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# Women in gynecological oncology, volume III: 2023

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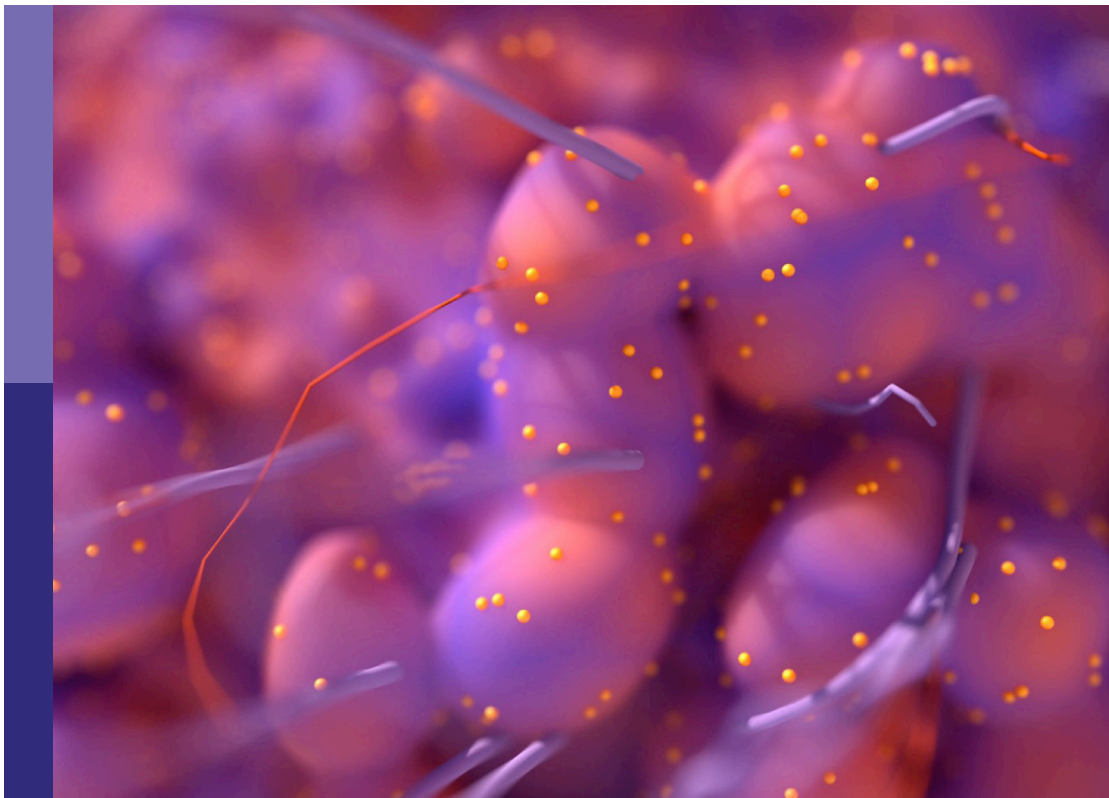

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# Women in gynecological oncology, volume III: 2023

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# Unraveling the relationship between the renin–angiotensin system and endometrial cancer: a comprehensive review

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Endometrial cancer (EC), the most common adenocarcinoma, represents 90% of uterine cancer in women with an increased incidence of occurrence attributed to age, obesity, hypertension, and hypoestrogenism. Being the most common gynecological malignancy in women, it shows a relation with the activation of different components of the renin–angiotensin system (RAS), which is predominantly involved in maintaining blood pressure, salt, water, and aldosterone secretion, thereby playing a significant role in the etiology of hypertension. The components of the RAS, i.e., ACE-I, ACE-II, AT1R, AT2R, and Pro(renin) receptor, are widely expressed in both glandular and stromal cells of the endometrium, with varying levels throughout the different phases of the menstrual cycle. This causes the endometrial RAS to implicate angiogenesis, neovascularization, and cell proliferation. Thus, dysfunctioning of the endometrial RAS could predispose the growth and spread of EC. Interestingly, the increased expression of AngII, AGTR1, and AGTR2 showed advancement in the stages and progression of EC via the prorenin/ATP6AP2 and AngII/AGTR1 pathway. Therefore, this review corresponds to unraveling the relationship between the progression and development of endometrial cancer with the dysfunction in the expression of various components associated with RAS in maintaining blood pressure.

KEYWORDS

RAS pathway, angiotensin I-II, ACE, immunosuppressor, endometrial cancer

**Abbreviations:** RAS, renin angiotensin system; EC, endometrial cancer; VEGF, vascular endothelial growth factor; ER, estrogen receptor; PRR, pro-renin receptor; ACE, angiotensin-converting enzyme; AT1R, angiotensin II receptor type 1; AT2R, angiotensin II receptor type 2; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; P13K, phosphoinositide 3-kinase.

# 1 Introduction

Endometrial cancer (EC), the most common female uterine cancer, mainly arises in post-menopausal women with an average diagnostic age of 60 years. According to the American Cancer Society, 2021 marked the occurrence of 66,570 new cases of EC in the US and more than 12,940 deaths. The increasing incidence of EC and its estimated growth rate in subsequent years have posed a great threat to the public health sector (1). It has been estimated that over 90% of uterine cancers are adenocarcinomas, of which ~80% are associated with an increased expression of estrogen under the influence of insulin resistance and obesity, whereas 20% are of unknown etiologies. The excess of exogenous and endogenous estrogens corresponds to the main risk factors for endometrial adenocarcinoma (2). Additionally, most of the past studies focused on evaluating the expression of estrogen and progesterone receptors (ER and PgR) in EC. These studies predominantly showed that 85%–90% of the well-differentiated ECs were positive for ER/PgR, whereas 70%–85% of moderately differentiated ECs expressed steroid receptors. Additionally, only 13% of poorly differentiated EC had detectable levels of ER/PgR, thereby assigning these receptors as predictive and prognostic biomarkers against anti-hormonal therapy in EC (3).

Among the various malignancies worldwide, EC has been rated as the seventh most common malignancy after breast, lung, and colorectal cancer. It differs into various histological subtypes based on varying frequency, clinical presentation, and prognosis as well as associated epidemiological risk factors (4). Endometrioid EC, serous EC, clear-cell EC, mixed EC, and uterine carcinosarcoma (UCS) correspond to different histological subtypes of EC. Endometriosis, significantly characterized by the presence of functionally active endometrial tissue, stroma, and glands outside the uterine cavity, is an estrogen-dependent inflammatory disorder of the endometrium affecting up to 11% of women of reproductive age worldwide, whereas its etiology remains largely unknown. However, among the several theories proposed to explain the pathogenesis of endometriosis, retrograde menstrual blood flow, coelomic metaplasia, and Mullerian remnants are famous (5). Apart from these theories, it has been reported that the components of the renin–angiotensin system (RAS), which are famously responsible for maintaining normal blood pressure and sodium homeostasis, are expressed in the endometrium during pregnancy. Moreover, emerging research suggests the potential implications of RAS in various gynecological cancers such as ovarian and breast cancer, indicating a broader role of RAS in influencing cellular processes critical to the progression of various gynecological malignancies. This RAS activity has been shown to stimulate angiogenesis, cell proliferation, and migration within the normal endometrium. Notably, dysfunction in the expression of these RAS components within the endometrium has also been associated with the development of endometrial cancer in women (6). A study conducted by Piastowska-Ciesielska et al. demonstrated that the levels of expression of AGTR1, AGTR2, vascular endothelial growth factor (VEGF), and estrogen receptor (ER)-α levels varied with the different stages of cancer, concluding a higher expression of AngII receptors in the early grade of EC (1). Additionally, Shibata et al. showed the levels of AngII, AGTR1, and VEGF peptides along with adipocyte-derived leucine aminopeptidase (A-LAP) as prognostic for EC (7). Significantly, (pro) renin receptor (PRR), a new biomarker for different types of cancer such as colorectal cancer, breast cancer, glioma, aldosterone-producing adenoma, urothelial cancer, and pancreatic ductal adenocarcinoma, has also been regarded as a potential prognostic and therapeutic biomarker for endometrial cancer (8). In various physiological and pathological pathways of tumorigenesis, (pro) renin receptor plays important roles via the Wnt/β-catenin, renin–angiotensin system, MAPK/ERK, and PI3K/AKT/mTOR pathways. Interestingly, our limited knowledge about PRR in cardiovascular and renal physiological functions and diseases has been updated with compelling pieces of evidence stating the prominent role of PRR in various cancers including endometrial cancer (9).

Moreover, renin–angiotensin–aldosterone system (RAAS) inhibitors, angiotensin-converting enzyme inhibitors (ACEIs), and antagonists of angiotensin receptors (ARBs) have been strongly associated with a considerable decline in the overall risk of gynecologic cancer. This system has a high potential to be expressed and/or triggered to promote aberrant cell growth and dissemination responsible for boosting angiogenesis, cell proliferation, and migration, a significant characteristic of endometrial cancer (10).

# 2 The renin–angiotensin system

The RAS is a complex and vital biochemical pathway that maintains plasma sodium concentration, arterial blood pressure, and extracellular volume. The RAS consists of three main components: angiotensinogen, renin, and angiotensin-converting enzyme (ACE) (Figure 1) (11). Renin, a hormone secreted by the kidneys, acts on its substrate to catalyze the transformation of angiotensinogen into angiotensin I. Angiotensin I is known to be a powerful vasoconstrictor that contributes to the development of hypertension. The conversion of angiotensin I to angiotensin II occurs under the influence of the ACE produced by the lungs. The RAAS is a group of genes and proteins that control the body's fluid balance and blood pressure. The RAAS network is responsible for releasing renin into the bloodstream, thereby catalyzing the conversion of angiotensinogen to angiotensin I. RAAS activation greatly shows its effect like controlled blood pressure, cell proliferation, inflammation, and fibrosis on every organ. Thus, an imbalance of renin and angiotensin II can be a leading cause of several chronic and acute diseases (12).

The prorenin sensor is a prominent regulatory protein synthesized by the kidneys that helps to regulate blood pressure by maintaining blood flow in the body's blood vessels and cells. It is also found in ovarian follicular fluid in extremely higher concentrations. Thus, the prorenin plasma levels increase transiently in blood during the menstrual cycle in the luteal phase. This provides a close relationship between ovarian prorenin levels in reproductive function, showing a deep relevance with female infertility, endometriosis, and toxemia during pregnancy. The gene REN in humans produces the

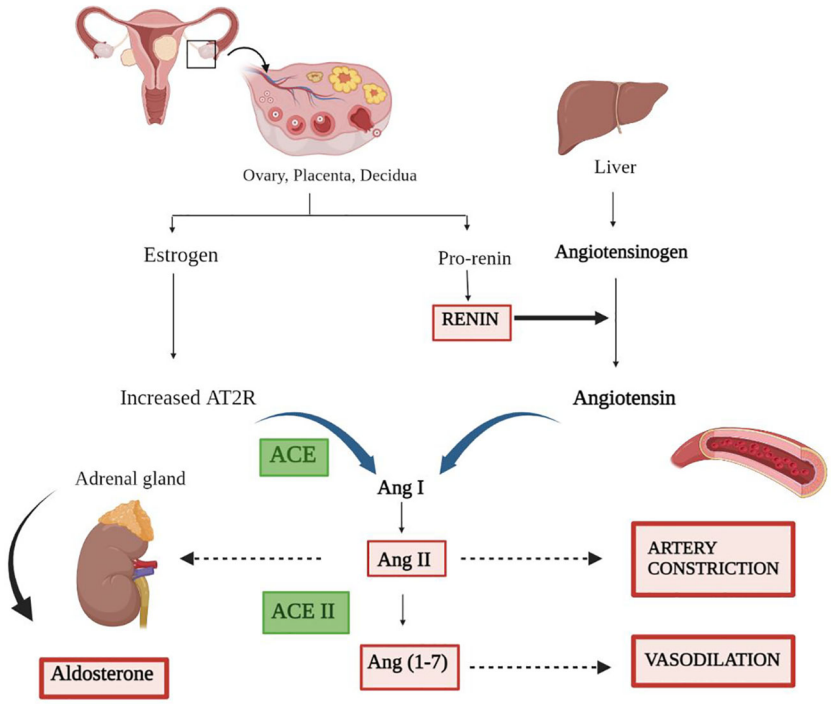


FIGURE 1
Representation of the renin–angiotensin system. The secretion of estrogen and pro-renin from the ovary, placenta, and decidua through a series of events results in artery constriction and vasodilation. Renin, the major candidate responsible for converting inactive angiotensinogen to active Angiotensin, is produced by the liver. Furthermore, the combined effect of angiotensin and receptor for angiotensin (AT2R) convert AngI to AngII via ACE. ACE II comes into play and catalyzes the final conversion of AngII to Ang (1–7), ending in a variety of responses along with the production of aldosterone.

protein known as the renin transmitter along with the renin receptor family protein (13).

Due to the presence of ovarian prorenin, the RAS is believed to play a role in the development of endometrial cancer when dysregulated (14). In the last few years, researchers have made great strides in understanding how cells communicate with each other and focused on understanding the response of cells to specific signals. One particularly important type of communication involves the crosstalk between the RAS pathway and the progression of endometrial cancer.

## 3 RAS and convergent signaling pathways in endometrial cancer

Different signaling pathways have an immense impact on endometrial cancer. The convergent pathways allow cells to share information more efficiently, and they are often responsible for coordinating the actions of multiple cells. One such convergent pathway involves cells communicating with endometrial cancroid cells. These cells are responsible for the growth and spread of cancer, and they use convergent signaling pathways to communicate with other cells in the tumor. In the previous section, details of the RAS pathway show the activation of different signaling molecules. RAS proteins are important for growth and survival in many cells, but they can also help cancer cells to spread (15). RAS proteins help the cells to break down their proteins and cells, whereas RAS inhibitors are anticipated to prevent the spread of tumors (16). Vasoconstriction, sodium–water retention, elevated arterial blood pressure, and enhanced myocardial contractility are the overall effects of RAAS activation, and they together increase the effective circulation volume. This section connects the convergent pathways with the RAS pathway to understand the inhibition of the apoptosis process with cell proliferation.

### 3.1 TNFα signaling pathway

Inflammation is crucially controlled by the TNF signaling system. The TNFα signaling pathway is composed of several steps, which are illustrated in Figure 2. This pathway mediates the stimulation of cytokines like TNF by pro-inflammatory inputs from cells like bacteria or viruses (17). These cytokines then initiate the activation of cells in the immune system, which, in turn, can lead to the inflammation of tissues. For the first time, the activation of the RAS in animals with a specific overexpression of TNF demonstrates that the RAS and proinflammatory cytokines have a functionally important cross-talk in endometrial cancer (18). The primary effect of the renin–angiotensin system is that angiotensin II majorly regulates the release of aldosterone. Additionally, ANG II interacts with the TNF signaling pathway to specifically enhance the functional tissue factors of cell surface activity (19). Aldosterone release is regulated by angiotensin II (Ang II), the primary effector of the RAS. Moreover, it was investigated that AngII and TNF-

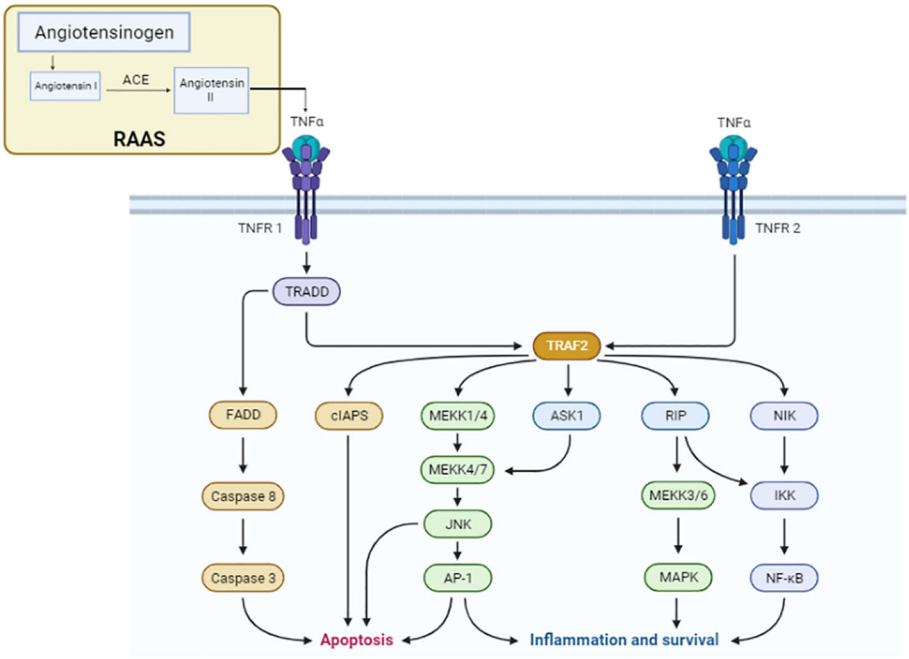


FIGURE 2
TNFα signaling pathway. The components of the renin–angiotensin system pathway involved in the conversion of angiotensin I to angiotensin II via ACE activate the TNFα receptor for the progression of the cascade of the signaling pathway for cancer development. In the TNF signaling pathway, TNFR1 transmits inflammatory signals by recruiting RIP1 and TRAF2 and apoptotic signals by recruiting FADD and caspase 8. To transmit signals related to inflammation, TNFR2 binds TRAF1 and TRAF2. Pro-caspase 8, the long isoform of FLICE-like inhibitory protein, and non-ubiquitylated receptor-interacting serine/threonine-protein kinase 1 (RIPK1) are additional apoptosis signaling pathways by which TNF can cause cell death (FLIPL).

might interact to specifically enhance the functional tissue factor (TF) cell surface activity. The activation of TNF, in murine hepatoblastoma cells and juxtaglomerular cells, expresses more angiotensinogen and renin mRNA. The first step involved in the activation of TNF receptors, located on the cell surface, is mediated by the primary component of RAS, angiotensin II, as discussed in Figure 2. This activation results in the release of the cytokines TNFα and IL-1β. Adipose cells, TAM, or tumor cells show the TNFα signaling pathway, which is responsible for the expression of aromatase. Thus, the upregulation of aromatase via the TNFα signaling pathway promotes the growth of cancer cells. Interestingly, TNFα boosts the production of estradiol, which binds to ER and encourages the growth of luminal cancer cells (20). Activated T lymphocytes, macrophages, and natural killer (NK) cells are the major producers of TNFα. By promoting psoriasis's mobilization of inflammatory cells, TNFα promotes the NF-B signal pathway-mediated keratinocyte proliferation and anti-apoptosis, ultimately resulting in the development of a micro-abscess (21). The TNFα signaling pathway is crucial for many pathological and physiological processes, including the control of immunological responses, the generation of inflammation, and cell proliferation, differentiation, and death.

## 3.2 TGF-β signaling

The TGF family of proteins is a sizable set of proteins with a shared structural makeup that functions in a variety of biological activities. The TGF proteins, which are made up of several amino acids and are split into two groups called TGF-1 and TGF-2, are known as a family of proteins. TGF proteins are essential for the growth of tissues, the formation of the embryo, and the preservation of the body's proper cell balance (21). Independent of blood pressure, the RAAS can increase TGF-β signaling via ANG II. Therefore, angiotensin II enhances the expression of TGF-β receptors, further amplifying the effects of TGF-β1. In general, elements of the RAAS can increase the concentration of TGF-β by affecting several pathways. Through a series of processes, angiotensin II (ANG II) increases TGF-β receptors and induces the production of TGF-β in the kidney. Thrombospondin-1 is stimulated by ANG II through the p38-mitogen-activated protein kinase and c-jun N-terminal kinase signaling, which increases the release of active TGF-β1 from the dormant latent complex (22)—for instance, renin via the (pro) renin receptor, angiotensin II via the AT1 receptor, and aldosterone via the mineralocorticoid receptor all boost TGF-β-1 expression (18). Without triggering TGF-β, ANG II can directly phosphorylate Smads. Mesangial cells and renal interstitial fibroblasts both express more TGF-β1 mRNA under the influence of ANG III which binds to AT1 receptors. Moreover, in these cells, this peptide promotes the production of extracellular matrix in the TGF-β pathway. TGF-β receptor is present in the cell membrane which activates the Ras/Raf/MEK/ERK pathway and Smad2/3 pathway for managing the tumor response in an organ. Finally, PI3K signaling is regulated and the action of apoptosis is inhibited (Figure 3). Moreover, TGF-β1, an isoform of TGF-β, inhibits phosphatase and tensin homolog (PTEN) transcription by binding to a combination of type I and type II

receptors. Thus, phosphorylating and activating receptor-regulated SMAD2/3 proteins, which then bind to common SMAD4 and move into the nucleus (23). The ligand–receptor complex then activates MEK, which phosphorylates and activates ERK1/2, thereby causing the suppression of PTEN protein post-transcriptionally. The TGF-β1-induced type II endometrial cancer cell migration is crucially mediated via PI3K–AKT signaling, which is enhanced by PTEN downregulation (Figure 4) (23).

## 3.3 RAAS and different receptors

A counter-regulatory mechanism includes neprilysin (NEP) and angiotensin-converting enzyme 1 (ACE1) for the formation of different angiotensinogens. Angiotensin (1–7) is formed via the cleavage of AngII by ACE2 and NEP, whereas Ang (1–9) is formed by ACE2 which activates AT2R, causing natriuresis. Additionally, angiotensin 1–7 binds to the proto-oncogene Mas receptor, causing vasodilation and antihypertensive and anti-fibrotic properties (Figure 5) (24). ANG II and ANG (1–7) are both important to induce nitric oxide formation via phosphatidylinositol 3-kinase (PI3K) which restricts cell proliferation (25). Angiotensin II (Ang II), a primary RAS component produced via the conversion of ANG I by ACE, induces angiogenesis and cell growth by binding to its receptor—angiotensin II type 1 receptor (AGTR1) (26). Moreover, the ACE2/Ang (1–7)/MasR pathway, another RAS route, competes with the Ang II/AGTR1 pathway, leading to angiogenesis. Furthermore, the binding of prorenin to the (P)RR causes cell proliferation and tumorigenic intracellular communications which are predominantly independent of Ang synthesis. Tumor growth and drug resistance are both influenced by the activation of the PI3K/AKT/mTOR pathways. Prorenin binding to (P)RR has been shown to activate the ERK1/2 signaling pathway and boost the VEGF protein level which is responsible for VEGFR activation in human diabetic retinopathy and several tumorous cancers enhancing angiogenesis. Hence, a positive correlation between (P)RR on VEGFR activation in the progression of cancer such as lung cancer, breast cancer, colorectal cancer, and endometrial cancer through angiogenesis via the PI3K pathway can be elucidated (27).

Endothelial cells and fibroblasts, which are significant elements of the tumor microenvironment, as well as tumor cells themselves, can produce and express RAS components that support angiogenesis. Ang II/AT1R, Ang II/AT2R, and Ang 1–7/MAS receptor axis signaling effects have primarily been investigated in tumor cells. The Ang (1,7)-MAS receptor and Ang II-AT2R pathways are believed to block many of the cellular actions of the Ang II–AT1R axis, in contrast to Ang II/AT1R, which mediates several psychopathic events associated with activated RAS, such as increased expression of cell proliferation, a decrease in apoptosis, motility, migration, invasion, and angiogenesis. Interestingly, PI3K signaling, a critical pathway for cell growth and metabolism, initiates cell proliferation and inhibits the activation of caspase 3 responsible for the activation of apoptosis (24). PI3K activity triggers the activation of numerous proteins, including those involved in the

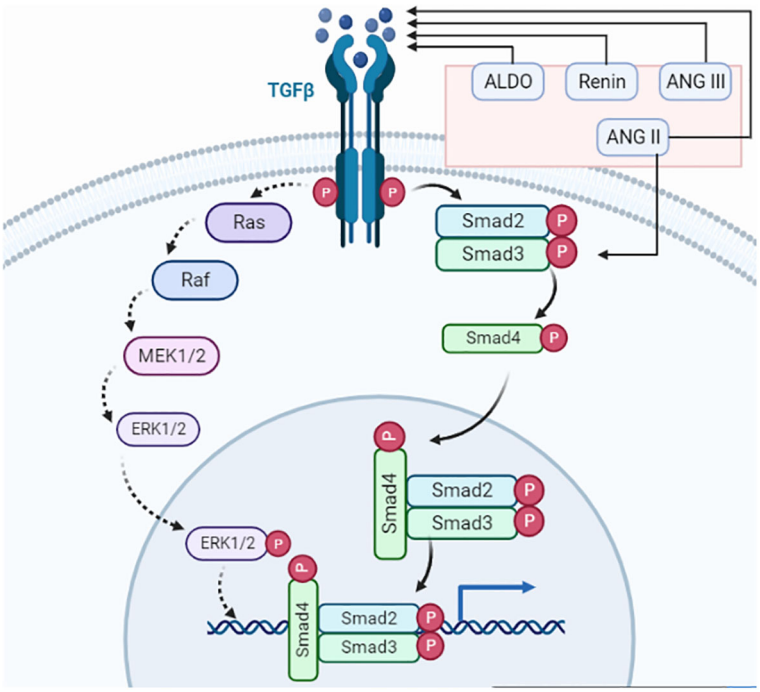


FIGURE 3
TGF-β signaling. The renin–angiotensin system primary component ANG II activates TGF-β and Smad3. TGF-ligand and TGF-receptor bind to form a complex. The receptor-induced phosphorylation of R-Smads leads to an interaction with cytoplasmic Smad2/3. Phosphorylated Smads combine with Smad4, which facilitates the transport to the nucleus where it connects with multiple transcription factors for transcriptional activities. Smad complexes start a negative loop, which makes Smad7 stop R-Smads from getting any more phosphorylation. TGF-receptors also phosphorylate TAK1 and CREB, which are involved in neuronal differentiation, axonal growth, cell cycle progression, and antidepressant effects.

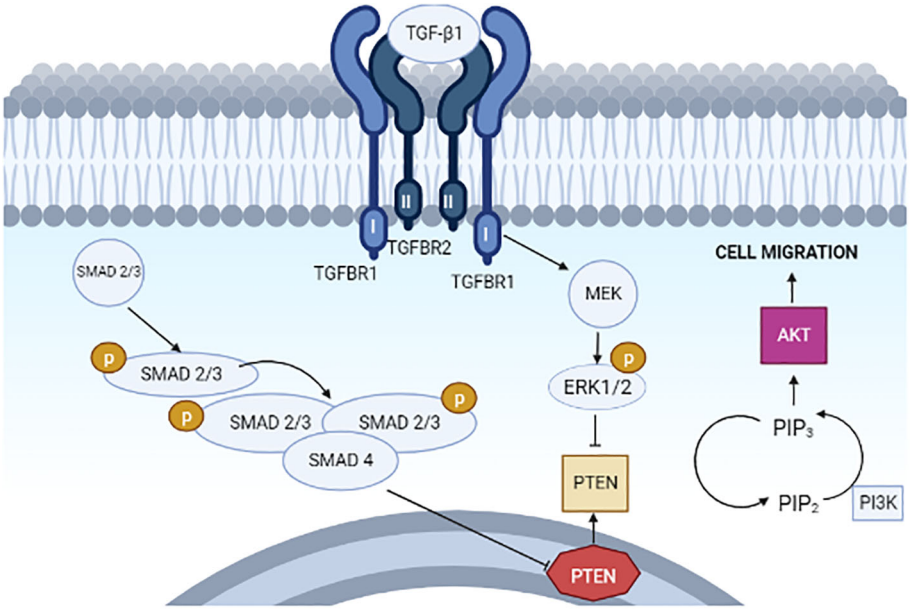


FIGURE 4
Proposed model for the actions of TGF-β1 on PTEN, PI3K–AKT signaling, and cell migration in type II endometrial cancer cells.

cell's energy production and metabolism (28). PI3K signaling is essential for cell growth and survival—for example, when cells are damaged or stressed, PI3K signaling can help them to recover (29), whereas other signaling pathways such as JNK and PKC initiate migration and invasion as per Figure 6. In addition, the cell's reaction to chemicals and cytokines is one of the critical processes that PI3K signaling controls.

## 4 AngII, AT1R, and AT2R and endometrial cancer

The pathogenesis of endometriosis is based on multiple data sets of genes which mainly include the AGTR1 gene responsible for encoding angiotensin II receptor type 1 (AT1R). The AngII possesses high binding affinity with two specific receptors, i.e.,

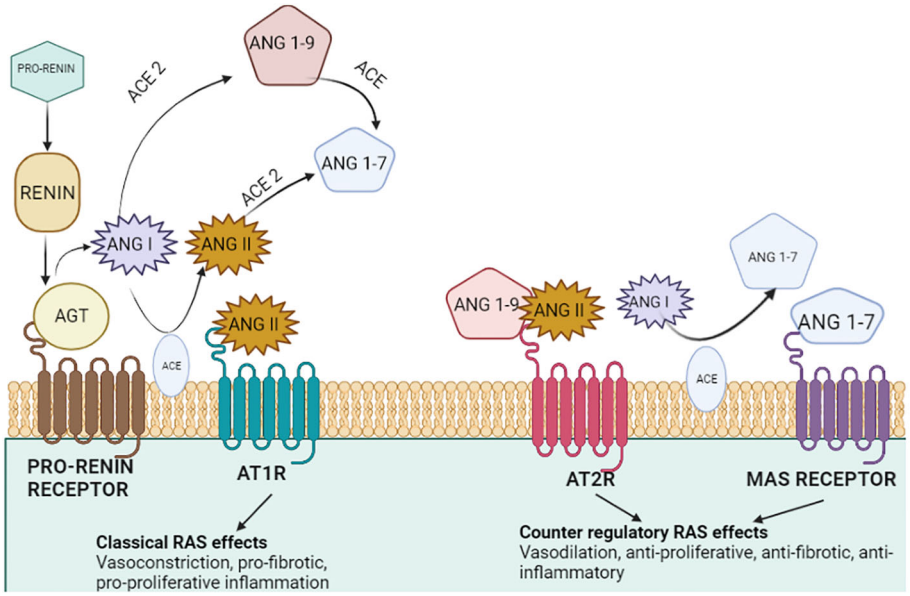


FIGURE 5
Tissue renin–angiotensin system in endometrial cancer. The physiologically active AngII may be produced with greater ACE1 abundance. The upregulation of the pro-angiogenic and pro-proliferative factors works together to boost the activation of the (P)RR and AGTR1-mediated intracellular signaling cascades, which would then drive the synthesis of TGFB1 and PI3KR1 and aid in the development of tumors. Through vascularization, the presence of MAS1 and increased ACE2 may promote tumor formation (24).

AT1R and AT2R (30). AT1R, involved in regulating the RAS, has a more prominent role in the development and progression of endometrial cancer due to the overexpression of angiogenic factors which affect the progression, proliferation, and apoptosis of EC cells (31). Apart from endometrial cancer, AngII/AT1R has shown a correlation with several other types of cancers such as breast, cervical, prostate, and lung cancer, thereby attributing to the overexpression of the pro-angiogenic and proliferative AngII/AT1R arm of the RAS (6). Additionally, targeting the AT1R proves to have a successful outcome, whereas several AT1R-blocking drugs such as losartan and telmisartan experimented through *in vitro* studies have shown a positive inhibition of EC cell growth, thus also blocking AngII actions (3). Interestingly, silencing of AT1R expression curbs the migration and invasion ability of EC cells. However, the complex role of AngII in the development of EC does not make silencing of angiotensin receptor 1 (AT1R) a successful approach to prevent the progression of endometrial cancer (31).

Angiotensin II, a potent RAS-derived vasoconstrictor peptide involved in tumor angiogenesis, is a prognostic predictor of endometrial endometroid adenocarcinoma along with AT1R, VEGF, and human A-LAP, a potential AngII degradation marker (4). Chidambaram et al. studied the positive correlation between RAS and the menstrual cycle. They noticed that RAS components such as AngII showed elevated levels in the luteal phase among the various phases of the menstrual cycle. Moreover, with the help of the immunostaining method, the localization of AngII, AT1R, and VEGF in corpora lutea indicates the role of AngII in luteal function (30, 31), whereas Ahmed et al. noticed the difference in the functioning of AngII, especially in the proliferative and secretory phases of the menstrual cycle, thereby suggesting the role of AngII in the regeneration of the endometrium after menstruation. Significantly, Piastowska-Ciesielska et al. noticed a high expression of AT1R and AT2R in the G1 stage of endometrial carcinoma, whereas a low expression was observed in the G3 stage. Ishikawa cells, i.e., EC cell lines, were reported to show a correlation between their cell cycle progression, proliferation, and expression of AT1R (31). Apart from the individual effect of AngII on endometrial cancer, the combined effect of microRNA 155 on inhibiting the translation of AT1R decreases the proliferation of EC cells. Thus, the AT1R-blocking drug, losartan, together with anti-mRNA-155, showed a synergistic effect in the reduction of the proliferative effect of AngII in endometrial cancer (31).

## 5 Angiotensin-converting enzyme 1 in endometrial cancer

The twin functions of the ACE, which converts dormant AngI to active AngII and degrades active bradykinin (BK), are well known and play a significant part in the regulation of blood pressure. The conversion of decapeptide angiotensin I to octapeptide angiotensin II is catalyzed by the angiotensin-I-converting enzyme (ACE), a monomeric, membrane-bound, zinc- and chloride-dependent peptidyl dipeptidase. The angiotensin-converting enzyme converts angiotensin I to angiotensin II in the blood. Blood arteries are immediately affected by angiotensin II, which causes them to contract and the blood pressure to rise. ACE1 is an enzyme that helps the body process angiotensin, a hormone that helps to control blood pressure. ACE1 is also responsible for breaking down other chemicals that can cause heart disease. ACE1

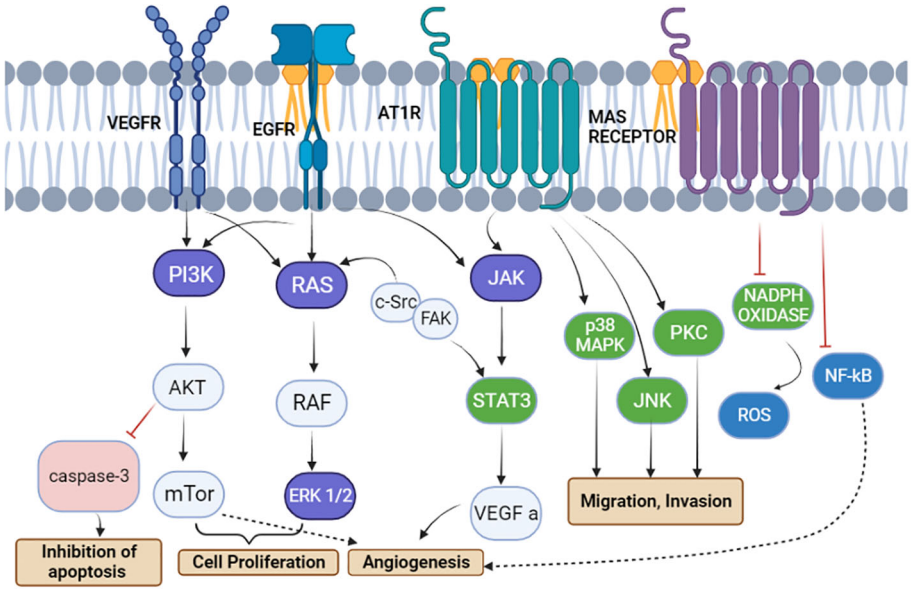


FIGURE 6
The renin–angiotensin system induces signal transduction pathways that are linked to cell division, migration, invasion, apoptosis suppression, and angiogenesis. Angiotensinogen type 1 receptor activation leads to a cascade of signaling pathways involving JAK, p38MPK, PKC, and JNK, causing the migration and invasion of tumor cells. PRR activates the vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor to activate the PI3K/AKT/mTOR and Ras/Raf/ERK pathways, respectively, for cell proliferation. Moreover, the inhibition of caspase 3 via the AKT pathway leads to inhibition of apoptosis, whereas VEGFR and MAS receptors are involved in angiogenesis (24).

is involved in the process of making cholesterol. Researchers have shown that ACE1 is linked to the development of endometrial cancer (32). In Caucasian and Asian groups, the I/D polymorphism of the ACE1 gene has received extensive research attention about cancer. Based on the evidence from several studies, researchers investigated the impact of ACE1 I/D polymorphism on the most prevalent benign pelvic tumor in women all over the world. The increased expression of AGTR1, ATP6AP2, and ACE1, crucial components of the RAS's pro-angiogenic/proliferative arm, raises the possibility that the RAS is involved in the development and progression of endometrial cancer (33). Consequently, drugs that are now in the market that block the RAS and are used to treat high blood pressure may one day be utilized to treat endometrial cancer. Selective AT1 receptor blockers and ACE inhibitors (ACEIs) are the two main groups of medications that target the RAS (ARBs). Even though both medication classes have angiotensin II as their target, the variations in their methods of action have an impact on how they affect other pathways and receptors, which could have therapeutic ramifications. By preventing the conversion of angiotensin I to angiotensin II, the ACEIs lessen the activation of the RAS and the AT1 and AT2 receptors. The pathogenic effects of angiotensin II, including vasoconstriction and other processes that raise the blood pressure as well as cause vascular hypertrophy, endothelial dysfunction, atherosclerosis, inflammation, and apoptosis, are mostly mediated by angiotensin II type 1 receptors. Angiotensin II reactivation has been linked to chronic treatment, even though acute treatment with ACEI decreases circulating angiotensin II to insignificant levels.

According to research done on endometrial cancer patients, the tumor tissues had higher amounts of AT1R, ACE1, and ACE2 mRNA than the surrounding non-cancerous tissues. It is unknown if ACEIs have a similar impact on the expression of ACE2, ACE1, and TMPRSS2 in the human endometrium. Hence, restoring the balance using ACE inhibitors is one of the main treatment approaches for halting the progression of the disease. The glandular epithelium (GE) and luminal epithelium were the primary sites of ACE1 protein expression (26). Rather than cancer, ACE1 and ACE2 had immense activity on the immunological imbalance which can lead to deadly diseases—for instance, there is a connection of imbalance in the ACE1 and ACE2 expression on the progression of COVID-19 which causes deadly long-term issues. By using Western blotting, the expression of the proteins ACE2, ACE1, and TMPRSS2 was examined and normalized to that of actin or tubulin. The expression of TIMPRSS2, ACE1, and ACE2 transcripts in Ishikawa cells exposed to a range of ACE1 and ACE2 inhibitor doses (0.3–30 M) for 24 h was also observed.

According to a previous study, ACE1 inhibitors decrease AngII production and Ang-(1–7) metabolism because ACE2 increases AngII metabolism. According to a recent study, for the treatment of chronic illnesses, people taking ACE1 inhibitors may also be at risk of contracting SARS-CoV-2 infection because of the increased expression of the ACE2 receptor (34). ACE1, ACE2, and TMPRSS2 transcripts and proteins are expressed in the human endometrium. For *in vitro* research on endometrial receptivity and embryo implantation, the commonly utilized human-sensitive endometrial Ishikawa and RL95-2 cells both express ACE1, ACE2, and TMPRSS2 transcripts and proteins. Since the ACEIs used in the current study did not affect the expression of ACE1, ACE2, and TMPRSS2 or the attachment of the spheroid to the Ishikawa cells, this suggests that using ACEIs to treat diseases may not have an impact on endometriosis and subsequent embryo implantation (35).

The RAS pathway involves a series of proteases that result in the production of several bioactive compounds. Angiotensinogen is broken down by renin, which is secreted by the juxtaglomerular cells of the kidney and primarily released by the liver, to create decapeptide angiotensin I (Ang I). Angiotensin-converting enzymes (ACE), which are expressed by endothelial cells in several organs including the lung, kidney, heart, and brain, change AngI into Ang II. The most important RAS pathway chemical, Ang II, works by activating the G-protein-coupled receptors AT1R and angiotensin II receptor type 2 (AT2R) (36).

The importance of ACE1 in endometrial cancer has been well documented. ACE1 is a key enzyme that helps control cell growth and division. When ACE1 is absent or poorly functioning, cells can grow unchecked and form tumors. ACE1 is also critical for the elimination of damaged cells. When cells are damaged, they can release inflammatory signals that can spur the growth of tumors. If ACE1 is absent or impaired, these inflammatory signals can build up and lead to the development of endometrial cancer. The role of ACE1 in endometrial cancer has been well studied, and the importance of this enzyme has been highlighted in many research studies.

Compared to limiting ACE, some researchers also think that blocking the AT1R may lessen the inflammatory mediators' reactions and decrease acute lung injury. Particularly, the ACE inhibitor captopril significantly decreased lipopolysaccharide, decreased the release of tumor necrosis factor and interleukin 6, decreased the ratio of AngII to Ang 1–7, and attempted to reverse the higher ratio of ACE to ACE2 in animal models of lung injury (37). Angiotensin I (AngI), which is traditionally created by the protease renin acting on liver-derived angiotensinogen, is then converted to AngII by the angiotensin-converting enzyme 1 (ACE1). Angiotensin II (AngII) can then attach to two separate GPCRs called AT1R and AT2R, which are responsible for this hormone's physiological effects. Interestingly, the cardiovascular-regulating activities of AngII are connected to AT1R activation (38). The communication that follows mediates the vasoactive effects of AngII. Nevertheless, excessive AngII : AT1R signaling may result in the clinical diseases listed above.

## 6 ACE2 and endometrial cancer

The whole RAS cascade stimulates the migration of cells, their proliferation, and angiogenesis in healthy endometrium. In the same way, if over-activated or over-expressed, it can lead to abnormal cell growth, setting the hallmark for endometrial cancer (39).

Whenever loss of blood occurs, which can be due to multiple reasons, the blood pressure drops instantly, and pressure-measuring

smooth muscles lining the renal afferent arteriole, called Polkissen cells, detect this variation in blood pressure, and this leads to a series of events with the target of being able to maintain the blood volume and blood pressure (40). A pivotal component of this cascade is angiotensin-converting enzyme 2 (ACE2), which facilitates vasoconstriction and maintains hydro-salinity balance. Its distribution in the female reproductive system also suggested that it may have a role in controlling follicle growth and ovulation as well as regulating luteal angiogenesis and degeneration (41).

A significant death rate is associated with one kind of uterine corpus endometrial carcinoma (UCEC), an endometrial epithelial malignant tumor. Its dependency on the hormone estrogen serves as the basis for division. Despite the limited occurrence of non-estrogen-dependent tumors, the prognosis, in this case, is bad due to the high malignancy. Additionally, ACE2 provided a positive prognosis (26).

## 6.1 Structure, function, and localization of angiotensin-converting enzyme 2

The open reading frame of the ACE2 transcript encodes an 805-amino-acid polypeptide (41). The extracellular catalytic region of the zinc metalloprotease ACE2 is 42% homologous to the N-terminal catalytic domain of ACE. Given that it is a protease, the enzyme can cleave angiotensin II into angiotensin (1–7) that then displays the necessary functions, as explained in Figure 7 (26, 39, 41).

The information at hand suggests that ACE2 is broadly expressed in the uterus, ovary, vagina, and placenta in addition to being localized in the lung epithelium. The oocyte and ovary levels were discovered to be relatively high (42). After examining information from HP Atlas and Gene Cards, the existence of ACE2 in the uterus and vagina was established (41).

In the menstrual cycle's proliferative stage, stromal cells and endometrial epithelium were both reported to express ACE2. However, it has been observed that expression rises throughout the secretory phase. In addition to oocytes from immature rat ovaries, the presence of ACE2 in stroma and granulosa cells has recently been discovered (43). Additional research has revealed that it exists in the granulosa and theca cells of cattle. In the human placenta, there was evidence of increased ACE2 expression (44). Its expression in primary and secondary placental villi endothelium and vascular smooth muscles, syncytiotrophoblast, and cytotrophoblast was found. Additional evidence of ACE2 was discovered in the atrial and venous endothelium as well as the smooth muscles of the umbilical cord, where ACE2 first manifests in early pregnancy. Moreover, ACE2 expression in the placenta is higher than that in the lungs (41).

As far as the function of ACE2 is put into consideration, it balances AngII and Ang in a synergistic manner (1–7) and later facilitates follicle development and ovary maturation via inducing steroid secretion. Studies suggested its contribution to follicular atresia, enhancing ovulation, maintaining corpus luteum progression, and promoting the production of estradiol and progesterone (41, 45).

Evidence points to its function in the progesterone-mediated differentiation process known as stromal cell decidualization, which primes uterine stromal cells for implantation. The same was demonstrated by the reduction of the decidualization response when ACE2-targeting siRNA was transfected into human endometrial stromal cells (43).

Additionally, AngII can induce angiogenesis, fibrosis, migration, invasion, and cell proliferation by acting on the AngII type 1 receptor (AGTR1) and activating growth factors and intracellular signaling pathways. ACE2 can further transform AngII to become Ang (1–7) and later works on its receptor Mas, which causes the earlier route way (AngII/AGTR1) to become antagonistic (46).

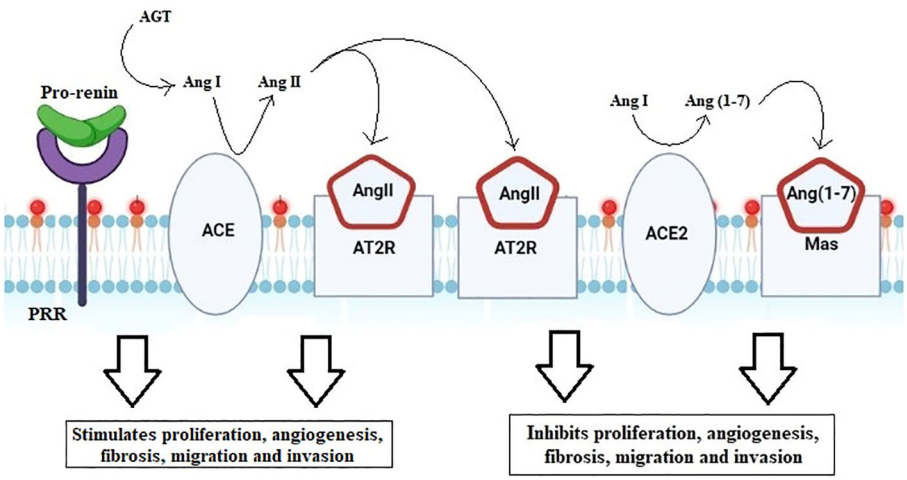


FIGURE 7
Renin–angiotensin system cascade. Activation of pro-renin by PRR forms angiotensin from angiotensinogen. ACE then converts AngI to the biologically active AngII. Angiotensin binds to either the angiotensin II type 1 receptor (AT1R) or the angiotensin II type 2 receptor (AT2R). The downstream pathway run, by binding to AT1R, stimulates angiogenesis, fibrosis, migration, and invasion, whereas binding to AT2R acts antagonistically, blocking proliferation, angiogenesis, migration, and invasion.

Vascular bed and endometrial regeneration are only a couple of the many functions that AngII plays in the endometrium. Through spiral artery constriction, it starts the menstrual cycle. The ideal proportion between AngII and Ang controls endometrial regeneration (1–7). AngII regulates menstrual periods in the endometrium as part of its normal function Dysfunctional uterine hemorrhage and hyperplastic endometrial polyps may be visible if the distribution and amount of AngII in the endometrium depart from normal (47).

Additionally, it was shown that the increased levels of ACE2 and AngII expression were associated with the development and metastasis of endometrial cancer. Increased ACE2 leads to increased conversion of AngI to Ang (1–7), increased Mas, and increased binding of AngII to AT2R, all of which are highlighted mechanisms to counteract the actions of AngII and ATR1 (48, 49). The major ways that AngII, ACE2, and Ang (1–7) operate during pregnancy are via controlling the blood pressure and fetal growth. They work together to preserve healthy uterine physiology in the meanwhile (41). Rat and human cell trophoblast invasions are induced by AngII. In early pregnancy (apoptosis, angiogenesis, and growth) and late pregnancy (uteroplacental blood flow), Ang (1–7) and ACE2 may function as local autocrine/paracrine regulators (41).

## 6.2 ACE2 mRNA expression levels in endometrium carcinoma

Immunohistochemistry was used to analyze the expression of downstream RAS pathway targets in endometrial cancer tissue and nearby non-cancerous endometrium, including phosphoinositide-3-kinase (PIK3R1), plasminogen activator inhibitor-1 (SERPINE1), VEGFA, and transforming growth factor beta 1 (TGFB1). Their expression was shown to be associated with that of RAS components. Using immunohistochemistry, which considers the protein by-products of all genes, all endometrial tumors and the surrounding non-cancerous tissues were identified. The tumor tissues exhibited significantly higher amounts of ACE1, AGTR1, and ACE2 mRNAs than the non-cancerous nearby tissue, although AGT mRNA did not differ between the malignant and non-cancerous bordering tissue (Figure 8) (41, 50).

ACE1, ACE2, AGTR2, AGTR1, and MAS1 protein immunostaining showed the glandular epithelium to be more intense. Additionally, it was found in the stroma, perivascular area, and endothelium. According to the study's findings, a higher risk of endometrial cancer recurrence is associated with higher levels of AGTR mRNA expression. All of this showed that AGT, by the synthesis of AngII and the overactivation of its receptors, contributes to the development and growth of tumors. The generation of AGT in the liver is estrogen-dependent. Adipose tissue may also express it and secrete it. Therefore, both high estrogen levels and obesity, which have the propensity to enhance AGT production, are substantial risk factors for endometrial cancer, thereby accelerating sickness. High ACEI mRNA levels in endometrial cancers tend to improve their ability to generate AngII.

Additionally, the most effective method for figuring out how the gene ACE2 is regulated is to look for TF binding sites in its promoter. To anticipate the probable TF binding sites, the well-known web-based application Mat Inspector from Geomatics was employed. This aided in the TFs in the ACE2 promoter's identification. The 1,947-nucleotide-long sequence of the ACE2 promoter, which was taken from PubMed with the NCBI reference sequence ID NG 068141 and is situated on chromosome X, is presented in a FASTA format. For the positive stand of the ACE2 promoter, a total of 51 TFs were identified. Among these 51 TFs, many were found to have high confidence binding sites (match factor greater than 0.9).

Tumor-prompting transcription factor has such a high confidence binding site at ACE2 promoter. Some of these transcription factors are B cell lymphoma 6 (BCL6), Wilm's tumor protein (WT1), signal transducer and activator of transcription 3 (STAT 3), Ying Yang-1 (YY1), ERG, AREB6, GKLF (or KLF4), and GATA TFs. All these transcription factors have been linked to the emergence of different malignancies, including endometrial cancer (41).

## 6.3 ACE2 and favorable prognosis

Further research was done on the association between ACE2 expression and prognosis in these cancers. No significant relationship between ACE2 expression and the prognosis of breast cancer and squamous cell carcinoma of the head and neck was found in the studies on the same topic (51). On the other hand, kidney renal papillary cell cancer and UCEC had much better prognoses due to high levels of ACE2 expression. Additionally, the relationship between tumor invasion and the prognosis was researched. Here immunological infiltration and ACE2 transcription levels in endometrial cancer were associated (52). The TIMER database has been employed for the same. The findings revealed that the expression of ACE2 was strongly linked with the

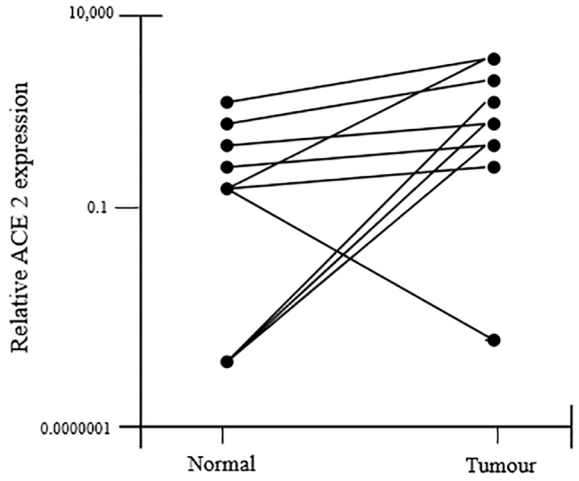


FIGURE 8
ACE2 mRNA expression was found to increase in tumor tissue as compared to the adjacent non-cancerous tissue.

degree of macrophage immune infiltration in renal papillary cell carcinoma. Similarly, it was discovered that ACE2 and the levels of CD4+ T cell, B cell, neutrophil, and dendritic cell immune infiltration in uterine corpus endometrial cancer are positively correlated (53).

## 7 The (pro) renin receptor in endometrial cancer

The pro-renin receptor is a crucial protein for both healthy cardiovascular and renal function as well as illness. However, P(RR) participates in several other crucial processes; thus, its function is not only restricted to the renal and cardiovascular systems. Over the last 5 years, the pro-renin receptor is irregularly expressed in several malignancies, including endometrial cancer, according to data from ongoing studies (48). It has been established via several trials that P(RR) plays a substantial role in a variety of cancer-causing cells, including endometrial cancer. When compared to healthy neighboring endometrial tissue, it has been demonstrated that P(RR) is over-expressed in human endometrial cancer tissue (54).

It is widely established that P(RR) stimulates angiogenesis and activates several cellular processes, including proliferation and migration, all of which are significant contributors to the onset and development of endometrial cancer (55).

The 350 amino acid sequences that make up pro-renin receptor's structural makeup make it a multifunctional protein. The N-terminus of this long trans-membrane protein faces the extracellular side of the cell, while the C-terminus faces the cytoplasm. It has a single-membrane-spanning domain. Both the proteases Furin and Adam 19 can cleave P(RR) in the Golgi complex and produce a shortened soluble protein. The soluble portion of the truncated protein is the N-terminus which can then be secreted as P(RR) into the body fluids, whereas the truncated C-terminus remains part of the transmembrane (56). ATP6AP2 which was found on the X chromosome regulates the activity of P (RR) (26).

In conjunction with the pro-renin receptor, the renin–angiotensin system works efficiently in women with a healthy endometrium during pregnancy. In the endometrium, under normal conditions, the main functions of RAS are to promote angiogenesis, cell proliferation, and migration. However, if overexpressed, it can promote irregular cell growth and metastasis, resulting in a classic case of endometrial cancer (57). The P(RR) receptor, as a component of vacuole ATPase, has the functional capability of acidifying the extracellular milieu (58).

In the RAS system, P(RR) plays a notable role. PRR can be bound by ligands like renin and its precursor pro-renin (RR). These molecules become highly active when they meet P(RR), which catalysis the conversion of angiotensinogen to angiotensin (ANG1). The cleaving function of ACE converts angiotensin 1 into angiotensin 2 (angiotensin-converting enzyme) (59). Angiotensin 2 is then produced and binds to its receptor, AGTR1, activating ANG-2 receptor-mediated signal transduction and accelerating processes like angiogenesis and cell division. By boosting the oncogenic factors, the upregulated activity of the ANG2/ANG2 receptor in conjunction with the pro-renin receptor causes cancer (60).

One of the crucial downstream factors generated by P(RR) signaling is TGF, which is thought to be required for the epithelial-to-mesenchymal transition. Since the RAS system activates TGF, there is a close relationship between the two. This TGF has an overexpression in tumor tissues when abnormal signaling is present. This pathway, therefore, suggests that P(RR) promotes cancer spread by activating TGF via RAS (61).

Pro-renin participates in intracellular signaling outside of the authorized route, which is unrelated to ANG2 synthesis and has the potential to be both proliferative and tumorigenic. In non-authorized signaling, P(RR) phosphorylates enzymes such as extracellular signal-regulated kinase 1/2 (ERK1/2) and mitogen-activated kinases (MAPK), which activate TGF. As a direct result of this signaling, P(RR) increases cell proliferation and encourages the unchecked spread and growth of this kind of cancer (Figure 9) (62).

When multiple sets of experimental data were seen, the proliferative ability of P(RR) in endometrial cancer was emphasized, highlighting P(RR) as an essential element of endometrial cancer formation. According to Delforce et al., an unbalanced RAS promotes the growth of endometrial cancer. Up to 30 different samples of endometrial cancer and its surrounding normal tissue were examined for protein and mRNA levels of various RAS components. The various RAS components' protein and mRNA levels were shown to be higher in tumor tissues than in the nearby normal tissue. The protein levels of P(RR) and the mRNA levels of P(RR), AGTR, ACE, and ACE2 in tumor tissues were considerably higher than in the surrounding normal tissue. Another factor closely linked to RAS, i.e., TGF, was found to be abnormally high in tumor tissues. Thus, the results show that pro-renin plays a substantial role in endometrial cancer due to the greater expression of multiple RAS components (Table 1) (58).

In this distinctive research, Jacinta et al., Sarah et al., and Riazuddin et al. monitored the mRNA and protein expression of P(RR) in different endometrial cancer cells, namely, Ishikawa cells (grade 1) followed by AN3CA cells (grade 3) and subsequently HEC-1-A cells (grade 2). The results from this study created a contradiction as it demonstrated that carcinoma grade and the mRNA levels of P(RR) had no co-relation, inferring that the mRNA levels of P(RR) functioned independently of the type of cancer (61).

Ishikawa cells were further subjected to siRNA transfection. Some proteins were found to be upregulated, while some were downregulated (the most significant among them being the pro-renin receptor). Notably, the most downregulated protein was found to be the pro-renin receptor, thereby demonstrating the efficiency of the siRNA knockdown procedure (61).

The MAX gene-associated protein (MGA) highlighted an increased expression in the siRNA-transfected Ishikawa cell lines. The functional activity of the MGA protein is a transcriptional activator/repressor which regulates the activity of genes that control cellular processes like proliferation (61). MGA and MYC are the binding partners of Myc-associated factor X (MAX), and upon dimerization of MAX with MGA/MYC, it dictates the transcription of target genes in non-tumorigenic cells. MYC-dependent cell

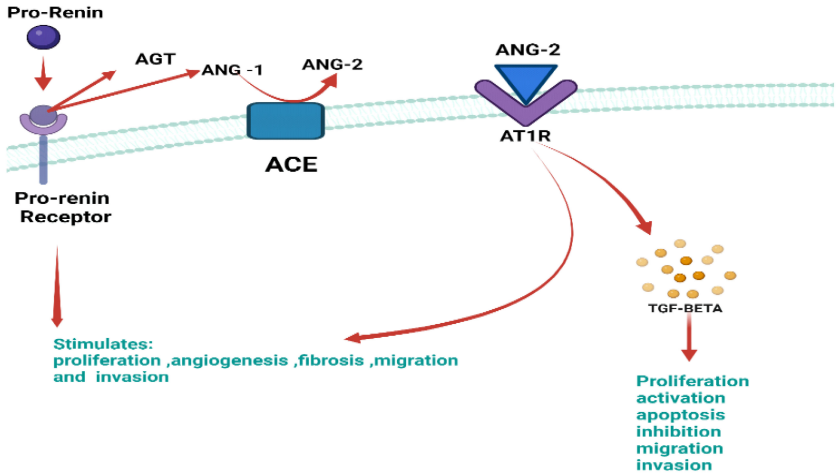


FIGURE 9
Illustration of the authorized P(RR) signaling with major key components which include pro-renin, ANG-1, ANG-2 AT1R, and TGF-BETA. Abnormally active pro-renin binds to its receptor P(RR), causing the formation of angiotensin-1 from angiotensinogen. Upon interaction of ANG-1 with ACE, it forms ANG-2, which binds to its respective receptor, i.e., AT1R causing TGF-beta formation. This erratic signaling stimulates proliferation, angiogenesis migration, and invasion.

transformation is negatively regulated by MAX and MGA heterodimers (62). Therefore, the over-expression of MGA by the knockdown of P(RR) will curtail the supply of MAX for MYC heterodimerization and stop the MYC-dependent cellular processes from shifting tumor cells from a rapidly dividing state to a more regulated state of division (8).

Extra-cellular tumor acidity is generally interlinked with cancer aggressiveness. Cancers usually show a typical pattern in which the intra-cellular pH is found to be higher than the extracellular pH due to the rapid efflux of $H^+$ ions. This rapid efflux could be linked to different modifications (such as expression and activity) in the targeted pH transporters (63). Four proteins involved in the acidification process were downregulated in the siRNA-treated Ishikawa cells which includes isoform 7 of sodium bicarbonate co-transporters (SLC4A7). The other protein that was downregulated in this study included ATP6VOA1 and ATP6VOD1, which are the two subunits required for putting together the V-type proton ATPase in its functional form. As P (RR) is also a component of V-ATPase, the downregulation of P (RR) therefore directly affects the levels of ATP6VOA1 and

TABLE 1 Different components of P(RR) signaling that show its effect in cancer, their mRNA expression, and the effect that each component has in cancerous signaling.

| S.No. | Pro-renin receptor components | Expression (mRNA and protein) | Outcome | References |
|---|---|---|---|---|
| 1. | P(RR) | Upregulated | Promotes angiogenesis and cell proliferation | (57) |
| 2. | ACE | Upregulated | Enzyme converting ANG1 to ANG 2 and promotes cell proliferation | (57) |
| 3. | AGTR1 | Upregulated | Receptor binding RAS components and promoting aggressive cell division | (58) |
| 4. | ANG 2 | Upregulated | Binds to the receptor and further activates components that take part in angiogenesis, cell proliferation, and metastasis | (58) |
| 5. | TGF β | Upregulated | Activation of proliferation, inhibition of apoptosis, and helps in the migration of cancerous cells | (59) |
| 6. | MAG protein | Upregulated | Limits the supply of MAX for Myc heterodimerization and negatively regulates Myc-dependent cell processes, thereby transforming tumor cells from a rapidly dividing state to a more regulated state of division | (60) |
| 7. | SLC4A7 | Downregulated | Component of P(RR)V ATPase and causes extracellular tumor acidity by changing the activity of PH transporters that facilitate hydrogen ion efflux | (60) |
| 8. | ATP6VOA7 and ATP6VOD7 | Downregulated | Assemble the components of P(RR) and contribute to cellular proliferation | (60) |

Components of P(RR) such as ACE, P(RR), AGTR1, ANG2, and TGF-beta are upregulated upon the knockdown of the pro-renin receptor. Certain proteins were downregulated.

ATP6VOD1. This provides sufficient evidence that the downregulation of SLC4A7, P(RR) ATP6VOA1, and ATP6VOD1 contributes to proliferation (64).

Overall, it is implicated from the provided data that the components of RAS and P(RR) both, in general, exhibit erratic signaling in endometrial cancer, highlighting unusually high levels in tumor tissues in contrast with the normal tissue which is used as a control. The mRNA and protein levels of P(RR) were higher in targeted cell lines like Ishikawa cells, AN3CA cells, and HEC-1-A cells, but the protein levels were found to be independent of the grade of the tumor. This research could pave the way for various effective treatment methods for endometrial cancer such as siRNA-treated P(RR) or monoclonal antibodies targeted against P(RR). Lastly, knocking down the P(RR) and its related proteins which are generally proliferative in cancerous tissue could prove to be a beneficial and curative strategy for targeting cancer of the endometrium (59).

## 8 The RAS components as a potential therapeutic target in endometrial cancer

One of the factors that have been associated with endometrial cancer is the Ras protein. Ras, which belongs to the family of GTPases, is involved in signaling pathways associated with cell growth and proliferation and regulates diverse cell behaviors. Any over-activation of this protein can bring alterations in the upstream and downstream components of signaling. It plays a vital role in tumor maintenance. Mutation in Ras has been shown by most human carcinomas and hence considered an appropriate target for cancer therapy.

The important molecular pathways, such as the PI3K/PTEN/AKT/mTOR and RAS/RAF/MEK signaling pathways, have been examined for their participation in the development of endometrial cancer (Figure 10) (65, 66). Ras mutations can activate PI3K. The PI3K–AKT pathway is one of the most dysregulated signaling pathways in endometrial cancer, which is caused by mutations in tumor suppressor genes, i.e., PTEN and PIK3CA (67). These pathways are triggered by a variety of cytokines and growth factors to avoid apoptosis and cell proliferation. Numerous tumor types exhibit the abnormal regulation of PIP3K and RAS pathways brought on by mutations in Ras and B-Raf as well as other genes (such as PTEN, Akt, and PI3K). Therefore, different parts of these pathways are considered potential biological targets for cancer treatment.

### 8.1 Deregulation of signaling components

Deregulation of PI3K/PTEN/Akt/mTOR and RAS/RAF/MEK/ERK signaling cascades is frequently caused by epigenetic silencing or mutations in either upstream signaling molecules like receptor tyrosine kinases (RTKs) such as HER2, EGFR, IGF-1R, PDGFR, VEGF, FGFR2/3 or, in other components of the pathway, such as RAS, BRAF, NF1, MEK1, PIK3CA, PIK3 (R1, R4, and R5), mTOR, PTEN, Akt, IRS4, TSC1, and TSC2, as presented in Figure 11. It was previously considered that the ERK and MEK genes were seldom

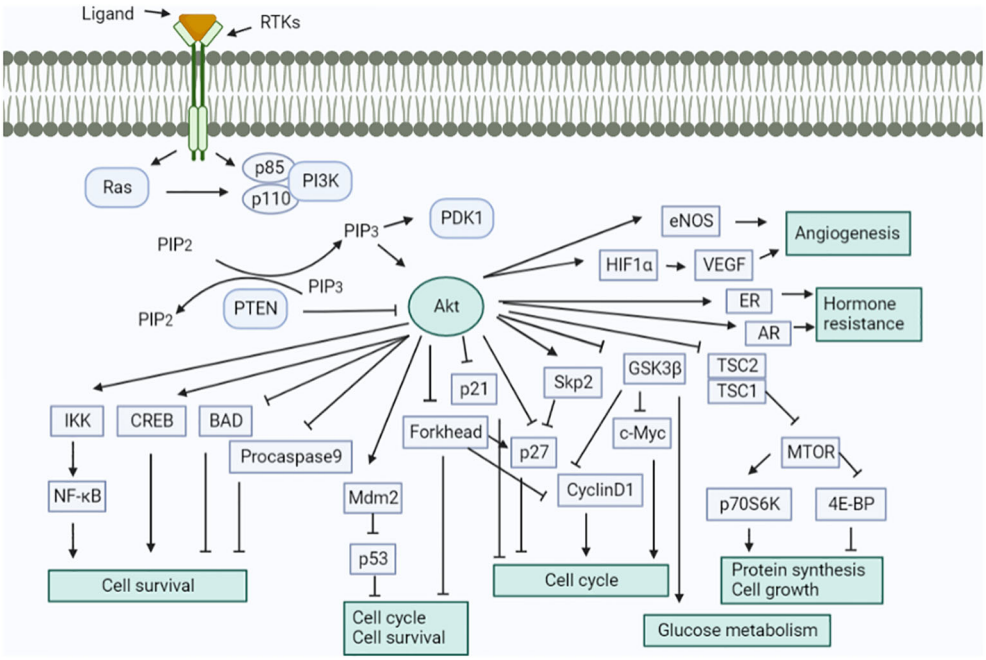


FIGURE 10
Schematic signaling shows the involvement of the renin–angiotensin system. Activation of the Akt signaling pathway in a variety of ways. This pathway performs numerous cellular tasks.

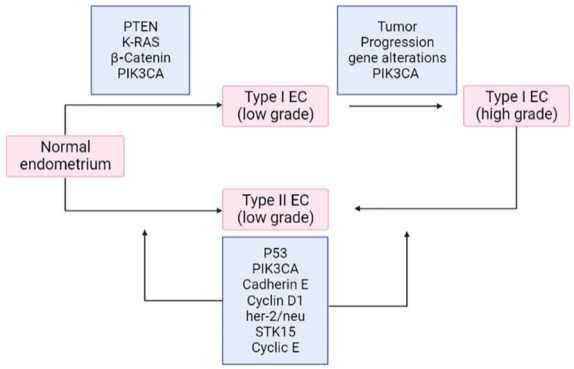


FIGURE 11
Frequent mutations in endometrial carcinoma. EC, endometrial cancer.

altered in human cancer, but according to recent research, MEK and MEK2 have been seen to be mutated in specific malignancies like in the case of ovarian and lung cancers. Deregulation of these components significantly impacts the differentiation pathways (68). It has been established that type I and type II endometrial cancers develop from different molecular alterations. Type I endometrial carcinoma shows mutations in K-RAS, PTEN, PIK3CA, and CTNNB1 (β-catenin) genes, whereas type II endometrial cancer has p53 changes, loss of heterozygosity, and other molecular modifications (p16, STK15, c-erb-B2, and E-cadherin). The RAS–RAF–MEK–ERK signaling pathway is crucial for tumorigenesis. The prevalence of K-RAS mutation varies from 10%–30%. It is believed that Ras effectors like RASSF1A offer an inhibitory growth signal that must be deactivated during tumorigenesis and remain inactivated. The increased activity of the RAS–RAF–MEK–ERK signaling pathway due to RASSFIA inactivation is caused by promoter hypermethylation (69). According to studies, the fibroblast growth factor (FGF) signaling pathway is significant in endometrial cancer. Endometrial cancer frequently exhibits the inactivation of the protein (SPRY-2) involved in the negative regulation of FGFR. Somatic mutations in FGFR2 receptor tyrosine kinase have also been reported to be around 6%–12%, specifically in type -I endometrial cancer (70, 71). Studies have concluded that mutations in PTEN and FGFR2 frequently coexist, whereas FGFR2 and K-RAS mutations remain mutually exclusive. In contrast, TP53 mutations occur more prominently in type II endometrial cancer than in type I, which significantly reduced the expression of c-erb-B2 (HER-2) and E-cadherin. Furthermore, variations in the STK15 gene have been indicated. The deregulation of E-cadherin is prominent in endometrial cancer and is brought on by promoter hypermethylation or loss of heterozygosity (72). The Akt pathway is negatively regulated by PTEN and its loss of function (due to deletion, mutation, or promoter methylation) leads to a rise in PIP3 concentration. The increased PIP3K substrate further results in the upregulation of the components of the PIP3K pathway, including Akt and mTOR.

## 8.2 Targeted therapies

There are no authorized targeted therapies for endometrial carcinoma. The prediction of advanced endometrial cancer in patients is still a problem. Recent developments in molecular targeted medicines have shown their potential to increase the cancer long-term survival rates of patients when used in conjunction with the right biomarkers. In this review, the anticancer effects of various pathway inhibitors are being clarified by preclinical and clinical investigations, although studies are ongoing in elucidating their effectiveness. Thus, Figure 11 represents a comprehensive approach to inhibitors via different pathways in endometrial cancer.

## 8.3 PI3K/Akt/mTOR pathway inhibitors

Many of the kinases found in the PI3K/Akt pathway provide excellent targets for the creation of small-molecule inhibitors. Additionally, multiple studies have demonstrated that compounds that block the PI3K/Akt pathway are likely to be utilized to treat not only endometrial carcinoma but also a variety of cancers (73). The inhibitors target the upstream regulators such as membrane receptors (like FGFR2) or directly block the steps of the pathway. The two varieties of PI3K inhibitor includes isoform-specific PI3K and Pan-PI3K inhibitors. The isoform-specific PI3K inhibitor such as buparlisib can inhibit all four isoforms of PI3K, whereas MLN1117 is a single specific isoform. Because of the major involvement of p110 (p110α and p110β) and p85 catalytic subunits, specific inhibitors that target them are considered for better safety assessment. The selective inhibitors for PI3K-p110α like INK1117 and NVP-BYL719 showed high efficacy in cell lines with PIK3CA mutations. The activity of PI3K-p110α inhibitors may be less effective against PTEN-deficient tumor cells. To overcome the reduced efficacy, GSK2636771 (p110β-specific inhibitor) or dual inhibitors of p110α and p110β could be employed [Figure 12 (74)].

mTOR kinase may be found in two complexes—mTORC1 and mTORC2, and their inhibitors are also available. Rapamycin inhibits mTORC1 and may inhibit mTORC2 in specific cell types after extended incubation. Rapamycin has been shown to suppress angiogenesis and is an antiproliferative and anticancer agent. It reduces excess VEGF production. However, other analogs of rapamycin showed higher efficacy in cell lines with PIK3CA and/or PTEN mutations under the clinical trials of everolimus, temsirolimus, and ridaforolimus (75).

Akt has major relevance in activating mTORC1 by phosphorylation of TCS2 protein and inhibiting the AMP-PK enzyme that, in turn, activates Rheb and mTOR complex (75). A few of the Akt inhibitors include competitive inhibitors: AZD5363, GDC-0068, GSK2141795, MK2206 (allosteric inhibitor), miransertib (competes for the ATP binding site of AMP-PK), and perifosine that induces apoptosis.

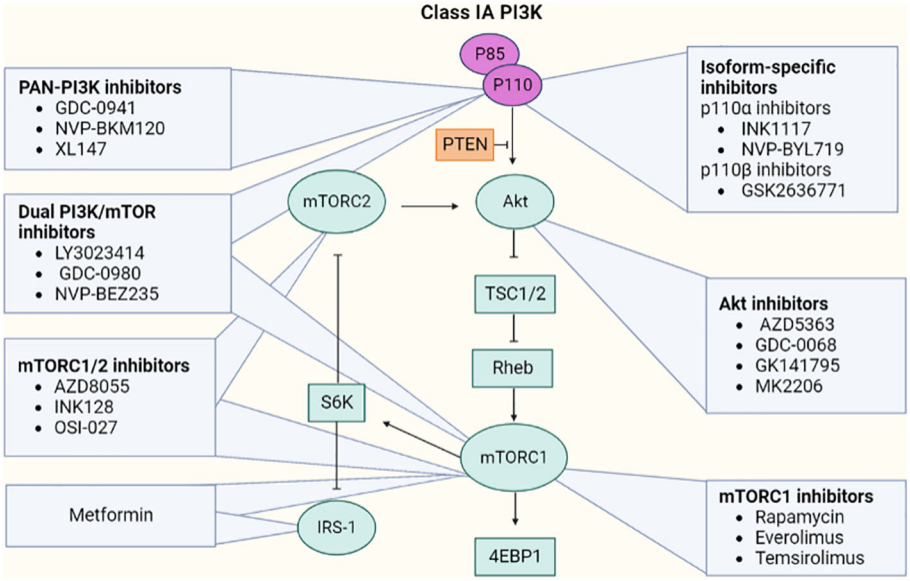


FIGURE 12
Representative inhibitors of the PI3k/Akt/mTOR pathway.

Investigations revealed that second-generation mTOR inhibitors have a significant benefit. These dual mTOR inhibitors rather than targeting individual components can suppress the whole PI3K/Akt/mTOR pathway. Drugs such as vistusertib and sapanisertib concurrently inhibit the phosphorylation of Akt and S6K1. Some other dual inhibitors including LY3023414 and dactolisib can inhibit both mTOR complexes (mTORC1 and mTORC2) and all four catalytic isoforms of PI3K (76). The clinical trials of FGFR inhibitors revealed that it may not always have an oncogenic function; hence, its inhibition could lead to negative consequences. Furthermore, it has been investigated that the overexpression of a transmembrane protein EphA2 was seen in the majority of type I endometrial carcinoma. A microtubule inhibitor coupled with an anti-EphA2 monoclonal antibody, namely, MEDI-547, was employed in tumor-associated mice models, demonstrating a significant anticancer efficacy.

Moreover, the loss of function of PTEN in the PI3K pathway led to a hampered homologous recombination which makes the cells vulnerable to poly(ADP-ribose polymerase) (PARP) suppression. Research investigating PARP inhibitors is still ongoing (77). Regardless of the presence of other risk factors for this disease, type II diabetes shows a possible link with endometrial cancer (78). To treat such malignancies, metformin (an anti-diabetic drug) is being implied by the researchers. Metformin is supposed to control the PI3K/AKT/mTOR signaling through the activation of AMPK (79). Moreover, its therapeutic efficacy in treating different carcinomas is still under investigation.

## 9 Future perspective

Endometrial cancer, one of the most common gynecological malignancies in women representing 90% of uterine cancer, develops high chances of occurrence due to age, obesity, hypertension, and hyperestrogenism. This fundamentally deadliest cancer shows great relevance with the RAS which governs and maintains blood pressure, salt, water, and aldosterone secretion, thereby playing a significant role in the etiology of hypertension. The components such as ACE-I, ACE-II, AT1R, AT2R, and Pro(renin) are predominant components involved in RAS whose dysregulation in expression can lead to endometrial cancer. Thus, devising an efficient drug targeting the components of RAS could prove to be a promising scope to control the progression or development of endometrial cancer. Further studies are required to understand the mechanism behind the development of endometrial cancer through the dysregulation of RAS, and effective drugs need to be discovered to target the RAS system effectively. Additionally, another challenge faced in limiting the progression of cancer is its early diagnosis. Thus, certain predictive biomarkers with high specificity and sensitivity are required for early diagnosis of endometrial cancer. Moreover, the new era of artificial intelligence could also be a promising approach, changing the future of science in various public health sector diseases worldwide including cancer. Hence, devising effective AI tools for the diagnosis of cell progression, cell migration, and tumors in the region of endometrium can help to mitigate progressive endometrial cancer in women at the early stage.

## 10 Conclusion

A crosstalk between the pathways involved in endometrial cancer and the renin–angiotensin system shows the great relevance of different components of RAS in the progression and development of endometrial cancer in women above the age of 60. The presence of Pro(renin) in the ovarian follicular fluid makes it an

effective target of choice to understand the various other pathways involved in the development of endometrial cancer through the dysregulation of RAS. Significantly, different pathways studied such as PI3K, TGF-β signaling, TNF signaling pathway, and ACEs are present to act as objectives of inhibitors and initiate endometrial cancer growth. The attributes of cancer such as tumor migration, invasion, and angiogenesis along with tumor adhesion to vascular endothelial cells are believed to be facilitated by angiotensin II. Various sets of genes involved in the pathogenesis of endometriosis include the AGTR1 gene. Thus, AT1R (receptor) plays a prominent role in the development and progression of endometrial cancer. Thereby, selective AT1 receptor blockers and ACE inhibitors (ACEIs) would target the RAS, providing therapeutic ramifications. Additionally, endometrial cancer is marked by the higher concentration of AT1R, ACE1, and ACE2 mRNA as compared to the surrounding non-cancerous tissues. ACE1 acts as a key enzyme in controlling the cell growth, division, and elimination of damaged cells; therefore, its dysregulation leads to uncontrolled growth and tumorigenesis. Prominently, ACE2 plays an important role in facilitating vasoconstriction and maintaining hydro-salinity balance and also in controlling follicular growth and ovulation. Moreover, Pro(renin) plays a versatile role in several malignancies, including endometrial cancer and therefore gets overexpressed in human endometrial cancer tissue, thereby stimulating angiogenesis and several other cellular processes such as proliferation and migration. The effectiveness and efficiency of RAS promote angiogenesis, cell proliferation, and migration in the endometrium under healthy conditions, especially during the menstrual phases, which concludes a positive relevance with the progression and development of endometrial cancer in women.

## Author contributions

NK, GR, DE, and ST played a role in designing the study as well as drafted the review paper. NK, AC, AF, AK, NS, and MK did the writing part. All authors contributed to the article and approved the submitted version.

## Conflict of interest

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

## Publisher's note



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# Habitat-based radiomics enhances the ability to predict lymphovascular space invasion in cervical cancer: a multi-center study

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**Introduction:** Lymphovascular space invasion (LVSI) is associated with lymph node metastasis and poor prognosis in cervical cancer. In this study, we investigated the potential of radiomics, derived from magnetic resonance (MR) images using habitat analysis, as a non-invasive surrogate biomarker for predicting LVSI in cervical cancer.

**Methods:** This retrospective study included 300 patients with cervical cancer who underwent surgical treatment at two centres (centre 1 = 198 and centre 2 = 102). Using the k-means clustering method, contrast-enhanced T1-weighted imaging (CE-T1WI) images were segmented based on voxel and entropy values, creating sub-regions within the volume ofinterest. Radiomics features were extracted from these sub-regions. Pearson correlation coefficient and least absolute shrinkage and selection operator LASSO) regression methods were used to select features associated with LVSI in cervical cancer. Support vector machine (SVM) model was developed based on the radiomics features extracted from each sub-region in the training cohort.

**Results:** The voxels and entropy values of the CE-T1WI images were clustered into three sub-regions. In the training cohort, the AUCs of the SVM models based on radiomics features derived from the whole tumour, habitat 1, habitat 2, and habitat 3 models were 0.805 (95% confidence interval [CI]: 0.745–0.864), 0.873 (95% CI: 0.824–0.922), 0.869 (95% CI: 0.821–0.917), and 0.870 (95% CI: 0.821–0.920), respectively. Compared with whole tumour model, the predictive performances of habitat 3 model was the highest in the external test cohort (0.780 [95% CI: 0.692–0.869]).

**Conclusions:** The radiomics model based on the tumour sub-regional habitat demonstrated superior predictive performance for an LVSI in cervical cancer than that of radiomics model derived from the whole tumour.

KEYWORDS

**cervical cancer, LVSI, radiomics, habitat, machine learning**

# 1 Introduction

Cervical cancer is one of the most prevalent gynaecological malignancies worldwide, ranking fourth in cancer incidence among women (1). In 2020, approximately 110,000 new cases of cervical cancer were diagnosed in China alone, representing 18% of the new cases of cervical cancer worldwide (2). In some developing countries, the prevalence and mortality rates of cervical cancer surpasses those of breast cancer (3, 4). In cervical cancer, lymphovascular space invasion (LVSI), the infiltration of tumour cells into the blood and lymphatic vessels, is closely associated with lymph node metastasis and serves as an independent risk factor for prognosis (5–7). According to the 2018 International Federation of Gynecology and Obstetrics (FIGO) staging and treatment guidelines, the treatment decision for patients with stage IA1 cervical cancer should take into account the LVSI status. Patients with LVSI-positive lesions should undergo adjuvant chemoradiotherapy or additional radical resection and lymph node dissection surgery to suppress the spread of lymph node micrometastases and improve prognosis (8). Therefore, determining LVSI status is important for making treatment decision, especially in women of childbearing age who wish to preserve fertility.

Considering the high heterogeneity of the malignancies, tumours exhibit diverse microenvironments and microstructures (9–11). Radiomics, which involves extracting numerous features from medical images to classify diseases using machine-learning techniques, offers the potential to deliver personalised medicine in an no-invasive manner. Traditional radiomic analysis typically focuses on the whole tumour and overlooks the sub-regional phenotypic variations within the tumour (12). Recently, a new approach called habitat, which divides tumours into sub-regions by identifying grayscale voxels with comparable imaging characteristics (12, 13), has shown the potential in improving the ability to distinguish between tumour heterogeneity (14–16). In this study, we intended to extract radiomic signatures from different sub-regions of cervical cancer using contrast-enhanced T1-weighted imaging (CE-TIWI) with habitat analysis to decode the LVSI status, thereby facilitating personalised therapeutic decision making.

# 2 Materials and methods

This study was approved by two medical ethics committees that conducted ethical reviews and waived the requirement for obtaining patient consent.

## 2.1 Patient population

We recruited 300 patients with pathologically confirmed cervical cancer, who underwent pelvicmagnetic resonance (MR) imaging within 1 month before surgery and without any anti-tumour therapy before MR. Among them, 198 patients from centre 1 constituted the training cohort, whereas the remaining 102 from centre 2 constituted the external test cohort. We collected and organised two distinct datasets of MRI images from female patients diagnosed with cervical cancer using a picture archiving and communication system. The training cohort comprised 104 LVSI-positive and 94 LVSI-negative patients and the external test cohort comprised 54 LVSI-positive and 48 LVSI-negative patients. We retrospectively analysed clinical data and laboratory indicators, including age, maximum tumour diameter, histological classification, degree of cellular differentiation, FIGO stage, CA125 and CA199 levels, squamous cell carcinoma antigen, and human papillomavirus infection status. The inclusion criteria for the study population were as follows: 1) patients who underwent pelvic MRI before surgery and 2) LVSI confirmed by postoperative pathological examination. The exclusion criteria were as follows: 1) pregnant women; 2) those who underwent cervical conization or loop electrosurgical excision; 3) those who had a history of radiotherapy or chemotherapy before the MRI examination; and 4) those with blurry diagnostic images.

## 2.2 MRI protocols

The scanning protocol and parameters are included in the Supplementary Material. The CE-TIWI images were downloaded from the picture archiving and communication system and transferred to a personal computer. Two radiologists, each with more than 5 years of experience in pelvic diagnosis, segmented the tumours layer-by-layer on the CE-TIWI images using the open-source software ITK-SNAP (version 3.6, www.itk-snap.org) to obtain the volume of interest (VOI) with the aid of diffusion weighted image (DWI). After 1 week, 30 sets of CE-TIWI images were randomly selected, and the outlining process was repeated. Features with intraclass correlation coefficients (ICC) value of<0.75 were retained for screening. Any differences in the outlining process were resolved by a radiologist with over 15 years of experience. The two radiologists were blinded to the patients' pathological diagnoses during the outlining process. A flowchart illustrating this process is presented in Figure 1.

## 2.3 VOI delineation and sub-region clustering

Habitat utilises voxel and entropy values derived from MR images to cluster VOIs into sub-regions (17–19). The voxel counts for each tumour VOI were determined using a traditional method, whereas the entropy values were computed for each layer of the MR images using the following formula:

$$V_{voxel} = \sum_{k=1}^{N_v} V_k$$

$$entropy = -\sum_{i=1}^{N_g} p(i)\log_2(p(i)+\epsilon)$$

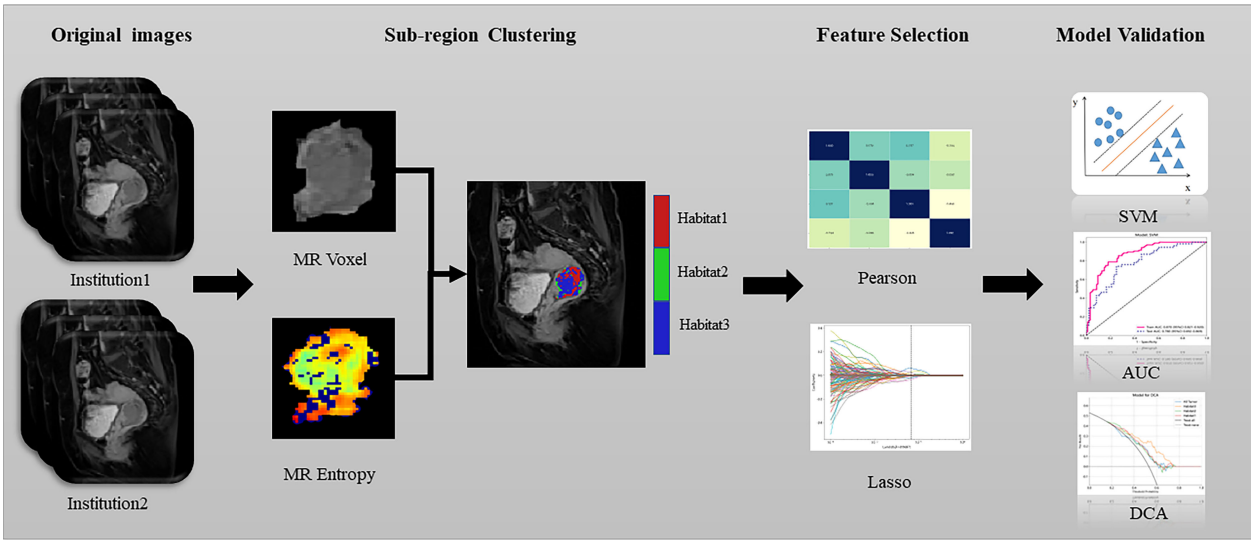


FIGURE 1
Flowchart showing the habitat analysis process.

The k-means method was employed to cluster the VOI regions at the patient level, forming multiple habitats, and the distance correlation between samples was calculated using the Euclidean distance (voxel values and entropy values). The number of habitats was tested from 2 to 10, and the optimal k-value was selected using the Consensus Cluster Plus method, which evaluated the consistency of clustering features by resampling multiple voxels in the cluster 1000 times in 80% of the samples to select the k-value corresponding to a well-separated and stable cluster. The optimal k-value served as the criterion for selecting the optimal number of clusters at the patient population level (Figure 2). The optimal k-value was found to be 3. Using the OnekeyAI platform, we imported each patient's VOI into the platform's components and classified the cervical cancer tumours into three classes named habitat 1, habitat 2, and habitat 3.

## 2.4 Feature selection and model development

To account for differences in imaging features caused by variations in the reconstruction layer thickness and pixel size, the images were resampled to 1×1×3 m^3 and normalized to a grayscale range of 0–255. Features were independently extracted from each of the four habitats, habitat 1, habitat 2, habitat 3 and the whole tumour using the PyRadiomics program package (20), which adheres to the imaging biomarker standardization initiative (21). Before the feature extraction, two filters, wavelet and log-sigma, were implemented to enhance the process, facilitating the extraction of various types of features, including first-order, shape, gray-level co-occurrence matrix, gray-level size zone matrix, gray-level run length matrix, neighbouring gray-tone difference matrix, and gray-level dependence matrix.

First, the features with ICC<0.75 were screened, and imaging histology features of different dimensions were subjected to Z-score processing, normalizing the data to mean of 0 and variance of 1. After normalising all the data, the correlation between features was calculated using the Pearson correlation coefficient. When the correlation exceeded 0.9, only one feature was retained between any two highly correlated features. Finally, the remaining features in the training dataset were filtered using the least absolute shrinkage and selection operator regression model.

A support vector machine (SVM) classification model was developed in the training cohort based on features extracted from habitat 1, habitat 2, habitat 3 and the whole tumour with five-fold cross-validation and finally validated in an external test cohort.

## 2.5 Statistical analysis

Clinical characteristics were compared using the chi-square test or Fisher's exact test for categorical variables and the t-test or Mann–Whitney U test for continuous variables.

The predictive performance of the models for LVSI in cervical cancer was evaluated using the area under curve (AUC) of the receiver operating characteristic curve. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were calculated. The model with the highest AUC was validated using an external test cohort. The generalisation of the model was assessed using the Delong test to compare the predictive performance of the training and test cohorts as well as the calibration curves. Ultimately, net benefit of the model's clinical usefulness was measured using the decision curve analysis. Statistical significance was set at $P< 0.05$.

# 3 Results

## 3.1 Clinical characteristics

Table 1 presents the clinical characteristics of patients with cervical cancer. A total of 300 patients from two centres, with the

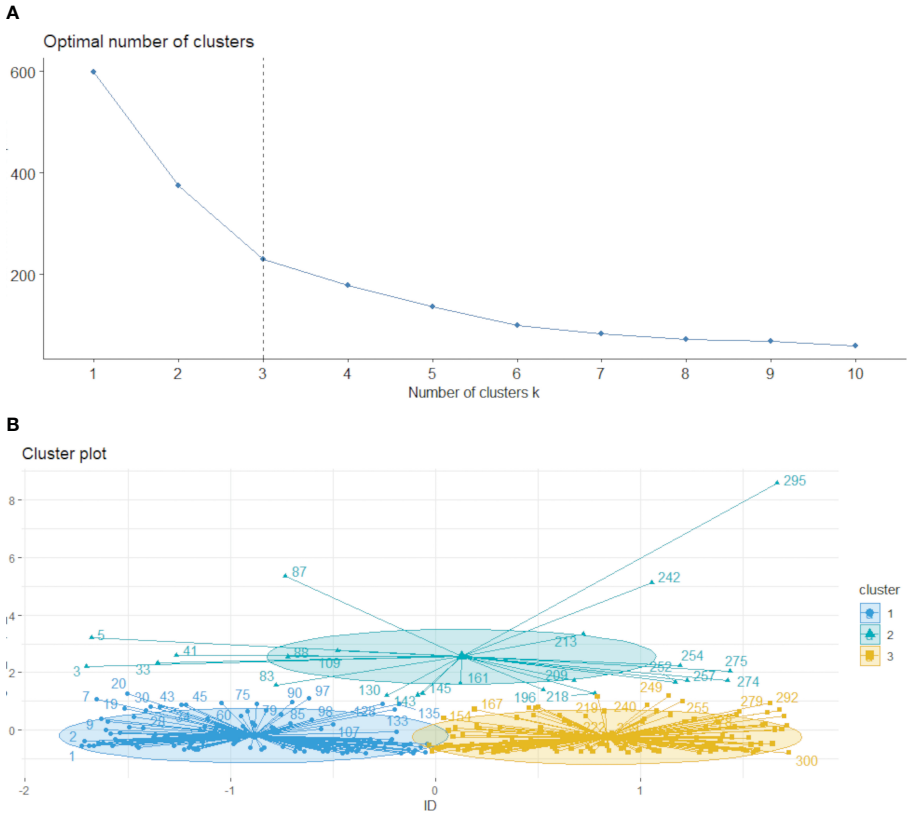


FIGURE 2
Based on the area change under the conditional density function curve. We observed that clustering separation was optimal at a k value of 3 **(A, B)**. This value corresponded to a sharp decrease in the area change under the receiver operating characteristic curve, which suggested that after this k value, further improvements in separability were negligible.

mean age of 51.48 ± 10.63 and 50.35 ± 9.77 years for the training and validation cohorts, respectively, were included in the study. Among them, 161 cases were classified as FIGO stage I, 105 cases as stage II, and 34 cases as stage III. Squamous cell carcinoma was present in 226 cases, adenocarcinoma in 54 cases, and adenosquamous carcinoma in 20 cases. Significant statistical differences were observed in maximum diameter, degree of cellular differentiation, CA125 levels, and FIGO stage within the training cohort. Maximum diameter, CA125 levels, and FIGO stage also demonstrated significant statistical differences within the validation cohort. Other clinical characteristics, including the difference between LVSI+ and LVSI- groups, did not show statistically significant differences in both training and external testing cohorts.

## 3.2 Feature selection

A total of 1016 histological features were extracted from the imaging data based on habitat 1, habitat 2, habitat 3, and the whole tumour. After screening the features using ICC values<0.75, the remaining number of imaging histological features based on habitat 1, habitat 2, habitat 3, and the whole tumour were 713, 617, 692, and 627, respectively. Pearson correlation coefficients were used for filtering, resulting in 190, 148, 170, and 155 features remaining for habitat 1, habitat 2, habitat 3, and the whole tumour, respectively. The remaining imaging histological features of the training cohort were screened using the least absolute shrinkage and selection operator regression method for model building, yielding 19, 18, 19, and 7 best imaging histological features based on habitat 1, habitat 2, habitat 3, and the whole tumour, respectively. These results are presented in the Supplementary Materials.

## 3.3 Performance evaluation of radiomics based on habitat imaging

We developed SVM machine learning models based on the most distinctive imaging histological characteristics of habitat 1, habitat 2, habitat 3, and the whole tumour. The prediction efficiency of each model is summarized in Table 2. Figure 3 illustrates the receiver operating characteristic curves of the SVM machine learning models, with area under the curves (AUCs) of 0.805 (95% confidence interval [CI]: 0.745–0.864), 0.873 (95% CI: 0.824–0.922), 0.869 (95% CI: 0.821–0.917), and 0.870 (95% CI: 0.821–0.920) for habitat 1, habitat 2, habitat 3, and the whole tumour, respectively. The external test cohort had AUCs of 0.629 (95% CI: 0.519–0.739), 0.683 (95% CI: 0.577–0.789), 0.649 (95% CI:

TABLE 1 Characteristics of cervical cancer patients in training and external test cohorts.

| Characteristic | Training cohort | | | Test cohort | | |
|---|---|---|---|---|---|---|
| | LVSI- | LVSI+ | P | LVSI- | LVSI+ | P |
| Age | 51.63 ± 10.84 | 51.35 ± 10.49 | 0.853 | 51.81 ± 8.73 | 49.06 ± 10.52 | 0.156 |
| Maximum diameter | 22.94 ± 11.66 | 34.31 ± 12.75 | <0.001 | 3.14 ± 1.45 | 3.85 ± 1.12 | 0.007 |
| Histological type | | | 0.161 | | | 0.717 |
| Squamous cell carcinoma | 64 (68.09) | 83 (79.81) | | 36 (75.00) | 43 (79.63) | |
| Adenocarcinoma | 24 (25.53) | 16 (15.38) | | 8 (16.67) | 6 (11.11) | |
| Adenosquamous carcinoma | 6 (6.38) | 5 (4.81) | | 4 (8.33) | 5 (9.26) | |
| Degree of cellular differentiation | | | <0.001 | | | 0.737 |
| Low | 10 (10.64) | 21 (20.19) | | 11 (22.92) | 16 (29.63) | |
| Middle | 68 (72.34) | 82 (78.85) | | 23 (47.92) | 23 (42.59) | |
| High | 16 (17.02) | 1 (0.96) | | 14 (29.17) | 15 (27.78) | |
| HPV | | | 0.275 | | | 0.868 |
| Negative | 48 (51.06) | 44 (42.31) | | 15 (31.25) | 15 (27.78) | |
| Positive | 46 (48.94) | 60 (57.69) | | 33 (68.75) | 39 (72.22) | |
| CA125 | | | 0.046 | | | 0.481 |
| ≤35 | 79 (84.04) | 74 (71.15) | | 36 (75.00) | 36 (66.67) | |
| >35 | 15 (15.96) | 30 (28.85) | | 12 (25.00) | 18 (33.33) | |
| CA199 | | | 0.878 | | | 0.456 |
| ≤27 | 75 (79.79) | 81 (77.88) | | 37 (77.08) | 37 (68.52) | |
| >27 | 19 (20.21) | 23 (22.12) | | 11 (22.92) | 17 (31.48) | |
| SCC | | | 0.622 | | | 0.582 |
| ≤1.5 | 44 (46.81) | 44 (42.31) | | 24 (50.00) | 23 (42.59) | |
| >1.5 | 50 (53.19) | 60 (57.69) | | 24 (50.00) | 31 (57.41) | |
| FIGO stage | | | 0.001 | | | 0.04 |
| I | 63 (67.02) | 47 (45.19) | | 29 (60.42) | 22 (40.74) | |
| II | 29 (30.85) | 43 (41.35) | | 15 (31.25) | 18 (33.33) | |
| III | 2 (2.13) | 14 (13.46) | | 4 (8.33) | 14 (25.93) | |

CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19-9; HPV, Human papillomavirus; FIGO, International Federation of Gynecology and Obstetrics; SCC, Squamous cell carcinoma antigen.

0.540–0.757) and 0.780 (95% CI: 0.692–0.869) for habitat 1, habitat 2, habitat 3, and the whole tumour, respectively. The habitat 3 model demonstrated superior performance than that of the whole tumour model in the external test cohort. Figure 4 displays the calibration curves for the training and validation cohorts, showing better calibration for both groups. Figure 5 presents the decision curve analysis curves for the external validation cohort of the model, with significant net gains observed for the habitat 3-based SVM model. Thus, the clinical importance of our model for early cervical cancer diagnosis was highlighted. Figure 6 presents the feature weight map and confusion matrix of the habitat 3 imaging histological model. The Delong test revealed statistically significant differences between habitat 3 and the whole tumour models in both the training and validation cohorts.

## 4 Discussion

In this study, three sub-regions were delineated based on voxel and entropy values from contrast-enhanced T1-weighted imaging (CE-T1WI) of cervical cancer using habitat analysis, which is a heterogeneous metric. The SVM models based on the three habitat sub-regions exhibited a higher predictive performance for LVSI in cervical cancer than those derived from the whole tumour. Notably, the highest AUC of 0.870 (95% CI: 0.821–0.920) was derived from habitat 3, and this performance was robust across different centres (the AUC of the model in the external test cohort was 0.780 (95% CI: 0.692–0.869), and the difference between the training and external test cohorts was not statistical significant. The performance of the models in predicting LVSI was compared, and

TABLE 2 LVSI prediction performance of SVM model.

| Models | Task | AUC | 95% CI | Accuracy | Sensitivity | Specificity | PPV | NPV | P |
|---|---|---|---|---|---|---|---|---|---|
| Habitat1 | train | 0.873 | 0.824 - 0.922 | 0.803 | 0.779 | 0.830 | 0.835 | 0.772 | 0.015 |
| | test | 0.683 | 0.577 - 0.789 | 0.686 | 0.963 | 0.375 | 0.634 | 0.900 | 0.346 |
| Habitat2 | train | 0.869 | 0.821 - 0.917 | 0.798 | 0.913 | 0.670 | 0.754 | 0.875 | 0.023 |
| | test | 0.649 | 0.540 - 0.757 | 0.647 | 0.833 | 0.438 | 0.625 | 0.700 | 0.729 |
| Habitat3 | train | 0.870 | 0.821 - 0.920 | 0.803 | 0.788 | 0.819 | 0.828 | 0.778 | 0.018 |
| | test | 0.780 | 0.692 - 0.869 | 0.745 | 0.741 | 0.750 | 0.769 | 0.720 | 0.006 |
| Whole tumour | train | 0.805 | 0.745 - 0.864 | 0.732 | 0.942 | 0.500 | 0.676 | 0.887 | ref |
| | test | 0.629 | 0.519 - 0.739 | 0.657 | 0.778 | 0.521 | 0.646 | 0.676 | ref |

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.
P values are derived from the DeLong's test of AUCs where AUC of whole tumour is the reference standard for comparison.

we observed that the prediction models built based on habitat 3 outperformed conventional overall tumour model in the training and external test cohorts with an AUC of 0.780 (95% CI: 0.692–0.869). This indicated that the tumour sub-regional radiomics model based on habitat analysis could enhance LVSI prediction in cervical cancer.

Cervical cancer primarily metastasizes through blood or lymphatic vessels to other body tissues (22). Previous studies have indicated that the presence of LVSI implies a higher risk of lymph node metastasis and a greater probability of lymph node micrometastasis when LVSI is positive (23). LVSI is widely recognised as a risk factor for cervical cancer and directly affects the prognosis of patients with cervical cancer (24). The treatment of cervical cancer varies according to the stage and the presence of LVSI in patients with clinical stage IA (8). In the absence of LVSI, cervical conization alone is necessary to avoid radical hysterectomy. Therefore, the preoperative evaluation of LVSI is essential (25–27).

In the final analysis, we included 300 patients. In the training and validation cohorts, the difference between FIGO staging and LVSI status was statistically significant. The probability of LVSI occurrence increased from 42.86% (69/161) in stage IB to 58.1% (61/105) and 82.35% (28/34) in stages II and III, respectively, suggesting a greater that the probability of LVSI occurrence increased progressively with the advancing stage of cervical cancer. In our study, squamous cell carcinoma was present in 226, adenocarcinoma in 54, and adenosquamous carcinoma in 20 patients. The difference between the histological type and LVSI in the training and validation cohorts was not statistically significant, thus indicating that the histological type of cervical cancer did not affect the occurrence of LVSI in patients with cervical cancer (28).

Compared to whole-tumour radiomics, habitat imaging, an approach focused on sub-region imaging omics analysis, offers better quantification of tumour sub-regions that are more relevant to tumour growth or invasiveness (15). Invasive sub-regions have been reported to be important for prognosis and treatment response (29, 30). Fang et al. utilised a variety of tumour habitat features within a radiomics model to predict treatment responses in patients with locally advanced cervical cancer before synchronous chemoradiotherapy (31). The model, consisting of three habitat features derived from multi-parametric MR images, demonstrated good predictive performance with AUCs of 0.820 and 0.798 in the training and test sets, respectively, outperforming a single MR parameter model. Cho et al. derived habitat images from dynamic contrast-enhanced magnetic resonance imaging of breast

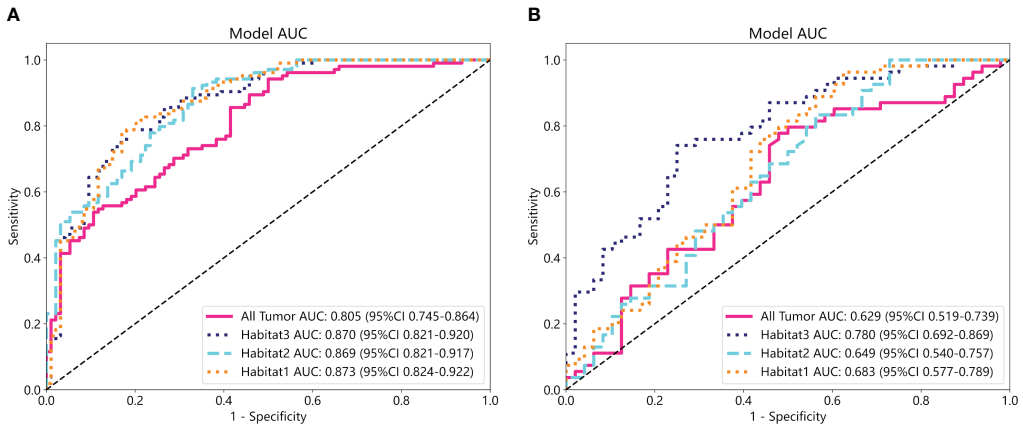


FIGURE 3
The ROC curves of the SVM machine learning models in the training **(A)** and external test cohorts **(B)**.

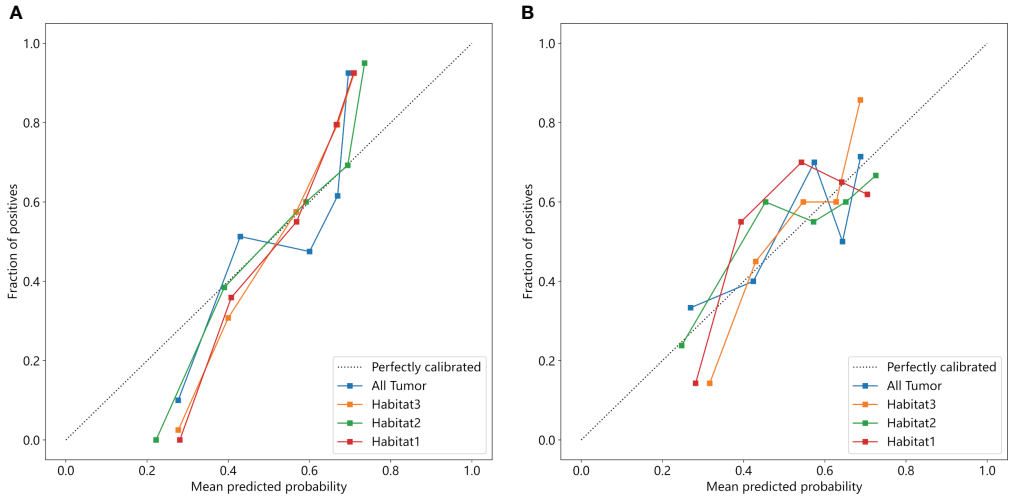


FIGURE 4
The calibration curves in the training **(A)** and external test cohorts **(B)**.

cancer and extracted radiomic features to establish a breast cancer habitat risk score that could accurately categorise patients into high-risk and low-risk groups (32). Choi et al. used multi-parametric MR to extract radiomic features from multiple habitats of the tumour and identified three different subtypes through consistent clustering, revealing different phenotypic subtypes of glioblastoma with clinical and genomic significance. This approach highlights the potential of radiomics as a prognostic biomarker by using multi-habitat imaging (33).

In this study, we employed CE-T1WI images to conduct a clustering analysis, enabling the effective evaluation of blood perfusion in the body by displaying vascular density and perfusion. Additionally, we measured the volume transfer constant, which relied on the permeability of tumour blood vessels (34). This approach provided more discriminatory information for predicting LVSI invasion in cervical cancer. Our prediction results indicated that the radiomics model based on habitat3 outperformed the whole tumour in both the training and external validation sets. The heterogeneous nature of solid tumours suggested that LVSI in cervical cancer might not be distributed uniformly and could exhibit variations at the microscale voxel level. After clustering the image voxels and entropy values, habitat3 was

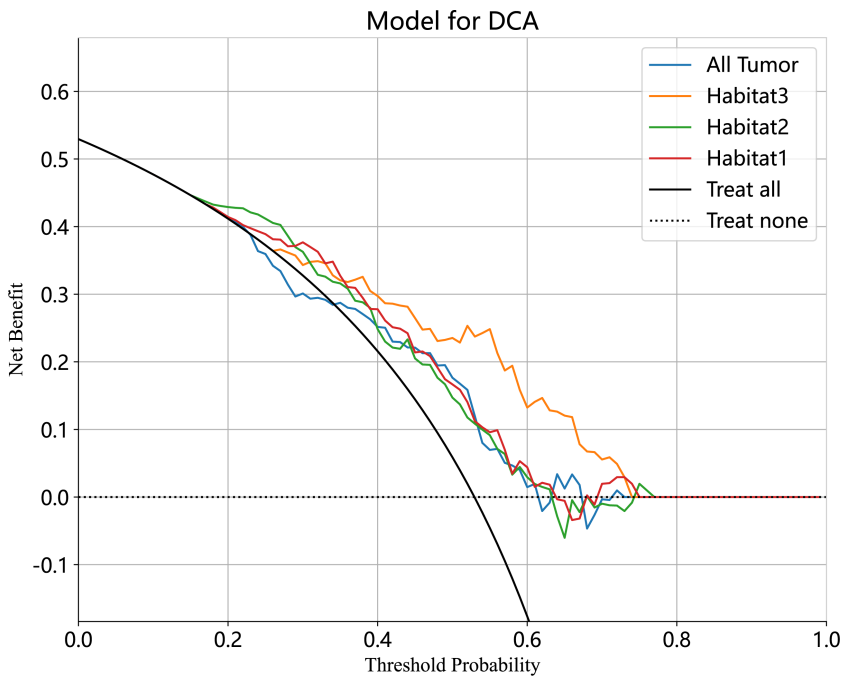


FIGURE 5
The decision curve analyses of the radiomic model in external test cohort. The Habitat3-based SVM model achieved a great net effect.

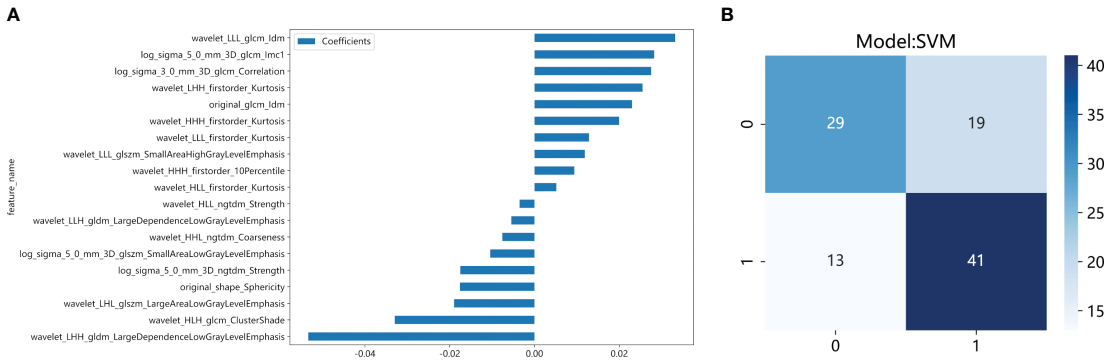


FIGURE 6
The feature weight map **(A)** and confusion matrix **(B)** of the Habitat3 radiomics model.

observed to contain more LVSI information, whereas the whole tumour comprised complete heterogeneous information. Our utilisation of habitat, a novel technology for clustering solid tumours in preoperative imaging and subsequently extracting radiomic features from the clustered tumour sub-regions, helped to avoid the inclusion of irrelevant areas that are not related to LVSI in cervical cancer in the feature extraction process, thereby improving the model's predictive performance.

This study had some limitations. First, although this study included a larger number of patients than that of previous studies, a larger prospective dataset will be required to further improve the model's performance. Second, the diversity in the settings of multi-centre MR devices could have introduced variability in MR images due to differences in equipment and scanning parameters. Thus, we made efforts to standardise and normalise the images as much as possible to eliminate the effect of equipment-related differences.

In conclusion, the sub-region-based approach could predict the LVSI status in cervical cancer demonstrating superior performance over traditional radiomics of the whole tumour, thus making it a promising non-invasive biomarker for predicting preoperative LVSI, especially in patients with cervical cancer. The external test cohort demonstrated the model's stable performance with a strong AUC.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by The Medical Ethical Committee of Shenzhen People's Hospital and Guangzhou Women and Children's Medical Center. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

JG: Conceptualization, Writing-review and editing; SW and XL: Data curation, Formal analysis, Resources, Software and Writing-original draft; XL and YW: Investigation; CJ and YL: Methodology and Validation; XT: Project administration; RW and XZ: Supervision. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1252074/full#supplementary-material

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# Outcomes of fertility preservation treatments in patients with endometrial cancer with different molecular classifications based on an NGS panel

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**Background:** The molecular classification of endometrial cancer has previously been shown to be associated with clinical outcomes. However, there are insufficient data to support the routine use of molecular classification for the treatment of patients seeking fertility preservation.

**Methods:** Here, we retrospectively investigated 90 patients received fertility-sparing treatment. We used a next generation sequencing (NGS) panel to classify these patients into four subtypes. All patients received hormonal therapy combined with hysteroscopy. Therapeutic effects were evaluated by hysteroscopy every three months during the treatment.

**Results:** Patients with POLE mutations had the highest disease progression rate (50.0%, P=0.013), while the microsatellite instability-high (MSI-H) group had the highest recurrence rate (50.0%, P=0.042). *PIK3CA* mutation (hazard ratio (HR): 0.61; 95% confidence interval (CI): 0.37–0.99; P=0.046), overweight (HR: 0.56; 95% CI: 0.32–0.96; P=0.033) and obesity (HR: 0.44; 95% CI: 0.20–0.95; P=0.036) were associated with a significantly lower cumulative complete response (CR) rate. The combination of gonadotropin-releasing hormone analogues (GnRH-a) and letrozole (HR: 3.43; 95% CI: 1.81–6.52; P< 0.001) was associated with a significantly higher cumulative CR rate. *KRAS* mutation was significantly associated with disease progression (P=0.002). In wild-type TP53 patients, *PTEN* and *PIK3CA* mutations significantly prolonged the duration of treatment to achieve CR (log rank P=0.034; P=0.018).

**Conclusion:** The implementation of molecular classification for EC patients undergoing fertility-sparing treatment is promising and can facilitate the selection of appropriate medical regimes to achieve better outcomes in patients with EC who require fertility preservation treatment.

KEYWORDS

endometrial cancer, NGS - next generation sequencing, fertility preservation treatment, molecular classifcation, molecular features

# 1 Introduction

The incidence rates of endometrial cancer (EC) have been rising over recent years, with an estimated 65,950 new cases and 12,550 deaths in the U.S.A in 2022 (1). Early-onset endometrial cancer (EOEC), diagnosed in patients under 50 years-of-age, is relatively uncommon, while recent studies have indicated that the incidence of EC is rising continually among young patients, particularly in women under the age of 45 (2, 3). According to the National Cancer Institute, the incidence rates of EC among women aged 20-34 years and 35-44 years were 1.8% and 5.3%, respectively (4). This implied that the proportion of cases managed by fertility-sparing treatment (FST) is increasing when compared to hysterectomy in young patients with early-stage endometrial cancer. Currently, the majority of FST studies related to EC focus mostly on assessing the treatment effects of various therapies and identifying clinical factors that impact FST outcomes (5–7). However, as research advances, studies of EC are transitioning toward a molecular-based approach.

The molecular classification of EC was first proposed by The Cancer Genome Atlas (TCGA) in 2013, which classified EC into four subtypes based on array and sequence technologies: (1) POLE ultramutated, (2) microsatellite instability hypermutated, (3) copy-number low, and (4) copy-number high (8). Subsequently, clinically applicable molecular classification systems were developed based on immunohistochemistry (IHC) or next generation sequencing (NGS) (9–12). NGS has been established to represent an effective method for the molecular classification of EC and shows high concordance with the final hysterectomy specimens when applied to curettage samples (13). According to NGS molecular classification, EC can be divided into four subtypes: (1) *POLE* mutated (*POLE* mut), (2) microsatellite instability hypermutated (MSI-H), 3. *TP53* wild-type (*TP53* wt), and (4) *TP53* abnormal (*TP53* abn). Both National Comprehensive Cancer Network (NCCN) and ESGO-ESMO-ESTRO have included molecular classification as a consideration to guide treatment strategies for EC patients undergoing surgery (14, 15). In addition, molecular classification has been encouraged in the newest ESGO/ESHRE/ESGE guidelines for EC patients receiving FST (15).

There were only a limited number of studies exploring the relationship between molecular classification and FST for patients with EC. However, these studies reported different outcomes. Some studies suggested that different molecular subtypes responded differently to conservative treatment, and that mismatch repair deficiency (MMR-D) may be associated with a longer treatment duration and a higher risk of recurrence than other subtypes (16, 17). However, another study indicated that molecular classification might not exert prognostic significance for EC patients undergoing FST (18). Consequently, there is an urgent need for further clinical research to confirm the significance of molecular classification for patients with EC undergoing FST.

In this single-center retrospective study, we aimed to evaluate the efficacy of FST among different molecular subtypes in patients with EC. Furthermore, we aimed to identify novel molecular prognostic factors for FST by comprehensively analyzing genomic changes in patients with EC by NGS testing.

# 2 Materials and methods

## 2.1 Study population

In this retrospective study, we investigated all EC patients receiving FST in the Obstetrics and Gynecology Hospital of Fudan University between January 2021 and January 2022. These patients include those who were initially diagnosed with EC and those who progressed to EC during the course of treatment. The study was approved by Ethics Committees of Obstetrics and Gynecology Hospital of Fudan University.

The diagnosis of EC was confirmed by at least two experienced gynecological pathologists according to the World Health Organization (WHO) Pathological Classification of EC (2014). Tissue specimens were obtained by dilation and curettage (D&C) with or without hysteroscopy.

The criteria for inclusion were as follows: (1) aged between 18 and 45 years, (2) a strong desire to preserve fertility, (3) histologically proven endometrial endometrioid carcinoma (EEC) upon initial diagnosis or progressed from atypical endometrial hyperplasia (AEH) during FST, (4) disease limited to the endometrium as observed and no suspicious or metastatic lesions by enhanced magnetic resonance imaging (MRI) or transvaginal ultrasound, (5) non-pregnant state, (6) no contraindication for

progestin treatment, and (7) molecular classification of an endometrial lesion obtained prior to the initiation of treatment. The criteria for exclusion were as follows: (1) a history of local or systemic hormone treatment for more than one month prior to initial evaluation and treatment in our center, (2) specimens had insufficient DNA quality for NGS, and (3) patients transferred to another hospital during the treatment.

## 2.2 Diagnosis and assessment

General information (including age, weight, height) and serum samples were collected prior to any form of treatment. All serum samples were collected and examined in the laboratory at the Obstetrics and Gynecology Hospital, Fudan University.

Body mass index (BMI) was calculated as weight (kg)/height (m2); a BMI ≥25 kg/m$^2$ was considered as overweight while a BMI ≥30 kg/m$^2$ was considered as obesity. According to our previous study (19), we considered a patient to be insulin resistant (IR) when the homeostasis model assessment-insulin resistance (HOMA-IR) was ≥2.95.

## 2.3 Treatment and evaluation

Patients who met the inclusion criteria received comprehensive evaluation, and a multidisciplinary team decided the therapeutic regimens for each patient. All patients received one of the following therapies: (1) oral megestrol acetate (MA) at a dose of 160 mg/d; (2) oral medroxyprogesterone acetate (MPA) at a dose of 250 mg/d; (3) levonorgestrel intrauterine system (LNG-IUS) insertion; (4) oral MA at a dose of 160 mg/d combined with LNG-IUS, or (5) gonadotropin-releasing hormone analogues (GnRH-a) at a dose of 3.75 mg/4w (intra-muscular [i.m.]) combined with oral letrozole at a dose of 2.5 mg/d. Some patients also received oral metformin at a dose of 1500 mg/d or rosuvastatin at a dose of 10 mg/d, depending on their medical complications.

Complete hysteroscopic evaluation was performed every three months during medical treatment to evaluate the efficacy of FST. Endometrial lesions were removed under hysteroscopy, and endometrium biopsy was performed if no obvious lesion was found. The cut-off points for analysis were extended to the 16$^{th}$ and 32$^{nd}$ weeks to account for slight variations in the timing of hysteroscopic evaluations.

The response to hormone treatment was assessed histologically using specimens obtained during each hysteroscopic evaluation. Complete response (CR) was defined as the absence of hyperplasia or cancer. Partial response (PR) was defined as pathological improvement. No response (NR) was defined as the persistence of EC. Progressive disease (PD) was defined as any appearance of disease with a higher degree, such as a higher histological grade, deep myometrial invasion, and/or extrauterine lesions. Recurrence was defined as atypical hyperplasia or carcinoma developing after CR was achieved. Time to CR was measured from the time point at which treatment was initiated to the time point at which CR was diagnosed pathologically by hysteroscopy.

Patients ceased or changed FST if unacceptable side effects occurred any time. Definitive hysterectomy was suggested if NR was evident after 6 months, PR was evident after 9 months, or disease progression occurred at any time during the treatment. For patients who refused hysterectomy, a multiple disciplinary discussion was held, and alternative treatments were considered. Once a patient achieved CR, the same regimen was continued for another 2–3 months for consolidation. Hysteroscopy was performed three months after the first CR. If CR was confirmed, patients were told to prepare for pregnancy or assisted reproductive technology as soon as possible.

## 2.4 Maintenance and follow-up

Low-dose cyclic progestin, oral contraceptives, or the LNG-IUS, were administered to patients without a recent pregnancy plan or after delivery to prevent recurrence. The patient was followed-up every 3 to 6 months. Ultrasound and endometrial biopsy was performed with a Pipelle to allow evaluation of the endometrium. All patients were followed-up until December 2022.

## 2.5 Molecular classification

Using the NGS classification panel, patients were classified into one of four molecular subtypes: (1) *POLE* mut, (2) *MSI-H*, (3) *TP53* wt, and (4) *TP53* abn (13).

## 2.6 Gene sequencing

Genomic DNA (tumor cell content ≥30%) from paraffin-embedded (FFPE) tissue samples was extracted, purified, and quantified using an Endometrial Cancer Molecular Classification Gene Mutation Detection Kit (Xiamen SpaceGen Co., Xiamen, China). DNA sequencing was performed on the NextSeq500 Illumina platform (Illumina Trading (Shanghai) Co., Ltd., Shanghai, China). The sequencing depth was up to 5000X, with an appropriate sensitivity to identify variants with a mutation frequency as low as 1%. We detected a range of genes related to the molecular classification of EC, including *POLE*, *TP53*, *MLH1*, *MSH2*, *PMS2*, *MSH6*, *EPCAM*, *PIK3CA*, *PTEN*, and *KRAS*. The Promega MSI Analysis System (version 1.2) was deployed on Biosystems 3500 and 3500xL Genetic Analyzers (Thermo Fisher Scientific) to identify microsatellite status. This sequencing strategy screened for mutations with a frequency > 1%, and pathological (P)/likely pathological (LP)/uncertain significant (VUS) variants were defined based on the current knowledge of relevant genes and clinical data (20, 21).

## 2.7 IHC analysis

IHC staining was performed on FFPE tissue specimens using a range of monoclonal antibodies: MLH1 (DAKO-ES05), PMS2

(DAKO-EPS1), MSH2 (DAKO-FE11), MSH6 (DAKO-EP49), p53 (DAKO-DO-7), and PTEN (DAKO-6H2.1), and utilizing a Leica Bond Max detection system. We also used two additional antibodies: estrogen receptor (ER) (Novocastra, NCL-ER-6F11) and progesterone receptor (PgR) (Novocastra, NCL-L-PGR-312). To analyze MMR (mismatch repair) proteins, the nuclear positivity of MMR proteins in more than 5% of cancer cells was used as a criterion for intact expression. Normal lymphocytes and/or stromal cells were used as internal positive controls. The overexpression pattern of p53 was defined as diffuse and strong nuclear staining in more than 80% of tumor cell nuclei; when no staining was observed, then we defined a sample as having a complete absence pattern. weak focal positive staining was defined as a wild-type pattern.

## 2.8 Statistical analysis

Continuous variables are given as medians and ranges. Categorical variables are presented as frequencies and percentages. Differences in the descriptive variables between the two groups were analyzed by the Student's t-test or the Mann–Whitney U test, and differences between than two groups were detected by one-way analysis of variance (ANOVA) or the Kruskal–Wallis H test where appropriate. Kaplan-Meier curves were used to estimate and present therapeutic durations and the differences between groups were compared by log-rank tests. The Cox regression model was used to estimate the hazard ratios (HRs) for CR. Statistical significance was considered as a P-value < 0.05 (two-tailed). All statistical analyses were performed in SPSS (version 25.0, IBM, Armonk, NY, USA).

# 3 Results

A study flow chart is presented in Figure 1. A total of 115 EEC patients receiving FST at the Obstetrics and Gynecology Hospital of Fudan University between January 2021 and January 2022 were retrospectively investigated. Overall, 25 cases were excluded, including eight patients who had a history of local or systemic progestin treatment for more than one month, four patients who

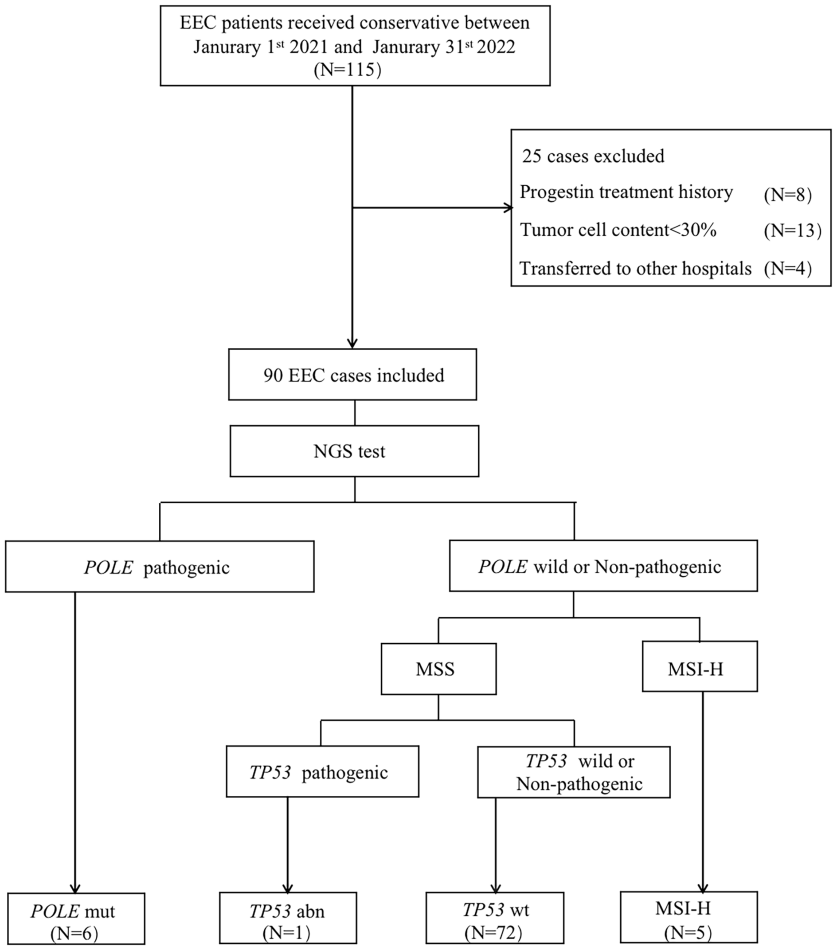


FIGURE 1
Flow chart showing the process used for patient selection. EEC, endometrioid endometrial cancer; NGS, next generation sequencing; *POLE* mut, DNA polymerase epsilon mutation; MSI-H, high microsatellite instability; MSS, microsatellite stable; *TP53* abn, *TP53* abnormal; *TP53* WT, *TP53* wildtype.

transferred to another hospital, and 13 patients whose specimens could not be sequences or had insufficient tumor tissue for DNA extraction. Ultimately, 90 cases were included in this study. Six (6.7%) patients had *POLE* mutation, five (5.6%) patients were classified as MSI-H, 84 (86.7%) patients were classified as *TP53* wt, and one patient (1.1%) was classified as *TP53* abn.

## 3.1 Patient clinical characteristics

The characteristics of the patients are presented in Table 1. In our study cohort, 36 patients were over 30 years-of-age at the time of treatment. Overweight patients accounted for 47.8% of the cohort, while obese patients accounted for 13.3% of the cohort. In total, 40.0% of patients had insulin resistance, and 60.0% had hyperlipidemia. All patients had positive estrogen and progesterone receptor expression prior to the first administration of hormone therapy. Overall, 70.0% of patients in our study cohort received MA as the main FST, while another 20.0% received a combination therapy of GnRH-a and letrozole. When considering the four subgroups, a significant difference was observed for the initial treatment regimens, with the highest proportion of patients in the *TP53* wt group receiving MA or GnRH-a combined with letrozole as the initial treatment (P=0.005). After 16 weeks of treatment, the *TP53* wt group featured 32.0% of patients achieving CR, although no statistically significant difference was observed compared to other subgroups. After 32 weeks of treatment, the CR rates for the four subgroups were as follows: *POLE* mut vs. MSI-H vs. *TP53* wt vs. *TP53* abn: 50.0% (3/6) vs. 60.0% (3/5) vs. 57.7% (45/78) vs. 0 (P=0.881). During follow-up, one patient in the *TP53* abn group did not achieve CR; in contrast, the rates of CR in the other three subgroups all exceeded 80.0%

TABLE 1 Patients' clinical characteristics in fertility-preserving patients (N=90) cohort according to NGS-based molecular classification.

| Variable | Total cohort N=90 (100) | *POLE* mut 6 (6.7) | MSI-H 5 (5.6) | *TP53* wt 78 (86.7) | *TP53* abn 1 (1.1) | P value |
|---|---|---|---|---|---|---|
| Age(years) | 31 (22-42) | 36.5 (26-42) | 36 (30-40) | 30 (22-42) | 33 | 0.107 |
| ≥30 | 36 (40.0) | 4 (66.7) | 5 (100.0) | 44 (56.4) | 1 (100.0) | 0.201 |
| BMI(kg/m$^2$) | 24.8 (15.9-40.9) | 26.3 (16.9-29.7) | 26.4 (22.5-30.0) | 24.52 (15.9-40.9) | 35.4 | 0.345 |
| 25-30 | 43 (47.8) | 4 (66.7) | 2 (40.0) | 25 (32.1) | 0 | 0.200 |
| ≥30 | 12 (13.3) | 0 | 1 (20.0) | 10 (12.8) | 1 (100.0) | |
| IR:N(%) | 36 (40.0) | 2 (33.3) | 2 (40.0) | 31 (39.7) | 1 (100.0) | 0.823 |
| MetS:N(%) | 21 (23.3) | 1 (16.7) | 2 (40.0) | 17 (21.8) | 1 (100.0) | 0.247 |
| PCOS:N(%) | 18 (20.0) | 0 | 0 | 18 (23.1) | 0 | 0.458 |
| Hyperlipidemia:N(%) | 56 (62.2) | 2 (33.3) | 3 (60.0) | 50 (64.1) | 1 (100.0) | 0.402 |
| ER expression:N(%) | | | | | | - |
| Negative | 0 | 0 | 0 | 0 | 0 | |
| Positive | 90 (100.0) | 6 (100.0) | 5 (100.0) | 78 (100.0) | 1 (100.0) | |
| PgR expression:N(%) | | | | | | - |
| Negative | 0 | 0 | 0 | 0 | 0 | |
| Positive | 90 (100.0) | 6 (100.0) | 5 (100.0) | 78 (100.0) | 1 (100.0) | |
| E2(pmol/L) | 154.0 (2.0-1324.0) | 187.0 (12.5-1324.0) | 155.0 (93.0-423.0) | 153.5 (2.0-1241.0) | 202.0 | 0.936 |
| P(nmol/L) | 1.21 (0.01-48.73) | 1.3 (0.06-3.66) | 0.41 (0.07-5.14) | 1.18 (0.01-48.73) | 2.91 | 0.571 |
| T(nmol/L) | 1.45 (0.01-4.86) | 1.2 (0.66-2.19) | 0.62 (0.03-1.95) | 1.49 (0.01-4.86) | 2.55 | 0.385 |
| CA-125(U/ml) | 18.29 (1.01-261.3) | 15.81 (10.66-51.52) | 13.48 (11.0-62.2) | 19.22 (1.01-261.3) | 47.57 | 0.420 |
| HE4(pmol/L) | 45.6 (24.2-281.0) | 47.1 (40.5-81.7) | 45.2 (27.9-55.5) | 45.6 (24.7-281.0) | 24.2 | 0.268 |
| Therapy | | | | | | **0.005** |
| MA | 62 (68.9) | 3 (50.0) | 2 (40.0) | 56 (71.8) | 1 (100.0) | |
| MPA | 2 (2.2) | 1 (16.7) | 1 (20.0) | 0 | 0 | |
| LNG-IUS | 3 (3.3) | 1 (16.7) | 1 (20.0) | 1 (1.3) | 0 | |

*(Continued)*

TABLE 1 Continued

| Variable | Total cohort N=90 (100) | *POLE* mut 6 (6.7) | MSI-H 5 (5.6) | *TP53* wt 78 (86.7) | *TP53* abn 1 (1.1) | P value |
|---|---|---|---|---|---|---|
| MA+LNG-IUS | 4 (4.4) | 0 | 1 (20.0) | 3 (3.8) | 0 | |
| GnRH-a+Letrozole | 19 (21.1) | 1 (16.7) | 0 | 18 (23.1) | 0 | |
| CR rate | | | | | | |
| 16w | 25 (27.8) | 0 | 0 | 25 (32.1) | 0 | 0.173 |
| 32w | 51 (56.7) | 3 (50.0) | 3 (60.0) | 45 (57.7) | 0 | 0.881 |
| Therapy outcomes | | | | | | |
| Overall outcomes | | | | | | |
| CR | 85 (94.4) | 5 (83.3) | 5 (100.0) | 75 (96.2) | 0 | **0.035** |
| Recurrence | 12 (14.1) | 0 | 3 (60.0) | 9 (12.0) | / | **0.037** |
| Progression | 12 (13.3) | 3 (50.0) | 2 (40.0) | 7 (9.0) | 0 | **0.013** |
| Oncological outcomes at 16weeks | | | | | | 0.123 |
| CR | 25 (27.8) | 0 | 0 | 25 (32.1) | 0 | |
| NR | 28 (31.1) | 3 (50.0) | 3 (60.0) | 21 (26.9) | 1 (100.0) | |
| PR | 34 (37.8) | 2 (33.3) | 2 (40.0) | 30 (38.5) | 0 | |
| PD | 3 (3.3) | 1 (16.7) | 0 | 2 (2.7) | 0 | |
| Oncological outcomes at 32weeks | | | | | | 0.325 |
| CR | 56 (62.2) | 4 (66.7) | 3 (60.0) | 49 (62.8) | 0 | |
| NR | 9 (10.0) | 2 (33.3) | 0 | 7 (9.0) | 0 | |
| PR | 22 (24.4) | 0 | 2 (40.0) | 19 (24.4) | 1 (100.0) | |
| PD | 3 (3.3) | 0 | 0 | 3 (3.8) | 0 | |
| Follow-up period(weeks) | 59.8 (19.1-104.0) | 39.4 (26.7-84.3) | 65.7 (49.1-100.4) | 59.9 (19.1-104.0) | 93 | 0.131 |

Values are presented as median (range) or number (%). P-value among different groups was calculated by one-way ANOVA, Kruskal-Wallis, Chi-square, or Fisher's exact test.
BMI, body mass index; IR, insulin resistance; MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; ER, estrogen receptor; PgR, progesterone receptor; E2, Estradiol; P, Progesterone; T, Testosterone; MA, megestrol acetate; MPA, medroxyprogesterone acetate; LNG-IUS, levonorgestrel intrauterine system; GnRH-a, Gonadotropin-releasing hormone analogues; CR, complete response; NR, no response; PR, partial response; PD, progressive disease; *POLE* mut, DNA polymerase epsilon mutation; MSI-H, high microsatellite instability; *TP53* wt, *TP53* wildtype; *TP53* abn, *TP53* abnormal.

(P=0.035). A total of 12 patients experienced disease recurrence after achieving CR, with a recurrence rate of 60.0% observed in the MSI-H group; this was significantly higher than that in the *POLE* mut and *TP53* wt subgroups (P=0.037). During the treatment process, disease progression occurred in 12 patients, featuring 50.0% and 40.0% of patients in the *POLE* mut and MSI-H subgroups, respectively; this compared to only 9% in the *TP53* wt subgroup (P=0.013). The median follow-up period for all patients was 59.8 weeks (range: 19.1-104.0 weeks).

## 3.2 Molecular and tppathological characteristics

The somatic mutation results for all patients in the study cohort are shown in Figure 2. Patients in the MSI-H group were all 30 years-of-age or older. One patient in the MSI-H group experienced recurrence and progression during FST, with the pathological type progressing to grade 3. Two patients were grade 2, both were TP53 wt. During follow-up, a total of five patients failed to achieve CR, one was classified as *POLE* mut, three were classified as *TP53* wt, and one was classified as *TP53* abn. In terms of IHC results, five patients showed a loss of MMR-related protein. All patients exhibited wild-type p53 expression.

When considering our target genes, *PTEN* was the gene with the highest frequency of P/LP mutations; this was detected in 57/90 (63.3%) patients; this was followed by *PIK3CA*, *KRAS*, *POLE*, *MMR*-related, and *TP53*, detected in 35/90 (38.9%), 14/90 (15.6%), 6/90 (6.7%), 5/90 (5.6%) and 2/90 (2.2%) patients, respectively. Fourteen patients did not have any P/LP mutations according to our gene panel. *PIK3CA* and *PTEN* mutations were more frequent in the *POLE* mut group; however, this was not

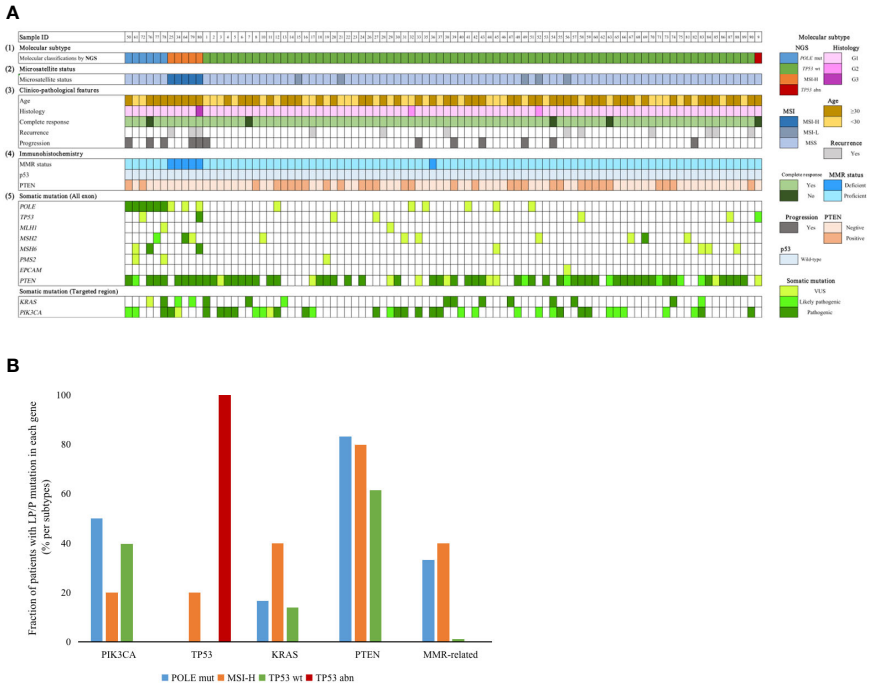


FIGURE 2
**(A)** Clinicopathological factors and mutation profiles in our cohort. (1) Molecular subtype by NGS. (2) Microsatellite status. (3) Clinical factors, CR status, recurrence status and progression status. (4) IHC staining. (5) Mutation profiles of the 90 EEC patients. **(B)** Distribution of different genes with P/LP mutations in the four subgroups. NGS, next generation sequencing; MMR, mismatch repair; *POLE* mut, DNA polymerase epsilon mutation; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stable; *TP53* abn, *TP53* abnormal; *TP53* WT, *TP53* wildtype.

statistically significant. It appeared that the presence of *TP53* P/LP mutations was an unfavorable factor for the outcome of FST. One MSI-H patient, with a concurrent *TP53* pathogenic mutation, experienced disease recurrence and progression after achieving CR, while another patient classified as *TP53*abn did not achieve CR during the follow-up period.

## 3.3 The effects of related factors on treatment outcomes

Table 2 shows the associations between variables and FST outcomes. LP/P *PTEN* mutations significantly reduced the CR rate at 16 weeks of treatment (P=0.002). The CR rate at 32 weeks of treatment decreased significantly with increasing BMI (P=0.004) and insulin resistance (P=0.005). Surprisingly, the combination of GnRH-a and letrozole as the initial treatment resulted in a 100.0% CR rate at 32 weeks (P<0.001); this was significantly higher than any of the other therapies. In our cohort, all patients who progressed had pathological progression, but no evidence of metastasis was found by enhanced MRI. There was a significant difference in disease progression rates among different initial therapies (P=0.011), with lower rates observed in the MA and GnRH-a combined with letrozole groups. At the molecular level, LP/P mutations in *POLE*, *KRAS*, and *MMR*-related genes were significantly associated with disease progression (P=0.013,

TABLE 2 Factors associated with fertility-sparing treatment outcomes.

| Variables | | 16-week CR rate | P value | 32-week CR rate | P value | Progression | P value | Recurrence | P value |
|---|---|---|---|---|---|---|---|---|---|
| Age≥30 | YES | 22.2% (12/54) | 0.150 | 53.7% (29/54) | 0.487 | 16.7% (9/54) | 0.411 | 18.0% (9/50) | 0.362 |
| | NO | 36.1% (13/36) | | 61.1% (22/36) | | 8.3% (3/36) | | 8.6% (3/35) | |
| BMI | <25 | 38.3% (18/47) | 0.066 | 72.3% (34/47) | **0.004** | 10.6% (5/47) | 0.143 | 8.7% (4/46) | 0.161 |
| | 25-30 | 16.1% (5/31) | | 45.2% (14/31) | | 22.6% (7/31) | | 24.1% (7/29) | |
| | ≥30 | 16.7% (2/12) | | 25.0% (3/12) | | 0 | | 10.0% (1/10) | |

*(Continued)*

TABLE 2 Continued

| Variables | | 16-week CR rate | P value | 32-week CR rate | P value | Progression | P value | Recurrence | P value |
|---|---|---|---|---|---|---|---|---|---|
| IR | YES | 16.7% (6/36) | 0.055 | 38.9% (14/36) | **0.005** | 16.7% (6/36) | 0.532 | 12.5% (4/32) | 0.991 |
| | NO | 35.2% (19/54) | | 68.5% (37/54) | | 11.1% (6/54) | | 15.1% (8/53) | |
| MetS | YES | 23.8% (5/21) | 0.643 | 42.9% (9/21) | 0.145 | 19.0% (4/21) | 0.608 | 21.1% (4/19) | 0.541 |
| | NO | 29.0% (20/69) | | 60.9% (42/69) | | 11.6% (8/69) | | 12.1% (8/66) | |
| PCOS | YES | 33.3% (6/18) | 0.556 | 50.0% (9/18) | 0.523 | 5.6% (1/18) | 0.485 | 22.2% (4/18) | 0.465 |
| | NO | 26.4% (19/72) | | 58.3 (42/72) | | 15.3% (11/72) | | 11.9% (8/67) | |
| Hyperlipidemia | YES | 28.6% (16/56) | 0.829 | 57.1% (32/56) | 0.907 | 14.3% (8/56) | 0.983 | 15.1% (8/53) | 0.991 |
| | NO | 26.5% (9/34) | | 55.9% (19/34) | | 11.8% (4/34) | | 12.5% (4/32) | |
| Therapy | MA | 21.0% (13/62) | 0.073 | 43.5% (27/62) | <0.001 | 12.9% (8/62) | **0.011** | 8.6% (5/58) | **0.005** |
| | MPA | 0 | | 50.0% (1/2) | | 50.0% (1/2) | | 0 | |
| | LNG-IUS | 33.3% (1/3) | | 66.7% (1/3) | | 66.7% (2/3) | | 100.0% (2/2) | |
| | MA+LNG-IUS | 25.0% (1/4) | | 50.0% (2/4) | | 25.0% (1/4) | | 50.0% (2/4) | |
| | GnRH-a+Letrozole | 52.6% (10/19) | | 100.0% (19/19) | | 0 | | 15.8% (3/19) | |
| Molecular classification | POLE | 0 | 0.173 | 50.0% (3/6) | 0.881 | 50.0% (3/6) | **0.013** | 0 | **0.037** |
| | MSI-H | 0 | | 60.0% (3/5) | | 40.0% (2/5) | | 60.0% (3/5) | |
| | TP53 wt | 32.1% (25/78) | | 57.7% (45/78) | | 9.0% (7/78) | | 12.0% (9/75) | |
| | TP53 abn | 0 | | 0 | | 0 | | | |
| LP/P somatic mutant | YES | 23.7% (18/76) | 0.090 | 52.6% (40/76) | 0.072 | 15.8% (12/76) | 0.242 | 14.1% (10/71) | 1.000 |
| | NO | 50.0% (7/14) | | 78.6% (11/14) | | 0 | | 14.3% (2/14) | |
| *PTEN* | YES | 16.4% (9/55) | **0.002** | 49.1% (27/55) | 0.069 | 16.4% (9/55) | 0.458 | 11.5% (6/52) | 0.591 |
| | NO | 45.7% (16/35) | | 68.6% (24/35) | | 8.6% (3/35) | | 18.2% (6/33) | |
| *PIK3CA* | YES | 22.9% (8/35) | 0.406 | 48.6% (17/35) | 0.216 | 17.1% (6/35) | 0.596 | 12.1% (4/33) | 0.919 |
| | NO | 30.9% (17/55) | | 61.8% (34/55) | | 10.9% (6/55) | | 15.4% (8/52) | |
| *KRAS* | YES | 21.4% (3/14) | 0.801 | 35.7% (5/14) | 0.085 | 42.9% (6/14) | **0.002** | 16.7% (2/12) | 1.000 |
| | NO | 28.9% (22/76) | | 60.5% (46/76) | | 7.9% (6/76) | | 13.7% (10/73) | |
| MMR-related | YES | 0 | 0.271 | 66.7% (4/6) | 0.932 | 50.0% (3/6) | **0.029** | 20.0% (1/5) | 0.542 |
| | NO | 29.8% (25/84) | | 56.0% (47/84) | | 10.7% (9/84) | | 13.8% (11/80) | |
| *TP53* | YES | 0 | 1.000 | 50.0% (1/2) | 1.000 | 50.0% (1/2) | 0.250 | 100.0% (1/1) | 0.141 |
| | NO | 28.4% (25/88) | | 56.8% (50/88) | | 12.5% (11/88) | | 13.1% (11/84) | |
| Maintenance Therapy | Dydrogesterone | – | | – | | – | | 20.0% (1/5) | **0.002** |
| | LNG-IUS | | | | | | | 27.8% (5/18) | |
| | Diane-35 + metformin | | | | | | | 6.7% (4/60) | |
| | Diane-35 + metformin + LNG-IUS | | | | | | | 100.0% (1/1) | |
| | none | | | | | | | 100.0% (1/1) | |

BMI, body mass index; IR, insulin resistance; MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; MA, megestrol acetate; MPA, medroxyprogesterone acetate; LNG-IUS, levonorgestrel intrauterine system; GnRH-a, Gonadotropin-releasing hormone analogues; LP, likely pathogenic; P, pathogenic; MMR, mismatch repair.
Bold values means the P-value < 0.05, there is a statistical difference.

P=0.002, P=0.029, respectively). In addition, MSI-H patients had a higher recurrence rate after CR (60.0%, P=0.005). There were significant differences in recurrence rates among different initial treatment and maintenance therapies; patients who were treated with MA as the initial treatment and Diane-35 plus metformin as the maintenance treatment after CR had a significantly lower recurrence rate (P=0.005; P=0.008, respectively).

Univariate Cox regression analysis demonstrated that overweight (HR: 0.48; 95% CI: 0.29-0.78; P=0.003), obesity (HR: 0.34; 95% CI: 0.17-0.70; P=0.003) and IR (HR: 0.56; 95% CI: 0.36-0.87; P=0.010) were associated with a lower cumulative CR rate. While GnRH-a combined with letrozole was significantly associated with a higher cumulative CR rate (HR: 4.81; 95% CI: 2.60-8.93; P<0.001) (Figure 3).

We selected factors with P<0.01 in the univariate Cox regression for further multivariate analysis. The adverse effects of overweight (HR: 0.56; 95% CI: 0.32-0.96; P=0.033) and obesity (HR: 0.44; 95% CI: 0.20-0.95; P =0.036) remained significant. It is worth noting that *PIK3CA* mutation was associated with a lower cumulative CR rate after multivariate Cox regression (HR: 0.61; 95% CI: 0.37-0.99; P =0.046). GnRH-a combined with letrozole was still considered a favorable factor for CR (HR: 3.43; 95% CI: 1.81-6.52; P<0.001).

## 3.4 The effects of different molecular mutants on the outcomes of oncological treatment

The treatment durations of the four molecular subtypes are presented in Figure 4A. Following multivariate Cox regression analysis, we found that the initial treatment significantly influenced the CR rate. Thus, our analysis excluded patients who received GnRH-a combined with letrozole as the initial therapy. No significant difference was found in the time to achieve CR when compared across the four subtypes (log rank test; P=0.086); however, we did find that only patients in the *TP53* wt group achieved CR at 24 weeks of treatment. Further analysis of the molecular characteristics of the *TP53* wt group (Figure 4B) showed that patients without targeted gene mutations had a shorter duration of treatment (log rank P=0.014), while *PTEN* and *PIK3CA* mutations prolonged the duration of treatment when conservative therapy was administered (log rank test: P=0.034; log rank test: P=0.018).

## 3.5 Patients with *POLE* mut, MSI-H, and *TP53* abn

Supplementary Table 1 presents the treatment details of patients with *POLE* mut, MSI-H, and *TP53* abn in our study. In our cohort, the P286R mutation accounted for 66.6% of all *POLE* mut cases, with M444K and V411L mutations in the remaining two cases. All five MSI-H patients underwent germline genetic testing, and one patient was diagnosed with Lynch syndrome (LS). Three *POLE* mut and two MSI-H patients changed therapy during treatment. One *POLE* mut patient received hysterectomy due to a failure to respond after nine months of treatment, while another LS patient underwent surgery due to disease recurrence and progression. Histopathological examination showed no deep myometrial invasion, lymphovascular space invasion (LVSI), or metastasis to the ovaries or lymph nodes.

## 3.6 Details of patients who underwent a change in therapeutic regimen

Next, we analyzed patients who changed their therapeutic regimen during treatment following multidisciplinary discussion. A total of 19 patients changed their therapy, with three (50%) in the *POLE* mut subgroup, two (40%) in the MSI-H subgroup, and 14 (17.9%) in the *TP53* wt subgroup, as shown in Supplementary Figure 1. At the final follow-up, 89.5% of patients achieved CR following a change in therapy. Two patients each in the *POLE* mut and *TP53* WT subgroups changed their treatment due to disease progression. Sixteen patients switched to GnRH-a combined with letrozole, while three patients switched to a combination therapy featuring Diane-35

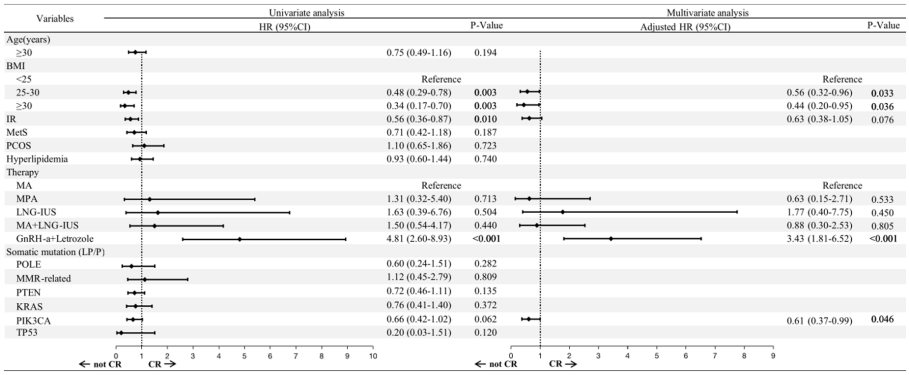


FIGURE 3
Risk factors associated with the FST outcomes, as determined by Cox regression. BMI, body mass index; IR, insulin resistance; MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; MA, megestrol acetate; MPA, medroxyprogesterone acetate; LNG-IUS, levonorgestrel intrauterine system; GnRH-a, Gonadotropin-releasing hormone analogues; LP, likely pathogenic; P, pathogenic; HR, hazard ratio; CI, confidence interval.

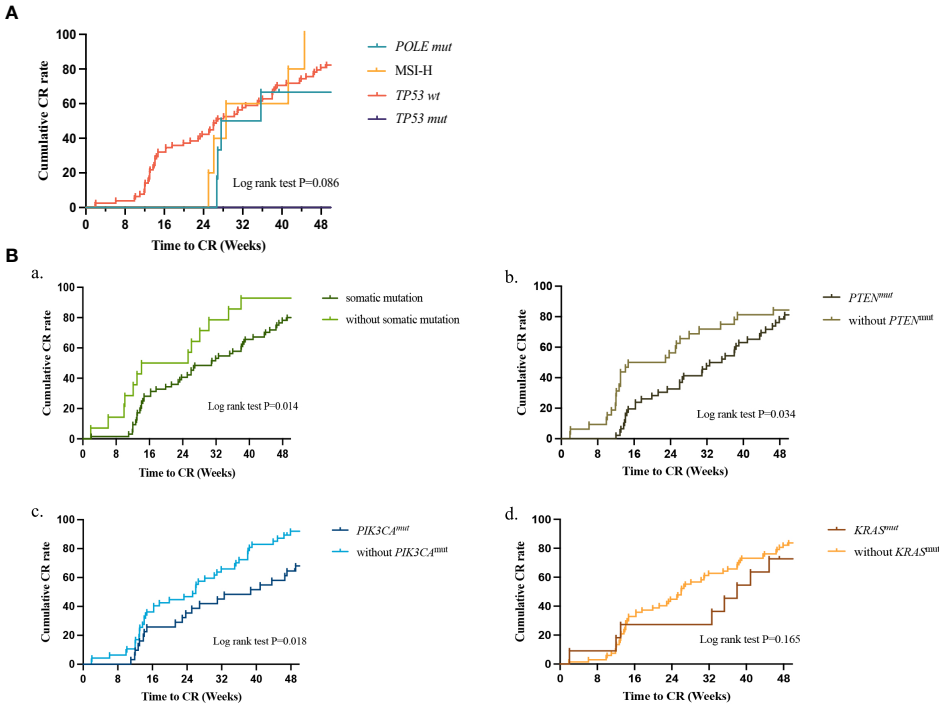


FIGURE 4
**(A)** Treatment duration to CR between different molecular classifications as determined by Kaplan-Meier analysis and compared by the log-rank test. *POLE* mut, DNA polymerase epsilon mutation; MSI-H, high microsatellite instability; *TP53* abn, *TP53* abnormal; *TP53* WT, *TP53* wildtype; CR, complete response. **(B)** Treatment duration to CR between different somatic mutants as determined by Kaplan-Meier analysis and compared by the log-rank test in *TP53* WT. (a) patients with or without somatic mutation; (b) patients with or without *PTEN* mutation; (c) patients with or without *PIK3CA* mutation; (d) patients with or without *KRAS* mutation. CR, complete response.

and metformin. We noticed that two patients still did not achieve CR within the follow-up period after changing therapeutic regime.

# 4 Discussion

## 4.1 Molecular characteristics and the outcomes of conservative treatment

In this study, we demonstrated that molecular classification can be used to predict the prognoses of patients with EC when treated conservatively. Our findings are different from those of previous studies in that patients with *POLE* mutation did not show a better response to progesterone therapy and were instead found to be insensitive to such treatment. Kaplan-Meier survival curve analysis did not reveal a significant difference among the four subgroups; this may have been related to a change of treatment and the small size of the patient cohort. In our study cohort, only one patient in the *POLE* mut group benefited from high-dose progesterone therapy. Furthermore, in the *POLE* mut group, patients with AEH were more likely to progress to EC during treatment; this was in stark contrast to the patients undergoing surgery. By analyzing existing reports, we only found three cases of conservatively treated EC patients with *POLE* mutations, two of whom received oral progesterone therapy but eventually underwent surgical treatment due to disease recurrence or progression; furthermore, only one patient achieved CR without recurrence after six months of treatment with LNG-IUS (16, 22). These data suggested that *POLE* mutation may be one of the unfavorable factors of FST in EC patients. Similar to surgical patients, those with *TP53* mutation were found to be associated with a poor outcome. One patient with EC was classified as a *TP53* abn in our study and did not achieve CR even after 90 weeks of treatment; this patient did not change her therapeutic regime due to irregular follow-up. Another patient with MSI-H and *TP53* mutation experienced disease recurrence and progression to high-grade EEC during treatment. In addition, we found that 60.0% (3/5) of patients in the MSI-H group experienced disease recurrence; this finding was consistent with previously reported research findings (17).

We found that patients in the *POLE* mut and MSI-H subgroups had a high tumor mutation burden (TMB), with the former showing a higher burden. In a recent study, Riggs et al. reported that the high TMB was associated with an increased frequency of *DACH1* gene mutation (23). The *DACH1* gene was positively correlated with progesterone receptor expression (24), thus suggesting that the insensitivity of patients in the *POLE* mut and MSI-H subgroups to progesterone may be related to mutation of the *DACH1* gene. Moreover, the detection of *DACH1* gene mutations can facilitate the identification of appropriate patients for FST. The lower TMB in the MSI-H subgroup when compared to the *POLE* mut subgroup may be correlated with the difference in long-term treatment efficacy between the two groups. Recently, Hu et al. reported that the expression of *PDGFC*, *DIO2*, *SOX9*, and *BCL11A*

was upregulated in progesterone-insensitive endometrial lesions when compared with progesterone-sensitive endometrial lesions, while the expression of *FOXO1*, *IRS2*, *APOE*, *FYN*, and *KLF4* was downregulated, as based on the integrated analysis of ATAC-Seq and RNA-Seq (25). By conducting differential gene enrichment analysis of TCGA data (the analytical data were not presented in this article), we found that the expression of *IRS2* was downregulated in *POLE* mut and MSI-H groups of EC patients compared with the *TP53* wt group. *IRS2* acts downstream of insulin receptor, activating the MAPK and PI3K/AKT pathways and inducing glucose uptake and membrane marker expression (26). Our study suggested that the downregulation of *IRS2* may be one of the reasons for the poor response to progesterone conservation therapy in the *POLE* mut and MSI-H groups.

Further analysis based on NGS results showed that patients with likely pathogenic/pathogenic gene mutations had longer treatment duration to achieve CR compared to those without any mutations, and patients with *PTEN* mutation had significantly lower response rates compared to those without *PTEN* mutation, consistent with our previous study (27). Additionally, our study found that *PIK3CA* mutation is also one of the factors that prolong the treatment duration.

In addition, AEH, a precursor to endometrial cancer, was found concurrent EC in approximately 32.6% of patients (28). However, studies on the molecular characterization of these patients are still limited. In a retrospective analysis conducted by Puechl AM and colleagues, of 37 patients with AEH, one of two (50%) patients with MMR-D demonstrated disease progression, one of four (25%) patients with POLE mutations experienced disease progression, and only two of 27 (7.4%) patients with p53wt demonstrated disease progression (29). Our study confirms these findings, suggesting that AEH patients with POLE mutations and MSI-H are more likely to experience disease progression. However, due to the limited sample size, further studies are essential to explore this patient subgroup in depth, potentially for providing valuable prognostic insights and facilitating the development of more personalized treatment and follow-up strategies based on molecular features.

## 4.2 Molecular characteristics and disease prognoses

For patients undergoing surgery, different molecular subtypes were associated with different prognoses, the *POLE* mut group was associated with a higher 10-year recurrence-free survival (RFS) rate, whereas the p53mut EC patients presented with a higher rate of distant recurrence and lower overall survival (30). In our study, LP/P mutations in *POLE*, *KRAS*, and MMR-related genes were associated with higher rates of disease progression. Following Cox multivariate regression analysis, *PIK3CA* was identified as a risk factor for achieving CR in endometrial cancer. Of the four subtypes, the *TP53* wt group had the largest number of patients. A previous study showed that patients with mutant *KRAS* and wild-type *ARID1A* were associated with a poorer 5-year recurrence-free survival in a *TP53* wt group (31). And our study identified *PTEN* and *PIK3CA* mutations as new molecular markers affecting the outcome of FST in patients with EC. *PTEN*, a tumor suppressor gene, was reported to be mutated in 57–83% of all cases of EC and is the most common molecular event in early endometrial cancer (32). *PTEN* is also a known negative regulator of the PI3K/AKT/mTOR pathway, and studies have reported a significant association between the loss of *PTEN* expression and metastatic disease (33). *PIK3CA* is also associated with disease invasion. For example, Hayes et al. proposed that EC with *PIK3CA* mutation should be considered as having invasive cancer, whereas those without this gene mutation would be candidates for a more conservative approach (34). In our present study, we found a correlation between *PIK3CA* mutation and poorer outcomes following conservative therapy. It has been reported that *KRAS* plays an early role in the progression of EC (30); similarly, our study cohort showed a higher rate of *KRAS* pathogenic mutations in the disease progression group.

Some patients with EC exhibited multiple molecular characteristics; previous studies showed patients with MMR-D and *POLE* mut carrying *TP53* mutations had better prognoses than single *TP53* mutation in surgical patients (35). In our study, one MSI-H patient who carried a pathogenic *TP53* mutation experienced disease progression during treatment; in this case, the histological type progressed to high-grade endometrioid carcinoma. This suggested that closer monitoring should be conducted for patients carrying *TP53* mutation. Currently, the outcomes of conservative treatment for patients with multiple molecular characteristics remain unclear and more clinical data need to be acquired and analyzed.

## 4.3 Other possible factors affecting the efficacy of conservative therapy

Our analyses confirmed the correlation between weight and FST outcome in patients with EC. Overweight and obesity were identified as independent risk factors that affect the duration of treatment in patients undergoing conservative treatment. Previous studies demonstrated a significant correlation between different BMI categories and the outcomes of conservative treatment (7). In our study cohort, the overweight populations in the *POLE* mut and MSI-H groups were higher than that in the *TP53* wt group, although no statistically significant difference was detected. The molecular classification of EC will allow us to focus on the correlation between different molecular characteristics and the outcomes of conservative treatment, and also considered the joint effects of molecular characteristics and metabolism on the outcomes of conservative treatment in patients with EC. The interaction between molecular characteristics and metabolism represents a significant research direction in the future.

## 4.4 The relationship between treatments and the outcomes of conservative therapy

GnRH-a, a gonadotropin-releasing hormone agonist, can suppress the pituitary secretion of gonadotropins, reduce the

secretion of ovarian hormones, inhibit ovarian function, and reduce the circulating levels of estrogen. In recent years, GnRH-a has been reported as an effective FST for patients with endometrial cancer (36, 37). Our analysis demonstrated that in the *POLE* mut subgroup, the use of GnRH-a combined with letrozole as the initial treatment method yielded significantly greater benefits than progesterone. This indirectly confirmed the insensitivity of *POLE* mut to progesterone.

Our study analyzed the prognostic value of molecular classification and other molecular features for patients with EC receiving FST. However, this study also had certain limitations that need to be considered. First, our analysis was limited by its retrospective nature and the use of a single institution database; this may have induced possible bias in the selection of patients. However, detailed data recording and strict adherence to inclusion and exclusion criteria for every AEH or EEC patient were applied throughout the study to avoid selection bias. Therefore, further prospective studies are now required to validate the full impact of molecular classification for patients with EC undergoing FST.

In conclusion, our study demonstrated that the molecular classification of EC represents a useful classification system applicable to patients receiving conservative treatment. However, its guidance for prognoses should be distinguished from that of surgical patients. In addition, we found that somatic pathogenic mutations of some other genes were also associated with the prognoses of conservative treatment, including *PTEN*, *KRAS*, and *PIK3CA*. The findings of our study indicated that molecular classification has the potential to differentiate EC patients with similar histological features but different prognoses, consequently providing direction for personalized therapeutic and monitoring regimens for patients with unique molecular profiles.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by Ethics Committees of Obstetrics and Gynecology Hospital of Fudan University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

YXu: Writing – original draft, Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – review & editing. MZ: Data curation, Formal Analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing, Software. LZ: Data curation, Investigation, Writing – review & editing. BW: Writing – original draft. TW: Data curation, Formal Analysis, Writing – review & editing. YXue: Data curation, Supervision, Validation, Writing – review & editing. ZX: Formal Analysis, Software, Supervision, Writing – review & editing. WS: Methodology, Project administration, Writing – review & editing. XC: Conceptualization, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. CW: Conceptualization, Funding acquisition, Project administration, Resources, Validation, Visualization, Writing – review & editing.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1282356/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Patients changed therapy in the study cohort. *POLE* mut, DNA polymerase epsilon mutation; MSI-H, high microsatellite instability; *TP53* wt, *TP53* wildtype; CR, complete response; PD, progressive disease; NR, No response; PR, partial response; GnRH-a, Gonadotropin-releasing hormone analogues.

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# The research progress on synchronous endometrial and ovarian carcinoma

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Synchronous endometrial and ovarian carcinoma (SEOC) is the most common combination of primary double cancer in the female reproductive system. The etiology and pathogenesis of SEOC remain unclear, and clinically, it is often misdiagnosed as metastatic cancer, affecting the formulation of treatment plans and prognosis for patients. This article provides a review of its epidemiology, pathological and clinical characteristics, risk factors, pathogenesis, diagnosis, treatment, and prognosis.

KEYWORDS

**endometrial neoplasia, ovarian neoplasia, synchronous, risk factor, prognosis**

## 1 Introduction

In the female reproductive system, primary double cancer is relatively rare, accounting for approximately 0.63-1.7% of all malignant tumors in the female reproductive system. Among them, synchronous endometrial and ovarian carcinoma (SEOC) is the most common, accounting for about 40-51.7% (1–3). Primary double cancer is often misdiagnosed as metastatic cancer in clinical practice, making it challenging for both clinicians and pathologists to accurately differentiate between primary and metastatic cases. This differentiation is of crucial importance for clinical management, treatment decisions, and patient prognosis. This article aims to provide a comprehensive review of the clinical and pathological characteristics, types, diagnosis, treatment, and prognosis of SEOC, with the goal of offering guidance for clinical diagnosis, differentiation, and personalized treatment.

## 2 Epidemiology

Synchronous Endometrial and Ovarian Carcinoma (SEOC) is a malignant tumor that occurs simultaneously in the endometrium and ovaries, and it is the most common type of primary double cancer in the female reproductive system. However, previous research reports have shown significant variations in its incidence, which can be attributed to factors such as ethnicity, geographical location, and small sample sizes. For example, Eisner et al. (1) studied

3,863 female reproductive tract cancer patients registered at the University of California, Los Angeles, between 1955 and 1986, and found 26 cases (0.7%) of primary double cancer, including 11 cases of SEOC (0.3%). In another study conducted in Turkey, SEOC accounted for approximately 0.89% (2). Previous research has reported SEOC incidence rates of less than 3% in ovarian cancer patients (4–7) and approximately 3.0-5.5% in endometrial cancer patients (5, 8–12).

## 3 Pathological characteristics

In SEOC, the histological types of tumors in both sites may be the same or different, but the most common type in both sites is endometrioid carcinoma. Other types, such as mucinous, clear cell, and mixed-type tumors, can also occur. It has been reported that endometrioid carcinoma in both sites accounts for about 45.5-86% of cases (11, 13–15). In the study of Zaino et al. (13), 74 patients with SEOC were included, almost 90% (67 patients) of tumors identified in the ovary and 88% (65 patients) of tumors identified in the endometrium were of endometrioid cell type (with or without foci of squamous differentiation), and the proportion of patients with endometrioid histological types at both sites was as high as 86%(64 patients), and most tumors were well differentiated. In 51% (38 patients) of these cases, both endometrial and ovarian tumors were histologically grade 1. In contrast, among epithelial ovarian cancers, endometrioid ovarian carcinoma represents only about 11% of cases (16).

## 4 The pathological diagnosis and differential diagnosis

In 1985, Ulbright and Roth (17) first attempted to use pathological features to differentiate between metastatic cancer and independent double primary cancers. The diagnosis of metastatic cancer was primarily based on the criteria of multiple ovarian nodules, with the following as secondary criteria: small ovaries (< 5 cm), involvement of both ovaries, deep myometrial invasion, vascular invasion, and involvement of the fallopian tube lumen. In 1998, Scully (18) modified and proposed the following clinical-pathological diagnostic criteria based on these criteria: 1, Histological differences between tumors.2, Endometrial tumors with no or only superficial myometrial invasion.3, Endometrial tumors without invasion of vascular spaces.4, No evidence of atypical endometrial hyperplasia.5, Absence of evidence of spread of other endometrial tumors.6, Unilateral ovarian tumor (80-90%). 7, Ovarian tumor located in the parenchyma.8, No invasion of vascular spaces, surface implantation, or primary location at the ovarian hilum.9, Absence of evidence of spread of other ovarian tumors.10, Ovarian endometriosis.11, Different ploidy or DNA index in the tumor (if it's non-diploid). 12, Different molecular genetic or cytogenetic abnormalities in the tumor. Many retrospective studies have confirmed the favorable survival outcomes of patients diagnosed using the Scully criteria for independent primary double cancer. This has led to the continued clinical and pathological use of these criteria to date. In 2018, Yang et al. (19) in a study conducted by the Affiliated Cancer Hospital of Tianjin Medical University in China, refined the Scully criteria and added clinical staging, foming 8 criteria (unilateral/bilateral ovarian involvement, ovarian tumor size, endometrial myometrium infiltration depth, lymphovascular infiltration status, involvement of other sites, endometriosis of ovary, atypical endometrial hyperplasia, and tumor staging), and gave each standard a value, making it a standard and regrouping 52 patients with SEOC. Then, they found that the new criteria can better distinguish metastatic cancer from primary double cancer compared with Scully standard. However, due to the small sample size and no control group in the study, this scoring standard was not widely used.

Previous studies have used some molecular detection techniques, including X chromosome inactivation pattern, gene mutation detection (P53, K-Ras, PTEN, PIK 3CA, POLE and CTNNB 1), immunohistochemistry, vimentin expression, loss of heterozygosity, microsatellite instability, etc.to assist in the diagnosis and differentiation of SEOC (20–25). The study conducted by Dirk Brinkmann et al. (26) in 2004, involving 62 SEOC patients, revealed that the genetic analysis of allelic loss and microsatellite instability demonstrated a consistency of only 53% with histological diagnosis. This highlights the discordance between genetic and histopathological diagnoses and underscores the limitations of histological diagnosis in SEOC cases. In 2008, Ramus, S. J. et al. (25) conducted a study involving 90 SEOC patients, where a combination of histological and genetic analysis was used. Out of the 88 patients with clear diagnoses, genetic analysis was able to diagnose 64 patients who might have been missed by relying solely on histology. This demonstrates the effectiveness of genetic analysis in distinguishing between primary double cancer and metastatic cancer cases. In recent years, with the rapid advancement of second-generation gene sequencing technology, sequencing analyses based on this technology have proposed a molecular clonality association in the vast majority of SEOC cases, indicating that most SEOCs are the result of primary tumors accompanied by metastasis. These molecular clonality studies help to gain a deeper understanding of the pathological mechanisms and origins of SEOC (27–29). In a study conducted by Michael S. Anglesio et al. (27), 18 SEOC cases were analyzed to investigate the relationship between endometrial and ovarian components. Utilizing targeted sequencing and whole exome sequencing, 17 cases showed clonal relationships, indicating primary tumors with metastasis. This included 10 out of 11 cases that were classified according to clinical pathological standards as primary double cancers. In another study, 23 cases of SEOC were collected and analyzed, all of which had endometrioid carcinoma as pathological type, 15 of which were classified as independent primary tumors by clinicopathology, 5/23 were analyzed by whole exome sequencing, and the remaining 18 were analyzed by large-scale parallel sequencing.Targeting 341 (n=4) or 410 (n=14) key cancer genes, the results showed that 22 sporadic SEOC were associated with cloning (28). On this basis, the concept of "microenvironment confinement" of metastasis has been

proposed in SEOC. This means that tumor cells in SEOC have the capability to detach from the primary lesion without undergoing apoptosis, spread spatially, and only re-locate within exclusive microenvironments without extensive metastasis. This distinguishes SEOC from endometrial or ovarian cancer, which often metastasize widely through lymphatic, hematogenous, or implantation routes. This phenomenon is also associated with the favorable prognosis observed in SEOC cases (27). While the studies mentioned above have provided evidence of clonality in SEOC, it's important to note that due to the limitations of markers and detection methods, they may not comprehensively assess or qualify the clonality. Additionally, these studies may not definitively determine the clone's origin and the direction of tumor metastasis, considering the potential heterogeneity within tumors. While the direction of metastasis is not definitively established, it is most likely that it occurs from the endometrium to the ovaries (30–32). Some research also suggests that the ovaries may be the preferred site of metastasis for tumors originating from various body parts (33). In a multicenter retrospective study conducted by Iacobelli, V. et al. (31) in 2020, it was found that the molecular characteristics of SEOC patients exhibited a remarkable similarity to the molecular profile of the uterine endometrial carcinoma tumor set from The Cancer Genome Atlas (TCGA) in 2013. This suggests that the endometrium might be the primary source of these cases rather than the ovaries. In clinical practice, when endometrial cancer spreads to the ovaries, it often presents as the invasion of the ovarian surface by multiple small tumor nodules and infiltration of vascular spaces.In contrast, primary endometrial tumors typically exhibit invasion into the deep layers of the uterine muscle and often extend into the fallopian tubes (34). Indeed, the clinical presentation of endometrial cancer spreading to the ovaries, as described, can be contradictory to Scully's diagnostic criteria for distinguishing between primary double cancer and metastatic cancer. This highlights the complexity and challenges in accurately diagnosing and differentiating SEOC cases, as various factors and criteria may need to be considered for a comprehensive assessment. While the research mentioned points toward an endometrial origin for SEOC, it's important to note that further extensive clinical studies are needed to provide definitive insights. This clarification is crucial for guiding surgical and subsequent adjuvant treatments to improve the prognosis and survival outcomes of SEOC patients.

# 5 Pathogenesis

As of now, the exact etiology and pathogenesis of SEOC remain unclear. Some researchers have proposed that the higher occurrence rate of SEOC compared to isolated endometrial or ovarian cancer may be due to the shared presence of several common risk factors, such as infertility and low parity (35). SEOC is more common in young, infertile and premenopausal women, indicating that the role of estrogen in the occurrence and development of SEOC is worthy of further investigation (14). Furthermore, the theory of embryonic origin supports the occurrence of SEOC (36–38). It suggests that the epithelial tissues of the cervix, uterus, fallopian tubes, and ovaries all originate from the Müllerian duct system. When these tissues are exposed to the same carcinogenic factors, it can lead to the independent development of multiple primary tumors. This further emphasizes the possibility that SEOC may involve the independent development of multiple primary tumors. Although endometrioid ovarian carcinoma represents a minority within epithelial ovarian cancers, it can account for up to 88% in SEOC (13). While most ovarian cancers occur at the fimbrial end of the fallopian tube, this location doesn't explain all ovarian tumors, and some of these tumors likely originate from components of the secondary Müllerian duct system (36).

# 6 Risk factors

## 6.1 Age

Previous research has shown that SEOC is common among young, premenopausal women (12). In the study by Soliman, P. T. et al. (14). The median age at SEOC diagnosis was 50 years, with over 50% of cases occurring in premenopausal women. This is about 10 years younger than the median age of onset for single endometrial or ovarian cancer (37). Similar findings were observed in the research by Kobayashi, Y. et al. (39). These results suggest a potential association between SEOC incidence and female hormonal factors.

## 6.2 Infertility and parity

Research reports indicate that over 50% of SEOC patients suffer from infertility (39). In another retrospective study involving SEOC patients under the age of 40, the proportion of infertility patients reached as high as 81% (9). Infertility and low parity are recognized risk factors for both endometrial and ovarian cancers (40). In the study by Herrinton, L. J. et al. (35), SEOC was more likely to occur in infertility and low parity. However, the precise relationship and underlying mechanisms require further research for a comprehensive understanding.

## 6.3 Obesity

Previous studies have found that SEOC patients are more common in obese women (14, 41). Obesity is a risk factor for the development of endometrial cancer (42). This is due to the excessive peripheral conversion of androgens to estrogens in adipose tissue, leading to a high estrogen state in the body, which can increase the risk of developing endometrial cancer. It has also been found that women who are overweight or obese during adolescence or young adulthood have an increased risk of ovarian cancer compared to women with a moderate body mass index (18.5-24.9 kg/m2) (43). More studies are needed to determine whether obesity is related to the occurrence of SEOC.

## 6.4 Genetic factors

It is well known that some cases of endometrial cancer or ovarian cancer are associated with Lynch syndrome, also known as hereditary non-polyposis colorectal cancer syndrome (HNPCC). Lynch syndrome is an autosomal dominant genetic disorder caused by mutations in mismatch repair (MMR) genes. Women who carry MMR gene mutations have an increased risk of developing gynecological cancers, with a 43% risk of developing endometrial cancer and a 12% risk of developing ovarian cancer. This risk is more common in premenopausal women, with the peak incidence occurring between the ages of 45 and 55 (44). In patients with HNPCC, the occurrence of multiple primary cancers is not uncommon. In the clinical management and assessment of SEOC patients, consideration should also be given to their genetic susceptibility, especially in young premenopausal women. In the study conducted by Soliman PT. et al. (45), using IHC analysis and MSI testing in SEOC patients, it was found that 7% of patients had clinical or molecular criteria suggestive of Lynch syndrome. All of these patients had a history of HNPCC or a first-degree relative with the disease. In another study involving 32 SEOC patients with characteristic analysis of MMR proteins, the results suggested that most SEOC cases are not caused by hereditary cancer due to germline mutations (39). Although these research findings suggest that only a minority of SEOC patients have a history of HNPCC and a family history related to genetics, MMR gene mutation testing holds significant importance in clinical decision-making for SEOC patients who are young and wish to preserve their fertility. In the future, more large-scale prospective studies are needed to confirm its impact on patient treatment and prognosis.

# 7 Clinical diagnosis

Synchronous endometrial and ovarian carcinoma (SEOC) is often challenging to clinically diagnose. It can be mistaken for metastatic cancer, impacting treatment decisions and prognosis. Diagnosis typically involves a combination of clinical evaluation, imaging studies, and pathological examination of tissue samples from both the endometrium and ovaries. An accurate diagnosis is crucial for guiding treatment and improving patient outcomes.

## 7.1 Clinical manifestations and signs

SEOC patients lack specific clinical symptoms and signs. Compared to early-stage primary ovarian cancer, which is often asymptomatic, most SEOC cases benefit from the typical symptoms of endometrial cancer in the early stages, namely abnormal vaginal bleeding. Approximately 90% of endometrial cancer patients experience symptoms of abnormal vaginal bleeding, sometimes accompanied by uterine pus accumulation. The most common symptom in SEOC patients, as indicated by the majority of research findings, is abnormal vaginal bleeding, followed by abdominal pain, abdominal distension, and pelvic abdominal masses (5, 14, 39, 41). In advanced stages, SEOC can also manifest as ascites, cachexia, and compressive symptoms. These symptoms and signs are not necessarily specific indicators of SEOC, as they can also be related to other gynecological issues.

## 7.2 Serological markers

The value of CA125 in preoperative detection of endometrial or ovarian cancer is limited. In most cases of early-stage ovarian cancer or endometrial cancer, CA125 levels may not be elevated. It is primarily used clinically for disease monitoring and treatment assessment (46). In a study by Jain, V. et al. (41), a retrospective analysis of preoperative CA125 levels in SEOC patients showed that approximately 80% of patients had elevated CA125 levels with a median level of 150 IU/ml. Another study retrospectively analyzed 347 patients with epithelial ovarian cancer involving the uterus and found that about 82.8% of patients had CA125 levels greater than 100 IU/ml (47). This suggests that the specificity and sensitivity of CA125 as a preoperative diagnostic tool in SEOC patients need further research.

## 7.3 Imaging examination

Imaging examinations can play a significant role in the clinical diagnosis of SEOC. Commonly used imaging tests include ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI).

### 7.3.1 Ultrasound examination

Transvaginal ultrasound is often more sensitive and helps identify abnormalities in the ovaries and endometrium. Color Doppler ultrasound can provide information about tumor blood supply. In a retrospective observational study from Italy in 2019, the ultrasound characteristics of SEOC patients were compared to those of patients with ovarian metastases from endometrial cancer. The ovarian masses in SEOC patients showed unilateral multilocular or solid masses, while the ovarian masses in the metastatic group were mostly bilateral solid masses. Endometrial lesions in the synchronous group presented more often with no myometrial infiltration and less often with a multiple-vessel pattern on color Doppler compared with the endometrial lesions in the metastasis group. These differences in morphological features may aid in preoperative identification of the two types of cancer (48).

### 7.3.2 Computed tomography (CT)

CT scans helps evaluate lymph node enlargement and the extent of spread within the abdominal cavity. For advanced-stage endometrial cancer, It can provide information about extrapelvic metastasis (46).

### 7.3.3 Magnetic resonance imaging (MRI)

MRI can be used for assessing the depth of myometrial invasion, involvement of the cervical stroma, and lymph node metastasis in endometrial cancer cases (46).

# 8 Treatment

Due to the low incidence of SEOC and the difficulty in establishing a preoperative diagnosis, along with the limitations of retrospective studies with small sample sizes, there is currently no unified standard or consensus for the treatment of SEOC. However, some studies suggest that for early-stage and low-grade SEOC patients, surgical treatment alone may lead to favorable outcomes (5, 49, 50). While there is no standardized treatment approach at present, these research findings provide valuable insights into SEOC treatment. Clinicians need to tailor individualized treatment strategies based on each patient's specific circumstances and pathological diagnosis. Furthermore, as further research and clinical practice progress, we may gain a better understanding of how to effectively manage SEOC. Clinical physicians can develop specific surgical plans based on the surgical standards for endometrial cancer or ovarian cancer, as well as the individual circumstances of the patient. After surgery, doctors will assess the risk factors to determine whether adjuvant treatment is necessary and develop specific adjuvant radiotherapy or chemotherapy plans as needed. This personalized treatment strategy can better meet the needs of patients, improve treatment effectiveness, and prognosis.

## 8.1 Surgical treatment

The preferred treatment for SEOC is surgery. The standard surgical approach involves staging surgery, which includes total hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymph node dissection, and omentectomy (5, 38). In a multi-center retrospective study from Turkey, which analyzed 63 cases of SEOC, it was proposed that the initial surgical standard for SEOC is optimal cytoreduction surgery (51). Some previous studies have suggested that lymph node dissection can improve the survival prognosis of SEOC patients through retrospective analysis, and they recommend lymph node dissection for all SEOC patients (52, 53). In primary endometrial or ovarian cancer, whether systemic lymphadenectomy improves survival is controversial (16, 46, 54).

Since SEOC is difficult to diagnose preoperatively, there may be cases where the surgical scope is insufficient. To date, there is no specific research to guide whether additional surgery or adjuvant treatment is needed after a clear pathological diagnosis following surgery. However, it may be determined based on the histological type and grading of the tissue, similar to the approach for single uterine or ovarian cancer. For example, if a surgery is performed preoperatively assuming endometrial cancer, postoperative comprehensive staging surgery and maximal cytoreductive surgery should be conducted, taking into account the histological subtypes of ovarian cancer. Afterward, adjuvant chemotherapy and maintenance treatment can be further determined (55).

## 8.2 Fertility preservation therapy

At present, fertility preservation therapy has been widely carried out in early stage and low-grade endometrial cancer or ovarian cancer for young women with fertility requirements, but there are few studies on fertility preservation therapy in patients with SEOC. In 2005, Morice, P. et al. (56) proposed that for patients with early stage and low-grade endometrial cancer who wanted conservative treatment, laparoscopic surgery should be performed to explore the adnexal and pelvic conditions to rule out extrauterine disease. Conversely, a multicenter retrospective study from Korean found that only 21 (4.5%) of 471 patients under 40 years of age with early endometrial cancer concurrent ovarian malignancy, and no synchronous cancer was found in low-risk endometrial cancer, so they do not advocate diagnostic laparoscopy in patients with early stage endometrial cancer who wish to be treated conservatively (9). In addition, study has also been reported two young patients with endometrioid borderline ovarian cancer whose final histopathological examination confirmed the diagnosis of invasive uterine cancer, suggesting that curettage is an essential way to rule out primary endometrial cancer before planned fertility-sparing surgery (57). The author believes that fertility preservation treatment for young SEOC patients needs multidisciplinary comprehensive evaluation, such as assisted reproductive technology, obstetrics, genetic counseling, etc., and the risk of subsequent disease progression and follow-up requirements of fertility preservation function should be fully informed. Existing studies have not mentioned such issues much, and more relevant studies are needed to guide clinical practice in the future.

## 8.3 Adjuvant therapy

Whether adjuvant therapy is necessary after surgery for SEOC is still a subject of debate. According to the study by Yoneoka et al. (58), SEOC patients with lesions limited to the uterine body and adnexa have a lower risk of recurrence and may not require adjuvant treatment. For patients with advanced stage, high-grade, poorly differentiated SEOC, and residual tumor tissue, aggressive adjuvant therapy is recommended (5, 53). Specific adjuvant treatment regimens can be tailored based on the adjuvant treatment methods used for endometrial cancer or ovarian cancer. For high-intermediate risk (HIR) subgroups in endometrial cancer, postoperative adjuvant radiotherapy is recommended as it can significantly reduce the risk of recurrence (46, 59). The HIR subgroup is defined by the Gynecologic Oncology Group (GOG) and the Post-Operative Radiation Therapy in Endometrial Cancer (PORTEC) for endometrial cancer. The definition of HIR in the PORTEC criteria includes the following two criteria: age greater than 60 years, grade 3 disease, or ≥50% myometrial invasion (MI). On the other hand, the GOG defines HIR based on a combination of age and the number of risk factors, including tumor grade2-3,

lymphovascular space invasion (LVSI), or involvement of the outer third of the MI. For patients aged at least 70 years, one risk factor is required. For patients aged at least 50 years, two risk factors are required, and for patients under 50 years of age, all three risk factors are needed. In early ovarian cancer, adjuvant chemotherapy after successful tumor cell reduction surgery has not been shown to improve survival outcomes; However, in advanced ovarian cancer, a first-line chemotherapy regimen is typically recommended, which often consists of a combination of platinum-based drugs (such as cisplatin or carboplatin) and paclitaxel (Taxol). This combination therapy is widely recognized and used in clinical practice to enhance treatment efficacy (16).

# 9 Survival and prognosis

## 9.1 Survival outcome

The survival outcomes of SEOC are favorable, with reported 5-year survival rates ranging from 83% to 85.9% and 10-year survival rates ranging from 80.3% to 96% (13, 60). In comparison, stage III endometrial cancer has a 5-year overall survival rate of approximately 57% to 66% (42), while stage II endometrioid ovarian cancer has a survival rate of around 82% (61). SEOC confined to the ovaries and uterine corpus has a favorable prognosis, which may be associated with the prevalence of early-stage, low-grade, and endometrioid histology tumors in both locations (62, 63). In a study by Matsuo, K. et al. (63) a retrospective analysis compared the survival rates between stage I endometrial cancer and stage I SEOC with tumors in both locations being of endometrioid histology. The study found that the survival outcomes were similar between the two groups. Some studies suggest that SEOC patients with tumors in both locations being of endometrioid histology have better survival rates compared to patients with non-endometrioid histology (14, 25, 41). On the contrary, there are also studies that indicate no significant difference in survival rates between patients with endometrioid and non-endometrioid histology (64).

## 9.2 Prognosis factors

### 9.2.1 Age and menopausal state

Age is an independent prognostic factor for SEOC patients (52, 64). A study in Italy analyzing 46 SEOC patients found that age affects patient prognosis, the 5-year survival rate for patients under 50 years old was 94.1%, while for those over 50 years old, it was 53.7% (64). Compared to premenopausal SEOC patients, postmenopausal patients have an increased risk of recurrence (52).

### 9.2.2 CA125

CA125 is widely used in the postoperative follow-up and monitoring of ovarian cancer. An elevated CA125 level has also been shown to be a predictive factor for poor prognosis in endometrial cancer patients (65). There are also studies reporting the impact of preoperative CA-125 levels on prognosis. In a multicenter retrospective study conducted in South Korea in 2014, patients with normal CA-125 levels had significantly better progression-free survival (PFS) and overall survival (OS) compared to patients with elevated CA-125 levels (11). The diagnostic value of preoperative CA125 levels in SEOC requires further research to confirm.

### 9.2.3 Lymphovascular space invasion

Lymphovascular space invasion (LVSI) refers to the presence of tumor cells within the capillary lumens of the lymphatic or microvascular drainage system of the primary tumor. A retrospective study from India analyzed 43 SEOC patients using a COX regression model in multivariate analysis and found that the presence of LVSI in both sites of the tumor is an independent prognostic factor for survival (41). In endometrial cancer patients, LVSI is an independent prognostic factor for lymphatic metastasis and distant recurrence, indicating an adverse survival outcome (66). LVSI is also an independent predictor of progression and survival in early-stage primary epithelial ovarian cancer patients (67).

### 9.2.4 Tumor histological grade

Most studies have confirmed that SEOC is more common in early stage and low-grade tumors. Whether it's endometrial cancer or ovarian cancer, a lower degree of tumor differentiation is typically associated with a worse prognosis. Research indicates a significant correlation between the histological grade of endometrial cancer and recurrence in SEOC patients (52). SEOC patients with high-grade lesions in both sites have a higher recurrence rate and significantly worse prognosis compared to those with low-grade lesions (13, 41, 64).

### 9.2.5 Tumer stage

Some studies suggest that the staging of ovarian cancer in SEOC is a factor influencing its recurrence and prognosis (5, 11, 41, 52). In the study by Song, T. al (11)., they analyzed the 5-year progression-free survival (PFS) and overall survival (OS) of 123 SEOC patients. They found that staging significantly influenced the prognosis of ovarian cancer (PFS P = 0.019, OS P = 0.003), but it did not have a significant impact on endometrial cancer (PFS P = 0.534, OS P = 0.651). This suggests that patients with ovarian cancer in stages II-IV have a higher risk of recurrence and poorer prognosis.

Bese et al. (52) analyzed and compared 13 patients with recurrent SEOC and 18 patients without recurrent SEOC, and found that omental metastases were present in 10 patients (77%) in the recurrent group, indicating that omental metastases were significantly correlated with recurrent SEOC.

A study from Japan in 2019 found that single-factor and multi-factor analyses showed that cervical stromal invasion had a significant impact on PFS (Progression-Free Survival) and OS (Overall Survival) (58). Based on this research, it was suggested that prognostic factors for SEOC (double cancer) patients might be different from those of endometrial or ovarian cancer patients. However, further research is needed to validate this finding and gain

a better understanding of the survival prognosis and related factors for SEOC.

There is controversy regarding whether lymph node metastasis affects the prognosis of SEOC patients. Some studies have shown that lymph node metastasis does not significantly affect the survival rate of SEOC patients (52). In the study by Turashvili et al. (68) a multifactorial analysis demonstrated an association between lymph node involvement (hazard ratio (HR) = 2.38, 95% CI 1.13-5.02, p = 0.023) and worse progression-free survival (PFS). This requires further research to better understand the impact of lymph node involvement on SEOC patients.

### 9.2.6 Residual lesion

Recurrence of SEOC was significantly correlated with residual lesions after surgery. In the Bese, T. study, 8 out of 31 patients with SEOC had residual tumors, and 7 of them had recurrence (52). If the initial surgery did not include staging or achieve satisfactory debulking, the necessity of a second surgery should be considered. It is also advisable to consider more aggressive adjuvant therapy to improve survival outcomes and prognosis. The size of residual lesions after the initial surgery is an independent prognostic factor in ovarian cancer, with smaller residual lesions associated with a better prognosis. However, there have been no similar studies in SEOC, and further research is needed to confirm this in clinical practice.

### 9.2.7 TP53 mutation

A multicenter retrospective study from the Netherlands in 2020 analyzed the molecular characteristics of SEOC patients and compared them with TCGA profiles. They found that SEOC patients had an enrichment of PTEN and CTNNB1 mutations and fewer TP53 mutations compared to cases with metastatic tumors. TP53 mutations are considered an independent predictor of poor prognosis. It is recommended to assess the TP53 mutation status in these patients using methods such as NGS (Next-Generation Sequencing) or immunohistochemistry. This can help stratify the risk in these patients for the consideration of systemic adjuvant therapy (31).

## 10 Conclusion

SEOC is clinically rare, characterized by early stages, low grade, and favorable prognosis. Accurate diagnosis and differentiation are of great significance for its management, treatment, and prognosis. Due to the difficulty of preoperative and intraoperative clinical diagnosis, reliance on postoperative pathological examination is necessary for diagnosis and differentiation, posing a significant challenge for clinical physicians in devising personalized diagnostic and treatment plans. With the continuous development of new technologies like genetic sequencing, there is hope for improved diagnostic accuracy, which in turn can aid in enhancing patient prognosis.

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# Rapid progression from complete molar pregnancy to post-molar gestational trophoblastic neoplasia: a rare case report and literature review

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**Background:** Post-molar gestational trophoblastic neoplasia (pGTN) develops in about 15% to 20% of complete hydatidiform mole (CMH). Commonly, pGTN is diagnosed based on hCG monitoring following the molar evacuation. To date, no detailed information is available on how fast can pGTN develop from CHM. However, the concurrence of CHM and pGTN is extremely rare.

**Case presentation:** A 29-year-old woman presented to the gynecology department with irregular vaginal bleeding and an elevated hCG serum level. Both ultrasound and MRI showed heterogeneous mass in uterine cavity and myometrium. Suction evacuation was performed and histologic examination of the evacuated specimen confirmed complete hydatidiform mole. Repeated ultrasound showed significant enlargement of the myometrium mass one week after the evacuation. pGTN with prognostic score of 4 was then diagnosed and multi-agent chemotherapy regimen implemented with a good prognosis.

**Conclusion:** In rare cases, CMH can rapidly progress into pGTN. Imaging in combination with hCG surveillance seems to play a vital role guiding timely diagnosis and treatment in the specific condition. Low-risk gestational trophoblastic neoplasia (GTN) should be managed stratified according to the individual situation.

KEYWORDS

**gestational trophoblastic disease, gestational trophoblastic tumor, hydatidiform mole, complete hydatidiform mole, ultrasound**

**Abbreviations:** GTN, Gestational trophoblastic neoplasia; pGTN, Post-molar gestational trophoblastic neoplasia; CMH, Complete hydatidiform mole; AVM, Arteriovenous malformation.

## Introduction

Post-molar gestational trophoblastic neoplasia, which is referred to as pGTN, is defined as the malignant change of hydatidiform mole, including invasive mole and choriocarcinoma (1). pGTN is signified by a plateaued or rising serum hCG concentration post-evacuation (2). Hydatidiform mole can be classified as complete or partial based on differences in morphology, karyotype, and malignant potential. Studies showed that the incidence of developing pGTN is approximately 15~20% from complete hydatidiform moles (CHMs) and less than 1~5% from partial hydatidiform moles (1, 3). Generally, a diagnosis of pGTN is made by hCG monitoring post-evacuation of the uterine molar tissue. The FIGO criteria for diagnosis of pGTN including: the plateau of hCG lasts for four measurements over a period of 3 weeks or longer; a rise in hCG for three consecutive weekly measurements over a period of 2 weeks or longer; histological evidence of choriocarcinoma (4). Although outcomes for most GTN arising from molar pregnancies are excellent, a few women die from the disease, mainly because of late diagnosis or drug resistance. Timely diagnosis and management of this disorder is of great importance, which contributes to the application of chemotherapy in-time and the reduction of severe complications and deaths.

To date, there is no detailed information regarding how fast malignant transformation occurs after molar pregnancy. Following a molar evacuation, studies reported that either an invasive mole develops after an average of 6 months in contrast to the development of a choriocarcinoma after an average of 13 months (4, 5). The risk of developing any pGTN from CHM within 1 month is low, only isolated cases had been reported (6–8). Here, we report an extremely rare case of a simultaneous presentation of CMH and pGTN, which may be misdiagnosed with choriocarcinoma arising from previous term delivery. Imaging played a vital role in the diagnosis of the case instead of usual hCG surveillance after molar tissue evacuation. Detailed imaging of the mass was provided, specifically the pelvic ultrasound images accompanying by uterine arteriovenous fistula. The patient was successfully cured by multi-agent combination chemotherapy regime and uterine arteriovenous fistula gradually disappeared during the course. This case makes us aware of the heterogeneous presentations of pGTN and highlights the following points: 1. CHM and pGTN can concurrently occur. In this rare condition, conventional hCG monitoring criteria fails to detect the pGTN timely. Imaging plays a crucial role in identifying and guiding the management of this rare condition. 2. GTN should be treated according to the individual situation, a part of low-risk GTN may need to be administered with multi-agent chemotherapy. 3. Uterine arteriovenous fistula can occur simultaneously with GTN, which may indicate an underlying association between the two disorders.

## Case presentation

A 29-year-old girl, G1P1, presenting with amenorrhea for 2 months, irregular vaginal bleeding for 1 month and elevated serum hCG level (83,962IU/L), was admitted to the gynecology department. The antecedent pregnancy of this patient was a full-term cesarean delivery 7 years ago. An ultrasound examination revealed a mixed echogenic mass measuring 3.2*4.5*3.9 cm in the uterine cavity, partially invading the myometrium, with honeycomb like internal echoes (Figure 1A), and color Doppler showed that it was filled with abundant blood flow signals (Figure 1B), and the spectrum of arteriovenous fistula was measured in pulsed-wave Doppler (Figure 1C). Meanwhile, the pelvic MRI scan depicted heterogeneous mass measuring 3.9*4.0cm in uterine cavity with hemorrhage and invasion of the myometrium (Figure 1D). There were no abnormal findings on gynecological examination, chest CT scan and other image examinations.

The patient's diagnosis caused controversy amongst the medical team. Someone considered it as GTN (choriocarcinoma) originating from the previous full-term pregnancy on the basis of the fact that GTN can emerge in many years or decades after a previous pregnancy. The patient, who is currently presented with abnormal vaginal bleeding, an elevated hCG level and positive images suggesting myometrial invasion, had an antecedent full-term cesarean section 7 years ago. While others disagreed with the above suspected diagnosis. They viewed it as a new onset of pregnancy which quickly progressed to a GTN, although the appearance was very rare. Because abnormal lesions occurred simultaneously in the uterine cavity and myometrium. In order to confirm the origin of the myometrial invasive mass, suction evacuation was performed after the discussion of the medical group. Histologic examination showed moderate to severe trophoblast proliferation accompanied with edematous villi (Figure 2A) and immunohistochemical staining identified p57 negative (Figure 2B), which indicated a complete molar pregnancy. The patient was reevaluated one week post evacuation. Although the hCG decreased to 26,444IU/L, ultrasound suggested honeycomb like mass filled with abundant fire-sea-like blood flow signals was seen within the uterine fundus measuring 5.3*4.8*2.7cm (Figure 3A). Additionally, pulsed-wave Doppler detected a high-speed, low-resistance arteriovenous fistula spectrum. (Figure 3B). Hence, pGTN was diagnosed as FIGO stage I with the prognostic score of 4(2 points for pre-treatment hCG concentrations, 2 points for the largest tumor mass diameter).

According to 2000 FIGO staging, a risk score of 6 and below is classified as low risk. Despite the patient's prognostic score being low risk, EMA/CO (etoposide, methotrexate, and dactinomycin alternating with cyclophosphamide and vincristine) multi-agent chemotherapy regimen was selected in order to avoid single-agent chemotherapy resistance, taking into account the patient's extensive uterine invasion, the high hCG level prior to chemotherapy, and the highly malignant potential of the tumor. The patient's HCG decreased drastically to 716.3 IU/L after the first chemotherapy course, to 8.9 IU/L after the second course, and was normalized after the third course. Additional 2 courses of chemotherapy were given to minimize the risk of recurrence. The last course of additional chemotherapy was replaced by methotrexate mono-chemotherapy due to severe bone marrow suppression caused by multi-agent chemotherapy. Ultrasound after each chemotherapy course showed gradual regression of the mass, with complete disappearance of the lesion and the arteriovenous fistula at the last chemotherapy course. The woman was followed up for one year and her hCG level was normal with regular periods.

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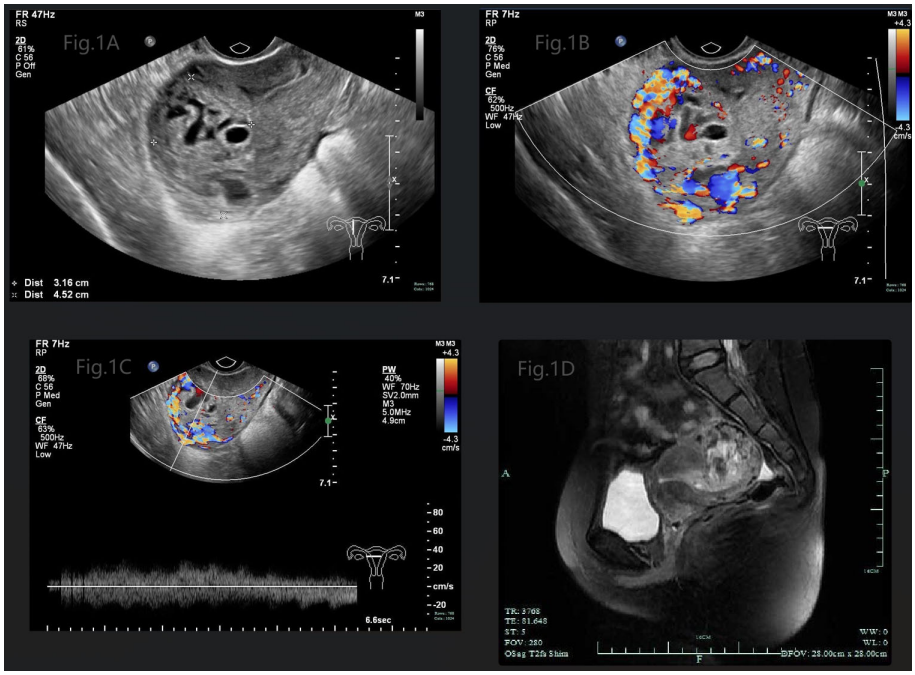


FIGURE 1
**(A)** Gray-scale pelvic sonography displayed a mixed echogenic mass in the uterine cavity, partially invading the myometrium, with honeycomb like internal echoes. **(B)** Color Doppler showed that the mass was filled with abundant blood flow signals. **(C)** Spectrum of arteriovenous fistula was measured in pulsed-wave Doppler. **(D)** Pelvic MRI scan depicted heterogeneous mass in uterine cavity with hemorrhage, invading to the myometrium.

## Discussion

GTN most commonly follows a molar pregnancy but may develop after any other type of pregnancy. The varied presentations of GTN can be irregular vaginal bleeding, an enlarged and irregular uterus, bilateral ovarian enlargement, or even asymptomatic (2). pGTN (usually invasive mole, occasionally choriocarcinoma) most commonly occurs following evacuation of CHM and is usually clinically diagnosed based on a plateaued or rising hCG concentration after evacuation of mole tissues. hCG is the first effective biomarker employed in the diagnosis and follow-up of the GTN (9). During post-molar follow-up, pGTN is diagnosed by a rising serum hCG levels ≥10% for 2 consecutive weeks or a plateau in serum hCG levels for 3 consecutive weeks (3), without histologic verification. In general, pGTN diagnosis can be timely confirmed if the patient complies with the hCG monitoring protocol after expulsion of uterine molar tissues. Delayed diagnosis of GTN can lead to life-threatening complications and raise the risk

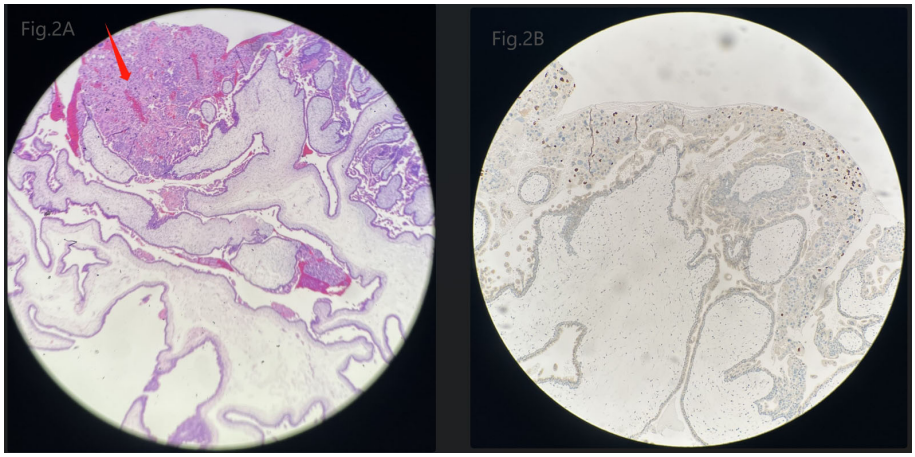


FIGURE 2
**(A)** Microscopic image of the evacuated mole tissues. Arrow points to moderate to severe trophoblast proliferation. **(B)** Immunohistochemical staining of the evacuated mole tissues identified a negative stain of p57.

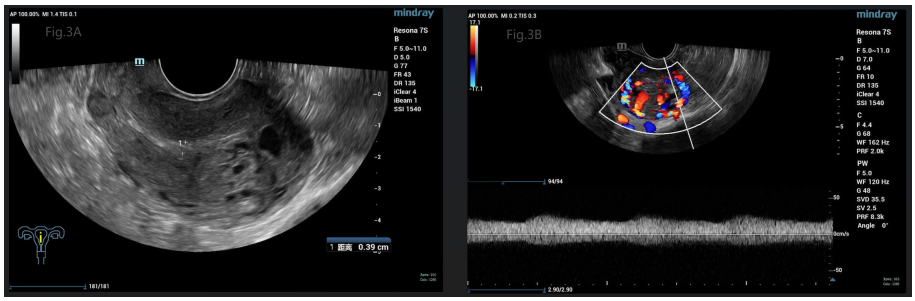


FIGURE 3
**(A)** Gray-scale pelvic sonography showed a large honeycomb like mass within the uterine fundus, close to the serosa. **(B)** Pulsed-wave Doppler detected a high-speed, low-resistance arteriovenous fistula spectrum.

of surgical intervention (10). Therefore, early diagnosis and timely implementation of chemotherapy are very important for the prognosis of GTN patients. In rare cases, pGTN can progress rapidly, presenting before the diagnosis of hydatidiform mole is confirmed, as in this case report.

Thus far, the pathogenesis of GTN still remains unknown and etiologic risk factors that contribute to the development of GTN are unclear. There are several factors that are known to influence the development of GTN from CMH including age >40 years, serum hCG>100,000 IU/mL, excessive uterine enlargement, and/or theca lutein cysts larger than 6 cm (1, 11). Prophylactic chemotherapy may reduce the incidence of pGTN for patients with above high-risk factors (1). Currently, there are increasing studies attempting to explore genetic and molecular biomarkers to predict pGTN. A cohort study demonstrated that heterozygous CHM had a higher potential for GTN than homozygous CHM (12). Braga et al. reported that the malignant transformation of CHM is closely linked to the apoptotic index, and this may be a useful biomarker to predict pGTN (13).

The wide availability of first trimester ultrasound can detect the suspicious molar pregnancy. Classic ultrasound findings of CHM include a heterogeneous uterine mass with cystic spaces (snowstorm appearance), no identifiable fetus or embryo, and no amniotic fluid. Sensitivity of pelvic ultrasound in diagnosing CMH ranges from 70% to 90%, and increase with gestational age (14–16). There is limited data on ultrasound morphologic features of gestational trophoblastic neoplasia. Pelvic ultrasound can determine the tumor burden and vascularity of the uterus and is usually conducted before treatment due to its availability and simplicity. Epstein et al. reported that the majority of uterine pGTN lesions were focal in the myometrium, with moderate to rich vascularization, which is in accordance with this case (17). Another fascinating result that was found is that tumor size larger than 4 cm was an independent predictor of methotrexate mono-chemotherapy resistance for GTN (17). This finding may support the choice of multi-agent chemotherapy in this case. The study of Lin et al. revealed that abnormal myometrial vascularization and lower uterine artery Doppler indices were correlated with GTN and lower uterine artery Doppler indices were associated with methotrexate resistance (18). Thus, ultrasound can be used both in the initial assessment in women with GTN, and to indicate the prognosis of GTN. It is worth noting that GTN lesions can only be detected by ultrasound at relatively high levels of hCG but may not be visualized at lower levels of hCG (19). In this condition, a lower uterine artery pulsatility index; presence of myometrial nodules within the myometrium or endometrium; or increased signal with power Doppler within the myometrium or endometrium may be predictive of GTN development (20). Magnetic resonance imaging, as a complementary investigation to Doppler ultrasound, is better in accessing tumor extension (21).

Although the first-line therapy for patients with low-risk GTN is single-agent chemotherapy, there are many studies that support further stratified management of low-risk GTN due to the challenge of single-agent chemotherapy resistance. A UK study showed that single-agent chemotherapy cure rate in low-risk GTN was significantly negatively correlated with the risk score, with a 45% cure rate in patients with a score of 4 (22). A US study reported that GTN patients with prognostic scores of 2-4 had a 2.02(P=0.027)times higher risk of single-agent chemotherapy resistance than those with scores of 0-2, and the risk of resistance with pretreatment hCG ≥10,000 IU/L was 2.62(P=0.002)times higher than those with less than 10,000IU/L (23). A study in China revealed that high risk factors for single-agent chemotherapy resistance in patients with low-risk GTN included a pre-chemotherapy hCG ≥ 4000IU/L, the presence of invasive lesions in the uterine corpus, and a FIGO prognostic score ≥ 5 (24). In this case, pre-chemotherapy hCG was 26,444 IU/L, the mass was extensive in the uterine corpus, and the malignancy progressed rapidly, taking these factors in to consideration, a multi-agent chemotherapy regimen was implemented and the efficacy was satisfactory.

Uterine arteriovenous malformation (AVM) following GTN is a rare condition. AVM can trigger chronic vaginal bleeding or life-threatening heavy bleeding, which can occur even after the complete regression of GTN after chemotherapy (25). The formation of uterine AVM in GTN is associated with a disorganized trophoblastic proliferation, increased angiogenesis caused by high levels of hCG, finally uterine curettage (26). The proliferation of trophoblastic tissue may destroy blood vessel walls and connect arteries and veins, thereby facilitating the formation of uterine AVM (27). In this case, the uterine AVM was detected by ultrasound at the patient's initial visit, with mild abnormal vaginal bleeding and disappeared at the fifth chemotherapy course. Because

of the rarity of uterine AVM and GTN coexisting, detailed information and related ultrasound images are included in this case presentation. More studies are expected to elaborate the role of uterine AVM in the diagnosis and management of GTN.

In summary, this case reported an unusual occurrence presenting with concurrence of CMH and pGTN. Despite being diagnosed with low-risk GTN, the patient received a combination chemotherapy regimen with EMA/CO due to the stratified management of low-risk patients. At the same time, this patient had a concomitant AVM, which is a very rare condition. Enlightenments from this case are revealed as following: 1. Although pGTN usually diagnosed weeks to months post evacuation of molar tissues by hCG monitoring, in rare cases, rapid progression from molar pregnancy to pGTN can occur just as this case. This case suggests that the usual hCG monitoring protocol for diagnosis of pGTN is unfavorable to the early diagnosis and management in this specific condition. Imaging, especially sonography, in combination with hCG monitoring, plays a key role in the diagnosis and treatment of this concern. 2. Low-risk GTN should be treated individualized. Multi-agent chemotherapy may be more favorable in low-risk GTN with a large tumor size, high hCG level, low uterine artery Doppler indices and high FIGO prognostic score. But further studies are expected to explore the concrete parameter. 3. AVM can occur simultaneously with GTN, which may indicate an underlying association between the two disorders.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by the ethics committee of Hangzhou First people's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

JQ: Resources, Writing – original draft. KG: Writing – review & editing. LS: Methodology, Writing – review & editing.

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## Conflict of interest

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

## Publisher's note



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# First-line monodrug chemotherapy in low-risk gestational trophoblastic neoplasia: a network meta-analysis

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**Objective:** The efficacy of the first-line monodrug chemotherapy has been generally established for low-risk GTN. Most patients can achieve a complete response after the first-line monodrug chemotherapy. However, which monodrug chemotherapy regimen is better for individual patients with GTN is not yet certain. This study aimed to assess the efficacy of first-line monodrug chemotherapy in low-risk gestational trophoblastic neoplasia (GTN).

**Method:** Databases, including PubMed, Embase, Web of Science, and Cochrane Library, were searched from inception to November 1, 2022, for case–control studies on first-line monodrug chemotherapy in GTN. Network meta-analysis was performed to compare the efficacy outcome of six monodrug chemotherapy regimens in GTN, with a complete response rate as the endpoint.

**Result:** Twenty-four studies were considered eligible, including 9 randomized controlled trials (RCTs) and 15 non-RCTs. A total of 3344 patients with low-risk GTN were involved. Six monodrug chemotherapy regimens were included and analyzed. In descending order of efficacy, these six regimens were VP-16 (5 days), ACT-D (5 days), MTX (5 days), ACT-D (1.25 mg/m$^2$), MTX (8 days), and MTX (30–50 mg/m$^2$) in all study, and five regimens were ACT-D (5 days), MTX (5 days), ACT-D (1.25 mg/m$^2$), MTX (8 days), and MTX (30–50 mg/m$^2$) in RCT.

**Conclusion:** Among the six first-line monodrug chemotherapy regimens for low-risk GTN in all study, VP-16 (5 days) was the best in terms of efficacy. And five regimens in RCT, ACT-D was the best. However, the finding needs to be validated through more high-quality clinical studies.

KEYWORDS

**first-line chemotherapy, low-risk gestational trophoblastic neoplasia, monodrug, network meta-analysis, GTN**

# 1 Introduction

Gestational trophoblastic neoplasia (GTN) is a rare malignancy originating from placental trophoblasts. Despite the high metastatic potential and lethal risk, GTN is associated with a response rate as high as 90% under most situations (1, 2). The International Federation of Gynecology and Obstetrics (FIGO)/World Health Organization (WHO) prognosis scoring system (2000) classifies GTN into low risk (≤6 points), high risk (>6–12 points), and ultra high risk (≥13 points), for which stratified treatment is recommended.

The FIGO 2021 guidelines (3) recommend monodrug chemotherapy for low-risk GTN and combination chemotherapy for high-risk GTN. For the former, the commonly used first-line agents are methotrexate (MTX) and actinomycin D (ACT-D). However, which monodrug or chemotherapy regimen is the best for individual patients has not yet been established. An intramuscular injection of MTX is a convenient and widely used MTX dosing regimen due to the prevalence of day care wards and family doctors in foreign countries. In China, textbooks recommend the 5-fluorouracil (5-FU)/floxuridine regimen. Even today, some Chinese grassroots-level hospitals, or even grade-3 first-class hospitals, are still using this regimen, although it has already been removed from the 2015 FIGO guidelines (4). The reason is that 5-FU is usually given for a long period, causing obvious adverse and toxic effects, for example, severe bone marrow suppression and ulceration of the intestinal mucosa, further leading to diarrhea. In some serious cases, pseudomembranous colitis induced by *Staphylococcus aureus* may even occur, leading to death. Seven monodrug chemotherapy regimens are more commonly used in low-risk GTN: (1) MTX (8 days) regimen: MTX 1 mg/kg or 50 mg, intramuscular (IM) or intravenous (IV), on days 1, 3, 5, and 7; FA 0.1 mg/kg, IM or oral, on days 2, 4, 6, and 8; (2) MTX (5 days) regimen: MTX 0.4 mg/kg or 25 mg, IM or IV, for 5 days consecutively; (3) ACT-D (1.25 mg/m$^2$) regimen: ACT-D 1.25 mg/m$^2$, intravenous injection (ivgtt;2 mg at most); (4) ACT-D (5 days) regimen: ACT-D 10–12 μg/kg or 0.5 mg, ivgtt, for 5 days consecutively; (5) MTX (30–50 mg/m$^2$) regimen: MTX 30–50 mg/m$^2$, IV; (6)VP-16 (5 days) regimen:VP-16 100 mg/m$^2$.d;(7) MTX pulse regimen: MTX 100 mg/m$^2$ IV, then 200 mg/m$^2$ ivgtt (over 12h); FA 15 mg. Six monodrug chemotherapy regimens are shown in Table 1.

The efficacy of the first-line monodrug chemotherapy has been generally established for low-risk GTN. Most patients can achieve a complete response after the first-line monodrug chemotherapy. However, which monodrug chemotherapy regimen is better for individual patients with GTN is not yet certain. A meta-analysis (5) comparing several first-line chemotherapy regimens included 7 RCTs, involving 667 patients with low-risk GTN. The results showed that ACT-D was significantly better than MTX in terms of effectiveness. The pulsed chemotherapy regimens using ACT-D and MTX did not differ significantly in side effects. Nevertheless, more high-quality evidence is needed to treat low-risk GTN. Given the diversity of the treatment regimens, a network meta-analysis can inform the choice of the optimal regimen for this condition. We performed a network meta-analysis with a complete response rate after the first-line monodrug chemotherapy to offer clues for the clinical choice of the chemotherapy regimen.

TABLE 1 First-line monodrug chemotherapy regimens.

| | |
|---|---|
| ACT-D (5 days) | 10–12 μg/kg or 0.5 mg, ivgtt, for 5 days consecutively |
| MTX (8 days) | 1 mg/kg or 50 mg, IM or IV, on days 1, 3, 5, and 7; FA 0.1 mg/kg, IM or oral, on days 2, 4, 6, and 8 |
| ACT-D (1.25 mg/m2) | 1.25 mg/m$^2$, intravenous injection (ivgtt;2 mg at most) |
| MTX (5 days) | 0.4 mg/kg or 25 mg, IM or IV, for 5 days consecutively |
| VP-16 (5 days) | VP-16 100 mg/m$^2$.d, for 5 days consecutively |
| MTX (30–50 mg/m2) | 30–50 mg/m$^2$ |
| MTX pulse regimen | 100 mg/m$^2$ IV, then 200 mg/m$^2$ ivgtt (over 12h); FA 15 mg |

# 2 Data and method

Network meta-analysis was performed according to the PRISMA guidelines (6, 7).

## 2.1 Literature retrieval

The Cochrane Library, PubMed, Embase, and Web of Science databases were searched using the Medical Subject Heading (MeSH)-term search strategy from inception to November 1, 2022. Literature search and screening were conducted by two researchers independently. The divergence of opinions was settled through a discussion between the two researchers. If the problem still persisted, a third researcher specialized in methodology was invited. The search words included the following: low-risk or low risk, gestational trophoblastic neoplasia, or gestational trophoblastic tumor. The flowchart of the literature search and screening is shown in Figure 1.

## 2.2 Inclusion and exclusion criteria

The inclusion and exclusion criteria were developed based on the Population, Intervention, Comparison, Outcomes, and Studies principles. The participants were confirmed with low-risk GTN, with a prognostic score ≤6 according to the FIGO/WHO prognostic scoring system; the studies were RCTs or non-RCTs; and the interventional treatments were first-line monodrug chemotherapy regimens, which included but were not confined to the following: MTX (8 days) regimen, MTX (5 days) regimen, ACT-D (1.25 mg/m$^2$) regimen, ACT-D (5 days) regimen, MTX (30–50 mg/m$^2$) regimen, and MTX pulsed regimen. The primary endpoint was the complete response rate after the first-line monodrug chemotherapy. Published studies written in English were included.

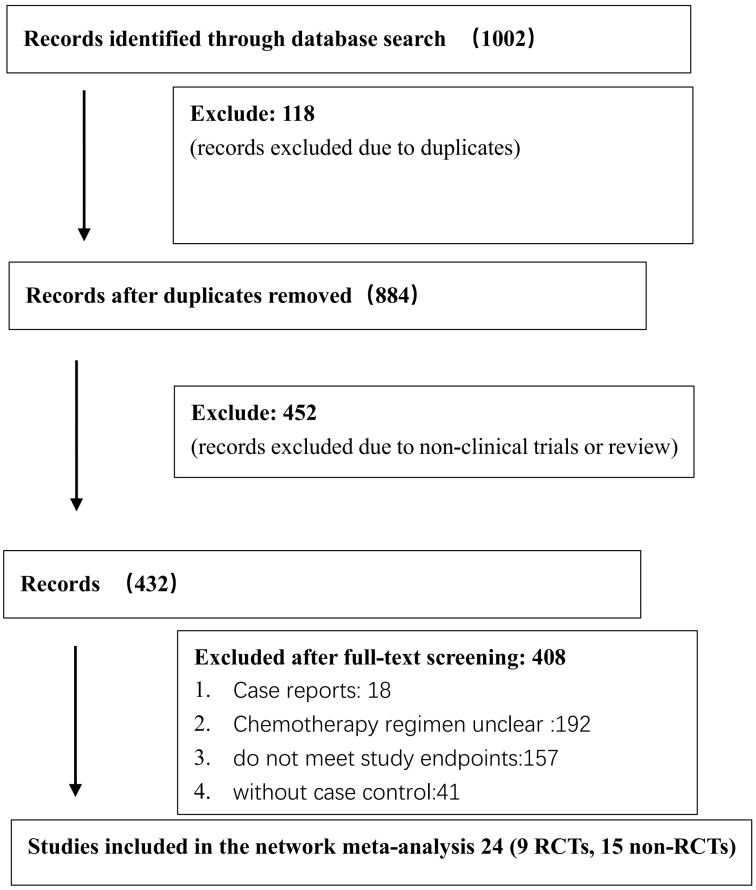


FIGURE 1
The flow diagram of patients.

Studies without controls, studies containing an unclear description of the chemotherapy regimens, studies using oral chemotherapy regimens as interventional treatment, and studies from which important endpoint data could not be extracted were excluded.

## 2.3 Data extraction

Two researchers independently extracted the following data from the included studies: authors, publication time, study site, study type, interventional treatment, subjects, sample size, and age. The complete response rate after the first-line monodrug chemotherapy was the outcome measure. Randomization scheme, blinding, and reporting were used as methodological indicators. The divergence of opinions was settled through a discussion between two researchers. If the problem still persisted, a third researcher specialized in methodology was invited.

## 2.4 Bias and quality assessment of the included studies

Bias and quality assessment was conducted for RCTs using Cochrane collaboration's tool for assessing the risk of bias (8). The following seven categories of indicator data were included for bias and quality assessment: (1) random sequence generation (selection bias); (2) allocation concealment (selection bias); (3) blinding of participants and personnel (performance bias); (4) blinding of outcome assessment (detection bias); (5) incomplete outcome data (attrition bias); (6) selective reporting (reporting bias); and (7) other bias. Bias and quality assessment was conducted for the included non-RCTs using the quality assessment tools for observational studies (9). The following 11 categories of indicator data were included for bias and quality assessment: (1) the source of information was defined (survey and record review), (2) inclusion and exclusion criteria were listed for exposed and unexposed participants (cases and controls) or previous publications were referred to; (3) time period used for identifying patients was indicated; (4) whether participants were consecutive if not population based was indicated; (5) whether evaluators of subjective components of study were masked to other aspects of the status of the participants was indicated; (6) any assessments undertaken for quality assurance purposes were described (e.g., test/retest of primary outcome measurements); (7) any patient exclusions from analysis were explained; (8) how confounding was assessed and/or controlled was described; (9) if applicable, how missing data were handled in the analysis were explained; (10) patient response rates and completeness of data collection were

summarized; and (11) what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained were clarified. Bias and quality assessment was conducted for each study based on the aforementioned criteria.

### 2.5 Data analysis

The data on the sample size and complete response rate under different interventional treatments were extracted from the included studies. Then, network meta-analysis was performed using R 4.2.2. The odds ratio was calculated for the enumeration data, and the measurements were expressed as mean ± standard deviation. First, the chi-square test for homogeneity was performed. $I^2$ ≤50% indicated small heterogeneity, and the study was considered eligible for meta-analysis. $I^2$ >50% indicated large heterogeneity. Thus, the sources of heterogeneity were identified and removed before the meta-analysis. Network analysis was performed by running the Markov Chain Monte Carlo algorithm.

## 3 Results

### 3.1 Basic features of the included studies

The search strategy was developed using the MeSH terms. The preliminary screening yielded 1002 studies, among which repeated studies and those not eligible for the meta-analysis were excluded, resulting in 24 eligible ones (10–33). Specifically, 9 RCTs (10, 14, 16, 17, 20, 21, 23, 24, 33) and 15 non-RCTs (11–13, 15, 18, 19, 22, 25–32) were finally included. A total of 3344 low-risk patients were involved. The following six first-line monodrug chemotherapy regimens were involved in studies: MTX (8 days) regimen, MTX (5 days) regimen, ACT-D (1.25 mg/m$^2$) regimen, ACT-D (5 days) regimen, MTX(30–50 mg/m$^2$) regimen, and VP-16 (5 days) regimen. The basic features of the included studies are presented in Table 2.

### 3.2 Bias and quality assessment of the included studies

The results of bias and quality assessment of the 9 RCTs are shown in Table 3, and those of the 15 non-RCTs are shown in Table 4. The included studies were mostly clinical trials of medium quality.

### 3.3 Complete response rate of first-line monodrug chemotherapy regimens in low-risk GTN

Six studies compared the ACT-D (5 days) regimen and the MTX (8 days) regimen; two studies compared the ACT-D (5 days) regimen and ACT-D (1.25 mg/m$^2$) regimen; two studies compared the MTX (8 days) regimen and the ACT-D (1.25 mg/m$^2$) regimen; five studies compared the ACT-D (5 days) regimen and the MTX (5 days) regimen; two studies compared the ACT-D (5 days) regimen and the VP-16 (5 days) regimen; two studies compared the MTX (5 days) regimen and the VP-16 (5 days) regimen; five studies compared the ACT-D (1.25 mg/m$^2$) regimen and the MTX (30–50 mg/m$^2$) regimen; three studies compared the ACT-D (1.25 mg/m$^2$) regimen and the MTX (5 days) regimen; five studies compared the MTX (8 days) regimen and the MTX (30–50 mg/m$^2$) regimen; two studies compared the MTX (8 days) regimen and the MTX (5 days) regimen; and one study compared the MTX (5 days) regimen and the MTX (30–50 mg/m$^2$) regimen.

Six monodrug chemotherapy regimens were included and analyzed. The probability ranking results show that VP-16 (5 days) is most likely to be the most effective treatment option. The probability is about 99%, followed by ACT-D (5 days)(78%), MTX (5 days)(45%), ACT-D (1.25 mg/m2)(43%). Subgroup analysis found ACT-D (5 days) is most likely to be the most effective treatment option, MTX (5 days), ACT-D (1.25 mg/m$^2$), MTX (8 days), and MTX (30–50 mg/m$^2$) in RCT(there are no RCTs on VP-16). The evidence graph and the results of network meta-analysis in all study are shown in Figure 2. The evidence graph and the results of network meta-analysis in RCT are shown in Figure 3.

Network meta-analysis has good consistency with traditional meta-analysis, the efficacy value and ranking probability do not change significantly, and the results are stable. GTN-node splitting analysis of inconsistency are presented in Supplementary Figure S1 and Supplementary Table S1. The analysis of heterogeneity are shown in Supplementary Figure S2 and Supplementary Table S2.

## 4 Discussion

The FIGO guidelines (3) recommend monodrug chemotherapy for low-risk GTN, and the options include MTX and ACT-D. The hCG level should be monitored once every 2 weeks before each cycle to guide the subsequent treatment. If the hCG level drops to normal after chemotherapy, two to three cycles of chemotherapy should be given before discontinuation. If a satisfactory initial response to chemotherapy is followed by a reduction in the hCG level to the plateau (down by <10% after three cycles of chemotherapy) or first a decrease and then an increase (hCG < 1000 μ/L), the regimen should be changed to the one different from the initial treatment. If MTX has been previously used, the monodrug therapy should be changed to ACT-D and vice versa.

Although MTX and ACT-D monodrug chemotherapy regimens are recommended as the preferred treatments by international guidelines, which one is better for individual patients is not yet certain. Studies have been conducted on combination therapies or other first-line monodrug chemotherapy regimens, but the controversy regarding the optimal dosing regimen for chemotherapy continues. Matsui et al. (12) compared the efficacy of MTX, VP-16, and ACT-D in 247 patients with low-risk GTN. The result showed that the complete response rate of the MTX (5 days) regimen, VP-16 (5 days) regimen, ACT-D (5 days) regimen, and MTX (8 days) regimen was 73.6%, 90.1%, 84.0%, and 60.0%, respectively. The complete response rate was significantly higher for the VP-16 and ACT-D regimens than for the

TABLE 2 Basic characteristics of research.

| study | type | regions | treatment regiments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | group1 | sample | group2 | sample | group3 | sample | group4 | sample |
| Lertkhachonsuk A 2009 (10) | RCT | Thailand | 1 | 22 | 2 | 27 | | | | |
| Lee YJ 2017 (11) | retrospective study | Korea | 2 | 53 | 3 | 18 | 1 | 5 | | |
| Matsui H 1998 (12) | retrospective study | Japan | 4 | 192 | 5 | 126 | 1 | 46 | 2 | 36 |
| Baptista AM 2012 (13) | prospective study | Brazil | 2 | 20 | 1 | 20 | 1 | 20 | | |
| Gilani MM 2005 (14) | RCT | Iran | 3 | 18 | 6 | 28 | | | | |
| Mu X 2018 (15) | retrospective study | China | 3 | 34 | 1 | 26 | | | | |
| Mousavi A 2012 (16) | RCT | Iran | 3 | 50 | 4 | 25 | | | | |
| Shahbazian N 2014 (17) | RCT | Iran | 3 | 15 | 6 | 15 | | | | |
| Korkmaz V 2022 (18) | retrospective study | Turkey | 2 | 53 | 6 | 20 | | | | |
| Maestá I 2018 (19) | retrospective study | Brazil | 2 | 151 | 6 | 174 | | | | |
| Kang HL 2019 (20) | RCT | China | 1 | 49 | 4 | 59 | | | | |
| Yarandi F 2016 (21) | RCT | USA | 4 | 32 | 3 | 30 | | | | |
| Schorge JO 2003 (22) | prospective study | USA | 2 | 5 | 4 | 20 | 6 | 7 | | |
| Osborne RJ 2011 (23) | RCT | Canada | 6 | 107 | 3 | 109 | | | | |
| Yarandi F 2008 (24) | RCT | Iran | 6 | 81 | 3 | 50 | | | | |
| Hoskins PJ 2020 (25) | retrospective study | Canada | 3 | 100 | 6 | 97 | | | | |
| Abrão RA 2008 (26) | retrospective study | Brazil | 1 | 42 | 4 | 42 | | | | |
| Uberti EMH 2015 (27) | retrospective study | Brazil | 2 | 115 | 3 | 79 | | | | |
| Roberts JP 1996 (28) | retrospective study | USA | 1 | 4 | 4 | 61 | | | | |
| Kang WD 2010 (29) | retrospective study | Korea | 2 | 59 | 6 | 48 | | | | |
| Matsui H 2005 (30) | retrospective study | Japan | 2 | 24 | 4 | 132 | 5 | 90 | 1 | 25 |
| Fülöp V 2021 (31) | retrospective study | Hungary | 2 | 304 | 1 | 109 | | | | |
| Xu J 2022 (32) | retrospective study | China | 1 | 88 | 4 | 122 | | | | |
| Anfinan N.M 2020 (33) | RCT | Jeddah | 2 | 26 | 6 | 34 | | | | |

group: 1. ACT-D: 10 Kg/kg per day intravenously for 5 days, every 2 weeks, 2. MTX: 1 mg/kg per day on days 1, 3, 5, and 7, alternating with intramuscular folinic acid 0.1 mg/kg per day on days 2, 4, 6, and 8, every two weeks, 3. ACT-D: pulse actinomycin-D (1.25 mg/m2 ) once every 14 days with a maximum dose of 2 mg, 4. MTX: 0.4mg/kg 5 days, 5. VP-16: 2.0mg/kg 5 days, 6. MTX: 30 -50 mg/m2 weekly.

TABLE 3 Assessment of risk of bias (RCT).

| study | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Jadad score |
|---|---|---|---|---|---|---|---|---|
| Lertkhachonsuk A 2009 (10) | Y | Y | unclear | unclear | unclear | Y | Y | 4 |
| Gilani MM 2005 (14) | unclear | Y | unclear | unclear | unclear | Y | Y | 3 |
| Mousavi A 2012 (16) | unclear | Y | unclear | unclear | unclear | Y | Y | 3 |
| Shahbazian N 2014 (17) | unclear | Y | unclear | unclear | unclear | Y | Y | 3 |
| Kang HL 2019 (20) | Y | Y | unclear | unclear | unclear | Y | Y | 4 |
| Yarandi F 2016 (21) | Y | Y | Y | Y | unclear | Y | Y | 6 |
| Osborne RJ 2011 (23) | Y | Y | unclear | unclear | unclear | Y | Y | 4 |
| Yarandi F 2008 (24) | unclear | Y | unclear | unclear | unclear | Y | Y | 3 |
| Anfinan N.M 2020 (33) | Y | Y | unclear | unclear | unclear | Y | Y | 4 |

1. random sequence generation, 2. allocation concealment, 3. blinding of participants and personnel, 4. blinding of outcome assessment, 5. incomplete outcome data, 6. selective reporting, 7. other bias.

other two conventional regimens. The complete response rate was significantly higher for the VP-16 regimen than for the other three regimens. Maestá et al. (19) analyzed the efficacy of different MTX dosing regimens in 325 patients with low-risk GTN, namely, MTX (30–50 mg/m$^2$) and MTX (8 days) regimens. Compared with the MTX (30–50 mg/m$^2$) regimen, the MTX (8 days) regimen was found to have a higher sustained response rate (84% vs 62%, $P < 0.001$). Although the latter also had a higher incidence of adverse reactions, nearly all of these reactions were controllable. The MTX (8 days) regimen was superior to the MTX (30–50 mg/m$^2$) regimen. Xu et al. (32) evaluated the efficacy of the ACT-D (5 days) regimen against the MTX (5 days) regimen in low-risk GTN. The results showed that the complete response rate was 72.73% in the ACT-D (5 days) regimen and 75.41% in the MTX (5 days) regimen, indicating no significant difference. Compared with the ACT-D group, the MTX monodrug group significantly reduced in the total number of chemotherapy cycles and average hospitalization cost ($P < 0.05$). No serious adverse reactions were reported in any group. However, the ACT-D monodrug group had a higher incidence of leukopenia (grade 1 or 2) (59.38% vs 17.39%). The MTX regimen (5 days) might be the preferred treatment option. This study compared six monodrug chemotherapy regimens involving three common agents using network meta-analysis. We intended to settle the controversy

TABLE 4 Assessment of risk of bias (non-RCT).

| study | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lee YJ 2017 (11) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Matsui H 1998 (12) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Baptista AM 2012 (13) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Mu X 2018 (15) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Korkmaz V 2022 (18) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Maestá I 2018 (19) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Schorge JO 2003 (22) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Hoskins PJ 2020 (25) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Abrão RA 2008 (26) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Uberti EMH 2015 (27) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Roberts JP 1996 (28) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Kang WD 2010 (29) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Matsui H 2005 (30) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Fülöp V 2021 (31) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |
| Xu J 2022 (32) | Y | Y | Y | N | N | N | Y | Y | Y | Y | Y | 8 |

1. Define the source of information (survey, record review), 2. List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications, 3. Indicate time period used for identifying patients, 4. Indicate whether or not subjects were consecutive if not population-based, 5. Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants, 6. Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements), 7. Explain any patient exclusions from analysis, 8. Describe how confounding was assessed and/or controlled, 9. If applicable, explain how missing data were handled in the analysis, 10. Summarize patient response rates and completeness of data collection, 11. Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained.

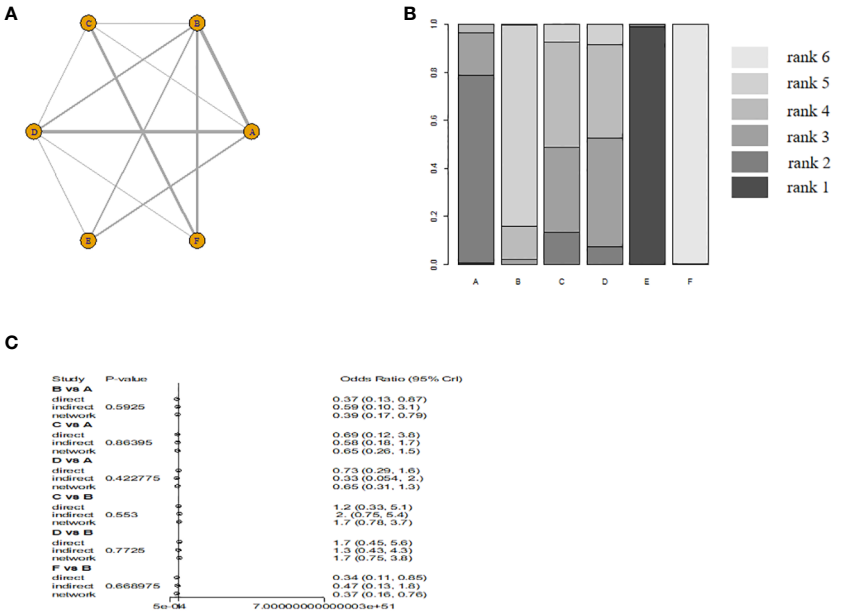


FIGURE 2
The evidence graph and the results of network meta-analysis in all study **(A)** The evidence graph, **(B)** ranks of treatments, **(C)** forest of network meta-analysis). A: ACT-D (10 ug/kg per day intravenously for 5 days,every 2 weeks), B: MTX(1 mg/kg per day on days 1, 3, 5, and 7, alternating with intramuscular folinic acid 0.1 mg/kg per day on days 2, 4, 6, and 8, every two weeks), C: pulse Act-D(pulse actinomycin-D (1.25 mg/m2) once every 14 days with a maximum dose of 2 mg), D: MTX(0.4mg/kg 5 day), E: VP-16(2.0mg/kg 5 day), F: MTX(30 mg/m2/weekly).

regarding the chemotherapy regimen most suited for individual patients with low-risk GTN. We found that VP-16 (5 days) regimen might be the preferred option in terms of efficacy when the complete response is used as the endpoint of the study, the probability ranking is about 99%, which is significantly higher than the other five chemotherapy options.

Network meta-analysis was performed to compare the efficacy outcome of six monodrug chemotherapy regimens in GTN, with a complete response rate as the endpoint. There may be other single-drug regimens or different ways of using the same drug regimen, which were not included in the analysis. For example, the MITO study compared clinical outcomes of patients diagnosed with low-risk

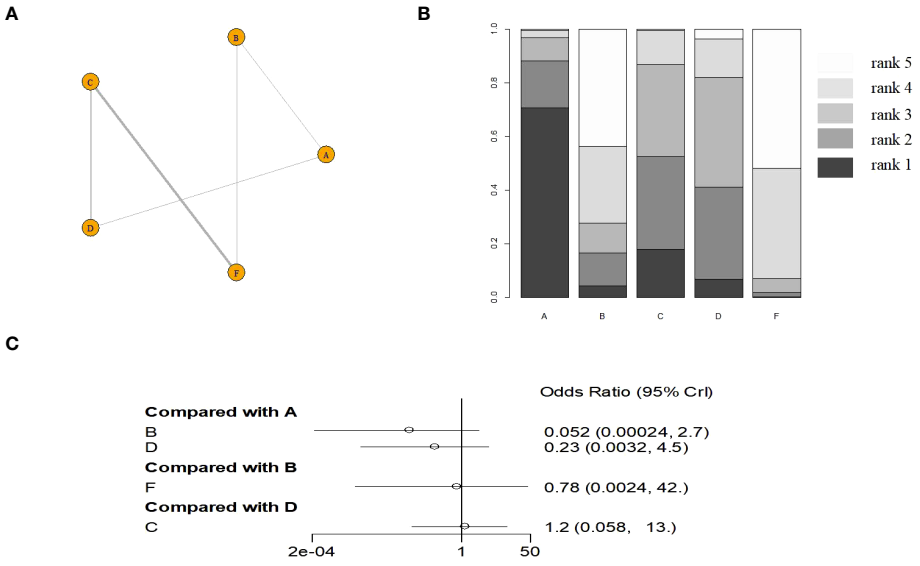


FIGURE 3
The evidence graph and the results of network meta-analysis in RCT **(A)** The evidence graph, **(B)** ranks of treatments, **(C)** forest of network meta-analysis). A: ACT-D (10 ug/kg per day intravenously for 5 days,every 2 weeks), B: MTX(1 mg/kg per day on days 1, 3, 5, and 7, alternating with intramuscular folinic acid 0.1 mg/kg per day on days 2, 4, 6, and 8, every two weeks), C: pulse Act-D(pulse actinomycin-D (1.25 mg/m2) once every 14 days with a maximum dose of 2 mg), D: MTX(0.4mg/kg 5 day), E: VP-16(2.0mg/kg 5 day), F: MTX(30 mg/m2/weekly).

gestational trophoblastic neoplasia (GTN) receiving intramuscular methotrexate 50 mg total dose/day versus 1 mg/kg/day in a 8-day methotrexate/folinic acid (MTX/FA) regimen. Because the both regimens in this study are MTX (8 days) regimen, the study was excluded (34). At the same time, we should also consider the feasibility of the treatment plan, applicability and disturbance to patients. Need to investigate patients' personal feelings. Choose a treatment plan based on multiple factors.

This study had certain limitations despite the clinical guidance it might provide. First, the included studies were mostly retrospective and of moderate quality. Considerable heterogeneity was found in the sample size across the included studies, which were published over a long period, leading to the risk of bias. Besides, we only included studies written in English at the expense of the loss of studies written in other languages. The conclusions drawn from the limited number of studies might contain some biases. The only endpoint discussed in the present study was the complete response rate. We did not analyze the incidence and severity of adverse reactions across the chemotherapeutic agents and dosing regimens, which might have affected the accuracy of the conclusions. The first-line monodrug chemotherapy regimens for low-risk GTN might differ in the incidence and severity of side effects. However, nearly all patients tolerated the associated adverse reactions. Very few cases of intolerance to adverse reactions were reported. The aforementioned results indicated that a complete response rate might be a more useful efficacy outcome compared with the incidence and severity of adverse reactions. These findings need to be further confirmed using high-quality evidence.

To conclude, we performed a network meta-analysis to compare the efficacy of six monodrug chemotherapy regimens in low-risk GTN. The evidence suggested that the VP-16 (5 days) regimen might be the preferred option in terms of efficacy, followed by ACT-D (5 days), MTX (5 days), ACT-D (1.25 mg/m$^2$), MTX (8 days), and MTX (30–50 mg/m$^2$). However, our conclusions should be verified through high-quality RCTs involving a large sample size. There are currently multiple registered clinical studies in progress, and the results of these studies will give us more clinical guidance (35).

## Author contributions

FZ: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. LK: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Writing – original draft.

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## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1276771/full#supplementary-material

SUPPLEMENTARY FIGURE 1
GTN-node splitting analysis of inconsistency (**A**: in all study, **B**: in RCT). **(A)** ACT-D (10 ug/kg per day intravenously for 5 days,every 2 weeks), **(B)** MTX(1 mg/kg per day on days 1, 3, 5, and 7, alternating with intramuscular folinic acid 0.1 mg/kg per day on days 2, 4, 6, and 8, every two weeks), **(C)** pulse Act-D (pulse actinomycin-D (1.25 mg/m2) once every 14 days with a maximum dose of 2 mg), **(D)** MTX(0.4mg/kg 5 day), **(E)** VP-16(2.0mg/kg 5 day), **(F)** MTX(30 mg/m2/weekly).

SUPPLEMENTARY FIGURE 2
analysis of heterogeneity(**A**: in all study, **B**: in RCT). **(A)** ACT-D (10 ug/kg per day intravenously for 5 days,every 2 weeks), **(B)** MTX(1 mg/kg per day on days 1, 3, 5, and 7, alternating with intramuscular folinic acid 0.1 mg/kg per day on days 2, 4, 6, and 8, every two weeks), **(C)** pulse Act-D(pulse actinomycin-D (1.25 mg/m2) once every 14 days with a maximum dose of 2 mg), **(D)** MTX (0.4mg/kg 5 day), **(E)** VP-16(2.0mg/kg 5 day), **(F)** MTX(30 mg/m2/weekly).

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# Evaluation of prognostic significance of lymphovascular space invasion in early stage endometrial cancer: a systematic review and meta-analysis

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**Background:** Studies evaluating the prognostic significance of lymphovascular space invasion (LVSI) in early stage endometrial cancer (EC) are conflicting.

**Objectives:** To evaluate whether LVSI identified in stage I EC is associated with worse survival.

**Search strategy:** A comprehensive literature search of three databases (Embase, PubMed, and Cochrane) was performed up to April 30$^{th}$ 2023.

**Selection criteria:** Cohort studies that have evaluated the relationship between LVSI and prognosis in patients with stage I EC were included.

**Data collection and analysis:** Two authors independently assessed the studies for inclusion, extracted the data of recurrence and survival, and conducted meta-analysis using random effects model. Heterogeneity was evaluated by $I^2$ test.

**Main results:** A total of 15 studies involving 6,705 patients were included in the meta-analysis. The overall pooled rate of LVSI was 14% [95% confidence interval (CI) CI 0.09-0.18] in stage I EC. LVSI was significantly associated with a higher risk of recurrence [odds ratio (OR) = 2.79, 95%CI 2.07-3.77], reduced overall survival (OS) [hazard ratio (HR)=5.19, 95%CI 3.33-8.07] and recurrence free survival (RFS) [HR = 5.26, 95%CI 3.45-8.02] in stage I EC patients. Similarly, LVSI was associated with an increased risk of recurrence [OR= 3.10, 95%CI 2.13-4.51], decreased OS [HR=5.52, 95%CI 2.16-14.09] and RFS [HR = 4.81, 95%CI 2.34-9.91] in stage IA grade 1 or 2 endometrioid carcinoma patients.

**Conclusion:** The presence of LVSI in stage I EC and in stage IA, grade 1 or 2 endometrioid carcinoma is associated with an increased risk of recurrence, lower OS and RFS.

**Systematic Review Registration:** https://www.crd.york.ac.uk/prospero/, identifier 42023425231.

KEYWORDS

endometrial cancer, lymphovascular space invasion, recurrence, overall survival, recurrence free survival

# 1 Introduction

Endometrial cancer (EC) is the most common malignancy of the female reproductive system in developed countries, with more than 65,000 new cases reported in the United States each year (1). The prognosis of EC is influenced by various factors such as age, clinical stage, tumor differentiation, and pathological type (2). Lymphovascular space invasion (LVSI), defined as the presence of tumor cells in lymphatic or small blood vessels outside the tumor core. Up to 35% of EC patients are reported to have LVSI (3). The National Comprehensive Cancer Network (NCCN) guideliness (version 1.2023, Uterine Neoplasms) recommend quantifying LVSI to guide postoperative treatment (4). Besides, the 2023 International Federation of Gynecology and Obstetrics (FIGO) surgical staging system include LVSI in staging (5).

Nevertheless, conflicting results have been reported regarding the impact of LVSI on survival outcomes in early-stage EC. Many studies have shown that LVSI-positive is associated with lower overall survival (OS), higher recurrence, and increased risks of lymph node and distant metastasis, and is a poor prognostic factor for EC (6–8). LVSI has been reported as a risk factor, even in cases of stage I EC without lymph node metastasis (9–11). Conversely, several studies have concluded that LVSI does not significantly affect survival rates in patients with early-stage EC (12–14). For instance, Okugawa et al. (14) reported that the 5-year EC specific survival rates for tumors in LVSI-positive and LVSI-negative patients were 97.0% and 98.9%, which do not show significant difference. Furthermore, there is a lack of consensus on whether adjuvant therapy is necessary for stage I EC patients with LVSI after surgery. It is crucial to determine which EC patients with LVSI, especially those in the low-risk group (defined as stage IA, grade 1 or 2 endometrioid carcinoma), would benefit from postoperative adjuvant therapy.

Therefore, we aimed to address the knowledge gap regarding the independent prognostic significance of LVSI in early-stage EC, particularly in the low-risk group. We investigated the impact of LVSI on survival and recurrence in stage I EC. The findings of this study will contribute to improving the management and treatment decisions for stage I EC patients, aiding in the identification of those who would benefit from adjuvant therapy.

# 2 Methods

This meta-analysis was conducted in strict accordance with the Systematic Review and Meta-Analysis Preferred Reporting Project (PRISMA) statements (15), and registered in the International Register of Prospective Systems Evaluation (CRD: 42023425231).

## 2.1 Literature search

We performed a comprehensive literature search of three databases: Embase, PubMed, and Cochrane, from database inception to April 30th 2023. Cohort studies that have evaluated the relationship between LVSI and prognosis in patients with stage I EC were included. The predefined search string was the following: (endometrial cancer OR endometrial carcinoma OR uterine cancer OR uterine carcinoma of endometrium) AND (early stage OR stage IA OR stage I OR FIGO I OR FIGO IA) AND (lymphovascular space invasion OR LVSI). Other literature resources, such as the reference lists of eligible studies, were reviewed to identify additional studies. We applied language restriction that only publications in English were included. There were no restrictions on the publication time, publication status or article type.

## 2.2 Study selection

Two authors (ZJ Qin and YS Wang) independently screened the titles and abstracts based on the inclusion criteria to determine relevant studies. After initial screening, the full text of all potentially eligible articles were independently reviewed by two authors for further assessment. Any discrepancies were resolved through discussion with the corresponding author.

The inclusion criteria for our research were as follows: (a) studies that are either prospective or retrospective cohort studies; (b) inclusion of EC patients who have undergone surgical pathological staging, FIGO stage I or IA, and any pathological type; (c) analysis of the prognosis of patients with and without LVSI; and (d) availability of the full text. The exclusion criteria were

as follows: (a) inability to extract valid outcome indicators from the literature; (b) non-English literature; (c) low scores in quality assessment; and (d) sample size less than 50.

## 2.3 Data extraction

Two authors (ZJ Qin and YL Chen) independently extracted the following data from each study: first author name, publication year, country, inclusion of EC staging and pathological type, time of initial treatment, median follow-up time, LVSI incidence, administration of adjuvant therapy, and outcome data related to the potential relationship between LVSI and prognosis, including recurrence, OS, and recurrence-free survival (RFS). Any discrepancies in data extraction were resolved through discussion with the corresponding author.

## 2.4 Quality assessment

The methodological quality of the studies included in the research was independently assessed by two authors (ZJ Qin and A Zheng) according to the Newcastle-Ottawa Quality Assessment Scale (NOS) (16). For the 'comparability of cohort based on design or analysis' criterion, studies that controlled for histological type of endometrioid adenocarcinoma received 1 star. For the 'adequacy of follow-up time in the cohort' criterion, a median follow-up time of more than 40 months was rated 1 star. Studies scoring 6 stars or more were considered high-quality and were included in our meta-analysis.

## 2.5 Statistical analysis

Statistical analysis was conducted using the metan, metabias, and metaprop software packages in STATA 15.0 (Statacorp, College Station, TX, USA). We calculated the odds ratio (OR) of recurrence, the hazard ratio (HR) of RFS and OS, as well as their 95% confidence intervals (Cls). In studies that only reported OS and RFS as Kaplan-Meier curves and where HR and 95% intervals could not be obtained from the original text, the necessary data were extracted using engage Digitizer 4.1 (http://sourceforge.net/projects/digitizer/), and HR was calculated using the tool recommended by Tierney et al (17).

Heterogeneity was quantified using the $I^2$ statistic: $I^2$ <30% was considered low heterogeneity, 30% <$I^2$ <50% was considered moderate heterogeneity, and $I^2$ >50% was considered high heterogeneity (18). Given the clinical heterogeneity of the studies included, we used a random-effects model for all meta-analyses. We created Galbraith radial plots to explore potential causes of heterogeneity. We also conducted subgroup analyses based on FIGO stage, histological type, and grade of differentiation. The Begg's test was used to assess the risk of publication bias, with a *p*-value of less than 0.1 considered as evidence of significant publication bias.

# 3 Results

## 3.1 Characteristics of the included studies

A total of 731 records were identified through the literature search. After removing duplicates, 463 articles underwent title and abstract screening, and 41 articles were selected for full-text screening. Ultimately, 15 studies (3, 6–14, 19–23) involving 6705 patients were included in the data analysis. The characteristics of the 15 included studies are shown in Table 1. The study selection process is illustrated in Figure 1. These studies were published between 2007 and 2022. All the included studies achieved a Newcastle-Ottawa Scale (NOS) score of 6 or higher, with the highest score being 8.

## 3.2 Rate of LVSI

A total of 15 studies involving 6,705 patients investigated the incidence of LVSI in early-stage EC. The pooled incidence of LVSI was 14% (95% CI 0.09-0.18; $I^2$ = 96.28%, $p$ < 0.001) in stage I EC (Figure 2A). Given the high heterogeneity observed among the LVSI meta-analyses of all studies, subgroup analyses were conducted based on country, sample size, publication year, and pathological subtype. However, these subgroup analyses did not clearly identify the source of heterogeneity, and further analysis of individual study characteristics failed to explain the observed heterogeneity.

## 3.3 Relationship between LVSI and recurrence

Nine studies (3, 6–9, 11, 12, 14, 21) involving 3,337 patients evaluated the relationship between LVSI and recurrence. The recurrence rate for patients with LVSI was 18.10% (72/398), compared to 6.16% (181/2939) for patients without LVSI in stage I EC. The meta-analysis showed that patients with LVSI had a significantly higher risk of recurrence (OR = 2.79, 95%CI 2.07-3.77; $I^2$ = 0%, $p$ = 0.926) in stage I EC (Figure 2B).

Further analysis revealed that the recurrence rate for patients with LVSI was 14.02% (30/214), compared to 5.03% (108/2148) for patients without LVSI in stage IA grade 1 or 2 endometrioid carcinoma. Similarly, patients with LVSI also had a significantly higher recurrence rate (OR = 3.10, 95%CI 2.13-4.51; $I^2$ = 23.8%, $p$ = 0.256) in stage IA grade 1 or 2 endometrioid carcinoma (Figure 2C).

## 3.4 Relationship between LVSI and survival

Five studies (8–10, 14, 20) reported on the OS of 2,880 patients. LVSI-positive patients in stage I EC had a 5.19-fold higher risk of death than LVSI-negative patients (95%CI 3.33-8.07; $I^2$ = 0, $p$ = 0.739; Figure 3A). In stage IA grade 1 or 2 endometrioid carcinoma patients, LVSI-positive had a 5.52-fold higher risk of death than LVSI-negative patients (95%CI 2.16-14.09; $I^2$ = 0, $p$ = 0.729; Figure 3B). Additionally, six studies (8–10, 20, 23) reported on the

TABLE 1 Characteristics of studies included in the meta-analysis.

| Study | Country | Study type | Inclusion criteria | Pathological type | N | LVSI + | LVSI- | Median follow-up | Quality assessment* |
|---|---|---|---|---|---|---|---|---|---|
| Okugawa(2021) | Japan | R | IA | mixed | 297 | 35 | 262 | 60m | 7 |
| Iida(2022) (12) | Japan | R | IA | mixed | 116 | 15 | 101 | 71.9m | 7 |
| Tortorella (2021) (3) | Italia | R | IA G1/G2 | Endometrioid carcinoma | 524 | 57 | 467 | 38m | 7 |
| Reis(2015) (20) | America | R | IA G1/G2 | Endometrioid carcinoma | 200 | 40 | 200 | 46.6m | 8 |
| Nwachukwu (2021) (11) | America | R | IA G1 | Endometrioid carcinoma | 222 | 14 | 204 | 20m | 7 |
| Güngördük (2018) (21) | Turkey | R | IA G1/G2 | Endometrioid carcinoma | 280 | 22 | 258 | 54-69m | 8 |
| Ayhan (2019) (19) | Turkey | R | IA G1/G2 | Endometrioid carcinoma | 912 | 53 | 859 | 42m | 8 |
| Ureyen(2019) | Turkey | R | IA | Endometrioid carcinoma | 720 | 60 | 660 | 48m | 8 |
| Pifer(2022) | Pittsburgh | R | I | Endometrioid carcinoma | 335 | 124 | 211 | 25.8m | 7 |
| Veade(2019) (7) | America | R | I | Endometrioid carcinoma | 275 | 48 | 227 | 54m | 8 |
| Cusano (2018) (9) | Canada | R | I | Endometrioid carcinoma | 400 | 54 | 346 | 66m | 8 |
| Bosse(2015) (22) | Netherlands | R | I | Endometrioid carcinoma | 926 | 70 | 856 | 89-160m | 8 |
| Aristizabal (2014) (23) | France | R | I | mixed | 384 | 112 | 272 | 38.7m | 6 |
| Gemer (2007) (10) | Israel | R | I | mixed | 699 | 40 | 659 | 39m | 6 |
| Yarandi (2023) (6) | Iran | R | I G1/G2 | Endometrioid carcinoma | 415 | 100 | 315 | NM | 6 |
| Total | | | | | 6705 | 844 | 5897 | | |

R, retrospective; G1/G2, grade1/2; NM, not mentioned.
*Quality assessment was measured using the Newcastle-Ottawa Quality Assesment Scale (NOS).

RFS of 2,595 patients. LVSI significantly influenced RFS in stage I EC (HR = 5.26, 95%CI 3.45-8.02; $I^2 = 0$, $p = 0.537$; Figure 3C) and stage IA grade 1 or 2 endometrioid carcinoma patients (HR = 4.81, 95%CI 2.34-9.91; $I^2 = 0$, $p = 0.596$; Figure 3D).

## 3.5 Publication bias

Publication bias was assessed based on the LVSI rate and was reported in most of the included studies. Ultimately, 15 studies were included, and the Begg's test ($p$=0.067) showed a potential source of publication bias. According to our funnel plot, studies with a lower LVSI rate are more likely to be published (Figure 4).

# 4 Discussion

Our analysis revealed that approximately 14% (95% CI 0.09-0.18) of stage I EC patients had LVSI, consistent with the reported range of LVSI frequency in the literature ranging from 6% (8) to 37% (13). This variability may be attributed to the inclusion criteria that did not limit the pathological type of EC, encompassing both endometrioid adenocarcinoma and other types of EC. Additionally, the clinical stages included both stage IA and stage I, introducing clinical heterogeneity in LVSI positive rates.

Importantly, our findings demonstrate that LVSI is significantly associated with a higher risk of recurrence, not only in stage I EC (OR= 2.79, 95%CI 2.07-3.77) but also in stage IA grade 1 or 2 endometrioid carcinoma patients (OR= 3.10, 95%CI 2.13-4.51). These findings are in line with previous investigations (3, 9, 10, 24), suggesting that the presence of LVSI increases the risk of tumor recurrence. Additionally, LVSI was found to significantly influence RFS in both stage I EC (HR = 5.26, 95%CI 3.45-8.02) and stage IA grade 1 or 2 endometrioid carcinoma patients (HR = 4.81, 95%CI 2.34-9.91). The presence of LVSI in early endometrial cancer patients increases the risk of tumor recurrence, Bosse et al. (22) and Güngördük et al. (21) illustrated the presence of LVSI was an independent risk factor for recurrence. Besides, LVSI is

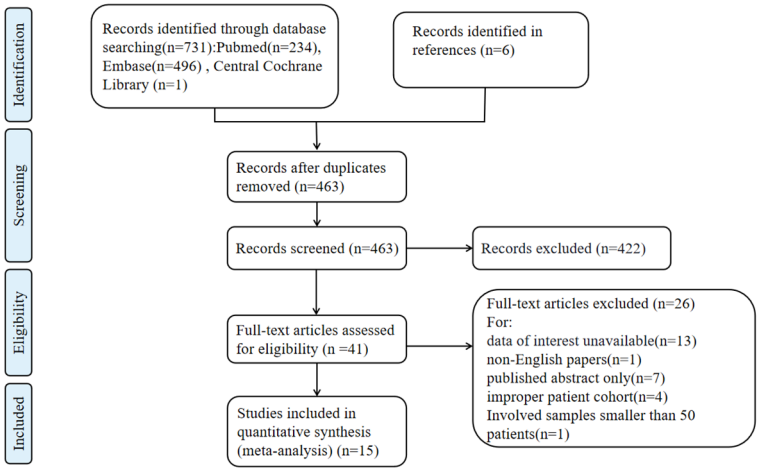


FIGURE 1
Flow diagram of literature searching and study selection.

independently associated with lymph node metastasis in women with early-stage endometrial cancer (25).

Furthermore, our meta-analysis demonstrated that patients with LVSI have worse OS in both stage I EC (HR=5.19, 95%CI 3.33-8.07) and stage IA grade 1 or 2 endometrioid carcinoma patients (HR=5.52, 95%CI 2.16-14.09) compared to patients without LVSI. Our research findings are in concordance with previous studies reporting a significant association between LVSI and decreased OS (3, 6, 7). However, it is important to note that there have been conflicting reports regarding the impact of LVSI on prognosis. For instance, Iida et al. (12) found no difference in prognosis between patients in stage IA and type 1 EC with and without LVSI, Okugawa et al. (14) reported that LVSI did not significantly affect cancer-specific survival or serve as a prognostic factor in stage IA endometrial cancer. The reason for this difference may be the specific study design that included the stage of early EC, pathological type, and the proportion of lymph nodes evaluated. The discrepancies in these findings highlight the need for further research to explore the prognostic value of LVSI in early EC.

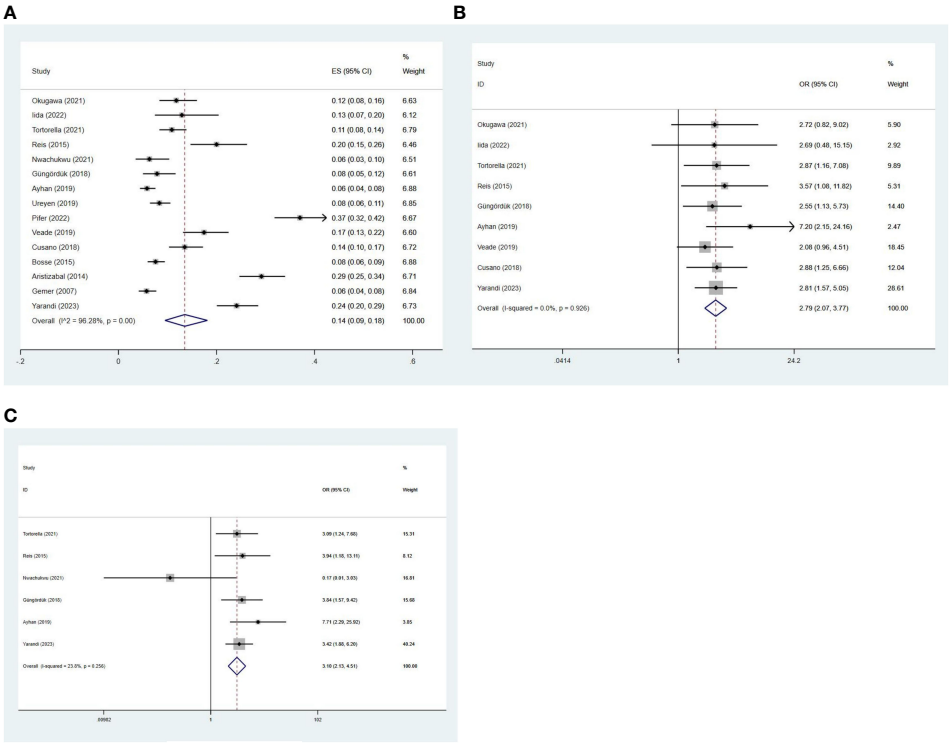


FIGURE 2
Forest plots of the **(A)** incidence of LVSI; potential relationships of LVSI with recurrence in **(B)** stage I and **(C)** stage IA G1/2 EC.

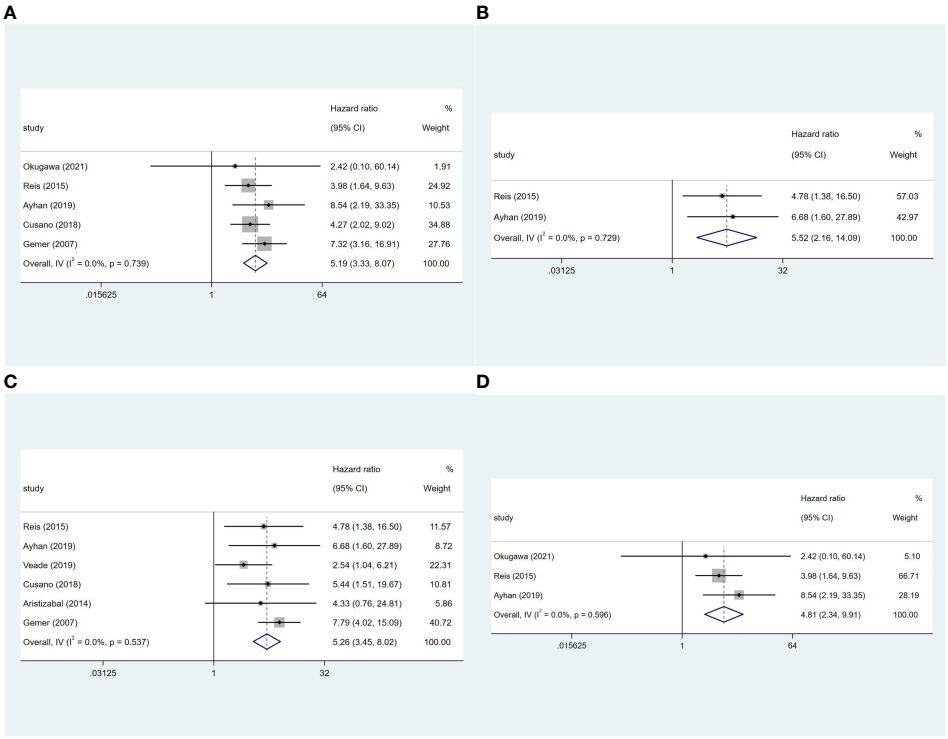


FIGURE 3
Forest plots of the potential relationships of LVSI with **(A)** overall survival in stage I and **(B)** stage IA G1/2 EC; **(C)** recurrence-free survival in stage I and **(D)** stage IA G1/2 EC.

It is crucial to determine which EC patients, especially those in the low-risk group, would benefit from postoperative adjuvant therapy. There is a lack of consensus on whether adjuvant therapy is necessary for stage I EC patients with LVSI after surgery. Notably, studies by Beavis et al. (26) and Son et al. (27) reported improved progression-free survival with adjuvant therapy compared to observation alone in stage I endometrioid EC patients with LVSI. However, none of the four randomized studies (28–31) showed that adjuvant radiotherapy improved survival in stage I EC patients. In fact, Johnson et al. (32) reported that external beam radiation therapy (EBRT) after surgery reduced the risk of local recurrence, but did not reduce the risk of distant metastasis or OS in stage I EC. Thus, the role of adjuvant therapy in LVSI subgroups of early-stage EC remains uncertain and requires further investigation. Our study shows that LVSI increases the risk of early EC recurrence and death. Postoperative observation and follow-up can be selected for endometrioid adenocarcinoma with LVSI in the low-risk group of stage IA, but EBRT supplementation may be considered when combined with other high-risk factors such as age over 60 years old, lesion diameter greater than 2cm or molecular classification indicating poor prognosis. Other stage I EC patients with LVSI are advised to consider EBRT.

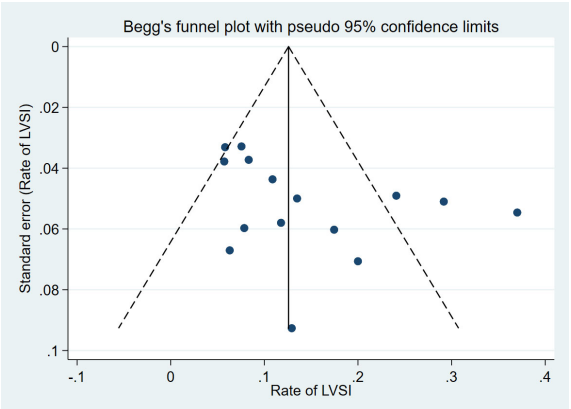


FIGURE 4
Funnel plot with pseudo 95% confidence limits of 15 included studies reporting the rate of LVSI.

This study also has certain limitations. First, clinical heterogeneity exists among the included studies due to differences in pathology types, surgical procedures, and adjuvant treatment methods. Second, the retrospective nature of the included studies introduces a risk of selection bias. Peters et al (33). have studied the correlation between the extent of LVSI and prognosis in patients with EC using samples from PORTEC trials. Their quantitative analysis revealed a significant correlation between the extent of LVSI and the risk of pelvic lymph node recurrence in EC patients. They propose defining clinically relevant LVSI in EC as the involvement of more than 4 LVSI-positive vessels in at least one H&E slide. However, due to insufficient LVSI grading data in the included literature, we were unable to perform subgroup analysis based on this parameter. Additionally, there may also be inconsistent diagnostic criteria for LVSI positivity, leading to

potential confounding bias in relevant studies. We only included English-language literature and publications and may have a publication bias risk.

In conclusion, our analyses demonstrates that the presence of LVSI in stage I EC and in stage IA, grade 1 or 2 endometrioid carcinoma is associated with an increased risk of recurrence, reduced RFS and OS. LVSI status should be considered as an important prognostic factor in early stage EC, and its presence should prompt closer postoperative clinical follow-up. The role of adjuvant therapy in LVSI subgroups of early-stage EC remains controversial and requires further investigation. Future research should focus on evaluating the survival benefits and potential risks of adjuvant radiotherapy in patients with LVSI to determine its optimal use in clinical practice.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary files, further inquiries can be directed to the corresponding author/s.

## Author contributions

Z-JQ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft. Y-SW: Data curation, Investigation, Methodology, Software, Writing – original draft. Y-LC: Formal Analysis, Investigation, Methodology, Software, Writing – original draft. AZ: Formal Analysis, Supervision, Visualization, Writing – review & editing. LH: Conceptualization, Formal Analysis, Supervision, Validation, Writing – review & editing.

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## Conflict of interest

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

## Publisher's note



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# Assessing the learning curve for transumbilical single-site laparoscopy for endometrial cancer

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**Introduction:** Applying transumbilical laparoendoscopic single-site surgery to endometrial cancers is worldwide, and the depiction of the learning curve is rarely described, which leads to the vagueness of young clinical practitioners. We accumulated the data to identify the completion of the learning curve by analyzing the operative and postoperative outcomes of the patients with endometrial cancer for transumbilical laparoendoscopic single-site surgery (TU-LESS).

**Methods:** This was a retrospective, consecutive single-center study of patients with endometrial cancer undergoing standard endometrial cancer comprehensive staging surgery (extrafascial hysterectomy, bilateral salpingectomy, and pelvic lymphadenectomy) through TU-LESS by an experienced surgeon from December, 2017 to June, 2021 in the Department of Gynecologic Oncology, West China Second Hospital, Sichuan University, China.

**Results:** After applying the inclusion and exclusion criteria, 42 patients were included in the study. The learning curve for this study was evaluated using both cumulative sum (CUSUM) and risk-adjusted CUSUM (RA-CUSUM) methods. Applying CUSUM and RA-CUSUM has grouped 42 cases into three phases. The prior five cases represented the learning period. The following six cases were needed to lay a technical foundation (cases 6–11). The third phase was regarded as achieving proficiency (cases 12–42). The operative time decreased drastically with the learning curve. There were no significant differences in terms of postoperative complications and lymph node retrieval among the three phases. More difficult patients were confronted in the third phase.

**Discussion:** In our study, the learning curve was composed of three phases. According to the results of our study, 11 cases were required for experienced surgeons to achieve a technical foundation.

KEYWORDS

**transumbilical laparoendoscopic single-site surgery, minimally invasive surgery, endometrial cancer, learning curve, CUSUM**

# Introduction

Endometrial cancer is the sixth most common cancer among women globally (1, 2). High-income countries exhibit a higher incidence of this malignant disorder, with 11.1 cases per 100,000 women (2). The incidence is still increasing (3). As endometrial cancer is frequently diagnosed at an early stage, an ideal outcome is expected with early medical intervention (4). Conventional laparoscopy is a widely recommended and accepted treatment modality as it achieves the same therapeutic results and causes less trauma to the patients compared to laparotomy (4). Transumbilical laparoendoscopic single-site surgery (TU-LESS) is an emerging technique that is the least minimally invasive substitution for conventional laparoscopy (5, 6). It is superior as it results in faster patient recovery and causes less pain, which shortens the time window before patients receive adjuvant therapy (7). Various clinical trials have verified its safety in terms of oncological outcomes (8). Previous reports have also demonstrated that a better subjective cosmetic result was obtained with TU-LESS (9, 10).

TU-LESS is performed through one incision. Hence, the surgeons may have trouble manipulating instruments (11, 12). Due to this, the procedure is likely to induce fatigue and is challenging to master. The learning curve can vary massively for different surgeons. Herein, we have presented the learning curve analysis of the 3-year cumulative experience of one surgeon at our institution for applying TU-LESS in endometrial cancer. This was done by analyzing the perioperative outcomes of patients with endometrial carcinoma undergoing TU-LESS.

# Materials and methods

## Patient population

From December, 2017 to June, 2021, a series of consecutive 71 endometrial cancer cases who experienced TU-LESS were to be performed by a single surgeon accompanied by experienced assistants at the Department of Gynecologic Oncology, West China Second Hospital, Sichuan University, China. To evaluate the surgical outcomes, 42 cases who went through extrafascial hysterectomy, bilateral salpingectomy (BSO), and pelvic lymphadenectomy were elected. Due to different surgeries, 29 cases were excluded. We examined the age, underlying disease, gestation and pregnancies, and family history of patients as background data. All patients were informed about the procedures, the advantages of TU-LESS, and the potential risks. Institutional Review Board approval was obtained.

## Data collection and definitions

All data were retrieved from West China Second Hospital, which were investigated and retrospectively viewed.

The data evaluated included patients' demographics, operative variables, and postoperative data.

Patient demographics include age, BMI, childbearing history, the presence of medical and surgical comorbidities, carbohydrate antigen 125 (CA 125), and carbohydrate antigen 19-9 (CA 19-9).

Operative variables studied included operative time, estimated blood loss, conversions to other techniques, and intraoperative complications.

Postoperative data included VAS score, hospital stay, time to first passage of flatus, postoperative complications, and pathology results.

The primary endpoint was operative time, which was used for cumulative sum (CUSUM) analysis. Operative time was identified as the duration from the first incision to the final closure. The second endpoints were conversions and short-term complications, which were used for the risk-adjusted CUSUM (RA-CUSUM) analysis. The pain score was acquired 12 h, 24 h, and 36 h after surgery, and we marked the according intervention and outcomes. Patients met the discharge standard if their temperature was normal, the catheter was removed with unblocked urination, and the first passage of the flatus was done without any abnormal complications or laboratory tests, which time frame was set as the enhanced recovery index (ERI). The hospital stay was identified as the interval from the surgery to the day of discharge.

Short-term complications were stratified in accordance with the Clavien–Dindo classification of surgical complications, which was applied to assess the success of surgeries. The pathological results and staging status were recorded based on the International Federation of Gynecology Oncology. We demanded patients be reviewed in outpatient clinics regularly.

## Surgical procedure

The patient undergoes a thorough cleaning and sterilization procedure at the umbilicus 24 h before the operation. Preoperative administration of antibiotics is conducted 30 min before the surgical procedure to decrease the risk of infection. The procedure is conducted using general anesthesia. Following the administration of anesthesia, the patients had standard disinfection using a towel, and a urinary catheter was inserted. Additionally, the assistants perform a resterilization procedure on the vulva and vagina.

Following the sterilization, the assistant inserts a simple uterine manipulator, which facilitates visibility of the surgical area. The patients were positioned supine, with the head lowered and feet elevated, throughout the procedure. Available visualization equipment options include the Olympus or the German STORZ laparoscopic system.

The traditional laparoscopic instruments include separation forceps, non-invasive grasping forceps, suction, scissors, curved forceps, needle holders, and others. The extended instruments consist of all 45-cm extended needle holders, suction, non-injury grasping forceps, and others. Additionally, energy devices are also

used. The energy instruments employed included the Johnson & Johnson Harmonic ultrasonic knife, an extended 45-cm ultrasonic knife, single and double electrocoagulation forceps, and BiClamp. The Johnson & Johnson SXPP1B401 barbed wire was utilized for vaginal suturing in the study.

Port preparation involves using a Kangji disposable single port, which has one inlet with a diameter of 10 mm, another with a diameter of 12 mm, and two inlets with a diameter of 5 mm each.

As for medical operations, we perform umbilicus sterilization, create a 2-cm incision in the center of the umbilicus aligned with the body's longitudinal axis, and sequentially cut through the layers of skin, where a port is inserted (Figure 1).

Following the creation of a pneumoperitoneum, a laparoscopic lens and surgical equipment are introduced into the port. A comprehensive examination of the pelvic and abdominal regions is conducted, and fluid from the pelvic and abdominal lavages is collected for analysis.

Additionally, the fallopian tubes are treated before the surgery. The surgery involves the removal of one or both fallopian tubes or ovaries, the complete removal of the uterus (the extent of which will be determined based on preoperative staging and intraoperative circumstances), and the surgical removal of lymph nodes. The "Zheng's 3C suspension method" is employed to aid with intraoperative exposure for individuals facing challenges in this regard (13).

Following the procedure, a T-tube is inserted into the pelvic floor to facilitate drainage of the vaginal canal, while the vaginal incision is sutured using barbed wire.

The umbilical incision is closed by applying traction to the apical line of the peritoneofascial incision, securing it with knots, anchoring it, and then shaping and beveling it to ensure proper closure. This process requires specialized techniques, which we invented and named "anchoring technique" (14). At last, we apply gauze to compress the umbilicus and use disposable dressings to recover a natural appearance.

## Evaluation of surgical performance

All procedures were performed by one skilled surgeon who had accumulated many experiences in conventional laparoscopy applied to malignant diseases and TU-LESS treating gynecological benign lesions. In order to identify surgical results, patient demographics, operative variables, and postoperative data were investigated.

## Statistical analysis

All statistical analyses were performed using SPSS version R26.0.0.0 and SAS@ studio. Normally distributed continuous variables were presented as mean and standard deviation. Categoric variables were exhibited as frequency. Differences in characteristics among groups were analyzed using the Chi-test with *post hoc* tests or Fisher's exact test (for categorical variables) and Bonferroni *t*-test (for continuous variables). Logistic regression was used to assess the second turning point of the learning curve. A *p*-value of $<0.05$ was considered statistically significant.

## CUSUM analysis

We used CUSUM analysis to determine which point was defined as the completion of the learning curve. CUSUM is widely accepted to evaluate the learning curve and distinguish the learning, proficient, and mastering phases (15). In this study, we calculated the CUSUM by ranking the cases chronologically from the first to the latest date of endometrial cancer using TU-LESS. CUSUM (1) = the first operation time OT (1) − the average operation time OT (mean), CUSUM ($n$) = OT ($n$) − OT (mean) + CUSUM ($n$−1), until the last CUSUM was calculated as 0. Furthermore, we established a trend line to show the change in the slope of the learning curve, based on which the inflection points

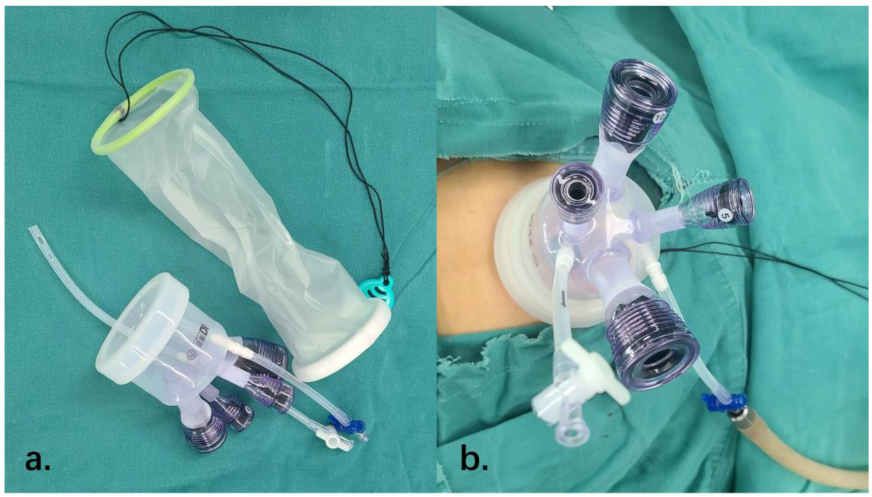


FIGURE 1
The establishment of transumbilical single-site laparoscopy. **(A)** The incision protective sleeve and the port we used to perform the surgery. **(B)** The establishment of transumbilical single-site laparoscopy.

were identified. The ascending phase of the trend line indicates it is located in the primary learning phase of this technique. The first inflection point demonstrated the completion of this procedure. The descending line showed the surgeon had laid a technical foundation.

## RA-CUSUM analysis

RA-CUSUM analysis was used to depict the success or failure of this technique. It was recognized as an extension of CUSUM to further assess the learning curve. In order to define the failure of surgery, we selected three parameters, including conversions, postoperative complications (Clavien ≥ III), and 30-day readmission. Any occurrence of one of these three events was defined as a surgical failure.

Univariate analysis was applied to analyze the risk factors, including all perioperative data and pathological results except the three events above. RA-CUSUM was defined as RA-CUSUM$\sum^{n}_{i=1}(x^{i}-\tau) + (-1)^{xi}P_i$. We used $x_i = 1$ to symbolize the presence of surgical failure, and $\tau$ represents the observed event rate. The expected rate is $P_i$, which is retrieved through the regression model. Therefore, the descending line refers to surgical success and the ascending line to surgical failure. However, due to the small sample size, we failed to filter multiple positive indexes to proceed with the calculation, so we incorporated operation time, surgical comorbidities, lymph nodes, and exhaust time, which were commonly seen as factors to influence the outcome and statistical analysis, all to finish the simulation.

# Results

## Patients' characteristics and surgical outcomes

We viewed 42 endometrial cancers between December, 2017 and June, 2021, with two conversions to porous laparoscopy in the second phase (Table 1). As to the pathologic results, after total clinical staging, there were 35 IA, four IB, two IIIA, and one IIIC. The average age for patients was 46.74 (SD = 10.33). The mean BMI is 24.62 (SD = 4.02) kg/m$^2$; 14 (33%) patients had a history of previous pelvic and abdominal surgery. The average operating time and blood loss were 207.45 (SD = 40.64) min and 97.38 (SD = 85.69) ml, respectively. The average time for the first passage of flatus was 2.57 (SD = 0.89) days. The hospital stay was 5.05 (SD = 1.36) days. The enhanced recovery index (ERI) was 2.97 (SD = 0.92) days. The reasons for two conversions to multiport laparoscopy were severe adhesion in the pelvic cavity and injury to the external iliac vein because of obesity, with extreme difficulty in exposing the field. Two cases with failed sentinel lymph node mapping were reported, which required a change of operation from resection of sentinel lymph nodes to lymphadenectomy. One lymphatic retention with infection appeared. After four phases of

TABLE 1 Preoperative parameters of three stages for TU-LESS in endometrial cancer (n = 42).

| | Total | First group (*n* = 5) | Second group (*n* = 6) | Third group (*n* = 31) | *p*-value | *p*1 | *p*2 | *p*3 |
|---|---|---|---|---|---|---|---|---|
| Demographics | | | | | | | | |
| Age | 48.12 (10.30) | 46.80 (10.05) | 45.33 (7.09) | 48.87 (10.99) | 0.720 | 0.818 | 0.453 | 0.684 |
| Manifestations | | | | | | | | |
| Irregular vaginal bleeding | 31 | 3 | 5 | 23 | | | | |
| Excessive menstruation | 7 | 0 | 1 | 6 | | | | |
| Vagina discharge | 3 | 1 | 0 | 2 | | | | |
| Infertility | 1 | 1 | 0 | 0 | | | | |
| Medical comorbidity | | | | | | | | |
| History of cancer | 2 | 0 | 1 | 1 | | | | |
| Hypertension | 6 | 0 | 0 | 6 | | | | |
| Diabetes | 2 | 0 | 1 | 1 | | | | |
| PCOS | 1 | 1 | 0 | 0 | | | | |
| Infertility | 1 | 1 | 0 | 0 | | | | |
| Surgical comorbidity | 0.57 (0.74) | 0.20 (0.45) | 0.83 (1.17) | 0.58 (0.67) | 0.371 | 0.164 | 0.447 | 0.290 |
| Laparotomy | 12 | 0 | 3 | 11 | | | | |
| Laparoscopy | 2 | 1 | 1 | 1 | | | | |

*(Continued)*

TABLE 1 Continued

| | Total | First group (*n* = 5) | Second group (*n* = 6) | Third group (*n* = 31) | *p*-value | *p*1 | *p*2 | *p*3 |
|---|---|---|---|---|---|---|---|---|
| Menopause | | | | | | | | |
| Yes | 11 | 2 | 1 | 8 | 0.423 | 0.317 | 0.405 | 0.132 |
| No | 31 | 3 | 5 | 23 | | | | |
| Childbearing history | | | | | | | | |
| Gestation | 2.83 (2.26) | 3.20 (2.59) | 3.33 (2.50) | 2.66 (2.22) | 0.749 | 0.924 | 0.516 | 0.628 |
| Pregnancy | 1.18 (1.03) | 1.2 (1.10) | 1.33 (0.82) | 1.13 (1.09) | 0.918 | 0.837 | 0.683 | 0.904 |
| Family history | 15 | 2 | 2 | 11 | 0.529 | 0.655 | 0.071 | 0.033 |
| BMI | 24.62 (3.89) | 23.17 (3.41) | 25.32 (3.57) | 24.73 (4.07) | 0.645 | 0.373 | 0.737 | 0.418 |
| Preoperative histology | | | | | | | | |
| Endometrioid G1 | 7 | 1 | 0 | 6 | | | | |
| Endometrioid G2 | 6 | 0 | 2 | 4 | | | | |
| Endometrioid G3 | 5 | 0 | 0 | 5 | | | | |
| Dysplasia | 12 | 4 | 2 | 6 | | | | |
| Serous | 1 | 0 | 0 | 1 | | | | |
| Mucinous | 2 | 1 | 0 | 1 | | | | |
| Carcinosarcoma | 1 | 0 | 0 | 1 | | | | |
| Mixed | 2 | 1 | 0 | 1 | | | | |
| Unidentified | 8 | 0 | 2 | 6 | | | | |

follow-up, only two patients reported successively observed lymphatic cysts by radiology, and they have been constantly monitored with no evidence suggesting a relapse ever since. No more late complications are observed.

## Assessment of creating a learning curve

Our learning curve is exhibited in Figure 2.

The first inflection was in five surgical cases (Figure 2). Two phases were primarily differentiated on the graph. Although the operation time tended to decrease after five cases, the descent time did not imply competence in TU-LESS. RA-CUSUM was introduced to further assess the learning curve (Figure 3). The three parameters were imported. According to the RA-CUSUM graph, the valley point was presented in the 11th case with minimal surgical failure, which was considered the achievement of competence. Combining the results of these two methods, the learning curve for TU-LESS in treating endometrial cancer was divided into three groups. The first group (cases 1–5) represented the initial learning period. The second group, which spanned six cases (cases 6–11), indicated the developed competence. The last group signified mastery and a challenging period.

## Assessment of the credibility according to the learning curve

As the inflection point was between five and 11 cases, patient backgrounds, preoperative pathophysiology, surgical results, and intraoperative and postoperative outcomes were examined and compared by dividing the patients into three phases. Each phase was analyzed for the potential learning curve effect. The baseline conditions were compared, and the three phases did not show significant differences. The mean operative time varied among the three phases. In the first two phases, it showed a drastic reduction. However, the third phase demonstrated an upward trend, exhibiting 275.20 (SD = 53.74) min, 194.83 (SD = 16.92) min, and 208.68 (SD = 31.99) min, respectively (Table 2). The average surgical estimated blood loss was 94 ml less, comparing 153.00 (SD = 153.56) ml to 60.00 (SD = 43.82) ml in the first two phases. No major intraoperative complications occurred, and only two cases were converted from single port to conventional laparoscopy due to severe pelvic adhesion. Postoperative outcomes did not show significant improvement over time among the three phases. No hospital readmission was observed in both groups for medical or surgical complications. Pathologic details were displayed in (Table 3).

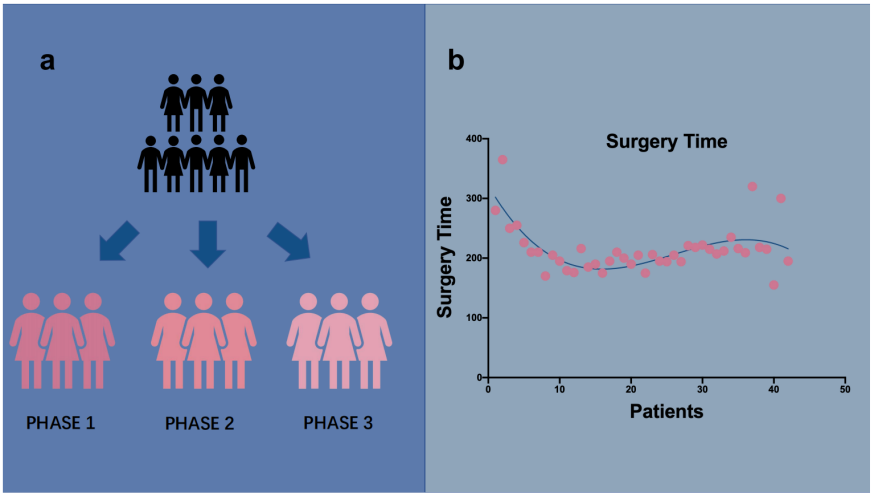


FIGURE 2
Group status and linear graph of operation time. **(A)** We separated patients in three groups In chronological order. **(B)** Surgery time is displayed in b which showed a declining trend.

## Discussion

TU-LESS is now being increasingly utilized to treat early endometrial cancer (8). A surgeon's learning curve provides us with a retrospective view of their performance. Although previous studies have assessed the learning curves of TU-LESS in endometrial cancer, they were restricted by simply arranging chronological cases into predefined segments (16). In the present study, we investigated 42 cases of endometrial cancer with LESS performed by a single surgeon at Sichuan University Second Hospital. We used the CUSUM and RA-CUSUM methods (Figures 2, 3) to evaluate our learning curve. The efficacy of this technique was achieved after five surgical cases.

TU-LESS was first widely utilized in urology and gastrointestinal surgery (17–19), where it was reported that surgeons entered their proficient stage after performing 30 cases (20). A previous study on endometrial cancer has described a learning curve with proficiency achievement after 20–40 cases (16).

It should be emphasized that our surgeon had performed over 53 procedures using TU-LESS for benign lesions and had already mastered surgical treatments for malignant tumors using conventional laparoscopy before conducting comprehensive

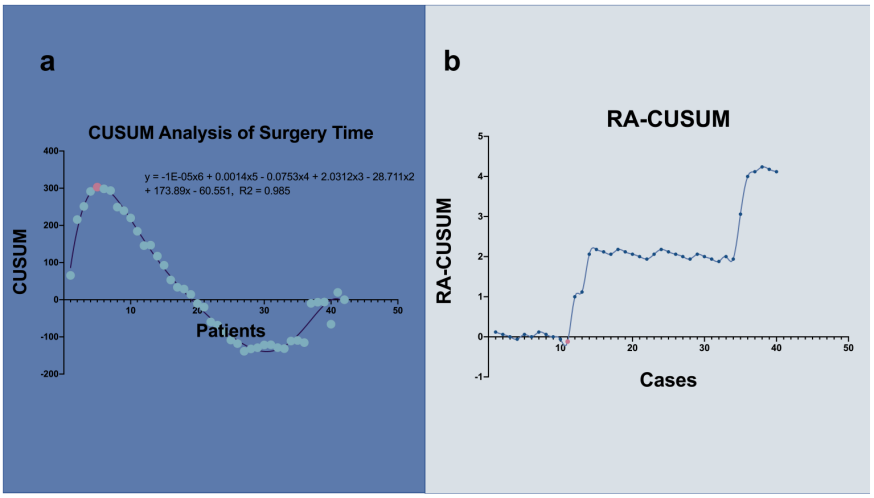


FIGURE 3
CUSUM and RA-CUSUM analysis of learning curve. **(A)** The inflection point separated the patients into 2 phases. The purple line represents the curve of best fit in a general model with equation, y = -1E-05x6 + 0.0018x5 - 0.091x4 + 2.3675x3 - 32.536x2 + 204.23x - 115.01, R2 = 0.9658. **(B)** The valley point divided the curve into 2 phases. 5 cases are needed to lay solid foundation and 11 to acquire proficiency. Corresponding points were marked with light pink.

TABLE 2 Perioperative surgical outcomes of three stages for TU-LESS in endometrial cancer (n = 42).

| Operative outcomes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | total | 1st group (n=5) | 2nd group (n=6) | 3rd group (n=31) | *P*-value | P1 | P2 | P3 |
| Surgical time | 214.62 (39.96) | 275.20 (53.74) | 194.83 (16.92) | 208.68 (31.99) | .000 | .000 | .000 | .359 |
| Blood loss | 132.02 (203.85) | 154.00 (153.56) | 60.00 (43.82) | 142.42 (228.25) | .653 | .457 | .377 | .908 |
| Surgical complications | | | | | | | | |
| Vascular complication | 3 | 0 | 0 | 3 | .392 | | .317 | .480 |
| Perioperative outcomes | | | | | | | | |
| Postoperative complications | | | | | .752 | | | |
| Lymphatic retention with infection | 1 | 0 | 0 | 1 | | | | |
| Sentinel lymph node | 2 | 0 | 0 | 2 | | | | |
| Conversion | 2 | 0 | 0 | 2 | | | | |
| Hospital stay | 5.05 (1.36) | 4.40 (5.55) | 4.83 (1.17) | 5.19 (4.47) | .452 | .872 | .840 | .490 |
| Enhanced recovery index | 2.97 (0.92) | 2.60 (0.89) | 2.67 (0.52) | 3.13 (0.99) | .331 | .151 | .041 | .533 |
| First passage of flatus | 2.57 (0.89) | 3.00 (0.71) | 2.50 (0.84) | 2.52 (0.97) | .527 | .656 | .999 | .538 |
| VAS pain score | | | | | | | | |
| 12 | | 2.2 (0.84) | 1.67 (0.82) | 1.79 (0.82) | .470 | | | |
| 24 | | 1.60 (0.55) | 2.17 (0.41) | 1.66 (0.55) | .115 | | | |
| 36 | | 0.80 (0.45) | 0.50 (0.84) | 1.17 (0.71) | .084 | | | |

staging surgery for endometrial cancer through TU-LESS. This could be why our surgeon only required five surgical cases to lay the technical foundation.

However, after the valley point in the RA-CUSUM graph, there was a trend of increasing surgical failure, which could be attributed to the surgeon operating on more challenging patients with higher risks.

Our surgeon required 11 cases to master this technique, a finding that is different from those of previous studies (21, 22). There was a significant reduction in operative time in the three groups. Pelvic lymph node retrieval and perioperative complications did not demonstrate significant differences. This result was observed because the procedure had been standardized and the baseline of the patients remained consistent.

Barnes et al. reported after including 110 patients that the average surgery time was 186 min (16) compared to the 208 min observed in our study. This could be due to the different operation modes in every institution and the unique anchor-suturing method (14) technique we applied. The difference was deemed acceptable.

During further exploration, we noticed that the experience of the assisting team, especially the assistant holding the laparoscope, could distort the vision, leading to disorientation. This could cause difficulty in identifying lesions, leading to prolonged operation time, potential injury to the vasculature, inadequate resection, etc. Our team had mastered laparoscopy with a relatively fixed and coordinated assistant.

Applying LESS for endometrial cancer is relatively easy for skilled operators. However, different operators may have entirely different learning curves because of their unique inline vision and relatively narrow space to operate instruments, including the difficulty of the operating handles in forming triangulations. With the change of approach, the position and postures of practitioners and the different angles of holding the instruments also vary from conventional laparoscopy. Various levels of adaptation add up to the diversity of the learning curves for different surgeons.

LESS for endometrial cancer is an advanced technique that requires the operator to be familiar with the anatomy of the pelvic cavity and proficient with laparoscopic manipulation. The learning curve is an individualistic study. We have concluded that beginners should start with simple surgeries, such as appendectomies and hysterectomies, to accumulate experience and skills. Beginners should skip ovarian cyst excisions at the start since any procedure involving suturing beneath the magnifier would enhance the difficulty.

This study might have limitations, such as a small number of patients, the insignificance of statistical results, and the absence of long-term results. It is essential to explore whether oncological outcomes might be compromised during the primary learning period. It was inevitable that the study population would be restricted to a smaller size. However, this study was an individualistic study to provide our experience in shortening the learning curve. Since combining the experiences of other

TABLE 3 Oncology outcomes of three stages for TU-LESS in endometrial cancer (n = 42)..

| Oncology outcomes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | total | 1st group (n=5) | 2nd group (n=6) | 3rd group (n=31) | P value | P1 | P2 | P3 |
| I | | | | | | | | |
| IA | 27 | 1 | 5 | 18 | | | | |
| IB | 2 | 0 | 0 | 2 | | | | |
| IA, G2-3, serous, mucinous, clear | 9 | 3 | 2 | 7 | | | | |
| IB, G2-3, serous, mucinous, clear | 1 | 0 | 0 | 1 | | | | |
| III | | | | | | | | |
| IIIA | 2 | 1 | 1 | 0 | | | | |
| IIIC | 1 | 0 | 0 | 1 | | | | |
| Pathology details | | | | | | | | |
| Peritoneal wash | | | | | | | | |
| Positive | 2 | 0 | 0 | 2 | .540 | | .157 | .257 |
| Negative | 34 | 3 | 6 | 25 | | | | |
| NE | 6 | 2 | 0 | 4 | | | | |
| LVSI | 10 | 0 | 0 | 10 | .030 | | .127 | .174 |
| Number of pelvic lymph nodes | 28.19 (10.10) | 25.6 (13.35) | 23.67 (5.13) | 29.48 (10.21) | .369 | .753 | .204 | .429 |
| Adjuvant therapy | | | | | | | | |
| Chemotherapy | 4 | 0 | 1 | 3 | | | | |
| Radiotherapy | 3 | 0 | 0 | 3 | | | | |
| Chemotherapy and radiotherapy | 2 | 0 | 0 | 2 | | | | |

practitioners in the same field could improve surgical outcomes, more studies from multiple centers should be carried out to help accumulate experience. Meanwhile, due to the early diagnosis and relatively good prognosis, quality of life and sexual function are emerging demands for patients receiving surgical intervention, which were previously underappreciated (23). We could expand on our findings by looking into this novel topic further.

Despite many obstacles, the emergence of robotic-assisted single-site laparoscopy has been advantageous (24, 25). It has been reported that the conversion rate could significantly decrease, and learning curves can be shortened with the assistance of a robot (26, 27). As stated earlier, we believe it is easy for experienced surgeons to master this technique. Our surgeon, who had excellent laparoscopy experience, mastered this technique after completing five endometrial cancer comprehensive staging operations with TU-LESS.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by West China Second Hospital Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because this was a retrospective study. This study was conducted after the patient was discharged. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article because This was a retrospective study, and when this study was conducted, the patient was discharged.

## Author contributions

FL: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. YZ: Funding acquisition, Resources, Visualization, Writing – review & editing. FY: Investigation, Supervision, Writing – review & editing. JL: Project administration, Writing – review & editing.

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## Conflict of interest

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

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# Malignant mixed mullerian tumors: a SEER database review of rurality and treatment modalities on disease outcome

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**Introduction:** Malignant Mixed Mullerian Tumors (MMMT) are rare and poorly understood sarcomas with limited research on risk factors, pathogenesis, and optimal treatments. This study aimed to address this knowledge gap and explore the impact of community size, patient characteristics, disease characteristics, and treatment modalities on MMMT outcomes.

**Methods:** Using the Surveillance, Epidemiology, and End Results database (SEER), the largest SEER cohort to date of 3,352 MMMT patients was analyzed for demographic factors, treatment modalities, and histologic characteristics. Data was processed, including the removal of incomplete entries, and analyzed in Python 3.1 using packages *scikit-learn, lifelines*, and *torch*; log-rank analysis and Cox proportional hazards models were used to evaluate a number of demographic characteristics and disease characteristics for significance in regard to survival.

**Results:** Our study found adjuvant radiotherapy and chemotherapy significantly improved survival, with modest benefits from neoadjuvant chemotherapy. Our findings also suggest age at diagnosis, disease grade, and suburban versus rural geographic locations may play key roles in patient prognosis. On multivariable analysis both disease Grade and surgical treatment were significant factors.

**Discussion:** MMMTs remain challenging, but appropriate treatment appears to enhance survival. The present findings suggest opportunities for improved outcomes and treatment strategies for patients with MMMTs.

KEYWORDS

**SEER analysis, mixed mullerian tumors, uterine carcinosarcomas, rural cancer, gynecologic cancer**

# 1 Introduction

Malignant Mixed Mullerian Tumors (MMMTs), also known as uterine carcinosarcomas, are rare and aggressive tumors that arise in the genital tract of postmenopausal women. They comprise 5% of all uterine neoplasms and 16.4% of all uterine cancer-related deaths (1, 2). Despite increased research interest in the pathologic mechanisms of MMMTs, risk factors, late diagnosis, and variable access to treatment have further contributed to a poor prognosis. Further prognostic factors include increasing age, lymph node metastasis, suboptimal surgical cytoreduction, the presence of heterologous features on histopathology, and heightened expression of VEGF, tumor protein p53, and p53 coupled with Wilms tumor 1 (WT1; 3). Current literature suggests that the five-year overall survival (OS) rate is less than 35%, which is a stark contrast to the 76% survival rate of endometrial stromal sarcomas (4, 5).

The biphasic histology of MMMT's entails both malignant epithelial and mesenchymal components commonly found in the uterus but sometimes arising in the ovaries, fallopian tubes, or vagina. The diagnosis of MMMTs is challenging due to its varied clinical presentation, which may include, but is not limited to, abnormal vaginal bleeding, bloody or watery discharge, abdominal or pelvic pain, and palpable pelvic masses (6).

The absence of any highly sensitive or specific clinical signs of this malignancy may inform why it is typically discovered relatively late on initial presentation: approximately one-third of patients possess clinical manifestations of positive regional lymph node metastasis, while the incidence of visceral metastasis at presentation is roughly 10% (7). Therapeutic options decrease with advanced disease; treatment in the presence of distant metastases is generally palliative (8, 9). Surgical intervention is indicated in masses > 6 cm or in symptomatic masses. Controversy exists regarding the notion that the epithelial component of the tumor drives metastasis as there is evidence for independent and separate metastatic potential of the two histologic components, and histological analyses of metastases have demonstrated mixed results (6, 10). The uncertainty surrounding the mechanism of metastases and the high mortality associated with disease spread underscore the importance of screening and diagnostic tools in identifying malignancy at earlier stages.

Previous systematic reviews have identified older age, Black race, obesity, long-term tamoxifen use, and prior pelvic radiation as risk factors for the development of MMMTs (9, 11–13). Previous SEER analyses have reviewed MMMT risk factors, such as medical predispositions, race, and socioeconomic variables. These studies compared the incidence, prognosis, and survival associated with different treatment modalities of uterine carcinosarcomas with carcinosarcomas of the cervix and ovaries and compared adjuvant chemoradiation to intraoperative lymphadenectomy for optimal disease control. However, none of these analyses have investigated variables such as geography, the influence of diagnostic methods of varying accuracy, treatment and survival in the non-surgical candidate, nor the role of neoadjuvant therapy. Furthermore, the largest SEER analysis of treatment trends prior to the present examination consisted of 1,541 patients (11). Our aim was to provide insight into the prognosis and OS of patients stratified by age, community residency, and available therapeutic options across chemotherapeutic, radiotherapeutic, and surgical modalities. We further offer brief analyses into treatment modalities that prolong survival in non-surgical candidates, current treatment trends in regard to adjuvant versus neoadjuvant chemotherapy and radiation, and provide an update to previously examined demographic, tumor, and treatment variables based on a twofold larger sample size on the most recent SEER data to date.

# 2 Materials and methods

This is a retrospective cohort study utilizing the Surveillance, Epidemiology and End Results (SEER) database sponsored by the National Cancer Institute (NCI; 14). This registry provides deidentified, detailed disease course data spanning approximately 28% of the US population. The registry was queried as a "Case Listing Session" for all 667 patient cases where variable "Histologic Type" was of "Malignant Mixed Mullerian Tumors". Patients were diagnosed with MMMT between 2000 and 2018. Exclusion criteria included patients with multiple primary fields, patients with an unknown or unclear diagnosis, and patients under 15 years old at the time of diagnosis.

Variables selected for review included patient demographics such as race, age, and geography, as well as tumor characteristics, including grade, tumor type, and histopathologic characteristics. Treatment modalities, such as surgical resection, radiation therapy, systemic therapy, and other adjunctive therapies were considered in conjunction with Kaplan Meier survival outcomes for various combinations and orders of the aforementioned treatment types. Outcomes based on tumor grade and histological characteristics were also analyzed. Data was processed, including the removal of incomplete entries, and analyzed in Python (Version 3.1; Scotts Valley, CA: CreateSpace) using packages *scikit-learn*, *lifelines*, and *torch*. Statistical analysis was conducted using the aforementioned Python packages and SPSS (Version 27.0; Armonk, NY: IBM Corp.), with a p-value of $< 0.05$ deemed statistically significant. Log rank analysis and Cox proportional hazards models were used to evaluate a number of demographic characteristics and disease characteristics for significance in regard to survival.

Diagnostic confirmation was interpreted in line with SEER coding standards. Briefly, tumors were coded as histologically diagnosed if microscopic diagnosis was based on fine needle aspirate, biopsy, surgery, autopsy, or dilation and curettage (D&C). Tumors were considered cytologically diagnosed if the patient was diagnosed via peritoneal fluid or cervical or vaginal smears. Histological diagnoses were also coded as higher priority compared to cytological diagnoses.

# 3 Results

## 3.1 Demographics

Utilizing selection criteria as detailed above, we obtained 3,352 cases of MMMTs. The median age at diagnosis was 64.3 years, ranging from patients between 15 and 20 years old to those older than 85. MMMTs were most prevalent in Caucasians, representing

84% of our cohort, followed by African Americans at 8.7%, Asian and Pacific Islanders at 6%, and American Indians or Alaska Natives at 0.7%. Race and other demographic information are summarized in Table 1. Patients with MMMTs had a median overall survival of 16 months with a range between zero months and 19 years (Figure 1). There were no differences in OS when comparing patients stratified by race (Figure 2). Exploring results categorized by rural/urban categorization provided deeper insight into the discrepancies between rural and urban communities. Kaplan Meier analysis demonstrated that nonmetropolitan counties not adjacent to a metropolitan area had the lowest

TABLE 1 Demographic Characteristics & Corresponding Survival of MMMT.

| Variable | N | Median Survival in months (min, max) |
|---|---|---|
| **Race** | | |
| White | 2822 | 16 (0, 227) |
| Black | 292 | 10 (0, 210) |
| Asian or Pacific Islander | 200 | 18 (0, 223) |
| American Indian / Alaska Native | 24 | 9.5 (0, 166) |
| **Urban-Rural Status** | | |
| Metropolitan Counties: > 1 million population | 676 | 16 (0, 354) |
| Metropolitan Counties: 250,000 to 1 million population | 422 | 18 (0, 279) |
| Metropolitan Counties: < 250,000 population | 110 | 13.5 (0, 351) |
| Nonmetropolitan Counties adjacent to Metropolitan area | 110 | 19.5 (0, 324) |
| Nonmetropolitan Counties not adjacent to Metropolitan area | 97 | 12 (0, 336) |
| **Age** | | |
| 25 - 29 year olds | 10 | 63 (2, 151) |
| 30 - 34 year olds | 10 | 86 (28, 220) |
| 35 - 39 year olds | 28 | 22 (1, 221) |
| 40 - 44 year olds | 85 | 29 (1, 226) |
| 45 - 49 year olds | 152 | 22 (0, 224) |
| 50 - 54 year olds | 283 | 21 (0, 227) |
| 55 - 59 year olds | 385 | 20 (0, 222) |
| 60 - 64 year olds | 458 | 18.5 (0, 210) |
| 65 - 69 year olds | 531 | 17 (0, 226) |
| 70 - 74 year olds | 495 | 15 (0, 219) |
| 75 - 79 year olds | 423 | 13 (0, 210) |
| 80 - 84 year olds | 312 | 10 (0, 194) |
| 85 + year olds | 176 | 5.5 (0, 154) |
| **Tumor Grade** | | |
| Well Differentiated; Grade I | 29 | 130 (0, 221) |
| Moderately Differentiated; Grade II | 51 | 43 (1, 219) |
| Poorly Differentiated; Grade III | 1045 | 19 (0, 226) |
| Undifferentiated; Grade IV | 695 | 18 (0, 226) |
| **Diagnostic Confirmation** | | |
| Positive Histology | 3245 | 16 (0, 227) |
| Positive Exfoliative Cytology, No positive Histology | 95 | 8 (0, 193) |
| **Cancer-Directed Surgery** | | |
| Surgery performed | 2942 | 18 (0, 227) |
| Not recommended | 324 | 5 (0, 224) |
| Not recommended; contraindicated due to other condition | 32 | 2 (0, 20) |
| Recommended but not performed; unknown reason | 20 | 17.5 (0, 98) |
| Recommended but not performed; refused | 12 | 5.5 (0, 27) |
| Recommended; unknown if performed | 14 | 19 (2, 61) |
| **Radiation** | | |
| Standard Fractionation External Beam Radiation Therapy | 98 | 21.5 (1, 222) |
| None/Unknown | 3219 | 16 (0, 227) |
| **Chemotherapy** | | |
| Yes | 2413 | 20 (0, 227) |
| None/Unknown | 937 | 5 (0, 226) |
| **Neoadjuvant vs. Adjuvant Therapy** | | |
| Radiation after surgery | 93 | 22 (1, 222) |
| Systemic therapy before surgery | 122 | 17 (0, 131) |
| Systemic therapy after surgery | 1314 | 21 (0, 153) |
| Systemic therapy both before and after surgery | 164 | 20.5 (0, 96) |
| Surgery both before and after systemic therapy | 6 | 25 (7, 34) |
| No radiation and/or cancer-directed surgery | 3250 | 16 (0, 227) |
| No systemic therapy and/or surgical procedures | 749 | 5 (0, 155) |

*(Continued)*

TABLE 1 Continued

Table depicting various demographic characteristics for patients diagnoses with malignant mixed Mullerian tumors based on analysis of the Surveillance, Epidemiology, and End Results (SEER) Program.

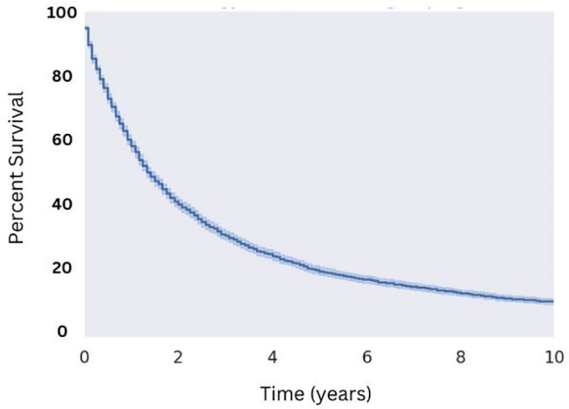



FIGURE 1
Overall Survival for Patients with MMMT. N=3,352 patients were extracted from the SEER database from the 2000 to 2018 reporting years with the diagnosis of MMMT, yielding a median OS of 16 months.

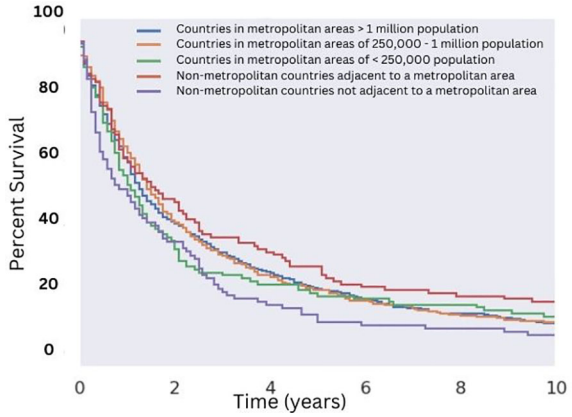



FIGURE 3
Overall Survival for Patients with MMMT by Urban-Rurality Status. OS of patients diagnosed with mixed mullerian malignant tumors in the 2000-2018 SEER database were stratified by Urban-Rurality county classification. Patients from non-metropolitan counties not adjacent to a metropolitan area had the lowest median survivorship (12 months). Patients from nonmetropolitan counties adjacent to a metropolitan area had the greatest median survivorship (20 months), which was significant compared to the aforementioned group ($p < 0.05$).

overall survivorship rates with a median survivorship of 12 months. Conversely, nonmetropolitan counties adjacent to a metropolitan area had significantly greater survival with a median of 19.5 months ($p < 0.05$) as illustrated in Figure 3.

## 3.2 Tumor characteristics

Regarding characteristics of the malignancy itself, we found a large majority of cases (86%) exhibited high tumor grade, and that grade and differentiation significantly impacted survivorship. Well-differentiated (Grade I) and moderately differentiated (Grade II) carcinosarcomas demonstrated greater OS relative to poorly differentiated (Grade III) and undifferentiated (Grade IV) carcinosarcomas (Figure 4). Patients with Grade I disease saw a significantly improved median OS among all groups (10.8 years), compared to a median OS of 43 months for patients with Grade II disease, 19 months for patients with Grade III disease, and 18 months for patients with Grade IV disease.

## 3.3 Diagnostic methods

Patients who received a histological diagnosis demonstrated a significantly greater OS compared to those who were diagnosed via cytology ($p < 0.05$). Only 20% of patients with a cytological

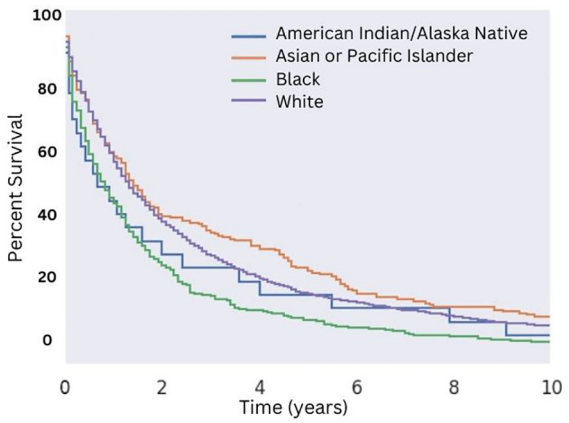



FIGURE 2
Overall Survival for Patients with MMMT by Race. OS of patients diagnosed with mixed mullerian malignant tumors in the 2000-2018 SEER database were stratified by race. There was no significant difference in median OS ($p > 0.05$) across all races. An increased median OS of 16 months versus 13 months for White patients and non-White patients, respectively, was seen ($p = 0.03$).

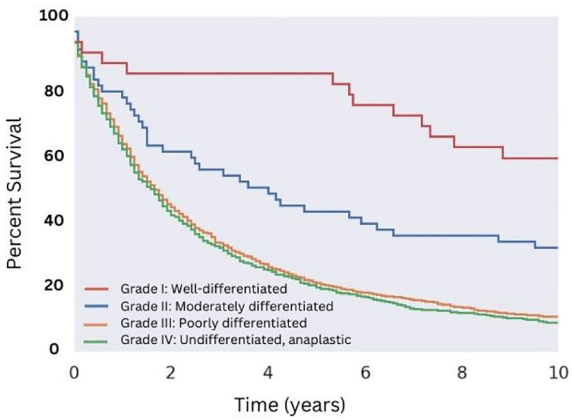



FIGURE 4
Overall Survival for Patients with MMMT by Histologic grade. OS of patients diagnosed with mixed mullerian malignant tumors in the 2000-2018 SEER database were stratified by histologic grade. Well-differentiated disease showed the highest median OS of 130 months ($p < 0.05$), followed by moderately differentiated disease (median OS of 43 months, $p < 0.05$). Both poorly differentiated and undifferentiated disease showed no difference in median OS of approximately 19 and 18 months, respectively ($p = 0.13$).

diagnosis received surgery, compared to 90% of patients with a histological diagnosis. 8% of patients with a histological diagnosis did not undergo surgery, while 62% of patients with a cytological diagnosis did not undergo surgery. There was no statistically significant difference in survival following surgery between patients who received a histological diagnosis and patients who did not. Cytological specimens were obtained via endometrial sampling from patients and submitted for diagnostic testing.

## 3.4 Treatment analysis

Patients who underwent surgical resection of well-differentiated tumors had significantly greater median OS compared with those that did not (18 months for those that received surgery compared to five months for those that did not; Figure 5). While there were a variety of radiotherapeutic modalities used to treat MMMTs, including External Beam Radiation Therapy (EBRT), brachytherapy, and the combination of the two, EBRT was by far the most popular (approximately 75% of patients who received radiation therapy). Patients who received EBRT lived significantly longer than those who did not (Figure 6A, $p < 0.05$ in regards to median OS). Systemic chemotherapy also prolonged survival, however, any survival benefit of chemotherapy did not appear to persist beyond 5 months (Figure 6B).

### 3.4.1 Adjuvant and neoadjuvant analysis

Radiation therapy was used as both adjuvant and neoadjuvant therapy, as well as delivered intraoperatively for the management of MMMTs. Due to low sample size of patients treated with intraoperative and neoadjuvant radiation (four patients total) this limited any analysis to determine if timing and or modality had a significant effect. Similarly, systemic chemotherapy was also given in both the adjuvant and neoadjuvant setting. Adjuvant chemotherapy appeared to be most effective in prolonging OS ($p < 0.05$), with an increase of about 8% at the five-year mark compared to those who received neoadjuvant therapy ($p < 0.05$; Figure 7). This trend of increased survival did not persist approaching the 10-year mark. Patients receiving both adjuvant and neoadjuvant therapy fared the worst, with all patients dying within eight years of diagnosis.

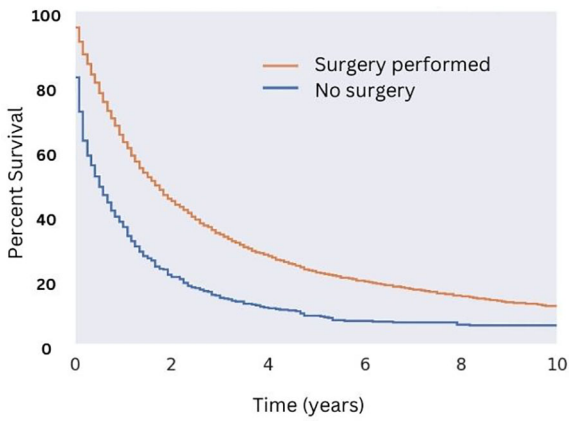



FIGURE 5
Overall Survival for Patients with MMMT by Surgical Status. OS of patients diagnosed with mixed mullerian malignant tumors in the 2000-2018 SEER database were stratified by presence or lack of surgical management. Patients who underwent surgery saw an increase in median OS (18 months versus five months, $p < 0.05$) and 10-year OS (10% versus 1% for those who did not undergo surgery; $p < 0.05$).

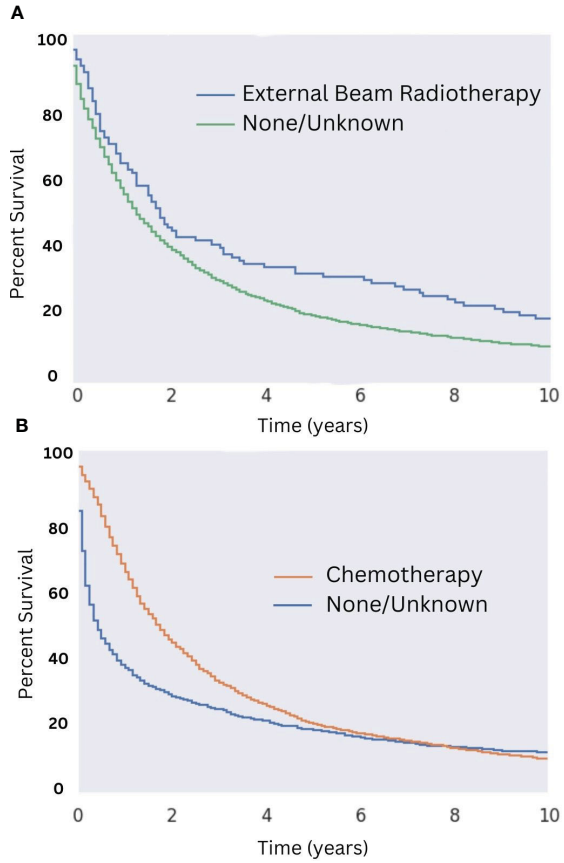



FIGURE 6
Overall Survival for Patients with MMMT by Radiotherapy and Chemotherapy Status in those who underwent Surgery. OS of patients diagnosed with mixed mullerian malignant in the 2000-2018 SEER database was stratified by presence or lack of radiotherapy **(A)** and chemotherapy **(B)** in those who underwent surgery. Patients who received external beam radiation therapy saw an increased 10-month OS of 17% compared to those who did not (10-month OS of about 9%; $p < 0.05$). Median OS was 22 months for patients who received radiotherapy, compared to 17 months for those who did not. No difference in 10-month OS was seen between patients who did or did not receive chemotherapy. Median OS in patients who received both surgery and chemotherapy was 21 months, compared to seven months for those who received surgery but did not receive chemotherapy.

## 3.5 Non-surgical candidate analysis

Among patients who were not surgical candidates, the available data suggest that chemotherapy (median survival of 12 months) but not radiation (median survival of 11 months) provided a survival benefit (see Figures 8A, B, respectively). The benefit of radiation among patients who did not receive surgery may be obscured by the low sample size, as only eight patients who did not undergo surgery received radiation. The survival benefit afforded by chemotherapy

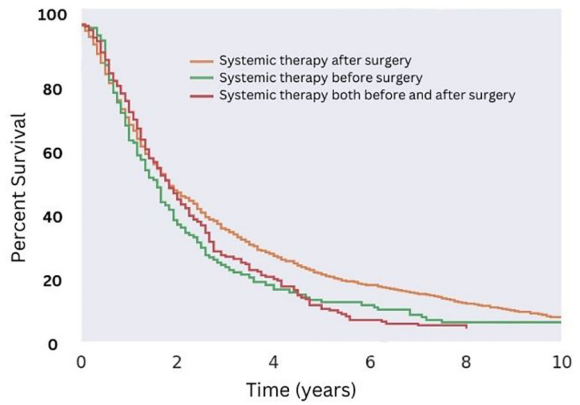



FIGURE 7
Overall Survival for Patients with MMMT by Neoadjuvant vs Adjuvant Chemotherapy Status. OS of patients diagnosed with mixed mullerian malignant tumors in the 2000-2018 SEER database were stratified by timing of adjuvant chemotherapy vs surgery. Patients received adjuvant, neoadjuvant, or both adjuvant and neoadjuvant chemotherapy. Adjuvant therapy showed improved OS at five years ($p < 0.05$) though this trend did not persist at the 10-year mark.

in this population was most notable in the first five years after diagnosis with median survival being one month for patients not treated with chemotherapy and 12 months for those that were (Figure 8B; $p = 0.44$). Of patients who did not receive surgery, 40% did not receive either chemotherapy or radiation. The median survival for this subset of patients was five months.

## 3.6 Overall trends and multivariable analysis

Univariant analysis was performed in regards to overall survival. Hazard ratios (HR) for selected variables in this analysis are displayed in Figure 9. It can be seen that patients older than 65 years of age (HR: 0.68; $p < 0.05$) and non-White patients (HR: 1.16; $p < 0.05$) saw decreased OS compared to patients diagnosed with MMMT at less than 65 years of age and White patients, respectively. Patients with Grade I or II disease saw increased OS compared with those diagnosed with Grade III or IV disease (HR: 2.60; $p < 0.05$). Patients treated with chemotherapy (HR: 3.00; $p < 0.05$) and radiotherapy (HR: 1.57; $p < 0.05$) both saw increased OS, compared to those that did not receive these treatments. Patients treated with surgery saw the greatest increase in OS for all treatment modalities (HR: 2.53; $p < 0.05$) compared to those that did not. Multivariable analysis controlling for treatment modality, disease grade, patient age, and patient race showed significance with only disease grade and surgical treatment of MMMT.

# 4 Discussion

## 4.1 Literature review

As of today, histological examination of tissue samples remains the gold-standard for diagnosis. In the literature, 75% of MMMTs are misdiagnosed as adenocarcinoma preoperatively given MMMTs biphasic nature and correct surgical staging often requiring large tissue samples (15). A negative endometrial biopsy does not rule out diagnosis as it does not obtain sufficient tissue (16). The use of early tumor imaging is of benefit for suspected patients with uterine cavities not readily accessible for biopsy but can be nonspecific in differentiating one tumor from another (17). Exfoliative cytology may provide confirmation if a biopsy is not available or appropriate. However, this method has variable and limited sensitivity, with 60% of tests correctly detecting some form of malignancy and only 8.6% of cytology specimens correctly detecting MMMTs specifically, as opposed to mistaking MMMTs for adenocarcinoma (17, 18). Therefore, histopathological confirmation is important even in those that are not surgical candidates as correct diagnosis has significant implication on prognosis and management.

Now considered an atypical histology by most treatment algorithms, MMMTs are overall treated similarly to high-grade endometrial adenocarcinoma. Endometrial adenocarcinoma is a

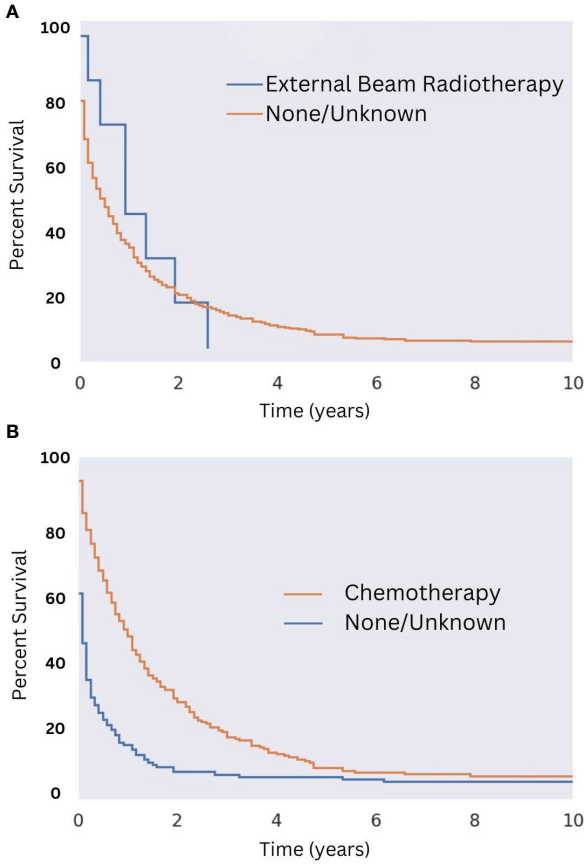
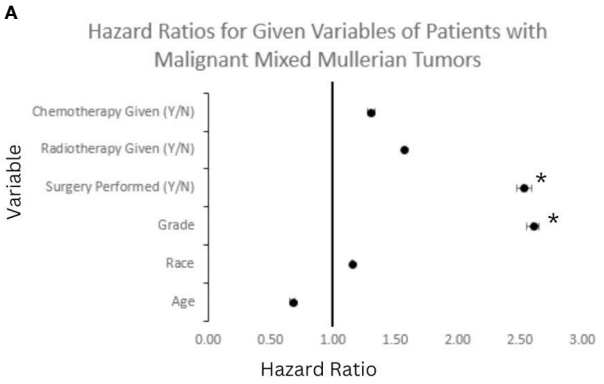



FIGURE 8
Overall Survival for Patients with MMMT by Radiotherapy and Chemotherapy Status in those not undergoing Surgery. OS of patients diagnosed with mixed mullerian malignant tumors in the 2000-2018 SEER database were stratified presence or lack of radiotherapy **(A)** and chemotherapy **(B)** in those who are not surgical candidates. Radiotherapy was not seen to improve OS ($n = 8$; $p = 0.44$). Chemotherapy improved OS slightly (2% improvement; $p < 0.05$). This survival benefit was most notable in the first five years following diagnosis with a median survival being one month for patients not treated with chemotherapy and 12 months for those who were ($p < 0.05$).

A




B

| Variable | Hazard Ratio (95% Confidence Interval) | p-value |
|---|---|---|
| Age (≥ 65 years vs < 65 years) | 0.68 (0.66, 0.70) | p < 0.005 |
| Race (White vs non-White) | 1.16 (1.15, 1.16) | p = 0.03 |
| Grade (I and II versus III and IV) | 2.60 (2.56, 2.66) | p < 0.005 |
| Surgery Performed (Y versus N) | 2.53 (2.48, 2.59) | p < 0.005 |
| Radiotherapy Given (Y versus N) | 1.57 (1.56, 1.58) | p < 0.005 |
| Chemotherapy Given (Y versus N) | 1.30 (1.28, 1.33) | p < 0.005 |

FIGURE 9
Forest Plot of Selected Variables and Multivariable Analysis. Hazard ratio (HR) calculations for selected variables based on overall survival (OS) of patients diagnosed with mixed mullerian malignant tumors between 2000 and 2018 based on analysis of the SEER database. **(A)** A Forest plot of selected variables highlights that on multivariable analysis surgical status and Grade I or II disease (denoted with a *) maintained significance in regards to OS. **(B)** Table depicting the breakdown of HR and 95% confidence intervals for each variable in the Forest plot above. All HR calculations met significance criteria.

relatively well-understood disease, and treatment guidelines fractionate patients very finely based on disease stage and grade. Inherent to their biphasic histology, MMMTs are more complex than endometrial adenocarcinoma, with treatment guidelines more nuanced than those of endometrial adenocarcinomas— indeed, differences in the pathophysiology of disease spread between high grade endometrial cancers and MMMTs have been recognized for nearly 20 years (19). MMMTs exhibit resistant behavior against conventional chemotherapies and have a high rate of recurrence—projected to be 37% and 46% at stages I and II respectively, with further increase as the disease progresses. This has been attributed to the disease's histological features, including high-grade epithelial and sarcomatous qualities (6, 20). While a complete surgical resection remains the primary approach, the ongoing debate regarding the optimal treatment regimen stems from suggestions to improve treatment paradigms by offering different modalities based on the stage at presentation and more precisely evaluating surgical candidacy in the context of patient risk factors (21, 22). Adjuvant treatment recommendations including chemotherapy and radiation, also remain unclear, as does disease prognosis in the setting of adjuvant treatments (23).

Due to their rarity, MMMTs have remained understudied in terms of identifying contributing risk factors and understanding their effects on pathogenesis. In an effort to address this knowledge gap, we conducted the largest study to date specifically focusing on MMMTs, with a particular emphasis on community size, the utilization of adjuvant/neoadjuvant treatments, and the care of nonsurgical candidates. We identify patients from suburban areas as having the best disease outcomes, and patients from rural areas as having the worst. We also address the paucity of data in the literature regarding the role of adjuvant and neoadjuvant therapies, finding significant improvements in survival associated with both adjuvant radiotherapy and chemotherapy, and a more modest improvement associated with neoadjuvant chemotherapy (9, 13). While there is existing literature and well-established guidelines concerning the utilization of combined therapies for endometrioid adenocarcinomas, our study aims to provide findings that encourage further exploration of similar treatment regimens for MMMTs (24). This knowledge has the potential to improve patient outcomes, guide treatment decisions, and pave the way for the development of more effective therapeutic strategies for this rare disease.

## 4.2 Rurality

Current literature focusing on cancer incidences and patient rurality statuses collapse the multilevel variability of rurality status into two broad categories (metropolitan vs. non-metropolitan) due to small numbers in some strata limiting the increasing number of subcategories (25). However, this measure of rurality may mask differences amongst subgroups within each group and could shroud potential variables differentiating various metropolitan and nonmetropolitan areas. Our study further explores the variable nuances of metropolitan and nonmetropolitan populations through the differentiation of various population sizes in metropolitan areas and the general proximity status to larger metropolitan counties for nonmetropolitan areas. Results show that nonmetropolitan counties adjacent to metropolitan counties did not show a significant difference in overall survival when compared to metropolitan counties. However, a statistically significant difference of overall survival was found between nonmetropolitan counties not adjacent to metropolitan counties and large metropolitan counties ($p < 0.05$).

These results could be the outcome of limited resource availability for isolated nonmetropolitan counties not adjacent to metropolitan counties when compared to nonmetropolitan areas with large metropolitan infrastructure nearby. Previous studies have supported that those residing in isolated rural communities have higher barriers to pivotal treatment modalities. These barriers, such as increased travel distance, decreased professional access, and decreased health literacy, often increase in severity and prevalence further rural communities are from larger metropolitan areas (26). Additionally, it has been found that limitations of access to modern cancer treatment modalities and screening mechanisms in isolated rural communities can lead to overall higher cancer staging at diagnosis and poorer average prognosis (27–29). In our study, this trend of rural patients presenting with later stage disease was noted but not significant, likely due to the low number of MMMT patients

who also lived in rural areas. It is probable that the many barriers of isolated patient rurality status contribute to the worse survival outcomes of patients from rural counties further away from higher population metropolitan counties.

## 4.3 Diagnostic methods

Cytology stands as a valuable initial screening tool owing to its non-invasive nature, rapid and ready accessibility and cost-effectiveness. However, cytology often fails to offer conclusive results or shows a limited picture of the condition at hand. This presents a challenge in its utilization compared to histology, notably due to the variable pathological presentation of MMMTs. Our research also demonstrated a positive histological diagnosis was associated with improved survival relative to patients diagnosed with cytology. Further investigation revealed patients with positive histology were significantly more likely to be offered surgery than patients with positive cytology. As a result of SEER coding convention, and in the clinical context of uterine and endometrial carcinomas, a histological diagnosis may refer to either a biopsy/D&C, or a histological examination of a surgical specimen. Furthermore, a patient who received both a cytological and surgical pathology diagnosis would be coded only as having received a histological diagnosis. Given this ambiguity, it is difficult to construct a causal understanding of the relationship between surgical candidacy and diagnostic method: it may be that all patients initially received a cytological diagnosis, and only the subset of those patients that were surgical candidates received a histological diagnosis in the form of a surgical pathology examination, thus making cytologic diagnosis a proxy of surgical candidacy. On the other hand, patients with only cytologic pathological analysis were likely not surgical candidates due to advance disease stage or comorbidities.

## 4.4 Treatment

Surgical resection, which by standard is a total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH-BSO) with or without nodal evaluation, is favored as the initial treatment approach and is generally considered a sufficient therapy option for patients diagnosed with an early stage MMMT (21, 22). Prior studies performed exploring the reasons behind cancer patient refusal of recommended cancer-directed treatment have shown that patients' socioeconomic conditions, reflected by marital status and medical insurance plan, can play a significant role in the decision-making process (30, 31). It is possible that patients are refusing therapies and surgeries due to the cost and resulting financial burdens these procedures can create. Other articles have also explored the effect of rurality on patient refusal. Particularly, patient residence in a rural county has displayed an association with increased health care avoidance behaviors (32), a specific association that was not able to be investigated in the present study

Chemotherapy was the most frequently utilized treatment modality within the subset of patients in our study who were not surgical candidates. Among non-surgical candidates, often attributable to metastatic disease, extensive pelvic nodal involvement, or presence of medical comorbidity, our data suggests chemotherapy may still offer a survival benefit of approximately six additional months. Examination of the prognosis of patients with recurrent MMMT revealed salvage therapy utilizing radiotherapy, chemotherapy, and chemoradiotherapy (CRT) all contributed to improved survival after recurrence (SAR) and cancer-specific survival (CSS). Notably, while surgical intervention improved CSS, it did not significantly improve SAR (33). In line with our own study findings, the literature supports the potential of chemotherapy to extend life in this patient population (33). Therefore, non-surgical modalities may be of benefit for patients with MMMT in recurrent circumstances.

Lastly, targeted radiation may have a role in the palliative setting based on tumor growth patterns and/or metastasis. The conclusions drawn from studies on MMMT indicate that the palliative care approach responds to individual patient needs rather than constituting an integral aspect of routine diagnostic care. Patients receiving any modality of treatment, be it for curative or palliative intent, with or without surgery, have been shown exhibit better overall survival rates (34). Palliative interventions to alleviate symptoms included radiotherapy and chemotherapy. Referrals for palliative care were frequent among patients experiencing recurrent or progressive disease rather than those demonstrating resistance to adjuvant therapy (35). This approach aimed to manage symptoms, facilitate hospice care, and assist in end-of-life care decision-making. As such, numerous non-surgical interventions remain available for patients with aggressive malignancies, but further research is warranted to explain these findings.

Advancements to treatment options bring hope in increasing OS relative to patients' age of diagnosis, initial MMMT staging, and post-surgical outcomes. Our findings suggest adjuvant radiation therapy provided an independent survival benefit, both overall and in the subpopulation of patients who received chemotherapy ($p < 0.05$). Our results also showed a survival benefit associated with chemotherapy among the subset of patients who were not surgical candidates. This suggests multimodal therapy may offer benefit for patients with MMMTs, and based on individual patient tolerability, perhaps even for patients with inoperable tumors.

The use of adjuvant chemotherapy and radiation therapy has both increased over time and improved the OS of patients with MMMTs (7). A study of older MMMT patients did not find sufficient data to suggest restricting adjuvant radiation ($p = 0.28$) or chemotherapy ($p = 0.61$) as further treatment options, even in late-stage disease (36, 37). Similarly, the present examination found adjuvant chemotherapy and radiation to both individually offer a survival benefit. Neoadjuvant chemotherapy was found to offer a small survival benefit, but given the larger benefit of adjuvant therapy, and the importance of not delaying surgical treatment, clinicians should exercise caution in therapeutic planning for the neoadjuvant setting. These results are in line with a study in Stage

IV MMMT patients, which found no significant improvement in OS when comparing the use of neoadjuvant therapy followed by resection in initially inoperable patients to candidates who underwent primary surgery (38).

### 4.5 Limitations and concluding remarks

Our study has several limitations that should be acknowledged. First, due to its retrospective nature, there are inherent limitations associated with analyzing previously collected data. There were instances of missing or unknown data, particularly regarding the grade and stage of tumors and cause of death, which, if there were a pattern to the missing values, could affect the accuracy of our analyses. Another weakness is that the SEER database is observational and cannot establish causality between variables, nor can it assess treatment details such as radiation doses, specific chemotherapeutic regimens, or medical comorbidity. For example, in non-operative patients there was no benefit to OS with radiotherapy, however, there is no way in the SEER database to distinguish if definitive radiotherapy was attempted vs palliation to a metastasis, diluting any potential effect that aggressive radiotherapy may have in non-operative patients. Further, patient follow-up data is limited and data regarding post-treatment complications, functional impairments, and patient-reported outcomes are not available through this database and may be a source of potential future investigations.

The present work indicates surgery and adjuvant chemotherapy and/or radiotherapy as important determinants for improved OS for MMMT. Neoadjuvant therapy remains relatively uncommon and offers only a modest benefit relative to the absence thereof and portends significantly worse outcomes compared to adjuvant therapies. For those who are not surgical candidates, the present analysis demonstrates that radiotherapy and/or chemotherapy improve OS in those who are not surgical candidates. Current risk factors for poor treatment outcomes have expanded in the present work to include rural patients adjacent to nonmetropolitan areas. Similarly, we have shown that increased grade plays a significant negative role in outcomes in this more up-to-date SEER population. In contrast to the analysis of the older SEER data (2) there were some similarities and differences encountered with the larger patent dataset: Nama et al. found that tumor histology, age greater than 40 years, Black race, disease grade (undifferentiated), and the presence of distant metastases were all independently associated with increased mortality. In contrast, this study found that age under 65 years, race being White (versus non-White), treatment with either surgery, radiotherapy, or chemotherapy, and disease Grade (Grade I or II versus III or IV) all conveyed an increase in OS on univariate analysis, though on multivariable analysis only disease Grade and treatment with surgery maintained this trend. In summary, MMMT is a complex and aggressive sarcoma subtype that continues to be characterized by poor prognosis, however this updated analysis of the present work indicated that this malignancy possesses an at least partial and possibly synergistic responsiveness to radiotherapeutic, chemotherapeutic, and surgical modalities.

## Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/seerstat/download/; see methods section for query criteria.

## Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

## Author contributions

NZ: Data curation, Writing – original draft, Writing – review & editing. AB: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Visualization, Writing – review & editing. VS: Conceptualization, Investigation, Methodology, Writing – original draft. MP: Writing – review & editing. GE: Writing – review & editing. ML: Project administration, Writing – review & editing, Investigation. MP: Project administration, Writing – review & editing, Methodology, Resources, Supervision, Validation.

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## Conflict of interest

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

## Publisher's note

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# Benign Brenner tumor of the ovary: two-dimensional and contrast-enhanced ultrasound features—a retrospective study from a single center

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**Objective:** Benign Brenner tumor (BBT) is a rare ovarian tumor, and there are few discrete reports about its manifestation in an ultrasound. This study sought to investigate the two-dimensional (2D) and contrast-enhanced ultrasound (CEUS) features of this entity.

**Methods:** This is a retrospective single-center study. The clinical manifestations, laboratory examination, and ultrasound data of 25 female patients with BBT were confirmed by pathology when they underwent 2D and/or CEUS examination at Ningbo First Hospital from January 2012 to June 2023. The ultrasound findings of the patients were analyzed using the terminology of the International Organization for the Analysis of Ovarian Tumor and were read by two senior sonographers who reached an agreement.

**Results:** Among the all 25 patients, most of them were unilateral, and only one patient was bilateral. Thus, 26 lesions were found: 44.0% (11/25) were in the left and 52.0% (13/25) were in the right. Moreover, 53.84% (14/26) were solid lesions, 15.38% (4/26) were mixed lesions, and 26.92% (7/26) were cystic lesions. Among the solid-type patients, 42.85% (6/14) of the cases were with calcification. Upon laboratory examination, 12.0% (3/25) of the patients had high carbohydrate antigen 125 (CA-125) level, and 19.04% (4/21) of the patients had an elevated carbohydrate antigen724 (CA-724) level in the serum tumor markers. In the hormone test, 14.28% (3/21) were found to have a high postmenopausal estrogen level and 14.28%(3/21) were found to have a high level of follicle-stimulating hormone (FSH). One patient with complex manifestations and three with solid manifestations were examined by CEUS to observe the microcirculation perfusion of the tumor. One with solid and cystic separation was rapidly hyperenhanced and cleared, and the filling subsided faster than the uterus. The postoperative pathological diagnosis was benign Brenner tumor with mucinous cystadenoma. The other three cases were solid adnexal lesions, which showed isoenhancement on CEUS and disappeared slowly, synchronizing with the uterus. The CEUS results were considered as benign tumors and confirmed by pathology.

**Conclusions:** BBT can show ovarian cystic, mixed cystic and solid type, and solid echo in 2D ultrasound. Unilateral ovarian fibrosis with punctate calcification is an important feature of BBT in 2D ultrasound. However, for solid adnexal masses and mixed cystic and solid masses with unclear diagnosis, if CEUS shows isoenhancement or hyperenhancement, the possibility of BBT cannot be excluded.

KEYWORDS

Brenner tumor, pathology, benign, diagnosis, surgery, ultrasound

# Introduction

MacNaughton-Jones was the first to report on Brenner tumors (BT) in 1898, and then Fritz Brenner described the tumor that bears his name in 1907 (1). It is a rare ovarian tumor derived from the transitional epithelium of the urinary tract and accounts for approximately 2%–3% of epithelial tumors in the ovary (2). Ovarian transitional cell tumor has been replaced by BT according to the latest classification of ovarian cancer by the World Health Organization (3). BT has three subtypes: benign Brenner tumor (BBT), which is by far the most common; borderline, also known as dysplastic BT; and malignant. Borderline and malignant BTs are extremely rare, accounting for less than 10% of BTs (4), and tend to occur in older patients (5). Most of the benign tumors are between 5 and 6 cm in diameter, and a few cases are more than 10 cm (6). Borderline and malignant BTs can reach a maximum diameter of 30 cm (7). Most patients are asymptomatic, but some may experience abdominal pain, vaginal bleeding due to estrogen activity, urinary retention, ascites, or pseudo-Meigs syndrome (8). BTs can occur at any age, but mostly at the ages of 50–70 years, and are often detected incidentally during routine physical and/or ultrasound examination (9). Preoperative ultrasound examination is very important for the treatment and differentiation of benign and malignant ovarian lesions. However, although the International Ovarian Tumor Analysis (IOTA) group has established the imaging criteria for ovarian cancer identification, the ultrasound appearance of BT overlaps with the appearance of a typical ovarian cancer, leading to image bias (10, 11). BTs are usually reported in individual cases. Some previous case reports have mentioned that the presence of calcification within the lesion is the imaging feature of this tumor (12). An accurate understanding of the ultrasound images of BBT before a surgery can reduce the patients' fear of ovarian cancer diagnosis, and appropriate surgical methods can prevent overtreatment. To date, the integrated ultrasound characteristics of BTs are still lacking. Thus, this study retrospectively analyzed the clinical data and ultrasound findings of 25 patients with BBT and explored the ultrasound features of this entity to provide evidence for clinical diagnosis and treatment.

**Abbreviations:** 2D, two-dimensional; BT, Brenner tumor; BBT, benign Brenner tumor; CDFI, color Doppler flow imaging; CEUS, contrast-enhanced ultrasound.

# Materials and methods

## Study population

This was a retrospective study conducted in a single center, namely, Ningbo First Hospital. Ethical approval for this study was obtained, and all the patients provided a written informed consent. A computerized search of the pathology records of this institution from January 2012 to June 2023 was performed. This resulted in the identification of 26 patients with a histological diagnosis of BBT. A second search was then conducted on the image archive and communication system to identify patients who underwent 2D and/or contrast-enhanced ultrasound (CEUS) examination before surgery. One was excluded due to the presence of the lesion at imaging the actual vagina, which was diagnosed as BBT by colposcopy. Therefore, a total of 25 patients were identified, and one of them had bilateral lesions. Finally, all the 25 patients with 26 lesions had 2D examination data, and four of them had the results of 2D and CEUS.

## Materials

A retrospective analysis was conducted on 25 patients with pathologically confirmed BBT and in whom 2D and/or CEUS examinations were performed in our hospital from January 2012 to June 2023. The patients aged from 32 to 77 years, with an average age of 61.37 ± 9.67 years. The clinical data included age at diagnosis, pre- or postmenopausal status, symptoms, presence of ascites, and the clinical stage [the IOTA consensus as described by Timmerman et al. (11)].

Laboratory examination such as the level of carbohydrate antigen 125 (CA-125), carbohydrate antigen 199 (CA-199), carbohydrate antigen 153 (CA-153), carbohydrate antigen 724 (CA-724), alpha-fetoprotein (AFP), estrogen levels (E2), follicle-stimulating hormone (FSH), and prolactin (PRL) in the blood, symptoms, and presence of ascites were according to the IOTA

consensus described by Timmerman et al. (11). The clinical data, laboratory examination, and 2D ultrasound image of the 25 patients with 26 lesions as well as the CEUS image of four patients were analyzed. The type of surgery (laparotomy or laparoscopy) and the extent of surgical resection, such as unilateral oophorectomy or more extensive total hysterectomy, or even bilateral salpingo-oophorectomy, were recorded and analyzed. The postoperative pathology of each patient was also recorded in detail. The clinical and ultrasound data were imported into Excel (Microsoft Office Excel 2010) and then analyzed. All patients signed the informed consent form, and the study was approved by the Medical Ethics Committee of Ningbo First Hospital (no. 2023RS125).

## Sonographic examination

GE E8, PHILIPS iU22, TOSHIBA Aplio500, and other Color Doppler ultrasound diagnostic instruments were used. For a comprehensive evaluation, transvaginal probe frequency of 5–9 MHz, transabdominal probe frequency of 3.5–5.0MHz, routine transvaginal scanning, and partial transabdominal ultrasonography were adopted. A comprehensive analysis was conducted according to the tumor's size, boundary, shape, location, internal echo, and blood flow as well as the presence of calcification, fibroids, polyps, and thoracoabdominal effusion. The ultrasound contrast agent in this study was SonoVue (Bracco, Milan, Italy). A 2.4-mL bolus of SonoVue was injected intravenously through the cubital vein, followed by a 5-mL saline flush to ensure that no residual contrast agent remained in the intravenous catheter. Imaging started immediately after injection and lasted for 2 to 4 min. The pathological diagnosis was the gold standard.

## Statistical method

SPSS 24.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analysis and chart drawing. Qualitative variables are represented using absolute values and percentages, and continuous variables are represented using mean ± standard deviation (SD).

# Results

## Clinical history

There were 26 nodules in 25 patients, of which one had bilateral nodules and the rest had unilateral nodules. Of the 25 patients, 52.0% (13/25) had BT in the right ovary and 44.0% (11/25) in the left ovary; 36.0% (9/25) showed symptoms of abdominal pain, abdominal distension, and vaginal bleeding; 64.0% (16/25) showed no symptoms and had normal physical examination results; 8.0% (2/25) had normal menstruation; and 92.0% (23/25) were menopausal.

## Laboratory examination

In the tumor marker test, 12.0% (3/25) of the patients had a high CA-125 level, which was 69.6 U/mL, with the normal level being 30 U/mL. Furthermore, 19.04% (4/21) of the patients had an elevated CA-724 level, which was 12.02 U/mL, with the normal level being 7 U/mL. Moreover, 4% (1/25) of the patients had a high CA-199 level, which was 29.9 U/mL, with the normal level being 25 U/mL. No obvious abnormality was observed in CA-153, alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), human chorionic gonadotropin (HCG), and CA-153 in the remaining patients who underwent the test in blood. Of the 25 patients, 21 underwent reproductive hormone examination, of whom 14.28% (3/21) were found to have a high postmenopausal estrogen level, 14.28% (3/21) were found to have a high FSH level, and 4.76% (1/21) were found to have a high PRL level. No obvious abnormality was observed in LH, P, and T of 21 patients who underwent hormone examination (Table 1).

## Ultrasound characteristics

Only one patient was pathologically confirmed to have bilateral BBTs, and 96%(24/25) of patients were pathologically confirmed to have unilateral BBTs with the following ultrasound characteristics: (1) site: left in 44.0% (11/25), right in 52.0% (13/25); (2) size (diameter of the lesion): 1.7–16.0 cm; (3) shape and boundary: round, quasi-round, or irregular, with clear boundaries; (4) internal echo and blood flow: cystic lesions in 30.76% (8/26), mixed cystic and solid lesions in 15.38% (4/26), and solid lesions in 53.84% (14/26). Among the eight cases of cystic lesions, three (37.5%) were unilocular with thin walls (Figure 1A). Thin hyperechoic septa were detected in two (Figure 1B). Through color Doppler flow imaging (CDFI), blood flow distribution was observed in the cyst wall.

Furthermore, 53.84% (14/26) of the lesions were mixed cystic and solid type (Figure 2A). The lesions were large, with light spots, and had a maximum diameter of 5.0–16.0 cm. Multiple thick hyperechoic separate and moderate echo protrusions, with irregular shapes, were also observed in these cystic lesions (Figure 3A). Through CDFI, rich blood flow was detected in the protrusions and solid part (Figures 2B, C, 3B), and PW demonstrated a low resistant index (Figure 2D). In addition, 38.46% (14/26) of the lesions were solid, with a maximum diameter of 1.7-4.5 cm (Figures 3D, E). Calcification of different sizes could be seen in six among 14 lesions, which could be unilateral or multiple (Figure 4A). Through CDFI, few blood flow signals were observed in the hypoechoic part of the lesion (Figure 4B); (5) abdominal effusion: 4.0% (1/25) of the patients had abdominal effusion, which was myxoid cystadenoma with BT; (6) complicated uterine fibroids: 25.0% (2/8) of the patients had cystic lesions, 25.0% (1/4) of the patients had mixed cystic and solid lesions, and 46.15% (6/13) of patients had solid lesions, which complicated with uterine fibroids, as confirmed by pathology; (7) intrauterine polyps: 14.29% (1/7) of cystic type, 50.0% (2/4) of capsule solid mixed type, and 30.77% (4/13) of solid type were pathologically confirmed to be the types of endometrial polyps present (Table 2); (8) CEUS features: CEUS examination was performed in four of the patients. One patient had mixed cystic and solid lesion, with the contrast agent infusing at 15 s in solid part after the injection. The contrast agent filling was highly enhanced in the separated and solid parts and then slowly washed out (Figure 3C), which was confirmed by pathology as

TABLE 1 Clinical characteristics and surgical management of the 25 patients with benign Brenner tumor (BBT).

| Parameters | | BBT patients (*n* = 25) |
|---|---|---|
| Age at surgery (years) | | 63 (32–77) |
| Serum CA125 level (U/mL) Reference range(0–30) | <30 | 88.0% (22/25) |
| | >30 | 12.0% (3/25) |
| Serum CA-724 level (U/mL) Reference range (0–7) | <7 | 80.95% (17/21) |
| | >7 | 19.04% (4/21) |
| Serum CA-153 level (U/mL) Reference range (0–25) | <25 | 100% (23/23) |
| | >25 | |
| AFP level (ng/mL) Reference range (0–25) | <25 | 100% (25/25) |
| | >25 | |
| CEA level (U/mL) Reference range (0–10) | <10 | 100% (25/25) |
| | >10 | |
| Serum CA-199 level (U/mL) Reference range (0–25) | <37 | 95.23% (20/21) |
| | >37 | 4.76% (1/21) |
| Estrogen levels (IU/L) | Normal | 90.47% (19/21) |
| | Abnormal | 9.53% (2/21) |
| Follicle-stimulating hormone (FSH) | Normal | 90.47% (19/21) |
| | Abnormal | 9.53% (2/21) |
| Prolactin (PRL) | Normal | 95.0% (19/20) |
| | Abnormal | 5.0% (1/20) |
| Menstruation | Premenopausal | 8.0% (2/25) |
| | Postmenopausal | 92.0% (23/25) |
| Symptomatology | Asymptomatic | 64.0% (16/25) |
| | Pelvic pain | 36.0% (9/25) |
| Surgery | Salpingo-oophorectomy laparoscopy | 24.0% (6/25) |
| | Total laparoscopic hysterectomy | 72.0% (18/25) |
| | Total abdominal hysterectomy | 4.0% (1/25) |
| Pathology | Pure Brenner | 72.0% (18/25) |
| | Brenner + mucinous cystadenoma | 12.0% (3/25) |
| | Brenner + serous cystadenoma | 4.0% (1/25) |
| | Brenner + seromucinous cystadenoma | 4.0% (1/25) |
| | Brenner + ovarian fibroma | 4.0% (1/25) |
| | Brenner + endometrioid cyst | 4.0% (1/25) |

Values are expressed as median (range) or n (%).

myxoid cystadenoma complicated with BT (Figures 5C, D). The others three were the solid type, which were confirmed by histopathological examination as pure BBT (Figures 5A, B). After injection of the contrast agent, the solid lesion in the left adnexal area began to infuse at 24 s, presenting equal enhancement. After reaching the peak value at 42 s, it simultaneously cleared with the uterus (Figure 3F).

## Pathological characteristics

A total of 25 patients underwent surgery, with 26 ovarian lesions removed. Among them, 72.0% (18/25) were treated with total laparoscopic hysterectomy and 24.0% (6/25) with salpingo-oophorectomy laparoscopy. Only one patient was treated with total abdominal hysterectomy for the huge volume of the tumor. After the surgery, it was confirmed by pathology that 18 cases were pure BBT, three cases were BBT combined with mucinous cystadenoma, and the remaining four cases were BBT combined with serous cystadenoma (Figure 5E), seromucinous tumor, ovarian fibroma, and endometrioid cyst, respectively.

# Discussion

BTs are derived from the germinal epithelium with multiple differentiation potentials on the surface of the ovary, also known as ovarian fibro epithelial tumors (12). They are mainly composed of special nests of epithelial cells and tightly arranged spindle cells or fibrous connective tissues surrounding the epithelial cells (12). BT can occur at any age but is more common in people older than 50 years. It occurs unilaterally more than bilaterally (13). Its clinical symptoms are non-specific, including non-specific abdominal distension, abdominal pain, mass, and irregular vaginal bleeding in some postmenopausal women (8). Some BTs are detected incidentally during routine examinations. This study included 25 patients, of whom 23 (92.0%) were postmenopausal, six (24%) had lower abdominal pain and discomfort, and three (12%) had vaginal bleeding; the remaining patients had normal physical examination results. The cause of vaginal bleeding in BT patients was the differentiation of epithelial cells with multi-directional differentiation potential into tissue components with hormone secretion function based on cytology (12). Therefore, some juvenile patients experience estrogen-related symptoms, such as early menstruation, breast development, genital development, and sexual precocity. In adults, the most obvious symptoms are functional uterine bleeding, amenorrhea, irregular vaginal bleeding following menopause, endometrial hyperplasia, and presence of endometrial polyps and uterine fibroids. In this study, 19 patients underwent sex hormone examination, of whom two were found to have elevated estrogen levels. Of the 25 patients included in this study, seven had polyps, including one with cervical polyps and nine with uterine fibroids.

BT is a rare ovarian epithelial tumor with a low incidence (4). It can be classified into benign, borderline, and malignant, of which

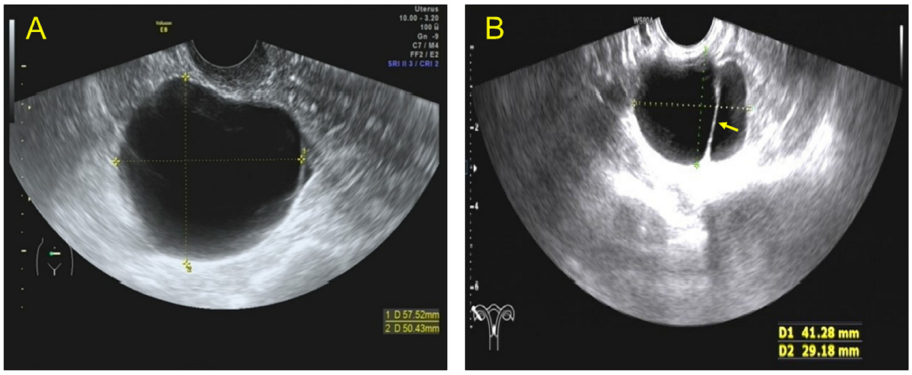


FIGURE 1
Transvaginal ultrasound images showing the cystic type lesions of benign Brenner tumor. **(A)** Transvaginal ultrasound (TVU) showing a case of unilocular lesions with thin walls. **(B)** TVU showing a case of cystic lesions with thin separation in the right ovary.

approximately 90% are benign and approximately 10% are borderline and malignant (4). The diagnosis and differential diagnosis of BT mainly depend on its pathology. The pathological features of BBTs are scattered epithelial nests and densely proliferating fibrous stroma with small nucleoli, broad and clear cytoplasm, squamous metaplasia in small foci, and local calcification (14). Borderline and malignant BTs are rare and have few related studies (15, 16). Histologically, BBT consists of a large number of densely proliferating fibrous stroma with various sizes of solid echoes, which are hypoechoic with posterior attenuation on ultrasound images. The local calcification in pathology, due to the different degrees of calcification, can be punctate, cluster, and patchy. Thus, the ultrasound shows various forms of hyperecho, followed by an acoustic shadow. For the detection of calcification, CT and MRI diagnosis were mostly reported in the previous literature (17). Nowadays, ultrasound can also show calcification well, and it is economical without radiation damage. In this study, 14 of 26 BBTs were of solid type, and six of them had calcifications. The diameter of the solid lesions was 1.7–11.0 cm, and the posterior echo attenuation was obvious; eggshell calcification was also observed. This is consistent with the study conducted by Moon et al. (18), who stated that most solid BTs

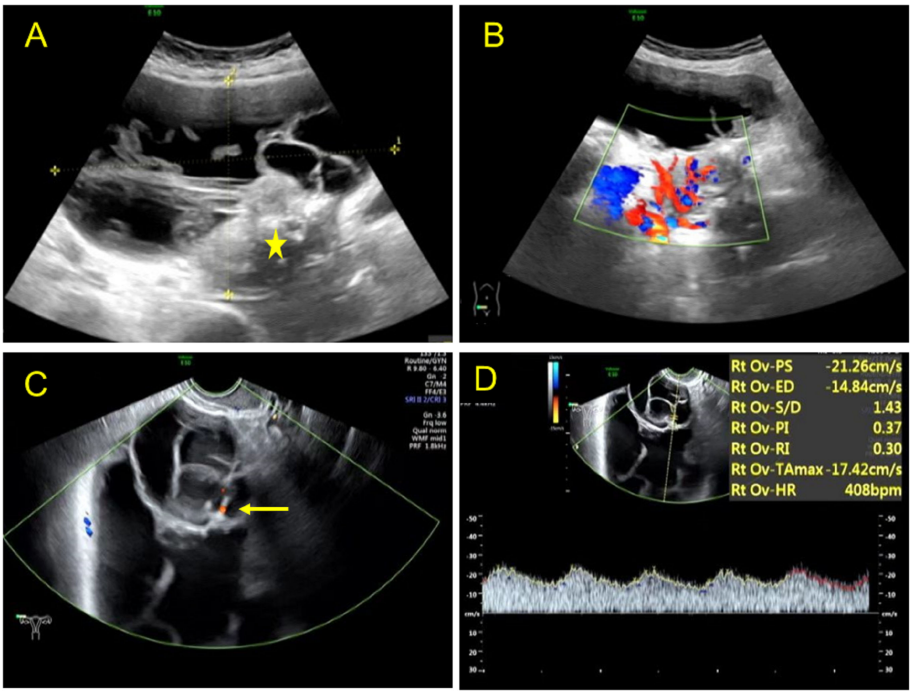


FIGURE 2
Ultrasound images of a benign Brenner tumor patient of the mixed cystic and solid type. **(A)** Transabdominal sonography showing a giant mixed cystic and solid (asterisk) lesion in the right lower abdomen with a diameter of 15.1 × 9.8 cm. **(B)** Color Doppler imaging revealed an abundant blood flow in the solid part of the lesion. **(C)** Transvaginal sonography showing a few flow signals in the separates with color Doppler (arrow). **(D)** Pulsed wave Doppler demonstrating a low resistant index of blood flow in the lesion.

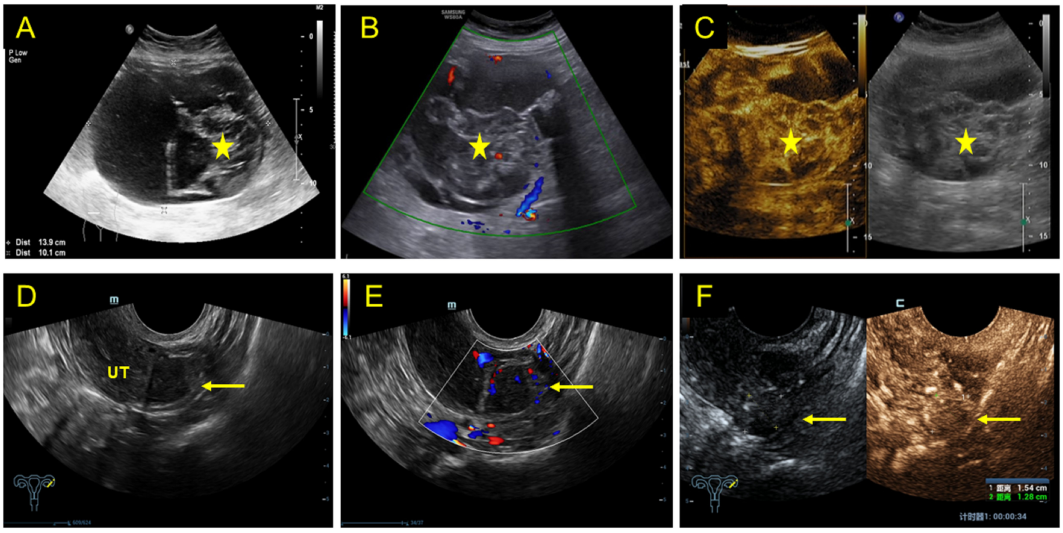


FIGURE 3
2D, CDFI, and CEUS characteristics in a mixed cystic and solid lesion (upper row) and a solid lesion (lower row). **(A)** 2D transabdominal ultrasound showing a mixed cystic and solid (asterisk) lesion in the right adnexal area with a diameter of 13.9 × 10.1 cm. **(B)** Rich blood flow was detected in the protrusions and solid part. **(C)** Transverse abdominal contrast-enhanced ultrasound showing the lesion significantly enhanced at 15 s after contrast agent injection and then slowly washed out. **(D)** Transverse transvaginal sonography showing a solid lesion (arrow) in the left ovary with a diameter of 1.70 × 1.34 cm. **(E)** Color Doppler image showing rich blood flow at the inner side of the solid lesion. **(F)** Transverse transvaginal contrast-enhanced ultrasound demonstrating that the solid lesion of the left adnexal area began showing equal enhancement at 24 s after contrast agent injection and peaked at 42 s.

are accompanied by an amorphous calcification and that extensive calcification within the solid component is the typical pathological manifestation of BT. This suggests that calcification is an important characteristic of BBT and that it should be considered when there is a solid lesion with calcification on ultrasound images.

As described above, BTs with solid calcification may be an important ultrasound characteristic of BBTs. On the contrary, non-calcified solid BTs are often misdiagnosed as malignant tumors due to their borders, morphology, and blood flow distribution. The solid type of tumor without calcification and ovarian malignant tumors were difficult to distinguish on ultrasound features in postmenopausal women. In this study, 14 patients were of the solid type, and eight of them were without calcification. Among the patients without calcification, three showed an irregular morphology and an unclear boundary in two-dimensional ultrasound images, and color Doppler showed the blood flow distribution. To further demonstrate the microcirculation, CEUS was performed all three patients, which indicated that the intratumoral blood supply was sparse and isoenhanced, which regressed synchronously with the uterus, and was suspected to be uncertain according to the IOTA system. The presence of sparse blood flow within the tumor in CEUS with enhancement and regression synchronizing with the myometrium was the reason for the diagnosis of uncertain rather than malignant. Therefore, CEUS played an important role in the diagnosis. However, the postoperative pathology results confirmed BBT. Of the six patients

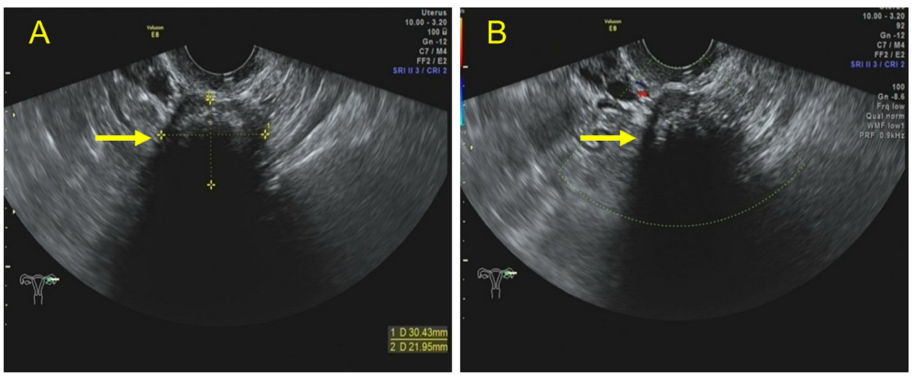


FIGURE 4
Transvaginal ultrasound images of a solid lesion with obvious calcifications in the left ovary. **(A)** Transvaginal ultrasound revealing a lesion with a diameter of 3.0 × 2.2 cm in the left ovary as well as arc-shaped calcifications in the nearer field and acoustic shadows in the rear. **(B)** Color Doppler imaging demonstrated a few flow signals in the hypoechoic part of the lesion.

TABLE 2 Two-dimensional and color Doppler ultrasound findings of the 25 patients with 26 benign Brenner tumor lesions.

| Ultrasonic classification | | Cystic (*n* = 8) | Capsule solid Mixed (*n* = 4) | Solid (*n* = 14) |
|---|---|---|---|---|
| Number | | 30.76% (8/26) | 15.38% (4/26) | 53.85% (14/26) |
| Position | Left | 12.0% (3/25) | 8.0% (2/25) | 24.0% (6/25) |
| | Right | 20.0% (5/25) | 8.0% (2/25) | 24.0% (6/25) |
| | Bilateral | | | 4.0% (1/25) |
| Maximum diameter (cm) | | 2.1–16.0 | 4.0–16.0 | 1.7–11.0 |
| Average diameter (cm) | | 5.37 ± 4.27 | 12.73 ± 0.78 | 2.27 ± 0.95 |
| Shape | Regular | 30.77% (8/26) | 15.38% (4/26) | 42.31% (11/26) |
| | Irregular | 0 | 0 | 11.54% (3/26) |
| Border | Clear | 30.77% (8/26) | 11.54% (3/26) | 42.31% (11/26) |
| | Unclear | | 3.84% (1/26) | 11.54% (3/26) |
| Cystic fluid | Clear | 87.5% (7/8) | | |
| | Unclear | 12.5% (1/8) | 100% (4/4) | |
| | No | | | 100% (14/14) |
| Separated | Yes | 62.5% (5/8) | 100% (4/4) | |
| | No | 37.5%(3/8) | | 100% (14/14) |
| Parenchymal echo and blood flow in the sac | Yes | 0 | 100% (4/4) | 0 |
| | No | 100% (8/8) | 0 | 100% (14/14) |
| Calcification | Yes | | 0 | 42.85% (6/14) |
| | No | 100% (8/8) | 100% (4/4) | 57.14% (8/14) |
| Associated with ascites | Yes | | 4.0% (1/25) | |
| | No | 32.0% (8/25) | 12.0% (3/25) | 52.0% (13/25) |
| Associated with uterine fibroids | Yes | 8.0% (2/25) | 4.0% (1/25) | 24.0% (6/25) |
| | No | 24.0% (6/25) | 12.0% (3/25) | 28.0% (7/25) |
| Associated with endometrial polyp | Yes | 4.0% (1/25) | 8.0% (2/25) | 16.0% (4/25) |
| | No | 28.0% (7/25) | 8.0% (2/25) | 36% (9/25) |
| Ultrasound diagnosis (IOTA system) | Benign | 30.77% (8/26) | | 42.30% (11/26) |
| | Uncertain | | | 11.54% (3/26) |
| | Malignant | | 15.38% (4/26) | |

Values are expressed as n (%).

with mixed BBT on ultrasound images, three were pathologically confirmed to have mucinous cystadenoma. The ultrasonographic characteristics of these three cases were summarized as follows. The main manifestation was a large cystic echo with the attachment of solid components, and blood flow could be detected in the solid parts, which overlapped with the ultrasound characteristics of ovarian malignant tumors. One case was suspected to be malignant due to the continuous hyperenhancement of the contrast medium in the separate and solid part of CEUS, but the pathological diagnosis was also mucinous cystadenoma combined with BBT. The reason for the enhancement such as CEUS in benign Brenner tumors may be caused by the incomplete basal layer of vascular endothelial cells in the tumor. The incomplete basal layer of vascular endothelial cells causes the contrast agent to pass through the gap of endothelial cells quickly, which shows rapid regression on contrast-enhanced ultrasound images (10).

In our study, 18 (72%) cases were pure BBT in pathology, which was consistent with the literature that the vast majority of BTs were benign (19). It was also reported that about 20% of BTs could be accompanied by mucinous cystadenoma, serous cystadenoma, benign cystic teratoma, or struma ovarii (20). Similarly, in our study, seven patients (28%) had concomitant benign ovarian tumors: four cases were BBT combined with serous cystadenoma, seromucinous tumor, ovarian fibroma, and endometrioid cyst,

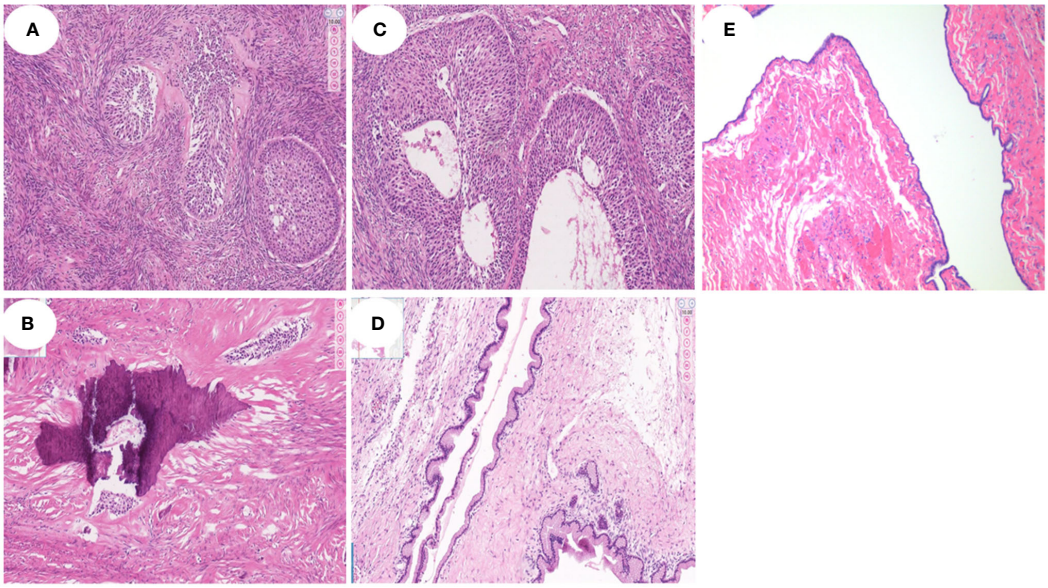


FIGURE 5
Histopathological findings of different types of BBT (HE staining, all ×100). **(A, B)**. Histopathological examination revealed one pure BBT patients, which demonstrated that nests of well-circumscribed ovoid or irregular transitional cells were seen in the dense fibrous stroma in the left ovary. And, significant calcification can be found in the tumor in figure **(B)**. **(C, D)**. Histopathological findings of a patient mixed myxoid cystadenoma component with BT, and the mucinous epithelial cells were seen in the cystic wall of tumor in the figure **(D)**. **(E)** The serous epithelial cells were seen in the cystic wall of a patient mixed serous cystadenoma component with BT.

respectively, and the other three were associated with mucinous cystadenoma, which was similar to what Roma and Masand reported (21, 22). Some scholars believe that the reason why mucinous cystadenoma and BBT can coexist is that they have a common clonal origin (23, 24). The other authors suggested that the combination of these two tumors is due to the differentiation of BBT cells (25). When the two coexist, the continuous secretion of epithelial cells in the cystadenoma leads to increased cystic fluid, which will push the BT tissue to the thinner side of the tumor. Thus, in the pathological diagnosis of large mucinous cystadenoma, careful observation of the surrounding tissues of the tumor is important to determine whether there is a small BT pathological tissue (26). The difference between BT and mucinous cystic tumors is that the latter have a single component, have no transitional epithelial nests, and are positive for the cytokeratin 20 (CK20) immunophenotype, whereas the former do not express CK20. The main basis for differential diagnosis is immunohistochemical analysis (27). However, CK20 can also be positive in patients with mucinous cystadenoma combined with BBT, and other immune markers should be combined for differential diagnosis (28).

However, the correlation between BBT and tumor markers is still unclear (29). There are studies investigating the predictive value of CA-125 level and malignancy, indicating that CA-125 alone is not suitable to differentiate between benign and malignant adnexal lesions (25). Until now, the correlation between BBT tumors and serum tumor markers, like CA-153, CA-724,and CA-199, has not been clearly concluded. These markers were elevated, which seemed more likely by chance, but the result was sometimes difficult to diagnose with ovarian malignancy, especially when the tumor showed mixed cystic and solid type or solid. Some of the mixed cystic and solid BBT tumors could be combined with cystadenoma. The association between BT and cystadenoma has also been reported by Abbas (26) in postmenopausal women. As for the increase in estrogen levels, the mechanism remains unclear. It has been reported that steroid hormones are produced by ovarian tumors probably via the following mechanisms. One is that the tumor cells themselves produce hormones, such as granular and Sertoli cell tumors. The other category is stromal cells, but not tumor cells producing hormones (30). In this study, two of the 25 patients had elevated serum estrogen levels following menopause, which may have been the cause of endometrial hyperplasia in these patients. However, whether the cause of estrogen elevation was tumor cells or stromal cells needs further study.

As for the choice of surgical methods, 72.0% (18/25) were treated with total laparoscopic hysterectomy and 24.0% (6/25) with salpingo-oophorectomy laparoscopy. Moreover, 4% (1/25) of the patients chose open surgery, which was inconsistent with the literature reports (31). The reason may be the large size of the tumor and the presence of potential malignancy. However, this procedure has a long recovery time. With the continuous development of laparoscopic technology, 96.0% (24/25) of the patients underwent laparoscopy. The surgical trauma is small, and the recovery is fast (5). Meanwhile, the surgical procedure will not cause spilling of potential malignant cells into the abdominal cavity. Laparoscopy is a very good choice for young women, especially those with fertility potential and desire. In our study, 24.0% (6/25) of the patients chose salpingo-oophorectomy laparoscopy to avoid overtreatment.

Due to the rare occurrence of malignant and borderline BTs, most of the ultrasound characteristics reported in the literature were mainly benign. It has been reported that malignant BT is usually

bilateral, presenting mixed cystic and solid components or dominated by cystic components, with abundant tumor blood supply and low spectral resistance (5). However, in our study, there was only one patient with a bilateral tumor that was benign. Zheng (32) reported that borderline BTs were usually large, unilocular, or multilocular and may be cystic or capsule solid. Distinguishing BBTs is difficult sometimes and relies only on immunohistochemistry (27). Due to the limited sample size of this study, confirmation by multi-center studies is warranted.

## Conclusion

The ultrasound manifestation of BBT is variable and diagnosis challenging. Features such as calcified acoustic shadow, solid components with sparse blood flow, and prevailing of the right appendage may indicate BBT. Unilateral ovarian lesion with punctate calcification is another important feature of BBT. For adnexal solid or mixed cystic and solid masses, if the CEUS result shows isoenhancement or hyperenhancement, the possibility of BBT cannot be excluded. Contrast-enhanced ultrasound can enhance the confidence of patients with benign ovarian tumors, reduce the psychological stress of patients, and allow them to choose rational surgery. We believe that this study will improve the understanding of the clinical manifestations, laboratory examination, and ultrasound manifestations of this rare entity.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

## Ethics statement

The studies involving humans were approved by the Bioethics Committee of Ningbo First Hospital, School of Medicine, Ningbo University, China (no. 2023RS125). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

MC: Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. SL: Formal analysis, Software, Visualization, Writing – original draft, Writing – review & editing. YC: Data curation, Supervision, Writing – original draft. MM: Formal analysis, Resources, Supervision, Writing – original draft. XJ: Data curation, Validation, Visualization, Writing – review & editing. SZ: Formal analysis, Methodology, Project administration, Software, Writing – original draft. YX: Conceptualization, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

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

## Publisher's note



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# Case report: A rare case of omental extrarenal rhabdoid tumor and review of the literature

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Extrarenal rhabdoid tumor of the greater omentum is extremely rare, with only sporadic reports and limited documentation of its ultrasonographic findings. Here, we report a case of an extrarenal rhabdoid tumor of the greater omentum in a 16-year-old girl and review the relevant literature. It was found that the disease mainly occurred in female children and adolescents, and mainly manifested as lower abdominal pain and a large abdominal cystic or solid hemorrhagic mass. The clinical characteristics include a high degree of malignancy and mortality. Ultrasound shows some malignant features, but it is not specific; thus, it is easy to be misdiagnosed in the clinic.

KEYWORDS

**greater omentum tumor, extrarenal rhabdoid tumor, extrarenal rhabdoid tumor of greater omentum, ultrasound, omentum**

## Introduction

Rhabdoid tumors are rare clinical malignant tumors, which occur widely, mostly in the kidney, but also in organs other than the kidney, collectively referred to as extrarenal rhabdoid tumor (1). The greater omentum is a common site of metastatic tumors, but primary tumors of the greater omentum are very rare (2). Primary extrarenal rhabdoid tumor in the greater omentum is extremely rare, and only sporadic reports have been documented in the literature. Because little is known about it, it is easily misdiagnosed as another type of tumor.

This paper reports a case of extrarenal rhabdoid tumor of the greater omentum treated in our hospital, and discusses its clinicopathological features and differential diagnosis by reviewing the publications, in order to further understand the disease and its diagnosis.

## Case presentation

A 16-year-old girl was hospitalized with severe pain that had developed from recurrent abdominal pain lasting over 10 days. The patient had been experiencing left lower abdominal pain for more than 10 days. The pain was localized and improved after rest without additional obvious symptoms, e.g., chills and fever, nausea and vomiting, diarrhea, hematochezia, and other discomfort. Seven days before the hospitalization, color ultrasound done in the other clinic revealed a pelvic mass of approximately 13 × 10 cm. After taking anti-inflammatory oral pills, the symptoms slightly improved and the pain was tolerable. Half a day before the admission to the hospital, the abdominal pain worsened, accompanied by malignant vomiting and palpitation, and the patient was admitted to the emergency department. Through anal finger examination and abdominal pressing, the huge abdominal mass is palpated to the two transverse fingers on the navel, reaching the midaxillary line on both sides. The boundary of the pelvic mass is clear, and the pain in the left lower abdomen is obvious during compression. The uterus is not palpable.

Color ultrasound on admission suggested that there was no obvious abnormality in uterus and ovary. A cystic solid mass of approximately 13 cm × 11 cm × 15 cm was found in front of the uterus. The wall of the cyst was locally thickened and not smooth with the thicker side measured approximately 0.8 cm. The light bands in different directions could be seen in the cyst, the sound transmission difference of cyst fluid was accompanied by dense and weak light spots, the boundary of the mass was clear, and the shape was not irregular. There were no obvious blood flow signals in the cyst wall and cyst (Figure 1).

Laboratory examination: white blood cell 12.1 $\times 10^9$/L,neutrophil count 9.36 $\times 10^9$/L, HR hemoglobin 112 g/L, and whole blood hypersensitive C-reactive protein 58.91 mg/L; carbohydrate antigen 12–5 1033 U/mL; postmenopausal Rome index 73.47%; six items of blood coagulation function: plasma fibrinogen 7.12g/L, D-dimer 8.43μg/mL, and fibrinogen degradation products 27.55μg/mL. The liver and kidney functions and infectious markers were normal.

The possibility of pedicle torsion of ovarian tumor is considered in the preliminary clinical diagnosis. The patient underwent transumbilical laparoscopic exploration under general anesthesia. Laparoscopic examination during the operation showed that the dense adhesion of the greater omentum and intestine formed an approximately 15 cm × 15 cm fillet-like mass, purplish red, with bleeding and necrosis (Figure 2). The mass adheres densely to the left abdominal wall and does not move. There is dark red blood accumulation in the mass, approximately 1,000 mL. After separating the adhesive band between the mass and the left abdominal wall, there was obvious pelvic congestion, and the mass adhered to the left anterior wall of the uterus. There was no obvious abnormality in the appearance of uterus and bilateral fallopian tubes and ovaries. When exploring the greater omentum, there were nodules scattered between 1.0 cm × 0.8 cm and 4 cm × 4.5 cm in size. The nodules were tough and purplish red, and the blood touch was obvious.

Intraoperative freezing suggested that epithelioid cells were found in "pelvic tissue" with necrosis and tended to be malignant to be analyzed with the paraffin section and immunohistochemistry. After consultation with gastrointestinal surgeons, the patient was treated with omentectomy, electroresection of rectal lesions, ovarian biopsy under direct vision, laparoscopic pelvic adhesion lysis, intestinal adhesion lysis, and abdominal puncture and drainage. During the operation, pelvic adhesion, intestinal adhesion, and pelvic congestion were obvious, and pelvic hemorrhage was approximately 1,500 mL.

Postoperative pathological examination showed that malignant tumors were found in "pelvic tissue", "greater omentum", "mesentery of small intestine", "abdominal wall mass", and "anterior rectal wall mass". The "bilateral ovarian biopsy tissue" showed ovarian cortex under the microscope, and no tumor was found. Microscopically, the tumor showed solid flake and nest distribution, infiltrative growth, round or oval tumor cells, rich cytoplasm, eosinophilia, large nucleus, deviation, vesicular chromatin, obvious nucleoli, easy mitosis, and poor adhesion of tumor cells in some areas. It can be seen that the rhabdomyoid cells with nuclear deviation and strong eosinophilic cytoplasm have necrosis. The results of immunohistochemistry showed the following: Bcl-2(-), CD34(-), CD45(LCA)(-), CK(AE1/AE3)(+), CK20(-), CK7(-), CR(calretinin)(-), Desmin(-), ERG(-), HMB45 (-), INI1(-), S-100(-), SMA(-), TLE1(-), Vimentin(+), and WT1 (Wilm's tumor)(-). After the general discussion in the Department of Pathology, combined with the results of immunohistochemistry, the malignant tumor of the greater omentum was consistent with the malignant extrarenal rhabdoid tumor (Figure 3).

After operation, it was diagnosed as rupture of a malignant extrarenal rhabdoid tumor with hemorrhage and necrosis. No other primary cancers or metastases were found by PET-CT after operation.

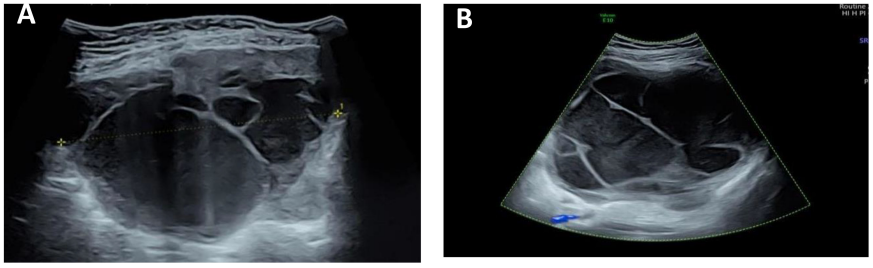


FIGURE 1
Ultrasound image of the tumor. **(A)** There is a separation in the mass, and the wall thickness is not smooth. **(B)** A weak light spot is seen in the mass, and there is no blood flow signal in the mass.

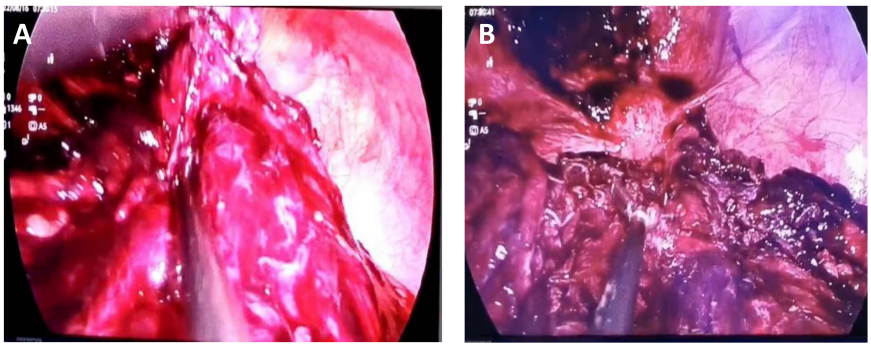


FIGURE 2
Seen during laparoscopy. **(A)** The omental tumor was large and ruptured bleeding was visible. **(B)** The uterus can be seen behind the omental mass, and metastatic nodules can be seen on the abdominal wall.

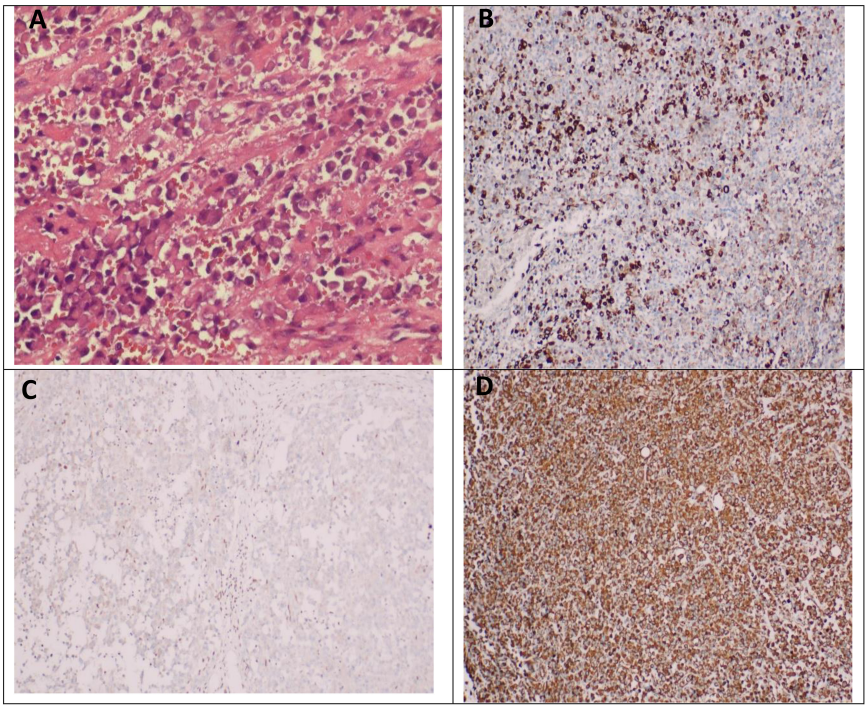


FIGURE 3
Pathological manifestations of extrarenal rhabdoid tumor of the greater omentum. **(A)** Microscopically, the tumor showed solid flake and nest distribution, infiltrative growth, round or oval tumor cells, rich cytoplasm, eosinophilia, large nucleus, deviation, vesicular chromatin, obvious nucleoli, easy mitosis, and poor adhesion of tumor cells in some areas. It can be seen that the rhabdomyosus-like cells with nuclear deviation and strong eosinophilic cytoplasm have necrosis. **(B)** Immunohistochemical results showed CK-pan positive in tumor cells. **(C)** The immunohistochemical results showed that the tumor cells were negative for INI1. **(D)** The results of immunohistochemistry showed that the tumor cells were Vimentin positive.

The patient was hospitalized again half a month after discharge. She was given symptomatic support treatment because of cancerous persistent blood loss and infection. She died 1 month after operation.

## Discussion

Renal rhabdoid tumor is a new subtype that was isolated from nephroblastoma and first reported by Beckwith in 1978 (3). Later, it was reported in other organs other than the kidney, including the heart (4), lung (5), liver (6), orbit (7), intestinal tract (8), vulva (9), and central nervous system (10). According to the location of the tumor, it can be divided into three types: malignant rhabdoid tumor of the kidney (MRTK), central nervous system atypical teratoid/rhabdoid tumor, and extrarenal extracranial rhabdoid tumor (EERT) (11). Malignant rhabdoid tumors are common among infants and children, and the non-kidney ones have a wide range of age and can occur in adults, but there are more cases in infants

and children; EERT cases are mostly common among late adolescence or young people (12).

The pathogenesis of extrarenal rhabdoid tumor is unclear. Eighty percent of the cases showed 22q11.2–12.2 deletion. The deletion or mutation of tumor suppressor gene SMARCB1/hSNF5/INI1 may play a role in the pathogenesis of extrarenal rhabdoid tumor (13). At present, the immunohistochemical results of almost all rhabdoid tumors show that INI-1 is negative, which has been used as one of the indicators for the diagnosis of rhabdoid tumors. Rhabdomyoid cells can be found in many kinds of tumors. The expression of INI-1 gene or INI-1 protein can be detected by immunohistochemistry as a marker for differential diagnosis of rhabdoid tumors and other tumors. With further research, researchers have proposed multiple hypotheses about the tissue origin of the tumor cells, such as primitive mesenchymal cells, neuroectodermal cells, and epithelial cells (14), without strong evidence. Hence, they are still classified as tumors of unknown tissue origin.

Most of the clinical features of extrarenal rhabdoid tumor are local mass, combined with compression or invasion of adjacent tissue. The general symptoms of greater omentum tumor are abdominal discomfort (45.5%), abdominal mass (34.9%), and abdominal distension (15.2%). Early adjacent tissue invasion or distant metastasis may occur. The prognosis is very poor, the mortality rate is high, and the 5-year survival rate is <50% (15). This patient presented with a pelvic mass with intermittent abdominal pain for 10+ days. The cause of abdominal pain may be related to the hemorrhage and necrosis of the mass. Adjacent tissue invasion and intestinal metastasis occurred at the time of onset, and the course of the disease progressed rapidly. She died in only 1 month.

The radiological features of extrarenal rhabdoid tumors were as follows: most of the tumors were large, showing solid or cystic-solid mixed masses; the distinction between cystic and solid was unclear; MRI mainly showed that inhomogeneous T1WI showed iso-signal or slightly low signal; T2WI showed high signal or slightly high signal; and solid part DWI showed high signal, inhomogeneous enhancement on the enhanced scan, and no enhancement in the cystic or necrotic area. Some of the lesions were associated with calcification, bleeding, local invasion, or distant metastasis (16, 17).

Extrarenal striated muscle of the greater omentum is very rare. There are only sporadic reports. We systematically reviewed the literature on extrarenal rhabdomyoma of the greater omentum by searching databases at home and abroad, i.e., PubMed, Zhiwang, and Wanfang, using keywords including omental tumor, extrarenal rhabdomyoma, and omental extrarenal rhabdoid tumor. As of August 2023, a total of three cases have been retrieved, and our case is the fourth one (Table 1).

Table 1 shows the clinical and imaging features of omental extrarenal rhabdoid tumor reported in the literature. The disease seems to be an adolescent disease. The maximum age of onset is 16 years old, the youngest age is 9 years old, the average age is 12.5 years old, the sex preference seems to be female, and all four patients are female. The clinical manifestations were lower abdominal pain; one had nausea, constipation, and anemia; one had rebound pain; and one had nausea, vomiting, and palpitation.

TABLE 1 Clinical and imaging features of omental extrarenal rhabdoid tumor reported in the literature.

| Case no. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Author | Wang Juan | So-Hyun Nam, et al. | Leonardo Suryawan, et al. | Li Hui, et al. |
| Year of publication | 2008 | 2014 | 2023 | This study |
| Age | 15 years old | 10 years old | 9 years old | 16 years old |
| Gender | Female | Female | Female | Female |
| Symptom | Lower abdominal tenderness, rebound pain, shifting dullness (+) | Lower abdominal pain | Abdominal swelling, pain and anemia, nausea, constipation, shifting dullness (+) | Lower abdominal pain, nausea and vomiting, palpitation |
| Ultrasound | Solid space on the left side of the cervix, abdominal and pelvic effusion | Large multi-segmental hematoma, enhanced peripheral blood vessels, and a small amount of hemorrhage in the abdominal cavity | N/A | There is a multilocular cystic space in front of the uterus with a weak spot |
| CT | N/A | Lobulated hemorrhagic mass, irregular enhancement around, and a small amount of hemorrhage in the abdominal cavity | Inhomogeneous enhancement of omental mass. Peritoneal effusion | N/A |
| Size | 8 × 8 × 6 cm | 9 × 6 × 6.5 cm | 8 × 6 × 8 cm | 13 × 11 × 15 cm |
| Intraoperative bleeding | 1,500 mL | N/A | 3,000 mL | 1,500 mL |
| Result | Died 2 months later | Died 9 months later | Died 3 weeks later | Died 1 month later |
| Distant metastasis | Yes | N/A | N/A | Yes |

N/A, Not applicable; (+), It indicates that the shifting dullness is positive.

Three cases had abdominal effusion before operation. All the masses had hemorrhage and necrosis. Except for one case without intraoperative bleeding, the intraoperative bleeding volume was larger in the other three cases, with the largest being 3,000 mL and the smallest being 1,500 mL. According to the longest diameter of the mass, the average size of the mass was approximately 10 cm, the largest mass was approximately 15 cm, and the smallest was approximately 8 cm. Most of the masses were hemorrhagic masses, including solid in one case, lobulated hemorrhagic mass in one case, cystic hemorrhagic mass in one case, and a mass of unknown nature in one case. Inhomogeneous mass enhancement was found in two cases by CT (18, 19), and CT scan was not performed in the other two cases. All patients received surgical resection. During the operation, one patient found that there were 24 lymph nodes with metastasis in 25 lymph nodes, one patient found greater omentum with pelvic and abdominal implants, and the other two cases had no lymph node or distant organ metastasis. Because of their poor condition, three patients did not receive radiotherapy and chemotherapy and died 3 weeks, 1 month, and 2 months after operation. Only one 10-year-old child received chemotherapy with a VDC/IE (VCR, doxorubicin, and cyclophosphamide/ifosfamide and etoposide) regimen. Although the VDC/IE regimen underwent four cycles of chemotherapy, she died 9 months after the operation because of the deterioration of the disease.

Ultrasonographic findings of extrarenal rhabdoid tumor are rarely reported. Of the two previously reported cases of extrarenal rhabdomyoma of the omentum and kidney, one case showed solid space occupying on the left side of the cervix (20), one case showed large abdominal hematoma (19), and both cases had peritoneal effusion. In our case, ultrasound revealed a huge tumor in the pelvic cavity with clear boundary and regular shape, mainly cystic, uneven thickening of the cyst wall, less smooth, multiple septum and dense weak light spots in the cyst, and no blood flow signal in the intracapsular and cyst wall. The dense and weak light spots in the capsule indicated that there is bleeding in the tumor. According to the consensus guide for the O-RADS ultrasound risk stratification and management system (21) published by the ovarian-adnexal reporting and data system (O-RADS), the mass in this case is larger than 10 cm in diameter, with an unsmooth inner wall, irregular septum, multilocular cyst, and a blood flow score of less than 4 points, which is in accordance with the O-RADS4 category, with a moderate malignant risk of 10% to 50%.

The ultrasonographic features of this case of omental extrarenal rhabdoid tumor should be differentiated from pelvic inflammatory mass, chocolate cyst, cystadenoma, and so on. (1) Pelvic inflammatory mass: mostly acute or recurrent history of pelvic infection. It is more common among sexually active people. In addition, it usually manifests as a lower abdominal pain without periodicity and possibly accompanied by a fever and leukocytosis, which can be subdued by antibiotic treatment. When hydrosalpinx or empyema occurs, it can be shown as a cystic mass like a pelvic intestine or a circuitous tube, with a uniform thickness of the cyst wall and thin light spots in the cyst. When pelvic empyema occurs, a cloud-like low echo area with an irregular shape and an uneven echo can appear in the pelvic cavity. The patient is a teenager without sex. Moreover, she did not have symptoms of fever and chills. (2) Chocolate cyst:Most of the patients had a history of dysmenorrhea, with round or oval cysts in the pelvis, which could be single or multiple; the outer edge of the cyst wall was clear, the inner wall was rough, and cloud-like dense light spots or irregular hyperechoic masses could be seen in the cyst. (3) Ovarian serous cystadenoma: most of the cysts were single-chamber cysts with a thin wall and a smooth inner wall, common nipple growth in multi-chamber cysts, and serous fluid in the cysts and transparent sound. (4) Ovarian mucinous cystadenoma: most of them are large multilocular cysts containing jelly-like mucus and clear fluid. Most of the cystadenoma show cloud-like light spots and nipples are rarely seen in the capsule.

## Conclusion

Extrarenal rhabdoid tumor of the greater omentum have the characteristics of high degree of malignancy, poor prognosis, and high mortality. Ultrasound has certain malignant features, but it cannot determine with certainty the source and characteristics of the lesion. Therefore, contrast-enhanced ultrasound can further determine the blood flow of the tumor and the relationship between the tumor and its periphery. For the diagnosis, we should consider both the typical histological characteristics of rhabdomyoid cells and the age of the patient. Because of the negative INI-1 protein expression in immunohistochemistry, when we detect that the abdominal cystic solid mass in children or adolescents has the characteristics of malignant tumor but bilateral ovaries are normal, we should suspect the disease, so as to provide more imaging evidence for clinical diagnosis and treatment.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by Ethics Committee of Suining Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

## Author contributions

H-L: Writing – review & editing, Writing – original draft, Data curation. X-HW: Writing – review & editing, Resources, Data curation. X-YF: Data curation, Resources, Writing – review & editing, Supervision, Project administration. Z-HW: Writing – review & editing, Supervision, Project administration.

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## Conflict of interest

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

## Publisher's note



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