N, Ziegler A. STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies. *Clin Chem*. 2015;61(12):1446-1452. doi:10.1373/CLINCHEM.2015.246280

70. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MMG, Sterne JAC, Bossuyt PMM. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536. doi:10.7326/0003-4819-155-8-201110180-00009/SUPPL_FILE/155-8-529-SUPPLEMENT.PDF

71. European Federation of Societies for ultrasound in medicine and biology. Minimum training requirements for the practice of Medical Ultrasound in Europe. *Ultraschall Med*. 2010;31(4):426-427. doi:10.1055/s-0030-1263214

72. Stegmann BJ, Sinaii N, Liu S, Segars J, Merino M, Nieman LK, Stratton P. Using location, color, size, and depth to characterize and identify endometriosis lesions in a cohort of 133 women. *Fertil Steril*. 2008;89(6):1632-1636. doi:10.1016/j.fertnstert.2007.05.042

73. Benagiano G, Brosens I. Review: The history of endometriosis: identifying the disease. *Hum Reprod*. 1991;6(7):963-968. doi:10.1093/OXFORDJOURNALS.HUMREP.A137470

74. Fenn Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Acad Emerg Med*. 1996;3(9):895-900. doi:10.1111/j.1553-2712.1996.tb03538.x

75. Hummelshoj L, De Graaff A, Dunselman G, Vercellini P. Let's talk about sex and endometriosis. *J Fam Plann Reprod Heal Care*. 2014;40(1):8-10. doi:10.1136/JFPRHC-2012-100530

76. Dinh T, Leonardi M, Espada M, Vanza K, Condous G. The Use of Ultrasound in Detecting Endometriosis: Endometriotic Nodule Detected on Ultrasound but not Visualized on Laparoscopy. *J Obstet Gynaecol Canada*. 2020;42(8):1016. doi:10.1016/J.JOGC.2019.12.013

77. Clement PB. The pathology of endometriosis: a survey of the many faces of a common disease emphasizing diagnostic pitfalls and unusual and newly appreciated aspects. *Adv Anat Pathol*. 2007;14(4):241-260. doi:10.1097/PAP.0B013E3180CA7D7B

78. Agarwal N, Subramanian A. Endometriosis - morphology, clinical presentations and molecular pathology. *J Lab Physicians*. 2010;2(1):001-009. doi:10.4103/0974-2727.66699

79. Walter AJ, Hentz JG, Magtibay PM, Cornella JL, Magrina JF. Endometriosis: correlation between histologic and visual findings at laparoscopy. *Am J Obs Gynecol*. 2001;184(7):1407-1413. doi:10.1067/MOB.2001.115747

80. Albee RB, Sinervo K, Fisher DT. Laparoscopic excision of lesions suggestive of endometriosis or otherwise atypical in appearance: relationship between visual findings and final histologic diagnosis. *J Minim Invasive Gynecol*. 2008;15(1):32-37. doi:10.1016/J.JMIG.2007.08.619

81. Koninckx PR, Meuleman C, Demeyere S, Lesaffre E, Cornillie FJ. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertil Steril*. 1991;55(4):759-765. doi:10.1016/S0015-0282(16)54244-7

82. Koninckx PR, Ussia A, Adamyan L, Wattiez A, Gomel V, Martin DC. Pathogenesis of endometriosis: the genetic/epigenetic theory. *Fertil Steril*. 2019;111(2):327-340. doi:10.1016/J.FERTNSTERT.2018.10.013