Harmsen Marissa J. (Orcid ID: 0000-0003-3842-5023)
Tellum Tina (Orcid ID: 0000-0003-2635-4504)
Van den Bosch Thierry (Orcid ID: 0000-0001-6777-5036)

Multidisciplinary view on uterine junctional zone in uteri affected by adenomyosis: explaining discrepancies between MRI and transvaginal ultrasound images on a microscopic level

M. J. Harmsen[1,2#], L. M. Trommelen[1,2#], R. A. de Leeuw[1,2], T. Tellum[3], L. J. M. Juffermans[1,2], A. W. Griffioen[4,5], I. Thomassin-Naggara[6], T. Van den Bosch[7] and J. A. F. Huirne[1]

#M. J. H. and L. M. T. contributed equally to this work.

[1] Department of Obstetrics & Gynaecology, Amsterdam UMC, location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[2] Amsterdam Reproduction and Development, Amsterdam, The Netherlands

[3] Department of Gynecology, Oslo University Hospital, Oslo, Norway

[4] Angiogenesis Laboratory, Department of Medical Oncology, Amsterdam UMC, location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[5] Cancer Center Amsterdam, Amsterdam, The Netherlands

[6] Department of Diagnostic and Interventional Imaging (IRIS), Sorbonne Université, Assistance Publique Hopitaux de Paris, Paris, France

[7] Department of Obstetrics and Gynecology, University Hospitals KU Leuven, Leuven, Belgium

Corresponding author: Dr J. A. F. Huirne

Amsterdam UMC, location VUMC, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

e-mail: j.huirne@amsterdamumc.nl

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Abstract

The uterine junctional zone is the subendometrial area in the myometrium that contributes to peristalsis and aids in spermatozoa and blastocyst transport. Alterations in appearance of the junctional zone on transvaginal ultrasound (TVUS) or magnetic resonance imaging (MRI) are associated with adenomyosis. Lack of uniform description of its appearance and ill-defined boundaries in both histology and imaging hamper understanding of the junctional zone's entity and limit its role in the diagnosis of adenomyosis. The objective of this state-of-the art review was to investigate the accordance on the definition of the junctional zone across different diagnostic approaches and examine how the imaging findings can be linked to histological findings in the context of adenomyosis diagnosis. A comprehensive literature review was conducted for articles describing the imaging appearance and histological structure of the junctional zone within the uterus. Our review suggests that the junctional zone is distinguished from the middle and outer myometrium by gradual changes in smooth muscle cell density, extracellular space, connective tissue, water content, and vascular properties. However, while the signal intensity from junctional zone to middle myometrium changes abruptly on MRI, the histopathological changes are gradual and its border may be difficult or impossible to distinguish on 2D TVUS. Moreover, the thickness of the junctional zone on MRI was larger than on TVUS. Thus, these two imaging modalities do not reflect exactly the same. Although a thickened junctional zone is often used to diagnose adenomyosis on MRI, the presence of adenomyosis can more accurately be described as interruptions of the junctional zone by endometrial tissue: direct features of adenomyosis, such as subendometrial lines and buds on 2D and 3D TVUS or bright foci on MRI. The histopathological criteria are based on enlarged uteri with severe adenomyosis, and might not reflect early stages. Clinicians should be aware that findings on MRI cannot readily be extrapolated to ultrasound. Understanding of this is necessary when investigating the potential relevance of the uterine junctional zone as a functional unit and the association between the visualization of direct features of adenomyosis in the junctional zone and clinical symptoms.

Introduction

While no unanimous definition exists, the myometrium can be subdivided into three layers; the inner myometrium or stratum subvasculare, the outer myometrium or stratum supravasculare between the vascular arcade and the serosa of the uterus, and the middle myometrium or stratum vasculare in between (Figure 1)[1, 2]. As early as 1898 the inner myometrial layer was suggested to be a separate entity and called the archimyometrium[3]. The inner myometrium, also known as the subendometrial area, gained attention in the 1980s when a difference in signal intensity or echogenicity in the myometrium was observed on magnetic resonance imaging (MRI) and ultrasound (US) respectively[4, 5]. This area was later called the junctional zone (JZ)[6]. Histologically, the JZ is seen as the inner third of the myometrium[7, 8], or the area of the myometrium below to the endometrium ranging 5 – 8 mm in thickness that is in direct contact with the endometrial glands without division by a basal lamina [9]. Remarkably, this is the only mucosal-muscle intersection in the body that lacks a basal lamina [10] . A clear definition of the JZ is lacking [11]. Essentially, the definition of the junctional zone varies among studies and diagnositic modalities (MRI, ultrasound, or histology), introducing the risk that the entity described might differ too.

The relevance of the assessment of the JZ is apparent from its physiological role and the problems related to alterations of the JZ. In physiology, the cyclic expression of estrogen and progesterone receptors in the JZ parallels that of the endometrium during the menstrual cycle, while the outer myometrial layers do not express these receptors in a cyclic pattern[2]. The functional and hormonal similarities of the endometrium and the JZ correspond to the proposed shared embryological origin from the Müllerian ducts, while the outer myometrial zones are of mesenchymal origin[2]. In the follicular phase, JZ peristalsis enhances transport of spermatozoa and in the luteal phase it contributes to embryo transport or implantation in the cavity[12, 13]. Alterations to the JZ, amongst others the presence of ectopic endometrial tissue in adenomyosis, are hypothesized to disturb uterine peristalsis and cause key problems associated with its function. Therefore, adenomyosis-related infertility might be due to dysperistalsis in the junctional zone [14-17]. The JZ is also hypothesized to play an important role in one of the hypothesis of the pathogenesis in adenomyosis. According to this theory microtrauma of the JZ is followed by a cascade of tissue injury and repair, accompanied by angiogenesis and inflammation and leading to myometrial invasion by the endometrial glands[15]. Another type of adenomyosis that does

not include the JZ, is seen in association with deep infiltration endometriosis and related to the invasion of ectopic endometrial cells from the outside into the serosa of the myometrium[18].

In essence, a major focus of interest is the correlation between changes in junctional zone morphology and adenomyosis. Lack of a uniform description of the junctional zone among diagnostic modalities questions its use as consistent diagnostic criterion for adenomyosis. The question arises which histological features inside the myometrium correlate with the appearance of the junctional zone on MRI and US images and which (pathological) changes can be visualized.

We hypothesize that the substrate of the definitions of the junctional zone by histology and imaging modalities is not uniform. We aim to investigate the accordance on the definition of the junctional zone across different disciplines (MRI, TVUS and histology), examine how the imaging findings can be traced back to histological findings and assess their relevance in diagnosing adenomyosis.

Methods

A comprehensive search was conducted in the PubMed, Google Scholar, and Web of Science databases up to June 8th 2022 for articles describing the imaging appearance and histological structure of the junctional zone within the uterus of premenopausal women. There was no restriction on the date of publication or study design, all articles and reviews written in English and published in peer-reviewed journals were included. Combinations of (MeSH) terms and synonyms for the junctional zone and uterus were applied (see Table S1 for full search strategy) and articles were added through snowballing. Studies that visualized the junctional zone using CT are not considered further, since CT is not commonly performed to assess the uterus because it produces suboptimal images of the uterus [19, 20]. We excluded the following topics from this review: studies on non-humans, postmenopausal women, malignant changes of the junctional zone, changes in pregnancy, non-uterus related diseases, effect of surgical treatment, or technical aspects of imaging techniques. All abstracts resulting from the search were screened for eligibility independently by two authors (MH and LT) using the Rayyan web application [21]. Full text articles were retrieved, assessed, and reviewed for eligibility. Conflicts on eligibility were discussed by two authors (MH and LT) until they reached an agreement. Data extraction was performed using a predefined standardized format and included number of patients, age range, condition, use of hormonal therapy, cycle phase, used definition of the junctional zone, study objective, methods used, and key findings.

Histologic appearance of the inner myometrium

Normal histological appearance

We identified 15 studies investigating the junctional zone in histology[2, 6-8, 11, 18, 22-30], of those six concerned the junctional zone with regards to adenomyosis[7, 8, 18, 24, 25, 27]. These studies used the terms inner myometrium and junctional zone interchangeably. In this section, we will consistently use the term inner myometrium to specify this anatomical layer. Most of the included studies make no distinction between the middle and outer myometrium. The terms 'stratum subvasculare, vasculare, and supravasculare' that refer to the three-layered myometrium, date from German anatomical studies on the human myometrium from the late 19th century[3, 31]. Of the (English) studies that were identified for this review, only one study mentions the distinction of three myometrial layer (JZ, myometrium proper and subserosal layer) [23]. Characteristics of all original articles on the histologic findings are summarized in Table S2. Most studies included less than 20 patients.

Depending on the staining method and visualized structure, similarities [7, 18, 25-28, 30] and differences[7, 8, 18, 23, 27-29] in the inner myometrium versus the outer myometrial zone were found (for details see Table 1). This is elucidated in Figure 2A.

With the conventional histopathological staining method, hematoxylin and eosin (H&E), the myometrium has a homogeneous appearance and no apparent differences in tissue or cell type were found between the inner and outer layers of the myometrium[6, 26, 30]. By visual inspection of this stained material a constant number of blood vessels across the total myometrium was found[26]. All three uterine layers consist primarily of smooth muscle cells (SMCs), and no difference in α-smooth muscle actin (α-SMA) of SMCs was found between the layers [18]. Staining of desmin and vimentin, intermediate filaments of SMCs, or elastin did not reveal an evident zonal pattern [18, 26, 27]. SMCs in both inner and outer myometrium had sparse cytoplasm, filled with myofilaments[7]. In the connective tissue of the inner myometrium versus the outer myometrium no difference in collagen distribution was found[26], nor any morphological differences in collagen differentiation[30]. The extent or intensity of collagen staining showed no differences between inner and outer myometrium[18, 25, 28]. Studies considering the role of menstrual cycle phase found no variation in ultrastructure at histological level between cycle phases[7, 27].

Differences in structures or cell types between the inner and outer myometrium layers were found. The SMCs in the myometrium differed when looking at the morphology of the cells: cell density[7, 8, 23, 24] and organisation[28, 29], nuclear size[7] and nuclear area[28, 29] of SMCs were increased in the inner versus the outer layers of the myometrium[27]. One study described the SMCs in the inner myometrium appeared longitudinally oriented, parallel to the endometrium glands, and gradually loosening towards the outer

myometrium[23]. The middle myometrium was described as an interwoven arrangement of SMC bundles, collagen bundles, and larger blood vessels, while the SMC bundles in the subserosal outer myometrial layer were arranged parallel to the uterine surface (Figure 2A). The nuclear area, attritubted to cell density, nuclear size, or a combination, was reported to differ between a 1.6-1.8-fold[23] to a 3-fold change[28], however this decrease seems gradual, without a distinct zonation[27, 28]. In various studies the extracellular space was smaller in the inner myometrium[23, 26, 28, 29]. The SMCs in the inner myometrium were closely interwoven in connective tissue, with a connective tissue-to-SMCs ratio of 40:60, whereas the SMC in the outer myometrium were widely spaced with a connective tissue-to-SMCs ratio of 60:40[7]. In contrast to the constant distribution by Von Giesen Staining[26], elastin stained by antibodies gradually increases from inner to outer myometrium, predominantly in perivascular tissue[11]. Additionally, the inner myometrium was negative for late differentiation markers (i.e. smoothelin and myosin heavy chain), and consisted of non-differentiated SMCs, whereas the outer myometrium is composed of terminally differentiated SMCs. The non-differentiated SMCs, similar to myofibroblast, have the potential to initiate cell proliferation[24]. Two studies demonstrated a gradual decrease in CD31-positive vascular endothelial cells from inner to outer myometrium, without a difference in volume fraction of the capillary walls[23]. The diameter of the vessels in the inner myometrium were smaller than in the outer myometrium, and the wall thickness thinner[22].

Appearance with adenomyosis

Traditionally, the histopathological diagnosis of adenomyosis is made when endometrial glands and stroma are found 2-2.5mm below the endometrial line[27, 28]. However, a definitive cut off for invasion depth to define adenomyosis has not been unanimously adopted[32]. At histological examination, the boundary between the inner myometrium and outer myometrium gradually changes. Additionally, as endometrium superficially penetrates the myometrium[23], physiological offshoots of the endometrium in the myometrium can complicate the exact diagnosis. Inner myometrium characteristics are difficult to compare between studies given the lack of consistent pathological definition[32]. Moreover, since a limited number of slices is examined on a hysterectomy specimen, focal lesions alongside areas of healthy myometrium may be missed[33]. Also, the histopathological studies were performed on uteri of women who needed a hysterectomy because of severe symptoms of dysmenorrhea and abnormal uterine bleeding[34], resulting in more severe or progressed adenomyotic uterine tissue.

The histological differences of the inner myometrium affected by adenomyosis versus the inner myometrium in absence of adenomyosis are found in Table 1, characteristics of the reviewed studies are found in Table S4. In adenomyosis, the inner myometrium becomes interrupted, with a loss of parallel orientation of the SMCs to the endometrium glands[24]. Basal endometrium glands and stroma are found invaginating in the myometrium, surrounded by type 1 collagen fibres in the extracellular

matrix (Figure 2B) [18, 24, 25]. Two studies saw no difference in α-SMA staining of SMC [18] and Desmin expression[27] between myometrium of uteri with or without adenomyosis, while one study found higher α-SMA staining in the inner myometrium of adenomyosis uteri versus non-adenomyosis. In the whole uterus affected by adenomyosis, but especially in the inner myometrium, various signs of cellular hypertrophy and metaplasia are seen: decreased cell density with increased nuclear size and cytoplasm [7, 27], a higher proliferation index in the inner myometrium than in the outer myometrium [27], higher collagen I labelling, as a marker for myofibroblasts in all myometrial layers [25], and less extracellular space and matrix[7, 8, 24, 25]. Staining of vimentin, the main component of intermediate filaments in mesenchymal cells (e.g. endometrial stroma and glands) and a minor component in smooth muscle cells was overexpressed in the inner myometrium[27]. Like in healthy uteri, these difference were gradual without zonation. The increased presence of immuno-labelled α-SMA and collagen I at the inner myometrium in uteri with adenomyosis compared to non-adenomyosis uteri suggests the presence of myofibroblasts and metaplasia as a response to chronic tissue injury and repair[27].

We conclude that, histologically, structural differences between the inner and outer myometrium can, at least partly, explain the concept of a junctional zone on imaging as presented in Figure 2. These histological differences are however gradual and not zonal. A serrated inner myometrium with invaginating endometrial glands, as well as cellular hypertrophy, or fibrosis in the inner and/or outer layers of the myometrium, are signs of adenomyosis [18, 24, 25].

The appearance of the uterine junctional zone using MRI

Normal MRI appearance

In 1983 a low signal intensity band interfacing endometrium and myometrium was first visualised by Hricak et al.[4]. Lee et al., using T2-weighted MRI (T2W MRI) recognized this 2-6 mm band between the high-intensity zone of the endometrium and the medium-intensity zone of the peripheral myometrium, as part of the myometrium and introduced the term uterine junctional zone[6]. This definition of the uterine junctional zone is considered standard for MRI today [23, 26, 28, 30, 35-37]. Further zonal discrimination into middle and outer myometrium was not described in the included MRI studies, so these layers are referred to as the outer layers in the description of the results. However, there are several MRI based studies, which were beyond the scope of this paper, that describe inner, middle, and outer myometrium[38, 39]. The findings per imaging technique are presented in Table 2, and the characteristics of all included original articles on MRI findings are summarized in Table S3. In this review, if not mentioned otherwise, we refer to the JZ on T2W MRI, as it is not clearly visualized in T1W MRI[4]. There are multiple theories explaining the differences in signal intensity of the uterine

layers. Two small studies showed that after drying hysterectomy specimen, the junctional zone was found to have a lower water content[26, 30] (figure 3A), but found contradicting results concerning the visibility of the junctional zone ex vivo. While one study found that the junctional zone remained visible on MRI performed ex vivo[26], the other found that it was less distinguished or had disappeared within 5.5 hours after hysterectomy[30]. The suggestion that blood volume, as an extracellular water containing space, could cause the difference in signal intensity between the different uterine layers was not convincingly refuted nor confirmed [23, 28]. Signs of reduced water content and increased cell density were also observed by a reduced apparent diffusion coefficient (ADC) in Diffusion Weighted Imaging (DWI) in the junctional zone compared to the outer myometrium [40, 41]. The role of blood flow was re-evaluated in more recent and larger studies by dynamic contrast enhanced (DCE) MRI (Table 2) [40, 41]Overall, these findings suggest a higher vascular perfusion rate, a higher velocity of tissue blood flow in the capillaries and a greater capillary permeability in the junctional zone than in the outer myometrium (Figure 3B1 and 3B2).

Another hypothesis for the observation of the junctional zone is the difference in orientation of fibres between the inner and outer layers of myometrium[23]. Diffusion Tensor Imaging (DTI)-MRI was used to investigate myometrial fiber architecture in 9 healthy women (Table 2)[42]. Although circular and longitudinal oriented muscular fibers were visible in the whole myometrium, the low ADC and the mean highest fractional anisotropy (FA) in in the junctional zone compared to the endometrium and OM, suggest good alignment of closely packed SMC fibres in the junctional zone[42] (figure 3C). Additionally, a lower amide proton transfer signal intensity (APT SI), reflecting a lower protein concentration, was seen in the junctional zone (Figure 3D) [43].

Apart from these hypotheses, there are also other factors that influence the JZ in MRI imaging. Some studies show an increasing thickness towards the secretory phase[44-46], others found the thickest JZ in the menstrual phase[47]. The variation in thickness correlated moderate-to-strong with serum estradiol levels[48, 49]. The ADC was higher in the luteal phase and lower in the ovulatory or menstrual phase, [47, 48, 50], and a moderate correlation was found between APT signal intensity and ADC in the junctional zone, which could be the result of increased cell proliferation in the secretory phase and increased peristalsis in the menstrual phase [43]. Peristalsis of the junctional zone is recognized on cine-MRI as a wave-like pattern and can mimic uterine pathologies such as adenomyosis and leiomyomas [51]. A positive correlation between the detectability of the junctional zone and the frequency of peristalsis was seen, but not between JZ thickness and frequency of peristalsis [52, 53]. Studies using blood oxygenation level-dependent (BOLD) MRI showed lower signal in the JZ in the menstrual phase [42, 43, 54]. This could be due to vascular spasms of the spiral arteries inducing menstruation [55], or due to contraction of SMCs in the junctional zone during peristalsis [51]. Age and hormonal medication were also found to be of influence

of the thickness and ADC value of the junctional zone [47, 48, 50]. The junctional zone thickness increased with age and was thickest at 41-50 years [50] with a drop in women over 50 years and in menopause [48, 50, 52, 56]. In premenarchal women, postmenopausal women and women with ovarian suppression caused by gonadotrophin-releasing hormone analogues, the border between the junctional zone and the outer uterine layers often becomes indistinct on MRI [52, 57]. Women using any form of hormonal contraception had a significantly thinner junctional zone, with a lower perfusion and interstitial volume compared to women who did not use this medication [36, 41, 57, 58].

These differences in blood flow, cellular density, water content of the extracellular space and fiber alignment, as well as the differences caused by cycle phase, hormonal medication, age and peristalsis are of influence on imaging and are summarized in Table 2.

MRI appearance with adenomyosis

On MRI, adenomyosis was first recognized as a trabeculated pattern of the myometrium with areas of high intensity interspersed with areas of medium intensity in three uterine specimens that were assessed ex vivo [6]. On T2W MRI a direct sign of adenomyosis is the presence of subendometrial cysts , seen as ill-defined, low-signal intensity masses within the myometrium[59-61]. They can appear on T1W MRI when hemorrhagic [62]. Several prospective studies on junctional zone characteristics found that a junctional zone thickness of ≥12mm, a maximum JZ thickness ratio of > 40% of the total myometrium, or a difference in junctional zone thickness of ≥5-7mm had a positive predictive value to diagnose adenomyosis [59-61]. The thickened junctional zone reflects SMC proliferation or hyperplasia surrounding the heterotopic endometrial tissue [52, 53]. This hyperplasia, spreading towards the outer myometrium, causes a wider area of dense smooth muscle cells, explaining the same signal intensity as the pre-existing junctional zone [42, 43, 54]. However, these findings were later contradicted by a prospective study, which suggested that a regular thickened JZ >12mm also appears in uteri without adenomyosis, and a thin JZ does not exclude adenomyosis[63]. One study suggested that differences in a thin layer of restricted water diffusion surrounding the endometrium on an ADC map of patients with adenomyosis, might be responsible for changes of the appearance junctional zone in adenomyosis. However, this layer was only seen in half of the patients and no pathologic substrate of changes in this layer was found after hysterectomy [64]. Due to physiological, peristaltic, and hormonal variations of the JZ in MRI a strict cut-off of the JZ thickness should be used carefully as a diagnostic criteria in the diagnosis of adenomyosis. Nonetheless, improvement of symptoms of adenomyosis and leiomyomas has been attributed to junctional thinning after treatment with gonadotrophin-releasing hormone analogue (GnRHa) [65]. Table 2 provides an overview of the MRI findings in the junctional zone in uteri affected by adenomyosis. The characteristics of all original articles describing the MRI appearance of the junctional zone in adenomyosis, are summarized in Table S4.

The appearance of the junctional zone on transvaginal ultrasonography

Normal transvaginal ultrasound appearance

The Morphological Uterus Sonographic Assessment (MUSA) group defined that the myometrium should be divided into three layers; the junctional zone (being synonymous with the inner myometrium), the middle myometrium and the outer myometrium (Figure 1). The middle myometrium spans between the junctional zone and the venous and arterial arcuate vessels of the uterus. The vascular arcuate can be used to orient between the uterine layers, since in most cases they can be distinguished on the sagittal plane in 2D TVUS or on the coronal plane in 3D TVUS, especially by using colour or power Doppler. The outer myometrium is located between the arcuate vessels and the uterine serosa. The findings of the junctional zone on TVUS are found in Table 2, characteristics of all original articles on US findings that were reviewed are summarized in Table S3.

The junctional zone in ultrasound (US) was first described as a hypoechoic halo within the myometrium surrounding the hyperechoic endometrium[29, 35]. The junctional zone can be assessed in both 2D and 3D TVUS, but only 3D TVUS offers an assessment of all planes, the sagittal, transverse, and coronal plane, as recommended by the MUSA group [66, 67]. 3D-TVUS, especially with the use of volume contrast imaging (VCI) in the multiplanar view, is suggested to visualize the junctional zone more accurately than conventional 2D-TVUS[68]. If visible, the junctional zone can appear regular and uninterrupted, thickened, irregular, or interrupted [67]. A focally thickened and irregular junctional zone, especially in the luteal phase, can be caused by physiological uterine peristalsis (1.2-1.7mm per second, 3-5 waves per minute) and be found in healthy uteri[69, 70].

While the appearance of the junctional zone on ultrasound and the link with histological findings has not been as extensively studied as in MRI (Table 2), known physical properties of ultrasound allow to formulate hypotheses. Ultrasound images are created by sending ultrasound pulses into tissue and reading the returning signal that echoed off structures. In general, a medium transmitting sound waves, such as fluid, appears black on US (hypoechogenic), while tissues reflecting or absorbing the US waves, such as bone or air, appear bright white (hyperechogenic)[71]. Therefore, the lower echogenicity of the subendometrial halo might suggest a higher water or blood content in this tissue. One small study tested this theory by investigating the uterine layers in uteri in vivo and ex vivo using ultrasound and did not find a change in the appearance of the junctional zone, suggesting that blood flow is not the reason for the appearance of the junctional zone[29]. However, US images are also a result of changes in tissue density and of acoustic wave propagation within the tissues. When adjacent tissues have a greater difference in tissue density or acoustic wave propagation, the ultrasound reflection will cause a bright echogenic signal, while a smaller difference will result in less echogenic signal. Therefore, the

differences in echogenicity between endometrium, junctional zone and middle/outer myometrium are probably less as a result of water content, but rather due to the difference in tissue density causing differences in acoustic waves absorption, refraction, or scattering[72]. In one study the visualization of the junctional zone using 3D TVUS, improved when there was a thicker endometrium[73]. Although the authors suggest that this is probably due to the improved ability to create the rendered coronal plane image using 3D TVUS, it might also be due to improved tissue contrast, in line with the explanation above.

Only few studies evaluated the influence of cycle phase, medication, or parity onJZ thickness on TVUS[74].. In contrast to the findings on MRI, no change in JZ thickness was found between menstrual cycle phases [35, 73, 75]. One 3D TVUS study in 30 women found that menopausal state, parity, and the presence of fibroids influenced the quality of visualization of the junctional zone [73] , while another 3D TVUS study in 82 women found no relation between JZ thickness and age, body mass index, parity, or use of hormonal contraception [75]. Only one study used 3D TVUS to investigate the junctional zone volume, and measured a greater volume in the secretory than in the proliferative phase [76]. Using 3D power Doppler TVUS, one study quantified the vascularity in the junctional zone[77]. A sharp increase in vascularity was seen in the JZ during the follicular phase with a pre-ovulatory peak and a post-ovulatory fall, then another increase at the end of the luteal phase [77]. These findings are in accordance with the DCE-MRI findings[40, 41].

Transvaginal ultrasound appearance with adenomyosis

Characteristics of all original articles on the transvaginal ultrasound appearance of the junctional zone in adenomyosis that were reviewed are summarized in Table S4. Direct, features of adenomyosis are hyperechogenic subendometrial lines or buds on 3D TVUS, and myometrial cysts on 2D TVUS, reflecting endometrial tissue invading the myometrium [78, 79]. Features of the junctional zone that are associated with adenomyosis are an irregular or interrupted junctional zone assessed on 3D TVUS [67, 80]. However, an irregular and interrupted junctional zone is also reported in endometrial cancer with myometrial invasion [81]. On TVUS, SMC hyperplasia caused by adenomyosis is thought to result in a thickened junctional zone and hyperechogenic islands [54, 82]. While a thickened junctional zone can be present in adenomyosis, evidence for the diagnostic and clinical relevance of a specific maximum thickness of the junctional zone for the diagnosis of adenomyosis on TUVS is lacking[67, 83]. One recent study found that the JZ thickness on 3D TVUS decreased in the first 12 weeks of treatment with an oral gonadotropin-releasing hormone receptor antagonist [84] It is important to note that assessment of the junctional zone requires technical expertise as shown in a study from 2019 by Rasmussen et al.[85]. In a cohort of 95

women with dysmenorrhoea or heavy uterine bleeding, the junctional zone was visible with 3D TVUS in only 44% of cases in the sagittal plane and 68% of cases in the coronal plane[86].

In conclusion, in transvaginal ultrasonography the junctional zone is best to visualize with 3D-TVUS, especially with VCI, however is not always visible in healthy nor adenomyotic uteri. The differences in echogenicity between the uterine layers might be explained by vascularity or differences in tissue density. An interrupted irregularly thickened junctional zone on 3D TVUS reflects the invading endometrial glands and SMC hyperplasia respectively.

Discrepancies between the junctional zone morphology on MRI and TVUS: interpretation in relation to histology findings

MRI and US are different technologies that do not use the same tissue properties to visualize anatomical structures. Therefore, the images of the myometrial layers obtained by each modality are likely to be method specific and show differences. These differences may provide interesting information on the essence of the junctional zone. Table 3 shows a comparison of our main statements concerning the characteristics of the JZ in these different modalities in absence and presence of adenomyosis.

While in MRI the junctional zone is generally measured in the sagittal plane, in many TVUS-based publications the junctional zone is displayed and measured in the coronal plane in 3D TVUS. TVUS and MRI images obtained from the same patients in the same cycle phase [35, 54, 76], showed a thicker junctional zone on MRI compared to TVUS images, as well as a thinner endometrium and outer layers of myometrium [35, 54]. In healthy women, the junctional zone thickness was measured as one third of the myometrium using MRI, while it was one forth on TVUS[54]. It seems that the endometrial layer is partly measured as junctional zone on MRI, or the junctional zone is partly seen as the endometrium in ultrasonography. In accordance with this theory, a greater endometrial volume and thickness was measured with 3D-TVUS compared to MRI. The largest discrepancies between 3D-TVUS and MRI measurements were seen in the luteal phase, explained by a change of contrast with the endometrium throughout the cycle on TVUS[76]. However a more recent 3D TVUS study found that cycle stage did not appear to influence the quality of visualization of the JZ[73]. Overall, the discrepancies between the measurements of the uterine layers visualised by MRI and TVUS suggest that what is described as the junctional zone in either imaging modality is not the same entity. Figure 4 depicts an example where the MRI image of the junctional zone includes a part of the middle myometrium instead of the endometrium.

Studies suggesting that the reported junctional zone is the same entity across histology and MRI, compared the direct morphometric measurements of the low-signal intensity band on MRI and the

area composed of dense smooth muscle fibres in the inner myometrium on histopathological slides [23, 28]. The full myometrium thickness on histology slides correlated well with the thickness of the combined low and medium signal intensity band on MRI[6]. However, as described above, on histology the outer part of the junctional zone seemed a transitional area with more loose SMCs and visualization of its border was dependent on whether only SMCs were stained or connective tissue as well [23]. The capacity to image this transitional area and the change in tissue density might be a reason for the difference in thickness observed between US and MRI. Moreover, these gradual morphological changes observed in histology leave no explanation for the sharp demarcation of signal intensity and echogenicity of the junctional zone in MRI and TVUS (Tabel 1). In histology and MRI there is barely mention of the middle myometrium as a separate entity from the outer myometrium, whereas ultrasound uses the vascular arcade to demarcate the middle from the outer myometrium. , Although the functional difference between the middle and outer myometrium is arguable, discriminating between the outer layers by notion of the vascular arcade can be useful when describing spread of disease or disease phenotypes, for example in adenomyosis.

Possible histological observations that could explain the morphological appearance of the junctional zone in contrast to the outer layers of myometrium on imaging are the increased density of SMCs [8, 27, 29], decreased extracellular space with a lower water content [23, 26] and higher vascular density [29]. First, lower interstitial volume on DCE-MRI and low APT-signal intensity in the junctional zone correspond with higher cellular density [23], low water content [26] and low cytoplasmic nuclear ratio [64]. Second, the higher vascular perfusion, higher tissue blood flow and capillary permeability in the junctional observed by DCE-MRI [2, 65], could be caused by the reported lesser blood vessel wall thickness and a gradually decreased elastin distribution in the junctional zone compared to the outer myometrium [11]. The vascular parameter differences between the uterine layers observed in imaging are in concordance with the observed fall in vascular gradient seen from inner to outer myometrium in histology [22]. We hypothesize that the abrupt change seen in the images, particularly in MRI, might be a result of a disproportional effect on the signal intensity that occurs when the cellular density, extracellular space, or difference in vascularity reach a certain threshold. Possibly, this threshold is different for MRI and US explaining the differences in junctional zone thickness.

The junctional zone to diagnose adenomyosis using different diagnostic modalities

Diagnosing adenomyosis solely on a diffuse or focal thickening of the junctional zone on T2 weighted MRI images is regarded as an outdated approach. Moreover, since MRI and TVUS seem to visualize the borders of the junctional zone differently, diagnostic criteria used in MRI cannot be extrapolated to TVUS. Whereas a thickened junctional zone on 3D TVUS [87], the difference between maximum and

minimum junctional zone thickness, and the ratio of the junctional zone to the total myometrium thickness on MRI were promising diagnostic markers, later studies could not confirm the diagnostic accuracy of a threshold for junctional zone thickness [63, 83]. Considering that thickening of the junctional zone could be a result of SMC hypertrophy, as well as of physiological factors such as peristalsis, vascularization, or water content, measuring the thickness of the junctional zone does not seem a useful marker for diagnosing adenomyosis[88].

Whether histological changes in the junctional zone are recognized as adenomyosis on TVUS or MRI, depend on the used definition and diagnostic criteria. Ultimately, the diagnosis of adenomyosis is defined by the presence of ectopic endometrium glands in the myometrium or infiltrating the junctional zone. These ectopic areas are visible on histology slides but can also be visualized on both TVUS and MRI (Figure 5). On T2-weighted MRI images, bright foci appear in the junctional zone or myometrium, representing foci of ectopic endometrial tissue, cystic dilatation of endometrial glands or haemorrhagic foci[80]. If the foci are haemorrhagic, they also appear bright on T1 FSWI images [53]. On TVUS, this is seen as as hyperechogenic lines and buds, interrupting the junctional zone, or hypoechogenic cystic areas in the middle or outer myometrium [54, 79, 82]. On TVUS, these features create a heterogeneous appearance of the myometrium[89] and often result in a poor defined junctional zone, while compared with MR-imaging of the same patient the pathological adenomyosis tissue is frequently reported as a thickened junctional zone (Figure 6) [90]. Using junctional zone thickness as the only diagnostic feature risks to miss earlier stadia of adenomyosis[63]. The features on TVUS and MRI that represent the presence of ectopic endometrium, such as bright foci on MRI and hyperechogenic lines or buds on TVUS, should be regarded as direct signs of adenomyosis (Table 3) [63, 67]. A meta-analysis identified both MRI, and 2D/3D TVUS as good and comparable methods for diagnosing adenomyosis when assessing all the direct and indirect features[83].

In theory these are expected to be visible in all modalities improving diagnostic accuracy and possibly also the association with clinical signs.

Conclusion

This review presents a critical appraisal of the definition of the junctional zone across the different disciplines, from reports on histology, ultrasonography, and MRI. Our review illustrates that the junctional zone is not a well-defined entity across and even within different diagnostic disciplines (Table 3). In histology the junctional zone cannot be visualized and differentiated from the outer layers of the myometrium. The histological morphology of the junctional zone is based on the differences between the inner versus the outer parts of the myometrium, not indicating a distinct boundary. Similarly, uniform features for demarcation of the junctional zone on TVUS or MRI are lacking. Although the junctional zone is described as the low-intensity signal band on MRI or the hypoechoic halo on US images, mostly no exact morphologic definition is given. Using more advanced histological techniques, gradual differences between the junctional zone and the outer layers of the myometrium were described, while in imaging the myometrium layers are often more distinct. This makes it difficult to compare histology and imaging morphology of the junctional zone. We therefore advocate to not use the terms “inner myometrium” and “the junctional zone” interchangeably. While the inner myometrial layer is always present and can be defined by a set measure, the junctional zone is not always visible in imaging.

Since the appearance of the layers derives from different structures per image modality observations or diagnostic criteria on MRI cannot be extrapolated to TVUS or vice versa. The question of what the definition of the junctional zone is, cannot be readily answered. Therefore, we propose to call the visible low-intensity signal band or the hypoechogenic halo by the modality used: the MRI-JZ or TVUS-JZ respectively.

Given the inconsistencies among imaging modalities, the thickness of the junctional zone cannot remain the golden standard in the diagnosis of adenomyosis anymore. Markers for adenomyosis, visible in all used modalities are the so-called direct signs of ectopic endometrium in junctional zone or myometrium affected by adenomyosis [67]. On histology, these are the spots of endometrial glands and stroma present in the myometrium, that are visualized on TVUS as subendometrial lines and buds and hyperechogenic islands in the myometrium and as an irregular or interrupted junctional zone by invaginated endometrium through the junctional zone, and on MRI as bright foci interrupting the junctional zone or myometrium (Figure 5). If these direct signs do not exist, a combination of indirect signs should be concordant (thickening of JZ, asymmetry, or big smooth uterus) which may suggest myometrial smooth-muscle hyperplasia/hypertrophy. This approach seems more promising to diagnose adenomyosis since they reflect the pathological substrate and possibly also better correspond with clinical features.

Harmsen Marissa J. (Orcid ID: 0000-0003-3842-5023)
Tellum Tina (Orcid ID: 0000-0003-2635-4504)
Van den Bosch Thierry (Orcid ID: 0000-0001-6777-5036)

Future perspectives

Considering our findings of the junctional zone we advise investigators to interpret any literature on the junctional zone with caution and be aware of the differences in imaging modalities and the definition used. Although conventional histology cannot show the functional properties of the junctional zone, strong indications of the function of the junctional zone are seen on TVUS and MRI .

Future studies should focus on the junctional zone as functional unit and could use advancements in diagnostic modalities, such as DTI-MRI/DCE-MRI or 3D TVUS, to investigate parameters such as vascularity, peristalsis, metabolism, and steroid receptivity. Hereby, the potential relevance of the junctional zone as a functional unit in relation to clinical symptoms (of adenomyosis) could be elucidated. In addition, consistent criteria across all diagnostic modalities are necessary to reliably diagnose and classify adenomyosis. We are not aware of any existing international guidelines or consensus statements in the pathology or radiology field concerning adenomyosis. In both histology and imaging the focus should be on the evaluation of direct features of adenomyosis, and their correlation with symptoms and reproductive outcomes. Future studies in this field should aim to formulate a uniform definition of adenomyosis that is based both on the direct features as well as on their required evaluation; e.g. how many sections should be obtained at hysterectomy, how many slices should be evaluated for the presence of direct features in histology, which planes should be assessed on 2D/3D TVUS and MRI, and how the amount of adenomyosis should be defined. Longitudinal studies, starting in young women or even adolescents, observing the natural progression of subtle changes in the JZ and their association with clinical findings and possible progression to adenomyosis, are warranted. Advanced insight into the anatomical as well as the functional properties of the junctional zone could finally convince clinicians of the relevance of the assessment of this uterine structure and aid in differentiation between pathology and physiology.

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FIGURE LEGENDS

Figure 1 – Schematic drawing of the sagittal plane of the uterus as visualized by two-dimensional transvaginal ultrasonography, and the uterine layers that may be discerned in orientation to the vascular arcade; the hyperechogenic endometrium, hypo-echogenic junctional zone (inner myometrium), the middle myometrium in between the junctional zone and the vascular arcade and the sub-serosal layer or outer myometrium.

Figure 2 - A - Schematic representation of the histological morphology of the smooth muscle and connective tissue (collagen) distribution in the inner versus the outer myometrium of the uterine wall. Inner myometrium (junctional zone) with a high density of smooth muscle cells (SMC) in a dense connective tissue and little extracellular space. Middle and outer myometrium with more loose SMC bundles with a higher ratio of connective tissue/SMCs. B – Schematic representation of the histological morphology of the inner myometrium (junctional zone) when affected by adenomyosis: lower density of SMCs with more cytoplasm, SMCs loose parallel orientation to endometrium and are interrupted by ectopic endometrium. Less prominent collagen fibers. This figure was created with BioRender.com.

Figure 3 - Schematic drawings of a coronal section through the uterine wall with the differences in morphology between the endometrium, junctional zone (JZ), middle myometrium, and outer myometrium that can be visualized by advanced MRI techniques.
A) T2-weighted MRI visualizes lower water content in the junctional zone (lower signal intensity) compared to high water content in endometrium and medium in outer myometrium. Diffusion weighted imaging (DWI-MRI) shows a lower apparent diffusion coefficient (ADC) in the JZ because of the lower water content in comparison to other layers. B1) Higher contrast exchange in the junctional zone, visible on dynamic contrast enhanced MRI. B2) Detailed image of a capillary in the JZ with a higher tissue blood flow (F), a lower contrast concentration in plasma (Vb) and interstitial space (Vi), a higher permeability-surface area product (Ps) and a longer lag time (Dt) in the junctional zone than in the outer myometrium. C) Lower ADC and highest fractional anisotropy (FA) using Diffusion Tensor Imaging (DTI)-MRI and Amide proton transfer (APT)-imaging, suggesting circular and longitudinal muscular fibres in the junctional zone versus the other layers. D) A lower APT-signal intensity in the JZ visualizes a lower protein concentration in the JZ, in comparison to the outer myometrium and endometrium visible through APT-imaging. This figure was created with BioRender.com.

Figure 4 – Transvaginal ultrasound image (top) and T2 weighted magnetic resonance image of a sagittal plane of the same uterus affected by adenomyosis in the same cycle phase. The junctional

zone is evidently thicker on MRI than on ultrasound. On ultrasound it also appears interrupted anteriorly, the endometrium and the outer myometrium appear to be thinner on MRI.

Figure 5 – Direct features of adenomyosis. From left to right: myometrial cysts, lining and budding, and hyperechogenic islands indicated through the junctional zone by schematic presentation of a coronal section of the uterus (row A), indicated by yellow arrows on sagittal and transverse planes on 2D TVUS (row B), and indicated by white arrow on sagittal and transverse planes on T2W MRI (row C). Figure adapted from van den Bosch et al 2019, and Agostinho et al 2017, and an example from research database of T. Tellum, and created with BioRender.com.

Figure 6 - Example from Amsterdam UMC clinical database of a patient with adenomyosis that underwent a TVUS and MRI. On MRI (left) the junctional zone is assessed as thickened, and a myometrial cyst is seen in the posterior wall. On the sagittal image of the uterus on 2D TVUS (middle), the junctional zone is very poorly defined and direct and indirect signs of adenomyosis are seen. On 3D TVUS (right) a interrupted JZ is seen, as well as direct signs.

Table 1 Overview of the histological findings of the inner myometrium described per immunohistochemistry technique			
Diagnostic tool Structures stained and technical specifications	**Inner myometrium in absence of adenomyosis**	**Inner myometrium affected by adenomyosis**	**References**
Hematoxylin and eosin staining (H&E) Broad range of nuclear, cytoplasmic, and extracellular matrix components	No distinction between inner and outer myometrium	-	Lee, 1985 McCarthy, 1989 Varpula, 1994
	Cell density, nuclear size, and nuclear area in the IM higher compared to OM	Lower cell density, larger nuclear size, lower total nuclear area	Mehasseb, 2011
Smooth muscle cells			
Feulgen stain Nuclear DNA	IM larger (total) nuclear area versus OM	-	Scoutt, 1991 Tetlow, 1999
α-SMA staining Smooth muscle cells	SMCs uniformly stained	No difference	Tetlow, 1999 Kishi, 2017
	More muscle mass (percentage area expressing α-SMA) in IM compared to OM	No difference	Mehasseb, 2011
		Higher in IM of adenomyosis versus controls	Ibrahim, 2017
Fluorescent actin filaments Cytoskeletal component	Dense compact SMCs in IM	-	Brown, 1991
Transmission electron microscopy Highly magnified image of SMCs using an electron beam	Extracellular space smaller in IM versus OM	-	Brown, 1991
	Gradual transition in SMC density towards outer myometrium with	-	Brown, 1991

	distinction between IM, MM and OM		
	Nuclear size, sarcolemmal plaques length larger in IM vs OM (not significant)	Nuclear size, sarcolemmal plaques length larger (significant)	Mehasseb, 2010
	Cytoplasm of SMCs sparse, filled with organelles	Cytoplasm abundant, less myofilaments	Mehasseb, 2010
	Myofilaments/cytoplasm ratio lower in IM versus OM	No difference	Zhang, 2014
	Orientation SMCs parallel to endometrium	SMCs diversely arranged	Brown, 1991 Ibrahim, 2015
	SMCs of IM uninterrupted	More evident microruptures	Ibrahim, 2015 Rasmussen, 2019
Connective tissue			
Transmission electron microscopy Highly magnified image using an electron beam	IM lower connective tissue to SMC ratio than OM	SMCs more separated in loose connective tissue	Mehasseb, 2010 Zhang, 2014
Mallory trichrome staining Connective tissue; collagen, cytoplasm, red blood cells	Better discrimination between between IM and middle myometrium	-	Brown, 1991
	Compact SMCs longitudinally oriented parallel to surface endometrium in IM	-	Brown, 1991
Masson's trichrome staining Keratin/muscle, collagen/ bone, cytoplasm, cell nuclei	No difference in collagen distribution between IM and OM	-	McCarthy, 1989

Type I, III, IV, V collagen **Type I: myofibroblasts** **Type III: interstitial ECM** **Type IV: basement-membrane ECM** **Type V: interstitial and basement-membrane ECM**	No difference in extent or intensity of collagen staining between IM and OM	Collagen I higher in IM of adenomyosis versus control IM Type I collagen > type III collagen Type I collagen higher in IM and OM vascular wall SMCs	Scoutt, 1991 Ibrahim, 2017 Kishi, 2017
Von Gieson staining Differentiation collagen (other connective tissue) and smooth muscle	No morphological difference IM and OM		Varpula, 1994
Elastic Von Gieson staining Elastin	No difference in elastin content or nature between IM and OM	-	McCarthy, 1989
Anti-elastin antibody +CD31 Elastin and vascular endothelium	IM shows less perivascular and extravascular elastin	-	Metaxa-Mariatou, 2002
Blood vessels			
CD31 Endothelial cells	Continuous decrease in vessel wall fraction from OM to IM No difference in volume fraction of capillary walls; representing microvascularity Vessels in IM small minimum diameter compared to OM	-	Aitken, 2006

	No obvious orientation of vessels		
	Greater total area stained with CD31 in IM	-	Tetlow, 1999
Morphometric analysis vessel size	Greater volume vessel wall, and larger cross-sectional areas in OM	-	Aitken, 2006
H&E staining, inspection of vessels	Constant number of vessels in total myometrium	-	McCarthy, 1989
Intermediate filaments			
Desmin	No distinct zonation	no difference compared to controls	Mehasseb, 2011 Ibrahim, 2017
	Desmin negative at IM + OM	Desmin negative SMCs at AM foci	Kishi, 2017
Smoothelin	Negative at IM + OM	Negative SMCs around glands	Kishi, 2017
Myosin heavy chain	Negative at IM + OM	Negative SMCs around glands	Kishi, 2017
Vimentin	More intense in connective tissue than SMCs, no cyclical change Weak diffuse cytoplasmic staining	More vimentin staining in IM Vimentin shows cyclical variation	Mehasseb, 2011

Abbreviations: AM adenomyosis, EMI endometrial myometrial interface, IM inner myometrium, MM middle myometrium, IM inner myometrium, SMC smooth muscle cells, OM outer myometrium

Table 2 Overview of the findings of the junctional zone described per imaging tool and technique				
Diagnostic tool Technical specifications	**Findings on junctional zone (JZ) in uteri not affected by adenomyosis**	**References**	**Differences in adenomyosis affected uteri**	**References**
Magnetic resonance imaging (MRI)				
T2-weighted – MRI MRI sequence investigating spin–spin relaxation time of protons in tissues	JZ shown as band of low signal intensity between the high-intensity zone of the endometrium and the medium intensity zone of the outer myometrium	Hricak, 1983; Lee, 1985 McCarthy, 1989 Mitchell, 1990 Bartoli, 1991 Scoutt, 1991 Varpula, 1994 Fusi, 2006	Diffuse AM: diffuse and (un-) even thickening of the JZ with homogeneous low signal intensity. Focal AM: localized, low signal intensity mass within the myometrium. 93% blended imperceptibility within the myometrium.	Mark, 1987 Byun, 1999
	JZ has a similar mean T2 value in uteri in vivo and ex vivo	McCarthy, 1989	Signal intensity varies: homogeneously low signal to interspersed sports of higher signal intensity resulting in inhomogeneous image	Lee, 1985 Kunz, 2000

	Greater contrast JZ versus endometrium and outer myometrium in uteri in vivo than ex vivo JZ indistinct or invisible ex vivo	Varpula, 1994	The mean thickness of the JZ was 0.7 cm in women without myoma or AM, and 0.9 cm in women with myoma and/or AM	Hauth, 2007
	JZ thickness or volume differs between cycle phases	Wiczyk, 1988 Mitchell, 1990 Hoad, 2005 Fusi, 2006 Meylaerts, 2017	Optimal value of JZ thickness to diagnose adenomyosis ≥12 mm diagnostic accuracy improved when JZdif ≥ 5-7 mm	Reinhold, 1996 Dueholm, 2001 Bazot, 2001
	Boundary sharpness of the endometrium to JZ and JZ to myometrium and highest single intensity ratio is in ovulatory phase	He, 2019	JZmax ≥ 12 mm not significantly associated with AM, Interrupted and/or irregular JZ strongly correlated with AM Regular JZ strongly correlated with not having AM. Only irregular JZ and myometrial cysts independent association with having AM	Tellum, 2019
	JZ was significantly thinner using hormonal contraception than without in all cycle phases	Lesny, 2004 Meylaerts, 2017	Decrease in JZ thickness during first 12 weeks of E2 suppression	Donnez, 2021
	The JZ thickness increases with age, to peak a 41-50 years and then drops.	Hauth, 2007 Kiguchi, 2016		

	Premenarchal girls, postmenopausal women and ovarian suppression with GnRH analogues have the same indistinct appearance of the junctional zone on MRI. Replacement therapy results in reappearance of the JZ.	Brosens, 1995 Demas, 1985 Lesny, 2004 Novellas, 2010		
Dynamic Contrast-Enhanced MRI (DCE-MRI) Exchange of low-molecular-weight contrast medium between intra- and extravascular extracellular space to examine microvascular parameters and perfusion	JZ shows higher tissue blood flow, higher vascular perfusion and greater capillary permeability than in the outer myometrium (proliferative phase) Lower volume of interstitial space compared to outer myometrium (secretory phase)	Thomassin-Naggara, 2010 Meylaerts, 2017		
Diffusion Weighted Imaging (DWI-MRI) Measures the apparent diffusion coefficient (ADC) of water molecules in tissue, which reflects cell density, cellular edema and microcirculation	ADC values are lowest in the JZ compared to endometrium and outer myometrium ADC values of the JZ are lowest in the menstrual phase, highest in the luteal phase	Fujimoto, 2013 Zhang, 2019	41/110 patients diagnosed with AM on MRI no decreased water diffusion at the JZ on ADC map Layer of restricted water diffusion visible, but no corresponding structures of this layer found in histologic specimens	Kido, 2016

Diffusion Tensor Imaging (DTI-MRI) Provides information about the anisotropy (change in direction) of water diffusion in tissues: this reflects structural orientation of tissues.	Mean fractional anisotropy highest in JZ Circularly oriented fibers at uterine fundus 69% in OM and 54% in JZ and in the uterine body 65% in OM and 70% in JZ. 6x more fibers in OM than JZ, volume of OM 4x larger than JZ, max. fiber length similar in OM and JZ	Fujimoto, 2013		
Amide Proton Transfer (APT) imaging Detects low concentration of endogenous proteins and amide chemical constituents in polypeptides. APT signal intensity (APT SI) depends on the exchange rate of amide protons and free water protons.	APT SI was significantly lower in JZ compared to EM and OM during all menstrual cycle phases Moderate correlation between APT SI and ADC could be the result of increased cell proliferation Highest APT SI of JZ, EM and OM in luteal phase, lowest in menstrual phase	Zhang, 2019		
Ultrasound				
Two-dimensional Transvaginal ultrasound (2D-TVUS) Uses differences in sound wave transmission to discriminate	JZ is a hypoechoic halo within the myometrium surrounding the endometrial cavity	Mitchell, 1990 Tetlow, 1999 Fusi, 2006	Halo focally disrupted	Kunz, 2000

between structures, transformed into a digital image. Both images and videos can be obtained.		Rasmussen, 2019 Van den Bosch, 2019		
	JZ thickness in all images and cycle phases greater on MRI than TVS Outer two third of myometrium smaller on MRI than in TVUS, significant in follicular phase	Mitchell, 1990	The JZ (halo on TVUS) of adenomyosis cases was smaller <31 yr than >31 year (P<0.0).	Kunz, 2000
	JZ remains visible as halo on TVS ex vivo	Tetlow, 1999		
	Mean diameter of JZ 3.5 ±1.1mm in healthy women with male infertility	Kunz, 2000	Mean diameter of halo 6.5 ±2.5mm in adenomyosis patients	Kunz, 2000
	The ability to visualize the JZ was significantly affected by age, parity and endometrial thickness.	Naftalin, 2012		
	No relation between cycle phase and JZ thickness	Mitchell, 1990	Decrease in JZ thickness during first 12 weeks of E2 suppression	Donnez, 2021
Three-dimensional Transvaginal ultrasound (3D-TVUS) Allows real time and offline evaluation of the volume of interest	3D enables the assessment of the lateral and fundal aspects of the JZ: minor changes are better visible.	Naftalin 2009, Van den Bosch, 2019	Best specificity and positive predictive value of US features of AM confirmed at targeted biopsy: max JZ ≥8mm, myometrial asymmetry, and hypoechoic striation	Luciano, 2013

(uterus) from any angle and in any plane. Power Doppler angiography can be added to visualize vascularity and blood flow.	Clearer visualization of the hypoechoic junctional zone in comparison to 2D imaging.		
	Quality of visualization differed between retroverted (31.7% unsatisfactory) and antroverted uteri (12.7% unsatisfactory).	Rasmussen, 2016	
	JZ thickness did not vary due to use of hormonal contraception or menstrual cycle phase	Rasmussen, 2016 Naftalin, 2012	
	3D with volume contrast imaging modality visualizes the JZ clearly in all planes in the multiplanar view, while it was poorly visualized in most 2D-TVUS images.	Exacoustos 2011	
	3D power Doppler demonstrated significant changes in vascularity in the JZ in the menstrual cycle, with a pre-ovulatory peak and post-ovulatory fall.	Raine-Fenning, 2004	

Abbrevations: AM adenomyosis, APT-SI Amide Proton Transfer signal intensity, MI endometrial myometrial interface, IM inner myometrium, JZ junctional zone, OM outer myometrium, NR not reported, TVS transvaginal sonography, IHC immunohistochemistry, EM endometriosis, ADC apparent diffusion coefficient

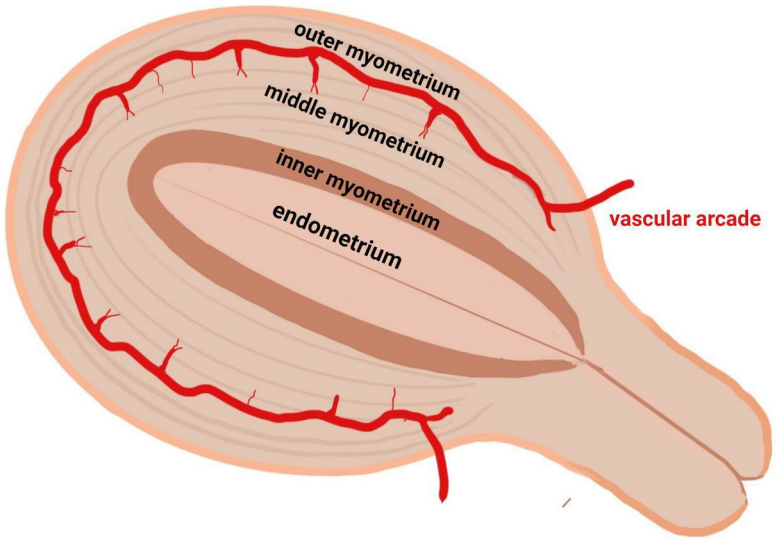
Table 3 Comparison of our main statements of the junctional zone in histology, MRI and ultrasound in uteri with or without adenomyosis

Characteristic	Histology	MRI	Ultrasound
			No adenomyosis
No distinct zonation	Gradual changes in cell density, from high in IM to low in OM. No measurements of IM thickness, because of the gradual changes.	Low signal intensity band between high intensity zone of EM and medium intensity zone of OM (T2W MRI). No zonation of MM and OM. JZ is measured thicker than in TVUS.	Hypoechoic halo surrounding the hyperechoic endometrium (best visible on 3D TVS and with VCI). Zonation of MM and OM by visualization of vascular arcade with 2D PD TVUS. JZTVS. not always visible. JZ is measured thinner than in MRI.
Low intra- and/or extracellular water content	The lower connective tissue-to-SMC ratio in IM than OM	Signs of reduced water content in the JZ vs OM.	Same appearance of JZ in dissected uteri, blood flow (extracellular water) does not seem to cause the appearance of the JZ.
Increased vascularization	No difference in number of blood vessels, but gradually increasing vessel wall thickness from IM to OM.	Higher vascular perfusion rate, higher velocity of tissue blood flow in capillaries and a greater capillary permeability in the JZ than OM.	Increase in vascularity in the JZ during the follicular phase towards a pre-ovulatory peak and a post-ovulatory fall in vascularity, increased again at the end of the luteal phase
Higher density fiber alignment	Longitudinal orientation of SMCs in IM parallel to EM, versus loose arrangement of bundles with SMC, collagen and blood vessels in OM	Better aligned SMC fibers with a higher cell density in the JZ than in the OM. Circular and longitudinal fibers in whole myometrium.	-

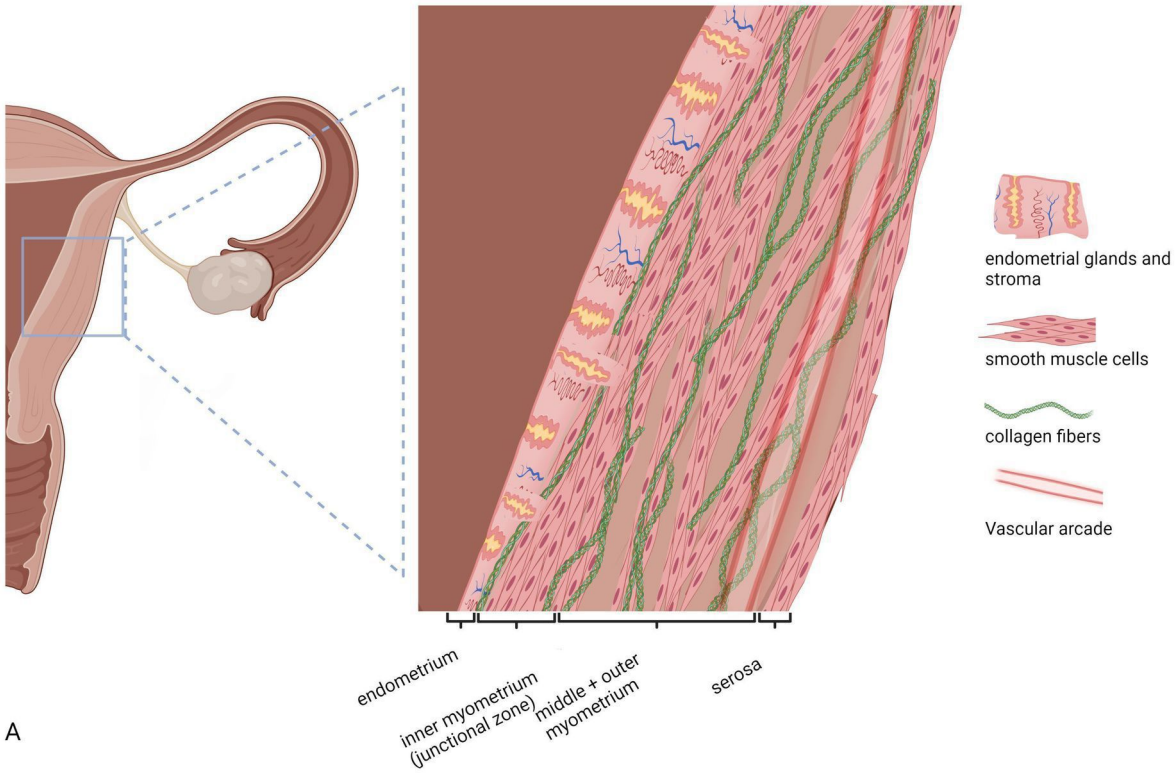
Influence of cycle phase, age, medication and/or peristalsis	No influence	Changes of the thickness and visibility of the JZ may be associated with medication use, cycle phase and age. Peristalsis is seen as a wave-like pattern of the JZ and can mimic uterine pathologies	No relation JZ thickness, but signs of JZ volume changes between cycle phases. Menopausal state, parity and fibroids have a negative effect on visualization. No effect contraception. Uterine peristalsis can cause transient irregularities or focally thickened appearance.
			Adenomyosis
Direct signs of adenomyosis	Endometrial glands penetrating the IM, without a definitive cutoff for invasion depth.	Hyperintensity (haemorrhagic) foci or cysts	Hyperechogenic subendometrial lines or buds on 3D TVUS and hyperechogenic islands myometrial cysts on 2D TVUS
Indirect signs of adenomyosis	Signs of SMC hypertrophy, fibrosis (myofibroblasts /collagen I) and non-differentiated SMCs. Loss of parallel orientation of SMCs	Low-signal intensity masses in the outer layers surrounding the ectopic endometrial tissue (reflecting SMC proliferation and hyperplasia) JZ thickness thinner after 12 week oral GnRH agonist treatment	Globular uterus, asymmetrical myometrium, fan shaped shadowing JZ thickness thinner after 12 week oral GnRH agonist treatment

Green area: modality agrees with main statement. Red area: modality disagrees with main statement

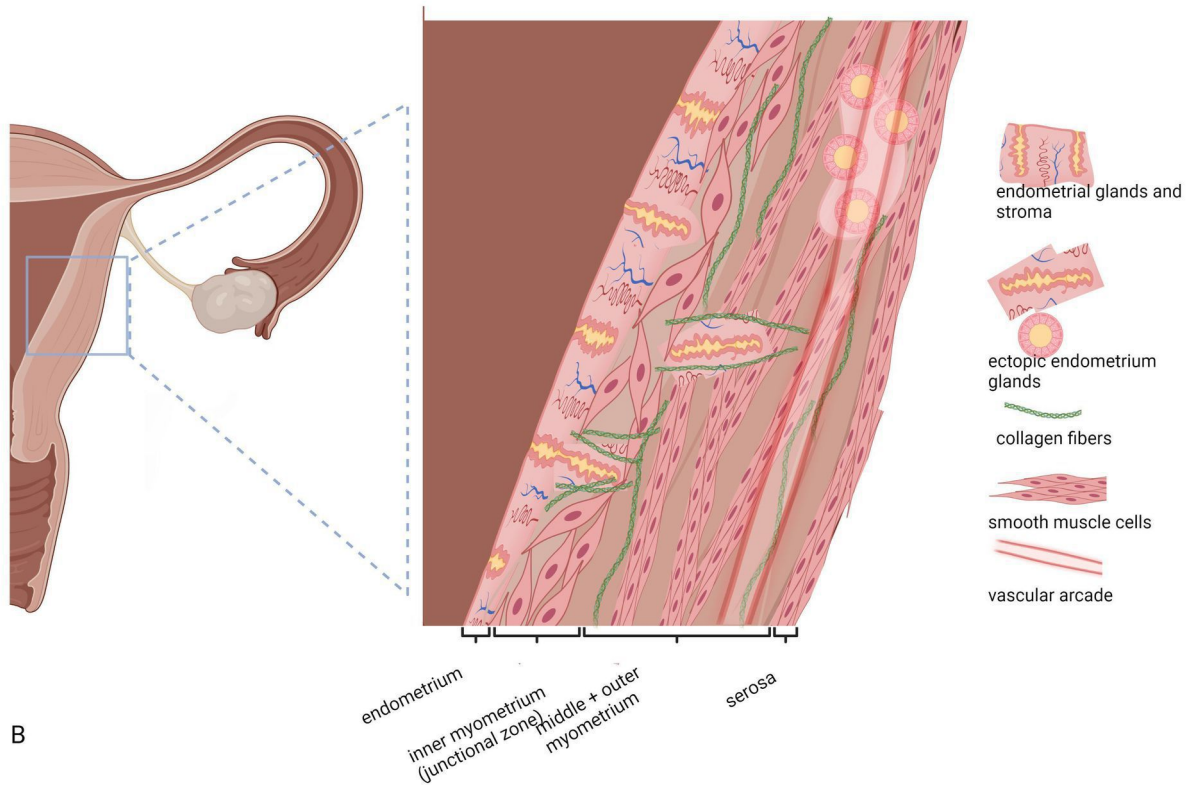
Abreviations: JZ junctional zone; IM inner myometrium; EM endometrium; MM middle myometrium; OM outer myometrium; SMC smooth muscle cell; VCI volume contrast imaging; PD PowerDoppler; GnRH gonadotropin-releasing hormone GnRH



UOG_26117_Figure 1 - US sketch.jpg



UOG_26117_Figure 2A. Cross section endo-myometrium.jpg



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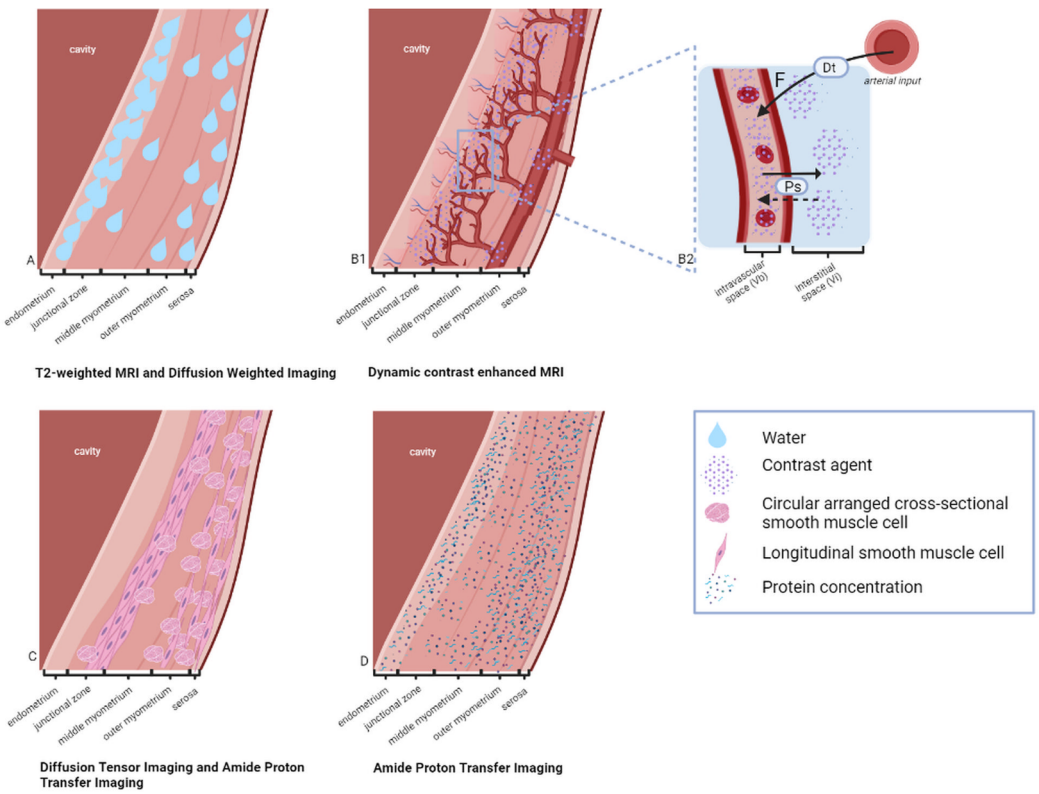
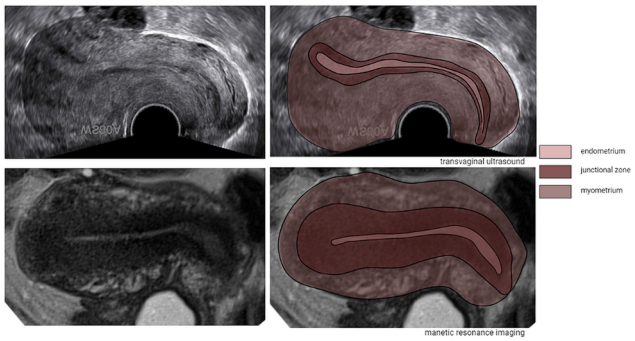
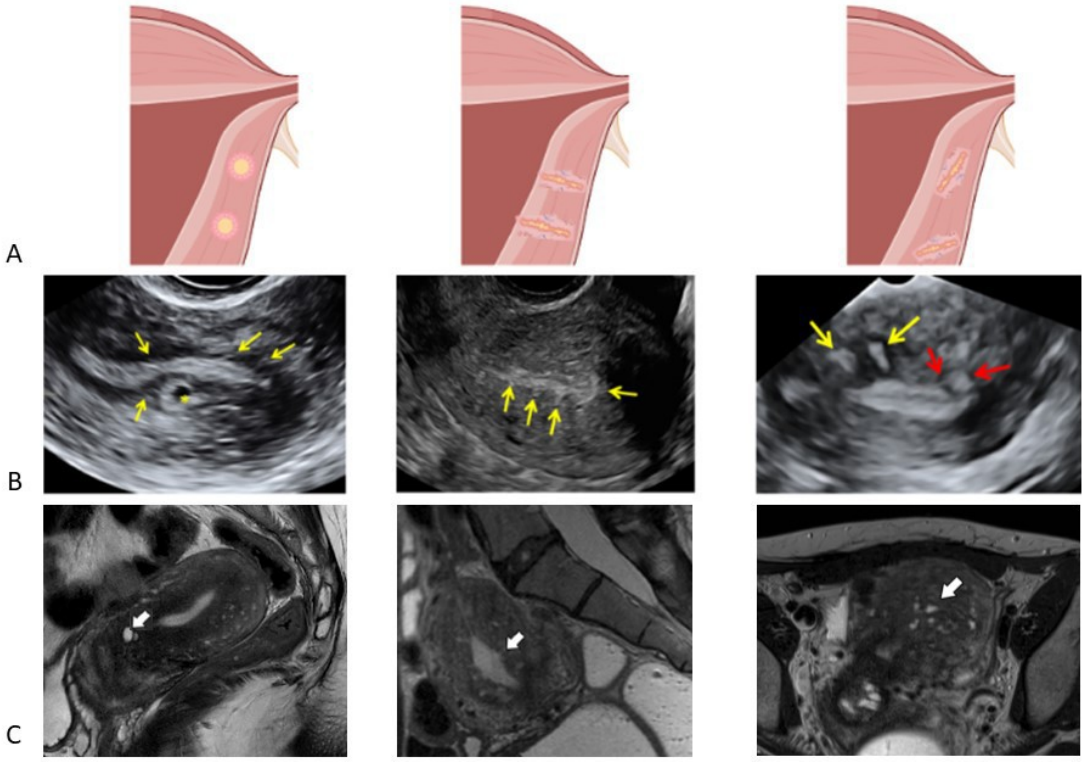


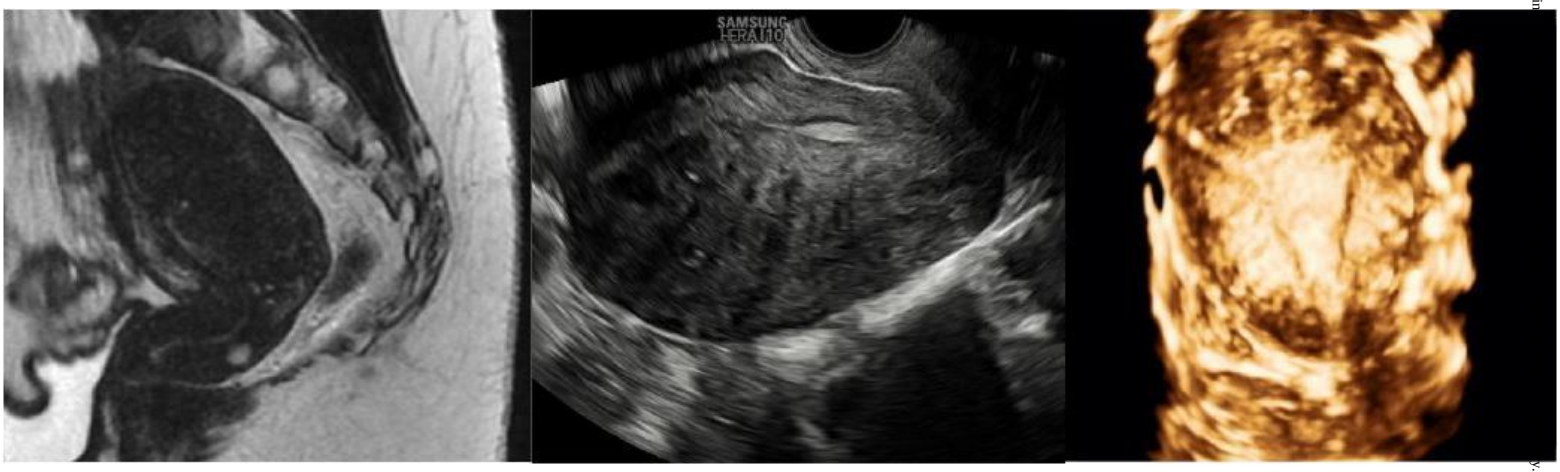
UOG_26117_Figure 3 Differences in morphology between uterine layes visualized by advanced MRI techniques.tif



UOG_26117_Figure 4. Difference in JZ thickness between a uterus images on TVUS and MRI.tif



UOG_26117_Figure 5. Direct features of adenomyosis.jpg



UOG_26117_Figure 6. Interupted JZ in US vs thickened in MRI_adenomyosis in posterior wall.JPG