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Advances in prognosis and treatment of endometrial cancers

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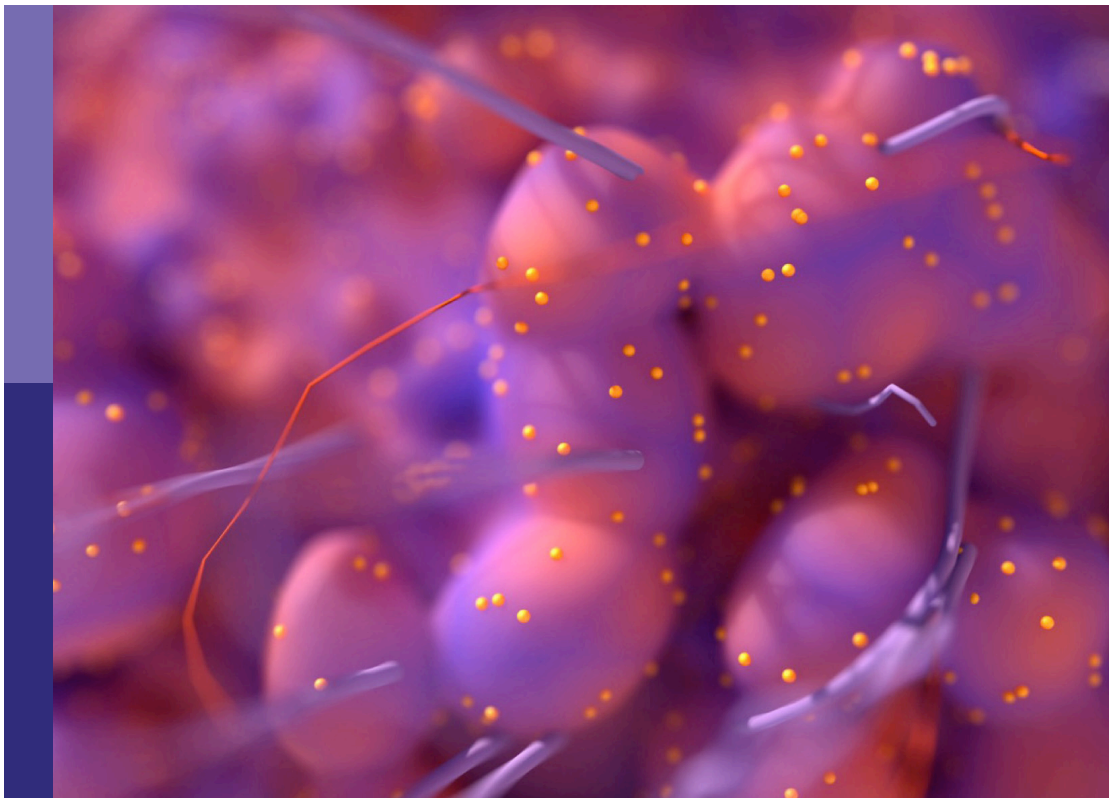

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Advances in prognosis and treatment of endometrial cancers

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ORIGINAL RESEARCH
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Metformin Regulates TET2 Expression to Inhibit Endometrial Carcinoma Proliferation: A New Mechanism

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Objectives: To investigate the relationship between TET2 expression and endometrial cancer's clinicopathological features and prognosis, and the effect of metformin on TET2 and 5hmC levels in endometrial cancer cells.

Methods: The clinical significance of TET2 expression in endometrial carcinoma was analyzed from TCGA public database. Eighty-eight patients with endometrial cancer and 20 patients with normal proliferative endometrium were enrolled in this study. TET2 and 5hmC were respectively detected by Immunohistochemistry and ELISA in endometrial tissues. Kaplan-Meier and Cox proportional hazard regression models were used to analyze relationships between TET2 and 5hmC and the overall survival of EC patients. Endometrial cell proliferation was assessed after TET2 gene knockdown. Western blotting and real-time PCR were used to detect the effect of metformin on TET2 expression and to explore whether AMPK is involved in metformin-mediated TET2 regulation.

Results: The clinical significance of expression of TET2 in endometrial cancer from TCGA public database confirmed that TET2 expression was significantly down-regulated in cancer samples and TET2 expression was also significantly different among different histopathological samples and TET2 is down-regulated in advanced, high-grade, and relapsed endometrial carcinoma tissues($P<0.05$). Immunohistochemical analysis showed that TET2 and 5hmC levels were significantly lower in endometrial adenocarcinoma($P<0.05$). TET2 expression was correlated with the degree of EC differentiation ($P < 0.05$). 5hmC levels were associated with clinical stage, differentiation, the depth of myometrial invasion, and lymph node metastasis ($P < 0.05$). The mean survival time of patients with negative staining for TET2 and 5hmC was shorter than that of patients with positive staining for both markers ($P<0.05$). Multivariate Cox regression analysis showed that TET2 expression was an independent risk factor for prognosis in patients with endometrial adenocarcinoma (HR = 14.520, 95% CI was 1.From 060 to 198.843, P = 0.045). siRNA-mediated TET2 knockdown increased the proliferation of EC cells. Metformin increased the levels of TET2 and 5hmC in EC cells. AMPK was involved in the regulation of TET2 by metformin.

Conclusions: TET2 may play an important role in EC development and may be a prognostic marker. Moreover, TET2 may be involved in a novel mechanism by which metformin inhibits EC cell proliferation.

Keywords: TET2, 5hmC, metformin, endometrial cancer, AMPK

INTRODUCTION

Endometrial cancer (EC) is one of the most common gynecological malignancies worldwide (1). The development of EC is a multistep process involving many molecular biological changes. EC has been shown to be a complex disease driven by abnormal genetic and epigenetic alterations, as well as environmental factors. Low levels of genomic methylation in cancer cells were first discovered by Feinberg et al. in 1983 (2). Since then, an increasing number of studies have shown that DNA methylation levels and patterns are disordered with the occurrence and development of tumors. Aberrant DNA methylation, characterized by genome-wide hypomethylation and regional hypermethylation, is common in various cancer forms and is closely associated with tumor initiation and progression (3).

DNA methylation (generating 5-methylcytosine [5mC]) and hydroxymethylation (generating 5-hydroxymethylcytosine [5hmC]) are epigenetic modifications that are frequently aberrant in cancer (4, 5). The conversion of 5mC to 5hmC occurs through an oxidative reaction catalyzed by the ten-eleven translocation (TET) protein family of dioxygenases (6–8). Previous studies have found that both the TET protein family and 5hmC play important roles in tumor development and progression (9). TET2 DNA dioxygenase plays an important role in regulating cell identity and inhibiting tumor development by regulating DNA methylation and the expression of a large number of genes. The expression level of TET2 is decreased in liver cancer, breast cancer, lung cancer, prostate cancer and other solid tumor tissues compared to normal tissues, and this downregulation decreases the content of its catalytic product 5-hmC, which is closely related to tumor development (10, 11). Changes in TET expression at the gene and protein levels and changes in the 5hmC level are thought to be associated with the development and progression of several cancer types, but there are little data related to EC. In this study, we detected the expressions of TET2 and 5hmC and analyzed their clinical significance in endometrial adenocarcinoma to explore the possible mechanism of TET2 in the development of endometrial cancer.

Metabolic diseases, such as central obesity, type 2 diabetes, and polycystic ovary syndrome (PCOS), are risk factors for type I EC. Diabetes increases the risk of EC by 2.8-fold. Metformin, which is safe and economical, is the first choice for treatment of type 2 diabetes. A large number of epidemiological and clinical observations have shown that metformin can reduce the incidence of a variety of tumors, improve the prognosis of patients with coexisting type 2 diabetes and tumors and improve the patient survival rate (12). Therefore, metformin is expected to become a new tumor treatment or adjuvant antitumor drug. Our previous studies have showed that metformin inhibits the proliferation of EC cells, but the exact mechanism remains unclear. Recent studies have revealed that the TET2 phosphorylation pathway mediated by the energy receptor adenosine monophosphate-activated protein kinase (AMPK) plays an important role in linking diabetes and cancer (13). Metformin is known to be an AMPK activator, so could metformin inhibit the proliferation of endometrial cancer by regulating TET2? In this study, we attempted to preliminarily explore the above possibilities through cytological experiments.

MATERIALS AND METHODS

Analysis of TET2 in the TCGA Public Database

The endometrial cancer dataset, including mRNA expression and clinical information, was obtained from The cancer genome atlas (TCGA, https://portal.gdc.cancer.gov/) database. The transcriptome data from TCGA was normalized and analyzed using the Limma package. Student t-test and Kruskal-Wallis test were applied to calculate the significance of expression differences between two or more groups, respectively. The univariate cox regression analysis was used to calculate the association between the expression level of TET2 and patient's overall survival (OS). Hazard ratios (HRs) and 95% confidence intervals (CIs) were computed based on the Cox regression analysis. Survival curves were estimated using the Kaplan-Meier method and were compared using the log-rank test. The significance was defined as a P value of<0.05.

Tumor Samples

Approval for patient sample analyses was obtained from the Ethics Committee of Xuzhou Central Hospital Affiliated Xuzhou Medical University. The studies were conducted in accordance with the Declaration of Helsinki. All samples were collected from Xuzhou Central Hospital (Xuzhou, Jiangsu, China). In all, 88 EC tissues and 20 normal endometrial tissues were included in the study. Normal endometrial tissues were obtained from women who were undergoing a hysterectomy (for conditions such as uterine fibroids or prolapse).

Immunohistochemical Staining

Immunohistochemical staining was performed using 4-μm thick paraffin-embedded tissue blocks. Blocking with 3% hydrogen peroxide was performed for 30 min to quench endogenous peroxidases. Tissue sections on slides were incubated with primary antibody (1:200 dilution) at 4°C overnight. Then, secondary antibody was added and incubated with the tissues at

37°C for 30 min. Diaminobenzidine tetrahydrochloride was used as a chromogen. As a negative control, phosphate-buffered saline (PBS) was used instead of primary antibody. TET2 positivity was visible as yellow-brown granules in the nucleus and cytoplasm, and 5hmC-positive staining was visible as a brownish yellow color in the nucleus. Each section was independently assessed by two pathologists without prior knowledge of patient data. The samples were assigned a mean score considering both the intensity of staining and the proportion of cells with an unequivocal positive reaction in the immunohistochemical analysis. Positive reactions were defined as those showing brown signals mainly in the cell nucleus. The staining index (range, 0-3) was determined according to the staining intensity and positive area. Scores of 0-3 were defined as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. For statistical analysis, scores of 0-1 were considered to indicate low expression, and scores of 2-3 were considered to indicate high expression.

Cell Culture and Reagents

The EC cell lines Ishikawa and HEC-1-A were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The cell lines were cultured in RPMI-1640 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and McCoy's 5A (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) medium containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) at 37°C in an atmosphere of 5% CO_2. The cells were passaged every 3-5 days. Metformin was purchased from Sigma-Aldrich; Merck KGaA. Primers were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The anti-phosphorylated (p)-AMPK (cat. no. BS4457P), and anti-AMPK (cat. no. BS4457) antibodies were purchased from Bioworld Technology, Inc. (St. Louis Park, MN, USA). TET2 primary antibodies were purchased from Abcam (USA) (ab94580, ab214728).

siRNA Transfection

The endometrial carcinoma cell lines were transfected with siTET2 or siControl *via* reverse transfection using Lipofectamine RNAiMAX (Invitrogen, USA). In parallel, 1.5 μL of Lipofectamine RNAiMAX was mixed with 50 μL of Opti-MEM. The solution mixture was mixed by gentle pipetting and incubated for 10-20 min at room temperature to allow siRNA/lipid complexes to form. EC cells were suspended in complete growth medium without antibiotics at 50,000 cells/mL, gently mixed with 100 μL of the transfection solution, and plated. The cells were incubated for 24-72 h at 37°C and then assayed for gene knockdown.

Cell Proliferation Assay (Cell Counting Kit-8; CCK-8)

The experiment was conducted according to the protocol of the Cell Counting Kit-8 Reagent Kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Cells transfected with siRNAs were seeded at 5,000 cells per well in 96-well plates and the TET2 knockdown cells and control cells were treated with 5mM metformin. They were both incubated in medium containing 10% FBS for 24 h, 48 h, 72 h and 96 h. After changing the medium without metformin, CCK-8 was added to each well, and the plates were incubated at 37°C for 1 h. Absorbance was measured at 450 nm using an automated microplate reader (Infinite 200; Tecan, Männedorf, Switzerland).

Western Blot Analysis

EC cells (1×10^5/dish) were plated in 10-cm dishes and treated with 1, 5, or 15 mM metformin for 24 h. The cells were collected, resuspended in cell lysis buffer for western blotting, incubated on ice for 30 min, and centrifuged at 12,000× g for 10 min at 4°C. The supernatant was collected, and the protein content was quantified using a bicinchoninic acid (BCA) protein assay kit (Beyotime). Protein samples (20 μg) were separated in a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore). After washing with PBST three times, the membranes were blocked with 5% nonfat milk for 30 min and then incubated with primary antibodies overnight at 4°C. The blots were then washed with PBST three times and incubated with the appropriate secondary antibodies. After washing with PBST three times, the protein bands were detected using an Odyssey Infrared Imaging system (Li-COR Biosciences). Primary antibodies against TET2 and AMPK were used at a dilution of 1:1,000, and secondary antibodies were used at a dilution of 1:2,000. The relative band intensity was analyzed with ImageJ software (version 1.47) (Schneider et al., 2012) and calculated as a ratio relative to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) band intensity. For evaluation of different blots, each band of the replicates was normalized to the GAPDH band intensity and then averaged. The averaged intensities were used for comparisons.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT–qPCR)

Ishikawa and HEC-1-A cells were plated at a concentration of 10^5 cells/well in 6-well plates for 24 h at 37°C and subsequently treated with metformin (0, 1, 5 and 15 mM). Total RNA was extracted from the harvested EC cells according to the manufacturer's instructions using TRI reagent (Sigma). The RNA concentration was determined by measuring the OD at 260 nm. First-strand complementary DNA (cDNA) was synthesized with a SuperScript II First-Strand Synthesis System for quantitative reverse transcription-polymerase chain reaction (qRT-PCR; Invitrogen). qPCR amplification was carried out using actin as an endogenous control. SYBR Green probes for each gene were used. The primers are listed in **Table S1**. Real-time PCR was carried out with 50 ng of cDNA and SYBR PCR master mix (TaKaRa) in an Agilent Mx3000P Real-time PCR System using the two-step procedure (95°C 2 min, 1 cycle; 95°C 15 s, 60°C 1 min, 30 cycles). Relative quantitation of the expression of each single gene was performed using the comparative threshold cycle method.

Quantification of Global DNA Hydroxymethylation (Indicated by 5hmC) *via* ELISA

The extracted genomic DNA was stored at −80°C. Global DNA hydroxymethylation (indicated by 5hmC) was assessed using a MethylFlash Global DNA Hydroxymethylation ELISA Easy kit (colorimetric) from EpiGentek according to the instructions provided by the manufacturer.

Statistical Analysis

All assays were repeated independently a minimum of three times (n ≥ 3), and three wells per assay were used for each treatment in each cell line. The experimental data are expressed as the mean ± standard deviation (SD). One-way analysis of variance was used for statistical analyses and was performed using SPSS software (version 22.0). Data were compared between the two groups using a least significant difference test. The log-rank test was used to compare differences in the overall survival rate. The Cox proportional hazard regression model was used for multivariate analysis. Statistical significance is indicated by * for $P < 0.05$ and ** for $P < 0.01$.

RESULTS

Clinical Significance of Expression of TET2 in Endometrial Cancer From TCGA Public Database

The differential expression of TET2 between normal endometrial tissue and endometrial cancer tissue was observed using the endometrial cancer data in TCGA database. TET2 expression was significantly down-regulated in cancer samples (P = 3.250346E-05) (**Figure 1A**). We also compared TET2 expression between different histopathological samples (Endometrioid endometrial adenocarcinoma, Serous endometrial adenocarcinoma, Mixed

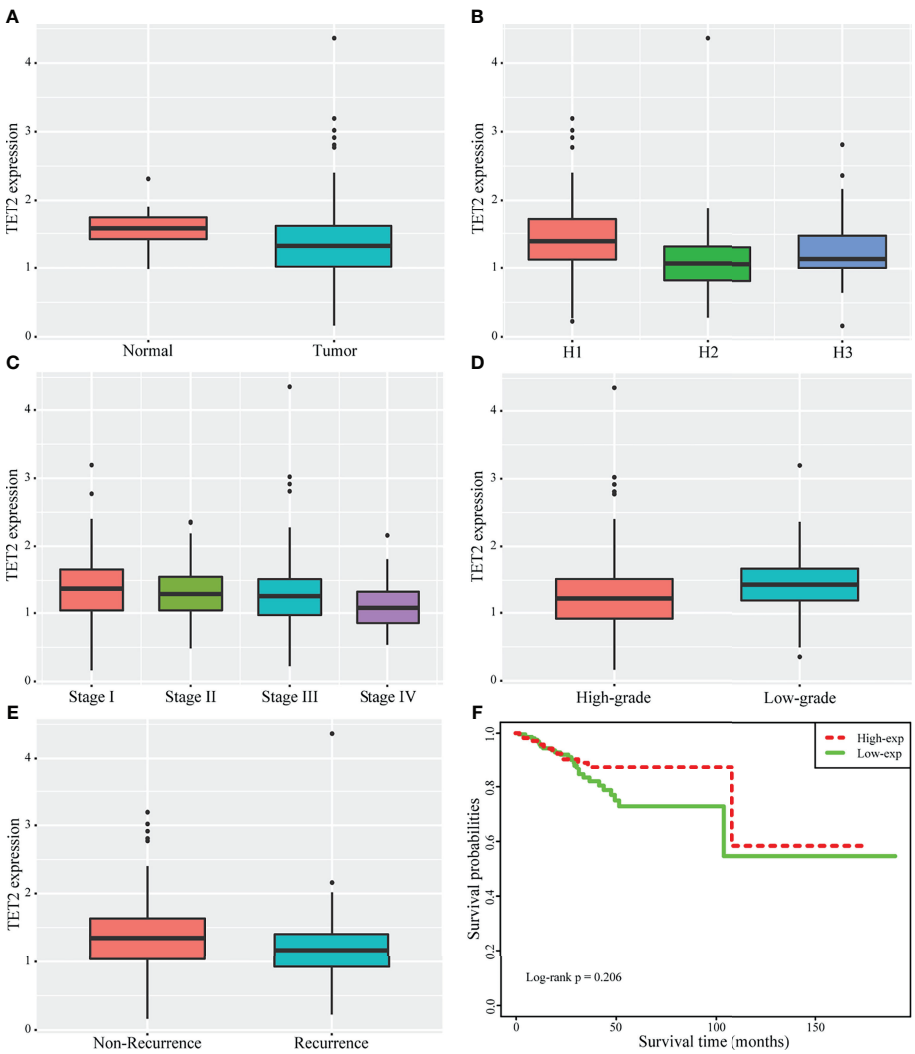

FIGURE 1 | Clinical significance of expression of TET2 in endometrial cancer from TCGA public database. The significant differences of TET2 expression between normal and endometrial cancer **(A)**. TET2 expression was also significantly different among different histopathological samples (1 stands for Endometrioid endometrial adenocarcinoma, 2 stands for Serous endometrial adenocarcinoma, 3 stands for Mixed serous and endometrioid) **(B)**. With the increase of stage, TET2 expression was continuously down-regulated (C: 1 stands for Stage I, 2 stands for Stage II, 3 stands for Stage III, 4 stands for Stage IV). TET2 was significantly down-regulated in the high-grade group **(D)**. TET2 was significantly lower in patients with recurrence **(E)**. TET2 expression is a protective factor, and the high expression group tends to have a better prognosis **(F)**.

serous and endometrioid). The results showed that the expression of TET2 was also significantly different among different histopathological samples, and it was significantly over-expressed in Endometrioid endometrial adenocarcinoma (P< 0.05) (**Figure 1B**). With the increase of stage, the expression of TET2 was continuously down-regulated, with significant differences in different stages(P=0.00495) (**Figure 1C**). Besides, TET2 was significantly down-regulated in the high-grade group (P=2.197738E-05) (**Figure 1D**). Comparison of TET2 expression between patients with and without recurrence showed that TET2 expression was significantly lower in patients with recurrence (P=0.0425) (**Figure 1E**). The correlation between TET2 expression and survival was observed by univariate Cox. The results showed that TET2 expression was a protective factor, but there was no significant correlation with overall survival (P =0.378; HR = 0.7257). Using the median expression value of TET2 as the dividing line, patients were divided into the high and low expression group (lower than the median is the green line, and higher than the median is the red line). There was no significant difference in survival between the two groups, but the high-expression group tended to have a better prognosis. (log-rank p = 0.206) (**Figure 1F**).

TET2 and 5hmC Are Expressed at Low Levels in Endometrial Carcinoma Tissue

TET2 expression among different histopathological samples (Endometrioid endometrial adenocarcinoma, Serous endometrial adenocarcinoma, Mixed serous and endometrioid) from TCGA public database showed that the expression of TET2 was also significantly different among different histopathological samples, and it was significantly over-expressed in Endometrioid endometrial adenocarcinoma (**Figure 1B**). Endometrioid adenocarcinoma accounts for 80-90% of the pathological types of endometrial cancer. Thus, we examined the levels of TET2 and 5hmC in endometrioid endometrial adenocarcinoma tissues. The conversion of 5mC to 5hmC occurs through an oxidative reaction catalyzed by the TET protein family of dioxygenases. We analyzed TET2 expression in a series of 88 endometrial carcinoma samples and 20 normal proliferative endometrium samples *via* immunohistochemistry. TET2 expression was observed in the nucleus and cytoplasm of cells as yellow-brown granules. 5hmC-positive staining was indicated by a brownish yellow color in the nucleus. We found that TET2- and 5hmC-positive staining was present at a higher level in patients with a proliferative endometrium than in patients with EC (**Figure 2**). These data suggest that TET2 may be associated with EC.

Relationships Between TET2 and 5hmC Expression and Clinicopathological Factors in EC

Correlations between TET2 and 5hmC expression and the clinicopathologic characteristics of endometrial carcinoma are shown in **Table 1**. TET2 expression in endometrial adenocarcinoma was correlated with the degree of differentiation (P < 0.05). The positive TET2 expression rate in poorly differentiated tissues was lower than that in highly differentiated tissues (P < 0.05). The 5hmC level in endometrial adenocarcinoma was associated with clinical stage, differentiation, depth of myometrial invasion, and lymph node metastasis (P < 0.05). The positive rate of 5hmC staining decreased with tumor malignancy (**Table 1**).

Relationships Between TET2 and 5hmC Levels in EC and the Survival Time of Patients

Among the 88 patients with endometrial adenocarcinoma, 58 (65.9%) survived, 12 (13.6%) died, and 18 (20.5%) were lost to

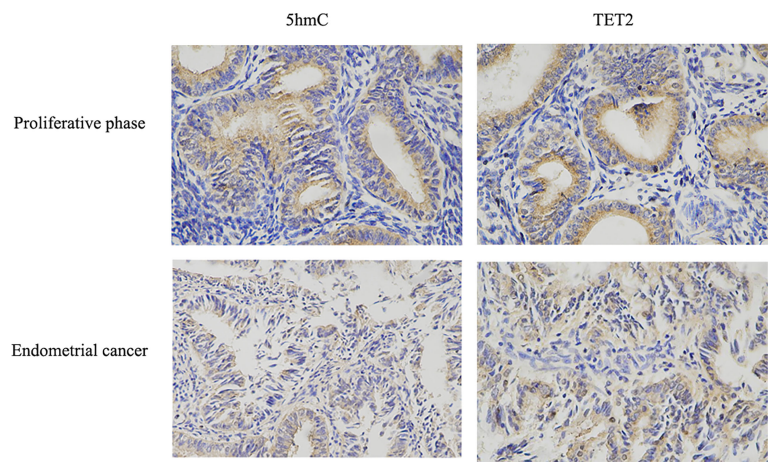

FIGURE 2 | TET2 and 5hmC were low-expressed in endometrial cancer. The expression of TET2 and 5hmC in proliferative phase endometrial tissue and EC tissue was assessed using IHC staining. Representative images were captured at ×400 magnification. TET2 and 5hmC IHC scores in EC tissues compared with proliferative phase endometrial tissues were presented.

TABLE 1 | Relationships between TET2, 5-hmC and clinicopathological factors in EC.

Grouping	TET2 positive [n (%)]	χ2	P	5-hmC positive [n (%)]	χ2	P
FIGO Stage		5.699	0.058		7.234	0.027*
I	40 (60.6)			45 (68.2)		
II	4 (57.1)			2 (28.6)		
III+IV	4 (26.7)			6 (40.0)		
Differentiation		6.145	0.046*		23.839	0.000**
Low	7 (31.8)			5 (22.7)		
Medium	9 (64.3)			6 (42.9)		
High	32 (61.5)			42 (80.8)		
Myometrial invasion		3.024	0.082		11.968	0.001**
<1/2	36 (61.0)			43 (72.9)		
≥1/2	12 (41.4)			10 (34.5)		
Lymph node metastasis		3.364	0.067		4.779	0.029*
No	47 (58.0)			52 (64.2)		
Yes	1 (14.3)			1 (14.3)		

*P < 0.05, **P < 0.01.

follow-up. Surviving patients were followed up for 60 to 89 months, and the patients who died had survival times ranging from 6 to 45 months. The mean survival time of patients was 58.00 ± 18.11 months, and the median was 66 months. The mean and median survival times of patients with positive TET2 expression were 66.57 and 68.22 months, respectively, and those of patients with negative TET2 expression were 46.74 and 44 months, respectively. The mean and median survival times of patients with positive staining for 5hmC were 64.47 and 68.33 months, respectively, and those for patients with negative staining for 5hmC were 48.09 and 46.50 months, respectively. The mean survival time of patients with positive TET2 and 5hmC staining was 63.46 months, and the median survival time was 67.50 months. The mean survival time of patients with negative staining TET2 and 5hmC was 41.35 months, and the median survival time was 36.50 months. The mean survival time of patients with negative TET2 and 5hmC staining was significantly shorter than that of patients with positive staining (P < 0.01). The 5-year survival rates of TET2-positive and TET2-negative patients were 97.7% and 57.7%, respectively, and those of 5hmC-positive and TET2-negative patients were 93.5% and 62.5%, respectively (**Figure 3**).

Multivariate Cox Regression Analysis of Prognostic Factors in Patients With EC

Multivariate Cox regression analysis revealed that stage, lymph node metastasis, and TET2 expression were associated with prognosis in patients with endometrial adenocarcinoma (P < 0.05). After adjusting for possible confounders, stage, lymph node metastasis, and TET2 expression may be independent prognostic factors in patients with endometrial adenocarcinoma [hazard ratio (HR) = 13.553, 95% confidence interval (CI): 1.509–121.677, P = 0.020; HR = 15.359, 95% CI: 1.284–183.783, P = 0.031; HR = 14.520, 95% CI: 1.060–198.843, P = 0.045] (**Table 2**).

Anticancer Effects of TET2 in EC Cell Lines

To explore the role of TET2 in EC cell lines, we knocked down TET2 using siRNA. The TET2 knockdown efficiency of the gene-specific siRNA was confirmed using real-time PCR and western blotting (**Figure 4A**). Knockdown of TET2 gene expression with siRNA significantly increased the proliferation rate of Ishikawa (P<0.01) and HEC-1-A (P<0.01) cells compared with that of cells transfected with nontargeting siRNA (**Figure 4B**). In addition,

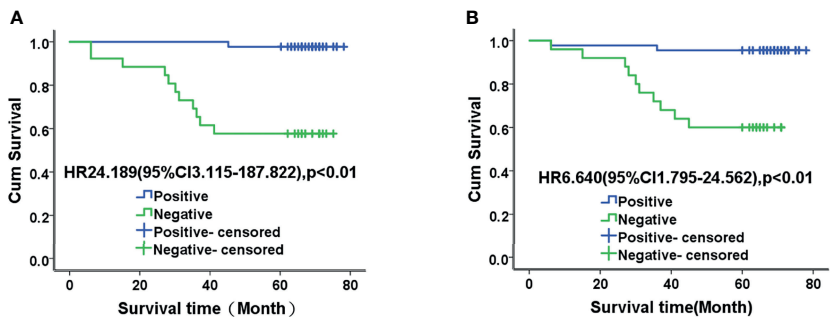

FIGURE 3 | Kaplan-Meier analysis of TET2 **(A)** and 5-hmC **(B)** expression and prognosis in patients with endometrial adenocarcinoma. The mean survival time of patients with negative TET2 and 5hmC staining was significantly shorter than that of patients with positive staining (P < 0.01).

TABLE 2 | Multivariate Cox regression analysis of prognostic factors in patients with EC.

Index	B	SE	Wald	*P*	HR	95% CI
Stage (ref = I)			6.024	0.049*		
II	3.214	1.87	2.954	0.086	24.875	0.637–971.197
III+IV	2.607	1.12	5.418	0.02	13.553	1.509–121.677
Differentiation (ref = low)			2.788	0.248		
Medium	-0.525	1.114	0.222	0.637	0.591	0.067–5.249
High	-2.209	1.335	2.737	0.098	0.11	0.008–1.504
Myometrial invasion	-2.92	1.557	3.519	0.061	0.054	0.003–1.140
Lymph node metastasis	2.732	1.266	4.653	0.031*	15.359	1.284–183.783
TET2	2.676	1.335	4.015	0.045*	14.52	1.060–198.843
5-hmc	9.773	115.514	0.007	0.933	17551.949	0.000–3.712

*$P < 0.05$.

we further examined the effect of TET2 on metformin-mediated inhibition of EC cell proliferation. TET2 knockdown cells were treated with 5 mM metformin for 72h, and the results showed that knockdown of TET2 attenuated the inhibitory effect of metformin on EC cell proliferation (**Figure 4C**).

Metformin Increased TET2 and 5hmC Expression in EC Cells

To examine the potential regulation of the expression and activation of TET2 and its substrates by metformin in endometrial carcinoma, two types of EC cells were treated with metformin at different concentrations for 24 h. In our study, western blotting and real-time PCR results showed that metformin treatment resulted in a potent increase in TET2 protein and mRNA expression, which occurred in a dose-dependent manner (**Figure 5A**). Metformin also increased the 5hmC level in a dose-dependent manner (**Figure 5B**).

TET2 and 5hmC Regulation by Metformin Depends on the Presence of AMPK

Metformin is known as a traditional AMPK activator. Therefore, we further tested whether AMPK is involved in the regulation of TET2 by metformin. We knocked down AMPK using siRNA. The AMPK knockdown efficiency of the gene-specific siRNA was confirmed using real-time PCR and western blotting (**Figure 6A**). At 24 h after siRNA knockdown of AMPK gene expression, TET2 expression and 5hmC level were significantly reduced (**Figure 6B**). Western blotting and real-time PCR results showed that metformin did not significantly increase TET2 or 5hmC expression in these cells after 72 hours of metformin treatment (**Figure 6C**). Thus, we speculated that metformin could regulate TET2 expression through the AMPK pathway.

DISCUSSION

According to a number of epidemiological studies, endometrial cancer (EC) is associated with chronic exposure to high levels of estrogen (14). However, beyond the involvement of estrogen, the mechanism of carcinogenesis in the endometrium remains unclear. In recent years, there has been a focus on epigenetic mechanisms, which involve regulation of gene expression through chromatin modification without a change in the DNA sequence. Aberrant DNA methylation plays an important role in tumor development, and disorder of DNA demethylation mediated by the ten-eleven translocation (TET) family is an important factor leading to DNA methylation imbalance. DNA 5-hydroxymethylcytosine (5hmC) is a major oxidation product of DNA 5-methylcytosine (5mC), and this reaction is catalyzed by the TET family of dioxygenases (6). To the best of our

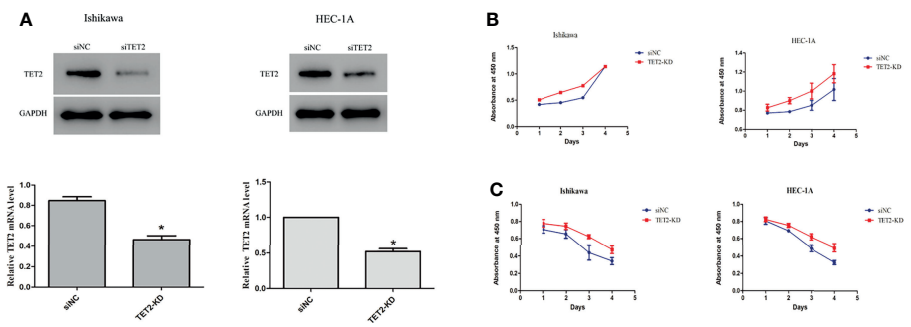

FIGURE 4 | Anticancer effects of TET2 in EC cell lines. The TET2 knockdown efficiency of the gene-specific siRNA was confirmed using western blotting and real-time PCR **(A)**. Knockdown of TET2 increased the proliferationin of EC cell lines **(B)**. The TET2 knockdown cells and control cells were treated with 5 mM metformin. The knockdown of TET2 attenuated the inhibitory effect of metformin on the proliferation of EC cells **(C)**. *P < 0.05, compared with control.

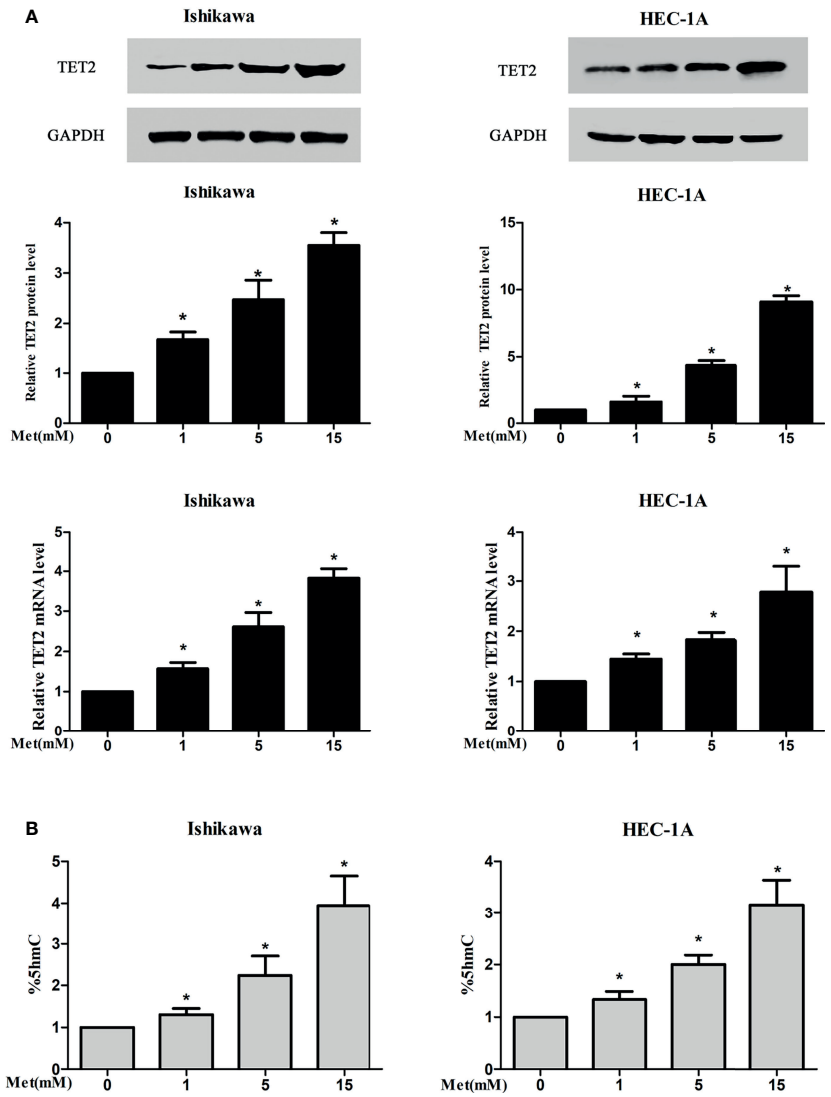

FIGURE 5 | Metformin increased the expression of TET2 and 5hmC in EC cells. Two types of EC cells were treated with metformin at different concentrations for 24 h The expression of TET2 was detected by western blotting and real-time PCR and 5hmC was detected by ELISA. Metformin increased the protein and mRNA expression of TET2 in a dose-dependent manner **(A)**. Metformin also increased the level of 5hmC in a dose-dependent manner **(B)**. *P < 0.05, compared with control.

knowledge, little studies have evaluated the role of TET2 in EC development and effect of metformin on TET2 expression. The results of the present study suggested that TET2 was associated with EC development and could inhibit EC cell proliferation. In addition, we found that metformin could increase TET2 protein expression through AMPK pathway in EC cells.

TET proteins, including TET1, TET2 and TET3, are α-ketoglutarate and Fe2+-dependent enzymes that can oxidize 5mC to 5hmC, which is an epigenetic DNA modification process (6, 15). DNA methylation (generating 5mC) and hydroxymethylation (generating 5hmC) are common epigenetic modifications in cancer (4, 5, 16). Previous studies have found that both the TET protein family and 5hmC play important roles in tumor development and progression (17). TET2 was first identified as a tumor suppressor gene in myelodysplastic syndrome (18). TET2 expression is decreased in liver cancer, breast cancer, lung cancer, prostate cancer and other solid tumors, leading to a decrease in the content of its catalytic product 5hmC, which is closely related to tumor development (10, 11, 19). The results of the present study suggest that the TET2 and 5hmC levels in EC tissues are significantly decreased compared with those in normal endometrial tissues from TCGA public database and immunohistochemical analysis. Our study showed a positive correlation between the 5hmC level and TET2 expression. Similar to the results of most studies of other malignant tumors, the results of our EC studies showed decreased TET2 and 5hmC expression in cancer tissues.

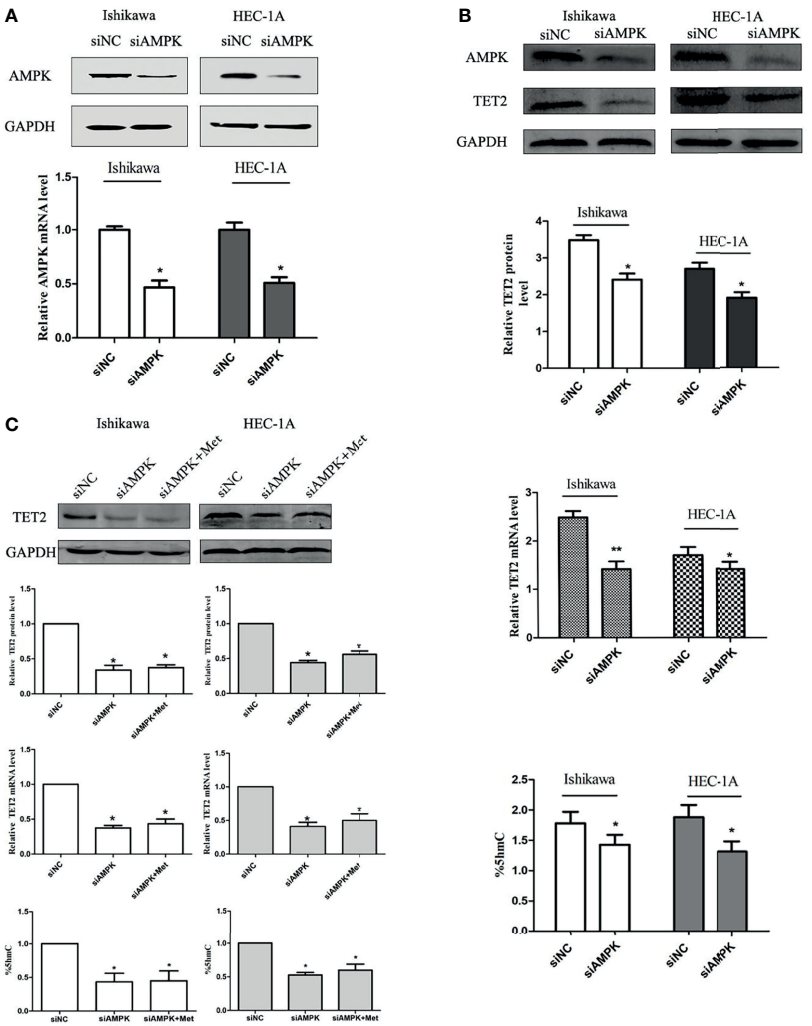

FIGURE 6 | The regulation of TET2 and 5hmC by metformin depends on the presence of AMPK. We knocked down AMPK using an siRNA. The AMPK knockdown efficiency of the gene-specific siRNA was confirmed using western blotting and real-time PCR **(A)**. The knockdown of AMPK gene expression with the siRNA significantly reduced the expression of TET2 and the level of 5hmC **(B)**. Western blotting and real-time PCR results showed that metformin did not significantly increase the expression of TET2 and 5hmC in these cells when knocked down AMPK **(C)**. *P < 0.05, **P < 0.01, compared with control.

In melanoma, a low 5hmC level is a marker of a poor prognosis and is associated with dysplastic cytomorphological features and tumor progression (20). In solid tumors, low 5hmC levels indicate poor overall survival and a high cumulative recurrence rate (21). In addition, 5hmC levels are highly correlated with tumor stage (22). In terms of the molecular mechanism, 5-hmC is a product of DNA demethylation of TET2, suggesting that loss of TET2 leads to loss of 5-hmC, which can promote cancer occurrence and progression by affecting gene expression patterns. The results of the present study showed that the TET2 expression rate in poorly differentiated EC tissues was lower than that in well-differentiated EC tissues. TET2 expression was significantly reduced in high-grade, advanced, and recurrent endometrial carcinoma from TCGA database. The expression of 5hmC in endometrial adenocarcinoma is related to clinical stage, the degree of differentiation, the depth of muscular infiltration and lymph node metastasis. The level of TET2 and 5hmC is widely decreased in cancer cells and can be used as a marker of the degree of cancer malignancy.

Alterations in genomic 5hmC levels and TET dioxygenase expression are closely associated with the survival rate of cancer patients (11, 23, 24) and are involved in breast (25), prostate, liver (26), lung, pancreatic, colorectal, gastric, small intestine, brain, kidney, and skin cancer and myeloid diseases (27–29). Our results also showed that the 5-year survival of EC patients with negative TET2 and 5-hmC staining was significantly reduced. Multivariate COX regression analysis revealed that TET2 might serve as an independent prognostic factor in patients with endometrial adenocarcinoma, and may be useful in predicting therapeutic effects. These above results suggest that TET2 may play an important role in EC development.

To further investigate the role of TET2 in EC, we assessed the proliferation of Ishikawa and HEC-1-A cells after TET2 knockdown. The results indicated that TET2 knockdown increased EC cell growth, suggesting that TET2 can inhibit EC cell proliferation. DNA methylation plays a key role in the regulation of genes involved in cell growth, proliferation and apoptosis in endometrial tissue (30). Thus, deregulation of the DNA methylation pattern can disrupt cell homeostasis in the endometrium and result in EC development (31). A recent study of colorectal cancer (CRC) indicated that genes with 5hmC in their promoters resist DNA hypermethylation, highlighting the important role that 5hmC plays in cancer cell proliferation (32). However, the mechanism by which TET2 deletion increases the proliferation of EC cells remains to be further studied.

Diabetes is a known risk factor for EC. TET2 is an important link between diabetes and cancer. Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer (13). Metformin is a first-line drug for diabetes treatment and has an antiproliferative effect on many types of cancer cells. Our previous studies have shown that metformin inhibits the proliferation of EC cells (33). We speculated that TET2 is involved in the inhibitory effect of metformin on EC cell proliferation. Then, we compared the proliferation of TET2 knockdown cells with that of control cells after metformin treatment. We found that TET2 knockdown significantly inhibited the antiproliferative effect of metformin on EC cells. Therefore, we hypothesized that metformin may inhibit EC cell proliferation by regulating TET2 expression. To test this hypothesis, we examined TET2 expression in EC cells treated with metformin at different concentrations. Our results suggested that metformin can increase TET2 expression and the 5hmC level in a dose-dependent manner. Adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved protein in mammalian cells and a "metabolism and energy receptor" of cells (34). Metformin is a traditional AMPK activator. Metformin inhibits the growth of ECC-1 cells and Ishikawa cells in a dose-dependent and time-dependent manner by activating AMPK and inhibiting the mTOR signaling pathway (35). Metformin failed to inhibit the proliferation of EC cells treated with AMPK siRNA or inhibitors (36). Two potential AMPK catalytic sites were identified by amino acid sequence analysis of TET2, and the TET2 protein was confirmed to be a substrate of AMPK. Activated AMPK can phosphorylate TET2 at serine 99, thus maintaining the stability of the TET2 protein (13). Our results showed that knockdown of AMPK gene expression with siRNA significantly reduced TET2 expression and the 5hmC level and attenuated the inhibitory effects of metformin on these factors. Therefore, we speculate that metformin may regulate TET2 expression by activating AMPK.

CONCLUSIONS

In summary, our current findings demonstrate the expression pattern and clinical significance of TET2 in EC. TET2 can repress EC cells proliferation. In addition, TET2 could be a useful biomarker for predicting the prognosis of EC patients and may represent a novel therapeutic target for EC treatment. Metformin increased TET2 expression and the 5hmC level. The results of the present study reveal that metformin regulates TET2 protein expression by activating AMPK. Our study provides new insight into the antiproliferative effects of metformin in EC.

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 authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Biomedical Research Ethics Review Committee of Xuzhou Central Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JZ and BZ contributed to the conception and design of the study. LK provided access to tissue and prepared tissue samples. YYL, QW, and HX provided clinical information. XZ, JL, and JZ performed cell culture, PCR and western blot. LK and QW carried out cell proliferation experiments. JZ and YL analyzed the data. YL performed TCGA data analysis. JZ and YL prepared the figures and drafted the manuscript. All authors read and commented on the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.856707/full#supplementary-material

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. *CA Cancer J Clin* (2020) 70:7–30. doi: 10.3322/caac.21590
2. Feinberg AP, Vogelstein B. Hypomethylation Distinguishes Genes of Some Human Cancers From Their Normal Counterparts. *Nature* (1983) 301 (5895):89–92. doi: 10.1038/301089a0
3. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, et al. Induction of Tumors in Mice by Genomic Hypomethylation. *Science* (2003) 300:489–92. doi: 10.1126/science.1083558
4. Baylin SB, Jones PA. A Decade of Exploring the Cancer Epigenome - Biological and Translational Implications. *Nat Rev Cancer* (2011) 11:726–34. doi: 10.1038/nrc3130
5. Ko M, An J, Rao A. DNA Methylation and Hydroxymethylation in Hematologic Differentiation and Transformation. *Curr Opin Cell Biol* (2015) 37:91–101. doi: 10.1016/j.ceb.2015.10.009
6. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1. *Science* (2009) 324:930–5. doi: 10.1126/science.1170116
7. Zhang H, Zhang X, Clark E, Mulcahey M, Huang S, Shi YG. TET1 is a DNA-Binding Protein That Modulates DNA Methylation and Gene Transcription *via* Hydroxylation of 5-Methylcytosine. *Cell Res* (2010) 20:1390–3. doi: 10.1038/cr.2010.156
8. He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-Mediated Formation of 5-Carboxylcytosine and its Excision by TDG in Mammalian DNA. *Science* (2011) 333:1303–7. doi: 10.1126/science.1210944
9. Roberti A, Valdes AF, Torrecillas R, Fraga MF, Fernandez AF. Epigenetics in Cancer Therapy and Nanomedicine. *Clin Epigenet* (2019) 11:81. doi: 10.1186/s13148-019-0675-4
10. Yang H, Liu Y, Bai F, Zhang JY, Ma SH, Liu J, et al. Tumor Development is Associated With Decrease of TET Gene Expression and 5-Methylcytosine Hydroxylation. *Oncogene* (2013) 32:663–9. doi: 10.1038/onc.2012.67
11. Haffner MC, Chaux A, Meeker AK, Esopi DM, Gerber J, Pellakuru LG, et al. Global 5-Hydroxymethylcytosine Content Is Significantly Reduced in Tissue Stem/Progenitor Cell Compartments and in Human Cancers. *Oncotarget* (2011) 2:627–37. doi: 10.18632/oncotarget.316
12. Martin-Castillo B, Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA. Metformin and Cancer: Doses, Mechanisms and the Dandelion and Hormetic Phenomena. *Cell Cycle* (2010) 9:1057–64. doi: 10.4161/cc.9.6.10994
13. Wu D, Hu D, Chen H, Shi G, Fetahu IS, Wu F, et al. Glucose-Regulated Phosphorylation of TET2 by AMPK Reveals a Pathway Linking Diabetes to Cancer. *Nature* (2018) 559:637–41. doi: 10.1038/s41586-018-0350-5
14. Farnell YZ, Ing NH. The Effects of Estradiol and Selective Estrogen Receptor Modulators on Gene Expression and Messenger RNA Stability in Immortalized Sheep Endometrial Stromal Cells and Human Endometrial Adenocarcinoma Cells. *J Steroid Biochem Mol Biol* (2003) 84:453–61. doi: 10.1016/s0960-0760(03)00066-9
15. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet Proteins can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science* (2011) 333:1300–3. doi: 10.1126/science.1210597
16. Jones PA. Functions of DNA Methylation: Islands, Start Sites, Gene Bodies and Beyond. *Nat Rev Genet* (2012) 13:484–92. doi: 10.1038/nrg3230
17. Gal-Yam EN, Saito Y, Egger G, Jones PA. Cancer Epigenetics: Modifications, Screening, and Therapy. *Annu Rev Med* (2008) 59:267–80. doi: 10.1146/annurev.med.59.061606.095816
18. Grossmann V, Kohlmann A, Eder C, Haferlach C, Kern W, Cross NC, et al. Molecular Profiling of Chronic Myelomonocytic Leukemia Reveals Diverse Mutations in <80% of Patients With TET2 and EZH2 Being of High Prognostic Relevance. *Leukemia* (2011) 25:877–9. doi: 10.1038/leu.2011.10
19. Lian CG, Xu Y, Ceol C, Wu F, Larson A, Dresser K, et al. Loss of 5-Hydroxymethylcytosine is an Epigenetic Hallmark of Melanoma. *Cell* (2012) 150:1135–46. doi: 10.1016/j.cell.2012.07.033
20. Larson AR, Dresser KA, Zhan Q, Lezcano C, Woda BA, Yosufi B, et al. Loss of 5-Hydroxymethylcytosine Correlates With Increasing Morphologic Dysplasia In Melanocytic Tumors. *Mod Pathol* (2014) 27:936–44. doi: 10.1038/modpathol.2013.224
21. Liu WR, Tian MX, Jin L, Yang LX, Ding ZB, Shen YH, et al. High Expression of 5-Hydroxymethylcytosine and Isocitrate Dehydrogenase 2 Is Associated With Favorable Prognosis After Curative Resection of Hepatocellular Carcinoma. *J Exp Clin Cancer Res* (2014) 33:32. doi: 10.1186/1756-9966-33-32
22. Chen ML, Shen F, Huang W, Qi JH, Wang Y, Feng YQ, et al. Quantification of 5-Methylcytosine and 5-Hydroxymethylcytosine in Genomic DNA From Hepatocellular Carcinoma Tissues by Capillary Hydrophilic-Interaction Liquid Chromatography/Quadrupole TOF Mass Spectrometry. *Clin Chem* (2013) 59:824–32. doi: 10.1373/clinchem.2012.193938
23. Jin SG, Jiang Y, Qiu R, Rauch TA, Wang Y, Schackert G, et al. 5-Hydroxymethylcytosine is Strongly Depleted in Human Cancers But its Levels do Not Correlate With IDH1 Mutations. *Cancer Res* (2011) 71:7360–5. doi: 10.1158/0008-5472.CAN-11-2023
24. Tan L, Shi YG. Tet Family Proteins and 5-Hydroxymethylcytosine in Development and Disease. *Development* (2012) 139:1895–902. doi: 10.1242/dev.070771
25. Zhong S, Li C, Han X, Li X, Yang YG, Wang H. Idarubicin Stimulates Cell Cycle- and TET2-Dependent Oxidation of DNA 5-Methylcytosine in Cancer Cells. *Chem Res Toxicol* (2019) 32:861–8. doi: 10.1021/acs.chemrestox.9b00012
26. Liu J, Jiang J, Mo J, Liu D, Cao D, Wang H, et al. Global DNA 5-Hydroxymethylcytosine and 5-Formylcytosine Contents Are Decreased in the Early Stage of Hepatocellular Carcinoma. *Hepatology* (2019) 69:196–208. doi: 10.1002/hep.30146
27. Albano F, Anelli L, Zagaria A, Coccaro N, Minervini A, Rossi AR, et al. Decreased TET2 Gene Expression During Chronic Myeloid Leukemia Progression. *Leuk Res* (2011) 35:e220–2. doi: 10.1016/j.leukres.2011.07.013
28. Chou WC, Chou SC, Liu CY, Chen CY, Hou HA, Kuo YY, et al. TET2 Mutation Is an Unfavorable Prognostic Factor in Acute Myeloid Leukemia Patients With Intermediate-Risk Cytogenetics. *Blood* (2011) 118:3803–10. doi: 10.1182/blood-2011-02-339747
29. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, et al. Impaired Hydroxylation of 5-Methylcytosine in Myeloid Cancers With Mutant TET2. *Nature* (2010) 468:839–43. doi: 10.1038/nature09586
30. Caplakova V, Babusikova E, Blahovcova E, Balharek T, Hatok J. DNA Methylation Machinery in the Endometrium and Endometrial Cancer. *Anticancer Res* (2016) 36:4407–20. doi: 10.21873/anticanres.10984
31. Ma X, Gao X. Epigenetic Modifications and Carcinogenesis of Human Endometrial Cancer. *Austin J Clin Pathol* (2014) 1:1014.
32. Uribe-Lewis S, Stark R, Carroll T, Dunning MJ, Bachman M, Ito Y, et al. 5-Hydroxymethylcytosine Marks Promoters in Colon That Resist DNA Hypermethylation in Cancer. *Genome Biol* (2015) 16:69. doi: 10.1186/s13059-015-0605-5
33. Zhang J, Xu H, Zhou X, Li Y, Liu T, Yin X, et al. Role of Metformin in Inhibiting Estrogen-Induced Proliferation and Regulating ERalpha and ERbeta Expression in Human Endometrial Cancer Cells. *Oncol Lett* (2017) 14:4949–56. doi: 10.3892/ol.2017.6877
34. Duca FA, Cote CD, Rasmussen BA, Zadeh-Tahmasebi M, Rutter GA, Filippi BM, et al. Corrigendum: Metformin Activates a Duodenal Ampk-Dependent Pathway to Lower Hepatic Glucose Production in Rats. *Nat Med* (2016) 22:217. doi: 10.1038/nm0216-217b
35. Cantrell LA, Zhou C, Mendivil A, Malloy KM, Gehrig PA, Bae-Jump VL. Metformin is a Potent Inhibitor of Endometrial Cancer Cell Proliferation–Implications for a Novel Treatment Strategy. *Gynecol Oncol* (2010) 116:92–8. doi: 10.1016/j.ygyno.2009.09.024
36. Milewicz T, Kialka M, Mrozinska S, Ociepka A, Krzysiek J. [Metformin–new Treatment Strategies for Gynecologic Neoplasms]. *Przegl Lek* (2013) 70:81–4.

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Management of Metastatic Endometrial Cancer: Physicians' Choices Beyond the First Line. A MITO Survey

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Background: Endometrial cancer (EC) therapeutic and diagnostic approaches have been changed by the development of a new prognostic molecular classification, the introduction of dostarlimab in microsatellite instability (MSI) high pre-treated advanced EC patients with further expected innovation deriving from lenvatinib plus pembrolizumab regardless MSI status. How this is and will be translated and embedded in the clinical setting in Italy is not known; this is why we developed Multicentre Italian Trials in Ovarian cancer and gynaecologic malignancies (MITO) survey on the current practice and expected future changes in EC.

Methods: We designed a self-administered, multiple-choice online questionnaire available only for MITO members for one month, starting in April 2021.

Results: 75.6% of the respondents were oncologists with a specific focus on gynaecologic malignancies and 73.3% of the respondents declared the availability of clinical trials in second line treatment for advanced EC. The therapeutic algorithm in second line was heterogeneous, being the most frequent choice administering anthracyclines followed by endocrine therapy or enrolling in clinical trials. While more

than half of the clinicians declared that they performed the molecular classification, only six/45 respondents (13.3%) ran all the tests needed for it. On the other hand, 80% of them declared regular assessment of MSI status with IHC as recommended. The therapeutic approach in MSI high advanced EC patients has changed since dostarlimab approval. Indeed the most frequent choice in second line has been chemotherapy (53.3%) before its availability, while dostarlimab has been preferred in more than three-fourths of the cases (75.6%) after its approval. As for MSS patients, 77.8% of clinicians would choose lenvatinib plus pembrolizumab for them in second line once approved.

Conclusions: Despite the selected sample of respondents from Italian MITO centres showing good knowledge of diagnostic and therapeutic innovations in EC, these are not fully implemented in everyday clinics, except for MSI status assessment.

Keywords: endometrial cancer, molecular classification, second line therapy, immune checkpoint inhibitors, MSI, survey

INTRODUCTION

In 2021, more than 400,000 new diagnoses of endometrial cancer (EC) have been estimated worldwide (1–3). Most of the new cases are early-stage malignancies because one of the most frequent symptoms, vaginal bleeding, is extremely precocious leading to early diagnosis with overall survival at 5 years of 81.1% (1, 2).

Nonetheless, patients with advanced and recurrent disease have a dismal prognosis with an expected 5-year survival of less than 20% and scarce treatment options (4). Indeed, patients with metastatic disease are candidates for a platinum-based chemotherapy with an expected median progression-free survival (PFS) of 13 months, while in second and further lines few studies are available and monotherapy with anthracyclines as well as platinum rechallenge, weekly paclitaxel, or endocrine therapy are usually the preferred choices, with low chances of response (4–6).

During the last years, both the diagnostic and therapeutic scenarios have changed dramatically in this field. From a diagnostic point of view, we overcame the traditional two-types classification based on Bokhman's clinical, metabolic, and endocrine features to a molecular and pathological driven definition of risk groups (7–10). Four subgroups have been identified by The Cancer Genome Atlas (TCGA) according to molecular features. An ultramutated group with frequent DNA Polymerase Epsilon (POLE) exonuclease mutations and a good prognosis, a hypermutated group with Microsatellite instable (MSI) cancers, harbouring a Mismatch repair deficiency (MMRd), a copy number low group, including most of the microsatellite stable (MSS) endometrioid cancers, and a serous-like group with frequent

Tumor Protein P53 (TP53) mutations (10). In addition to the prognostic role of this classification, it might help drive therapeutic choices. Specifically, serous-like tumours have the worst prognosis and are characterized by a low immune infiltrate while POLE and MSI cancers are characterized by a high predicted neo-antigens load, overexpression of PD-1 and PD-L1, and massive CD3+ and CD8+ Tumour-associated lymphocytes infiltration, thus suggesting that these two subgroups might be the best candidates for immunotherapy (9–11). Several studies independently demonstrated that the diagnostic algorithm can be implemented using a few immunohistochemical markers [p53, MutS Homolog 6 (MSH6), and PMS1 Homolog 2 (PMS2), at least, though the gold standard is the assessment of the four MMR proteins: MutL Homolog 1 (MLH1), MutS Homolog 2 (MSH2), MSH6, and PMS2), and only one molecular test (mutation analysis of the hotspots in the exonuclease domain of POLE) to identify prognostic groups, which mostly overlap the TCGA molecular-based classification (12–17). These studies did not only show the feasibility of this approach but also confirmed the prognostic role of this classification, above all in early-stage EC (12–17). Of note, to classify an EC sample according to this molecular classification all the diagnostic tests described above need to be performed (4). Up to now, the molecular classification plays an important role in the choice of adjuvant treatment, and it is recommended, when feasible, by the new ESMO-ESGO-ESTRO Guidelines in all early-stage EC (4). Moreover, the universal screening for MSI/MMR status is of uppermost importance, since it is the first step to find patients and thereafter relatives (healthy carriers) with Lynch Syndrome (18, 19). In these healthy carriers, genetic counselling and an intensified follow-up is recommended to detect malignancies at an early stage (18). On the other hand, the therapeutic role of this classification in late disease has been explored in the last few years, with the beginning of the immunotherapy era also in EC. Indeed, for patients with MMRd tumours, the current treatment algorithm in advanced disease has been revolutionized by the introduction of checkpoint inhibitors (20, 21). First pembrolizumab and then

Abbreviations: CD3, Cluster of differentiation 3; CD8, Cluster of differentiation 8; EC, Endometrial Cancer; IHC, Immune Histochemistry; MITO, Multicenter Italian Trials in Ovarian cancer and gynecologic malignancies; MLH1, MutL Homolog 1; MMRd, Mismatch Repair Deficiency; MSH2, MutS Homolog 2; MSH6, MutS Homolog 6; MSI, Microsatellite Instability/Instable; MSS, Microsatellite stable; PCR, polymerase chain reaction; PD-1, Programmed cell death protein 1; PDL-1, Programmed death-ligand 1; PFS, Progression Free Survival; PMS2, PMS1 Homolog 2; POLE, DNA Polymerase Epsilon; PTS, patients; TCGA, The Cancer Genome Atlas; TP53, Tumor Protein P53.

dostarlimab, with a large phase Ib trial, demonstrated activity in patients with MMRd tumours (20–22). Specifically, 104 patients received dostarlimab as a single agent in second or further lines with an objective response rate of 42.3%, including 12.7% confirmed complete response and a median duration of response which was not reached at a median follow-up of 11.2 months (21). This lead to the approval of dostarlimab by the U.S. Food and Drug Administration (FDA) and received conditional marketing authorisation by European Medicines Agency (EMA), thus being available in Italy within an expanded access program in January 2021 (23, 24). A further reshaping of the treatment algorithm is expected also in patients without MMRd tumours after the release of Study 309/KEYNOTE-775 results, a phase III trial conducted in patients pre-treated with a platinum doublet, showing improvement in terms of PFS and overall survival (OS) with the combination of pembrolizumab and lenvatinib, compared with a standard treatment irrespective of MSI status, with a manageable safety profile (25, 26).

How much of this knowledge has been transferred and is available in Italian everyday diagnostic and therapeutic algorithms is not known as well as we cannot predict if and how much the new combination of lenvatinib and pembrolizumab would be the chosen regimen for EC patients. Therefore, we led a survey among Multicenter Italian Trials in Ovarian cancer and gynaecologic malignancies (MITO) centres to evaluate the current management in EC, how the new discoveries have impacted the daily clinical practice, and the expected changes across Italy in 2021. The main objective of the investigation was to evaluate current practice in EC among different centres.

METHODS

We developed a survey which was a self-administered online questionnaire. The survey was developed by GG and GV, reviewed and discussed by the MITO scientific committee; submitted to and approved by the MITO internal review board. Thereafter, it was available on the MITO website only for MITO members from April 12, 2021 to May 7, 2021. Specifically, the survey was composed of 25 multiple choice questions (see the list of questions in the **Supplementary Table S1**). The first nine questions focused on the characteristics of the respondents and on the number of patients treated in each centre; nine questions dealt with the therapeutic algorithm in second line (and how it changed or was expected to change due to the introduction of immune checkpoint inhibitors), and six with the diagnostic algorithm, while one question asked about COVID19 impact in this setting. We analysed one answer form per each centre. All replies were anonymized. Descriptive analyses are detailed in the results session.

RESULTS

An invitation to complete the survey was sent to 691 MITO members, for a total of 175 centres. Among them, 284 clinicians (41.1%) opened the invitation, 52 (7.5%) clicked on the link, and 49 (7.1%) completed the survey. In three cases, more than one respondent per centre was recorded and we analysed only one questionnaire per centre. A total of 45 responses (25.7% of the MITO centres) were therefore analysed. Most of the respondents were aged 40 or more (34/45, 75.6%) and worked in a public hospital (17/45, 37.8%) or university hospital (15/45, 33.3%). More than 75% of the respondents (34/45) treated mainly but not exclusively patients with gynaecological cancers, being most of the questionnaires completed by medical oncologists (34/45, 75.6%) (see **Table 1**). The physicians completing the survey were well distributed across the country with 20 of them (44.4%) working in hospitals located in the North of Italy while 15 (33.3%) and 10 (22.2%) were from the Centre and the South of Italy respectively (see **Table 1**). Most of the responders had a medium volume of EC patients. Indeed 25 (55.6%) clinicians had 5 to 10 new diagnoses of EC per month with seven (15.6%) and six (13.3%) of them treating 11 to 25 and more than 25 new cases of EC, respectively, per month. More than half of the respondents (24/45, 53.3%) treated 5 to 10 advanced or metastatic EC patients per month with 16 (35.6%) and 5 (11.1%) of them seeing in everyday clinic less than five patients and more than 10 patients, respectively. In second and further lines, the volume is similar, with 22 (48.9%) physicians seeing five to 10 EC patients in this setting per month while 15 (33.3%) and eight (17.8%) respondents treated less than five patients per month and more than 10 per month, respectively.

More than 75% of patients received second line treatment in the experience of 23 (51.1%) of them while 20 (44.4%) respondents offered second line treatment to 50%-75% of their EC patients. The most frequent reasons for not proposing an active treatment were frail general conditions in 22 (48.8%) and a combination of comorbidities and bad performance status in 16 (35.6%) cases while two (4.4%) clinicians said they did not candidate patients to second line because of the absence of effective treatments. Thirty-three respondents (73.3%) confirmed the availability, for patients treated at their institution, of clinical trials in this setting, while 12 (26.7%) did not (**Figure 1A**). We asked which were the preferred treatments (requiring a maximum of two answers). The drugs administered in second line were extremely heterogeneous in our cohort being the most frequent choices anthracyclines (31 cases, 68.9%), endocrine therapy (16 cases, 35.6%), enrolment in a clinical trial (13 cases, 28.9%), weekly paclitaxel (or another taxane), or a rechallenge with platinum (12, respondents, 26.7%, each) (**Figure 1B**). Nearly all the responders confirmed that they evaluated hormonal receptor (oestrogen and or progesterone receptors) (42/45, 93.3%) using immune histochemistry (IHC) while 25 (55.6%) of them said that they performed the molecular classification in their centre. Nevertheless, 6/45respondents (13.3%) ran all the tests needed for it (POLE hotspots sequencing, IHC for MMR proteins or MSI status defined using polymerase chain reactions -PCR- and p53 IHC). Thirty-three of 45 respondents (73.3%) evaluated p53 and MMR proteins using IHC, being p53 IHC the only performed test for four interviewees (13.3%) (**Figure 2A**).

The most frequent approach to evaluate MSI/MMR status was IHC (36 cases, 80%) for all the four proteins (MLH1, MSH2,

TABLE 1 | Respondents' characteristics.

Respondents characteristics		
Feature	**Number**	**Percentage**
Age		
<40 years old	11	24,4
>40 years old	34	75,6
Years in practice (focus on gynaecological cancer)	14,8 years (average)	
Health organizations where the respondents work		
Public hospital	17	37,8
University Hospital	15	33,3
Istituto Di Ricovero e Cura a Carattere Scientifico (Italian institutes for research and care)	10	22,2
Private Hospital	2	4,4
Other	1	2,2
Location of the Hospital		
North of Italy	20	44,4
Centre of Italy	15	33,3
South of Italy, Sicily or Sardinia	10	22,2
Medical training		
Medical Oncology	34	75,6
Gynaecology	10	22,2
Other	1	2,2
Clinical focus		
Only gynaecological cancers	9	20,0
Mainly gynaecological cancers	34	75,6
Other	2	4,4
Cumulative number of new EC diagnoses per month		
Less than 5	7	15,6
5-10	25	55,6
11-25	7	15,6
More than 25	6	13,3
Cumulative number of recurrent, locally advanced (unresectable) or metastatic EC patients treated per month		
Less than 5	16	35,6
5-10	24	53,3
More than 10	5	11,1
Cumulative number of pretreated metastatic EC patients treated per month		
Less than 5	15	33,3
5-10	22	48,9
More than 10	8	17,8

[a]*EC, endometrial cancer.*

MSH6, PMS2) with one respondent (2.2%) evaluating only MSH6 and PMS2 (**Figure 2B**). Six clinicians (13.3%) used PCR as a second step approach for indeterminate cases at IHC while it was performed upfront in five cases (11.1%) (**Figure 2B**). Only six respondents (13.3%) evaluated MLH1 methylation status (**Figure 2B**). We asked in which moment of the patient journey MSI/MMR status was assessed, and 33 clinicians (73.3%) responded that it was screened universally in every patient with a new diagnosis of EC while it was evaluated in second or further lines to define the best treatment choice in eight cases (17.8%). Once a deficiency in MMR machinery was detected on the tumour specimen, genetic counselling was

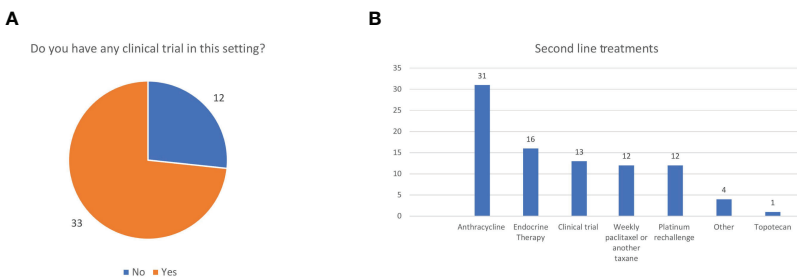

FIGURE 1 | Clinical trials **(A)** and treatment choices **(B)** in second line.

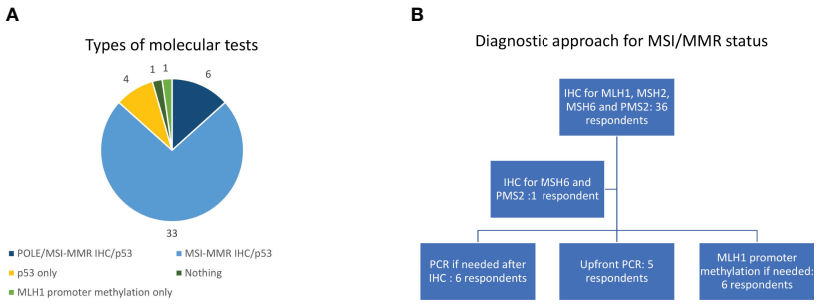

FIGURE 2 | Types of molecular tests **(A)** and diagnostic approach for MSI/MMR status **(B)**. IHC, immunohistochemistry; MMR, mismatch repair; MSI, Microsatellite instability; PCR, Polymerase chain reaction.

planned before the blood sampling for the germline testing in 22 centres (48.9%), after the germline confirmation of a Lynch Syndrome in eight centres (17.8%) and in patients with both confirmed germline MMRd or a high suspect of Lynch Syndrome according to their family history in six centres (13.3%). Only eight respondents (17.8%) said they referred for genetic counselling all EC patients with a family history suspicious for Lynch Syndrome even before testing MSI/MMR status on the tumour sample.

The therapeutic approach in MMRd patients has been changed according to the respondents in the last year with the availability of the expanded access program of dostarlimab (**Figure 3**). Indeed, before its availability, most of them (24, 53.3%) treated patients with a single agent chemotherapy in second line while 20 out of 45 (44.4%) proposed a checkpoint inhibitor off-label, paid by the hospital, or a clinical trial (10 respondents each, 22.2%) (**Figure 3A**). Since dostarlimab approval, 34 respondents (75.6%) think that it is the best option for MMRd EC; only five respondents (11.1%) are continuing to administer a monotherapy with another cytotoxic agent in this setting, and the remaining respondents are preferring a checkpoint inhibitor off-label (3,6.7%), a clinical trial (1, 2.2%) or other treatments (2, 4.4%) (**Figure 3B**). During the 5 months of dosarlimab availability, 13 clinicians (28.9%) said they have never prescribed dostarlimab and 21 (46.7%) had no patients on treatment with dostarlimab while 11 (24.4%) clinicians had one to five patients receiving dostarlimab at time of the survey. This new drug has changed the MSI/MMR status screening only in 20 (44.4%) cases, with the introduction of this test in the advanced setting. No changes were declared from the remaining respondents because there was a universal screening system before dostarlimab availability (19 cases, 42.2%) or because it continued to be proposed in selected cases (5 cases, 11.1%).

As for MMR proficient (MMRp) patients, 35/45 clinicians (77.8%) affirmed that the combination of lenvatinib plus pembrolizumab, according to KEYNOTE-775 results, was going to become the preferred choice for the second line setting, when available.

Lastly, we asked how COVID-19 pandemic impacted EC management with 33 interviewees (73.3%) saying it did not impact at all on the treatment of EC patients; 12 (26.7%) clinicians responded that they modified the follow-up (longer interval and/or phone calls instead of in-clinic visits) while no difference was recorded in treatment indications or administration.

DISCUSSION

This survey is a snapshot of the diagnostic and therapeutic choices for advanced pre-treated EC in Italian MITO centres. It highlights how the new molecular classification has not been

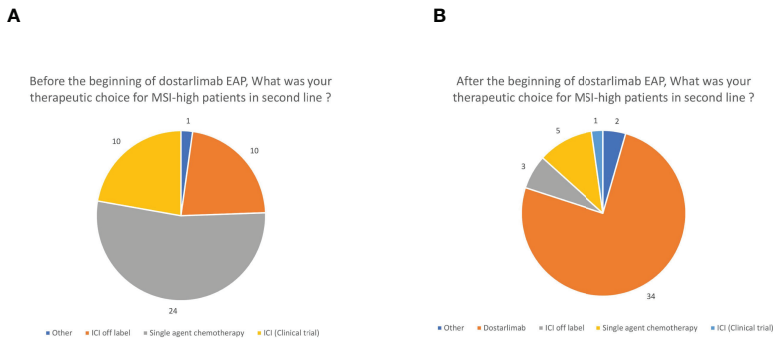

FIGURE 3 | Therapeutic choices before **(A)** and after **(B)** the beginning of dostarlimab expanded access program (EAP). ICI, immune checkpoint inhibitor other than dostarlimab.

extensively implemented in the clinical setting. Moreover, it confirms that the therapeutic approach beyond first line is extremely heterogeneous. Indeed, since its availability, dostarlimab has been the preferred choice for MMRd patients but, probably for the short timespan between its approval and our survey and the low number of patients with pre-treated MMRd EC, a small number of women were receiving or had received this treatment at the time of the survey, with more than one-fourth of the respondents having never prescribed it.

An important point that should be underlined regards the features of the interviewed population. We administered this questionnaire *via* the official web site and newsletter of MITO group, which involves centres with a focus on gynaecological cancer and who are keen to enroll gynaecological patients in clinical trials. However, only around 25% of the MITO centres responded to this survey and most of the responses were from medical oncologists.

This implies a possible selection bias and makes it difficult to generalize our results to all Italian hospitals but, on the other hand, the respondents were well distributed across the country, most of them with a long experience and a medium to high volume of EC patients, being a low number of them focused only on gynaecological malignancies. We believe that this is the most frequent setting in which a woman with a relapsed EC is treated or to which she is referred.

Most of the EC patients were candidates to second line of treatment and the reasons not to propose a further treatment are usually comorbidities instead of an expected lack of benefit from drugs administered in pre-treated women (27). The response rate in this setting is lower than 20% but, on the other hand, the availability of clinical trials in nearly three-fourth of the centres suggests once again that there are more therapeutic options for these hospitals and that the positive attitude toward administering experimental treatments is extremely solid (5). The heterogeneity of drugs prescribed in second or further lines is concordant with the literature, in the absence of head-to-head comparisons between single agents or between chemotherapy and endocrine therapy, with the last one being the preferred option in grade 1 slow progressing EC (4, 5, 28–30). Interestingly, our results are similar to a German survey in which chemotherapy was preferred to progestins, although a wide variability in the choices was recorded (31).

In our survey, most of the centres performed oestrogen and progesterone receptor assessment which has a prognostic role but does not drive therapeutic choices (32). On the other hand, slightly more than half of the interviewees stated that they have implemented the EC molecular classification in clinical practice. Surprisingly though, only in six hospitals, all the required diagnostic tests are run together leading to two conclusions (4). The first one is that we are far from the optimal setting in which treatment decisions can be driven by an accurate assessment of molecular characteristics of each EC, being difficult and expensive to implement it also in dedicated settings such as the MITO centres. The second one is that we probably need to increase the knowledge on how the molecular classification is performed, perhaps supporting educational meetings with pathologists and lab researchers, being a field in which the well-known IHC is side-by-side to novel sequencing techniques (PCR and hotspot sequencing) (4). On the other hand, universal screening for Lynch Syndrome is performed by more than three-quarters of the respondents as suggested by international and national guidelines but only six of them have appropriate facilities performing MLH1 promoter methylation assessment, thus reducing the number of unnecessary genetic referrals (4, 19, 33). Moreover, the timing for the genetic referral is quite variable though around half of the interviewees refer patients right after the assessment of MSI/MMR status on tumour specimens.

How both diagnostic and therapeutic implementations reflect into the treatment choices is quite impressive. Before the availability of dostarlimab, most of the clinicians administered a cytotoxic agent also to MMRd patients in second line, although around 40% of them had the possibility to propose an immune checkpoint inhibitor (off-label or in the setting or a clinical trial). After the beginning of the expanded access program, more than one-fourth of them are choosing to prescribe dostarlimab. Notwithstanding, a low number of patients have been treated with this drug so far, which is probably due to the rarity of the setting and the short timespan between the approval and the end of the survey.

It is moreover expected a change in the therapeutic algorithm also in MMRp patents, with nearly 80% of the respondents believing that the preferred treatment in this setting will be lenvatinib plus pembrolizumab which has been approved by EMA in December 2021 regardless MMR status.

Lastly it seems that COVID-19 had little effect on therapeutic management of EC patients. Previous surveys suggested that the pandemic impacted the treatment choices above all focusing on ovarian cancer patients, thereafter it would be interesting to record and evaluate EC patient outcomes during these years in which, on one hand, new therapeutic options are available after decades but, on the other, the challenge of a global threaten is faced, redirecting resources for research and treatment to this emergency (34, 35).

Our study has several limitations; the most important ones are the possible selection biases deriving from the low number of MITO members who filled in the questionnaires, with feedbacks from one-fourth of the MITO centres. Moreover, the interviewed centres have a focus on gynaecological malignancies and there was prevalent participation of oncologists, while the treatment of these women is carried out by both gynaecologists and oncologists in Italy. As for the questionnaire, to avoid heterogeneity, we chose closed-ended questions in most of the cases, which do not allow to represent the various nuances of the therapeutic and diagnostic pathways.

In addition, these results are too premature to evaluate and weight the changes in treatment for MMRd EC and the survey was available only for one month. We are expecting, in view of the answers collected, that the therapeutic scenario will be improved for all patients with advanced EC and that a better classification of early ones will allow us to personalize the adjuvant treatment and further reduce the risk of recurrence. This is why a follow-up survey will be administered to all MITO members with the aim of evaluating if there has been an improvement, with better knowledge and wider availability of these tools in the clinical setting over the last year. How these changes will impact the quality of life and survivorship of women who have usually important comorbidities is not known. It is,

indeed, of uppermost importance to plan real-life studies which will evaluate if there is an implementation of the molecular assays in these centres, how dostarlimab treatment is managed, which are the long-term outcomes and toxicities, and if there is any impairment in quality of life.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Authors' contributions: GG and GV: Conceptualization; all authors: resources, GG and DC: Data curation; GG and GV: Formal analysis, Software and Methodology; GV: Funding acquisition; GG and GV: Investigation and Project administration; GV: Supervision, Validation and Visualization; GG and GV: drafting of the manuscript; GG, GV, MDM, and DL: review & editing; all authors: final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.880008/full#supplementary-material

REFERENCES

1. *SEER.Cancer.Gov* . Available at: https://seer.cancer.gov/statfacts/html/corp.html.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer J Clin* (2018) 68 (6):394–424. doi: 10.3322/caac.21492
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
4. Concin N, Creutzberg CL, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP Guidelines for the Management of Patients With Endometrial Carcinoma. *Virchows Arch* (2021) 478(2):153–90. doi: 10.1007/s00428-020-03007-z
5. Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, Son CH, et al. Current Recommendations and Recent Progress in Endometrial Cancer. *CA Cancer J Clin* (2019) 69(4):258–79. doi: 10.3322/caac.21561
6. Miller DS, Filiaci VL, Mannel RS, Cohn DE, Matsumoto T, Tewari KS, et al. Carboplatin and Paclitaxel for Advanced Endometrial Cancer: Final Overall Survival and Adverse Event Analysis of a Phase III Trial (NRG Oncology/Gog0209). *J Clin Oncol* (2020) 38(33):3841–50. doi: 10.1200/JCO.20.01076
7. Kurman RJ, Visvanathan K, Shih Ie M. Bokhman's Dualistic Model of Endometrial Carcinoma. Revisited. *Gynecol Oncol* (2013) 129(2):271–2. doi: 10.1016/j.ygyno.2013.03.029
8. Bokhman JV. Two Pathogenetic Types of Endometrial Carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7
9. Gargiulo P, Della Pepa C, Berardi S, Califano D, Scala S, Buonaguro L, et al. Tumor Genotype and Immune Microenvironment in POLE-Ultramutated and MSI-Hypermutated Endometrial Cancers: New Candidates for Checkpoint Blockade Immunotherapy? *Cancer Treat Rev* (2016) 48:61–8. doi: 10.1016/j.ctrv.2016.06.008
10. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated Genomic Characterization of Endometrial Carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12113
11. Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, et al. Association of Polymerase E-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. *JAMA Oncol* (2015) 1 (9):1319–23. doi: 10.1001/jamaoncol.2015.2151
12. Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-Stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. *Clin Cancer Res* (2016) 22(16):4215–24. doi: 10.1158/1078-0432.CCR-15-2878
13. Bosse T, Nout RA, McAlpine JN, McConechy MK, Britton H, Hussein YR, et al. Molecular Classification of Grade 3 Endometrioid Endometrial Cancers Identifies Distinct Prognostic Subgroups. *Am J Surg Pathol* (2018) 42(5):561–8. doi: 10.1097/PAS.0000000000001020
14. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A Clinically Applicable Molecular-Based Classification for Endometrial Cancers. *Br J Cancer* (2015) 113(2):299–310. doi: 10.1038/bjc.2015.190
15. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A Simple, Genomics-Based Clinical Classifier for Endometrial Cancer. *Cancer* (2017) 123(5):802–13. doi: 10.1002/cncr.30496
16. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series. *Ann Oncol* (2018) 29 (5):1180–8. doi: 10.1093/annonc/mdy058
17. León-Castillo A, de Boer SM, Powell ME, Mileshkin LR, Mackay HJ, Leary A, et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. *J Clin Oncol* (2020) 38(29):3388–97. doi: 10.1200/JCO.20.00549
18. Yurgelun MB, Hampel H. Recent Advances in Lynch Syndrome: Diagnosis, Treatment, and Cancer Prevention. *Am Soc Clin Oncol Educ Book* (2018) 38:101–9. doi: 10.1200/EDBK_208341
19. Stjepanovic N, Moreira L, Carneiro F, Balaguer F, Cervantes A, Balmana J, et al. Hereditary Gastrointestinal Cancers: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Updagger. *Ann Oncol* (2019) 30 (10):1558–71. doi: 10.1093/annonc/mdz233
20. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/JCO.19.02105
21. Oaknin A, Tinker AV, Gilbert L, Samouelian V, Mathews C, Brown J, et al. Clinical Activity and Safety of the Anti-Programmed Death 1 Monoclonal Antibody Dostarlimab for Patients With Recurrent or Advanced Mismatch Repair-Deficient Endometrial Cancer: A Nonrandomized Phase 1 Clinical Trial. *JAMA Oncol* (2020) 6(11):1766–72. doi: 10.1001/jamaoncol.2020.4515
22. O'Malley DM, Bariani GM, Cassier PA, Marabelle A, Hansen AR, De Jesus Acosta A, et al. Pembrolizumab in Patients With Microsatellite Instability–High Advanced Endometrial Cancer: Results From the KEYNOTE-158 Study. *J Clin Oncol* (2022) 40(7):752–61. doi: 10.1200/JCO.21.01874
23. EMA. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/jemperli.

24. FDA. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/761174Orig1s000TOC.cfm.
25. Makker V, Colombo N, Herráez AC, Santin A, Colomba E, Miller D, et al. A Multicenter, Open-Label, Randomized, Phase III Study to Compare the Efficacy and Safety of Lenvatinib in Combination With Pembrolizumab Versus Treatment of Physician's Choice in Patients With Advanced Endometrial Cancer. *Gynecologic Oncology : An International Journal* (2021) 162:S4.. al MVe. doi: 10.1016/S0090-8258(21)00657-0
26. Makker V, Colombo N, Casado Herráez A, Santin AD, Colomba E, Miller DS, et al. Lenvatinib Plus Pembrolizumab for Advanced Endometrial Cancer. *N Engl J Med* (2022) 386(5):437–48. doi: 10.1056/NEJMoa2108330
27. Eggemann H, Ignatov T, Burger E, Costa SD, Ignatov A. Management of Elderly Women With Endometrial Cancer. *Gynecol Oncol* (2017) 146(3):519–24. doi: 10.1016/j.ygyno.2017.06.029
28. Humber CE, Tierney JF, Symonds RP, Collingwood M, Kirwan J, Williams C, et al. Chemotherapy for Advanced, Recurrent or Metastatic Endometrial Cancer: A Systematic Review of Cochrane Collaboration. *Ann Oncol* (2007) 18 (3):409–20. doi: 10.1093/annonc/mdl417
29. Vale CL, Tierney J, Bull SJ, Symonds PR. Chemotherapy for Advanced, Recurrent or Metastatic Endometrial Carcinoma. *Cochrane Database Syst Rev* (2012) 8):CD003915. doi: 10.1002/14651858.CD003915.pub4
30. Kokka F, Brockbank E, Oram D, Gallagher C, Bryant A. Hormonal Therapy in Advanced or Recurrent Endometrial Cancer. *Cochrane Database Syst Rev* (2010) 12):CD007926. doi: 10.1002/14651858.CD007926.pub2
31. Battista MJ, Schmidt M, Eichbaum M, Almstedt K, Heimes AS, Mallmann P, et al. Management of Recurrent or Metastatic Endometrial Cancer in Germany: Results of the Nationwide AGO Pattern of Care Studies From the Years 2013, 2009 and 2006. *Arch Gynecol Obstet* (2015) 292(6):1355–60. doi: 10.1007/s00404-015-3786-y
32. Jerzak KJ, Duska L, MacKay HJ. Endocrine Therapy in Endometrial Cancer: An Old Dog With New Tricks. *Gynecol Oncol* (2019) 153(1):175–83. doi: 10.1016/j.ygyno.2018.12.018
33. Tibiletti MG, Carnevali I, Calò V, Cini G, Lucci Cordisco E, Remo A, et al. Universal Testing for MSI/MMR Status in Colorectal and Endometrial Cancers to Identify Lynch Syndrome Cases: State of the Art in Italy and Consensus Recommendations From the Italian Association for the Study of Familial Gastrointestinal Tumors (A.I.F.E.G.). *Eur J Cancer Prev* (2021) 31 (1):44–9. doi: 10.1097/CEJ.0000000000000677
34. Cioffi R, Sabetta G, Rabaiotti E, Bergamini A, Bocciolone L, Candotti G, et al. Impact of COVID-19 on Medical Treatment Patterns in Gynecologic Oncology: A MITO Group Survey. *Int J Gynecol Cancer* (2021) 31 (10):1363–8. doi: 10.1136/ijgc-2021-002784
35. Martinelli F, Garbi A. Change in Practice in Gynecologic Oncology During the COVID-19 Pandemic: A Social Media Survey. *Int J Gynecol Cancer* (2020) 30 (8):1101–7. doi: 10.1136/ijgc-2020-001585

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Radiomics Nomogram in Assisting Lymphadenectomy Decisions by Predicting Lymph Node Metastasis in Early-Stage Endometrial Cancer

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Background: Lymph node metastasis (LNM) is an important risk factor affecting treatment strategy and prognosis for endometrial cancer (EC) patients. A radiomics nomogram was established in assisting lymphadenectomy decisions preoperatively by predicting LNM status in early-stage EC patients.

Methods: A total of 707 retrospective clinical early-stage EC patients were enrolled and randomly divided into a training cohort and a test cohort. Radiomics features were extracted from MR imaging. Three models were built, including a guideline-recommended clinical model (grade 1-2 endometrioid tumors by dilatation and curettage and less than 50% myometrial invasion on MRI without cervical infiltration), a radiomics model (selected radiomics features), and a radiomics nomogram model (combing the selected radiomics features, myometrial invasion on MRI, and cancer antigen 125). The predictive performance of the three models was assessed by the area under the receiver operating characteristic (ROC) curves (AUC). The clinical decision curves, net reclassification index (NRI), and total integrated discrimination index (IDI) based on the total included patients to assess the clinical benefit of the clinical model and the radiomics nomogram were calculated.

Results: The predictive ability of the clinical model, the radiomics model, and the radiomics nomogram between LNM and non-LNM were 0.66 [95% CI: 0.55-0.77], 0.82 [95% CI: 0.74-0.90], and 0.85 [95% CI: 0.77-0.93] in the training cohort, and 0.67 [95% CI: 0.56-0.78], 0.81 [95% CI: 0.72-0.90], and 0.83 [95% CI: 0.74-0.92] in the test cohort, respectively. The decision curve analysis, NRI (1.06 [95% CI: 0.81-1.32]), and IDI (0.05 [95% CI: 0.03-0.07]) demonstrated the clinical usefulness of the radiomics nomogram.

Conclusions: The predictive radiomics nomogram could be conveniently used for individualized prediction of LNM and assisting lymphadenectomy decisions in early-stage EC patients.

Keywords: endometrial cancer, early-stage, lymph node metastasis, radiomics nomogram, lymphadenectomy decision

INTRODUCTION

Endometrial cancer (EC) is the most common gynecologic malignancy in industrialized countries (1). Tumor size, tumor grade, histological subtype, depth of myometrial invasion (MI), lymphovascular space invasion (LVSI), and lymph node metastasis (LNM) are known prognostic factors of EC (2). According to the International Federation of Gynecology and Obstetrics, complete pelvic and para-aortic lymphadenectomy was the recommended surgical treatment for stage II-IV EC patients (3). However, the therapeutic value of lymphadenectomy in early-stage EC is still in debate, as no improvement in disease-free survival or overall survival (OS) was found in early-stage EC with or without lymphadenectomy (4).

Lymphadenectomy is not recommended by the Gynecologic Oncology Group (GOG) in early-stage EC patients with grade 1 or 2 and superficial MI (<50% MI) (5). Furthermore, based on a landmark GOG-33 staging study, an overall 9% risk of LNM was reported in clinical early-stage EC (6). In addition, lymphadenectomy resulted in longer operating times, more blood loss, higher transfusion rates, and longer hospital stays (7). Thus, preoperative evaluation of early-stage EC is clinically useful in helping with lymphadenectomy decion-making for these patients.

Magnetic resonance imaging (MRI) is a useful tool which allows for noninvasive visualization of anatomic structures with high spatial resolution and soft tissue contrast. However, a meta-analysis indicated that MRI has low sensitivity and specificity in diagnosing LNM in EC patients (8). Metastasis in a normal-sized lymph node (LN) can be missed, and inflammatory LN enlargement cannot be reliably distinguished from LNM by conventional MRI (9). Radiomics, a method of high-throughput extraction of quantitative medical image features, might improve standard visual image analysis and offer valuable information for diagnostic and prognostic purposes (10). A previous study showed that MRI-based radiomics are efficient in helping the radiologists in identifying LNM preoperatively (9). In addition, a radiomics nomogram combining the radiomics features and clinical risk factors could be conveniently applied to help clinical management decisions (11).

We assumed that the radiomics nomogram could be a useful tool in helping clinical management decisions in early-stage EC. Thus, the aim of this study was to develop and validate a clinical- and radiomics-based nomogram for the preoperative prediction of LNM individually in assisting lymphadenectomy decisions in patients with early-stage EC.

MATERIALS AND METHODS

Patients

This retrospective study was reviewed and approved by the Institutional Review Board of Obstetrics & Gynecology Hospital of Fudan University (No. 2020-10). All patients signed the informed consent. In total, 707 patients from January 2016 to May 2021 were included in this study. All patients met the following inclusion criteria (1): histopathology confirmed EC and pelvic/aortic LNM status (2); patients underwent a dilatation and curettage (D&C); (3) patients underwent MRI planning including T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), diffusion-weighted imaging (DWI), apparent diffusion coefficient (ADC) maps, and contrast-enhanced (CE) T1WI within 30 days before the surgery. Patients were excluded if: (1) tumor less than 2 slices on the MRI scan; (2) insufficient imaging quality to obtain measurements or insufficient clinical information; (3) cervical infiltration or extra-uterine tumor showed on MRI. The flow chart of inclusion and exclusion criteria is shown in **Figure 1**.

The 707 patients were randomly divided into a training cohort and a test cohort, according to the ratio of 5:5. Clinical information of all patients were obtained from the medical records, including D&C tumor grade, MRI-reported MI status, age, metabolic syndrome, and cancer antigen 125 (CA125). Patients with pelvic LN > 8 mm or abdominal LN > 10 mm, or with non-homogeneous enhancement and central necrosis on CE-T1WI images were considered as MR-report LNM positive (12). For the patients with total hysterectomy and bilateral salpingo-oophorectomy without lymphadenectomy, the follow-up of at least two years was used to confirm if the patient had LNM or not.

Development of the Clinical Model

The clinical model for preoperatively deciding whether a patient required lymphadenectomy was built according to the recommendation from the Society of Gynecologic Oncology Clinical Practice EC Working Group (5). Patients with grade 1-2 endometrioid tumors (by D&C), less than 50% MI (on MRI without cervical infiltration), and tumor of 2 cm or less require no lymphadenectomy.

MRI Acquisition and Segmentation

MRI was performed using a 1.5-T MR system (Magnetom Avanto, Siemens, Germany). The following sequences were obtained: axial spin-echo (SE) T1-weighted imaging (T1WI)

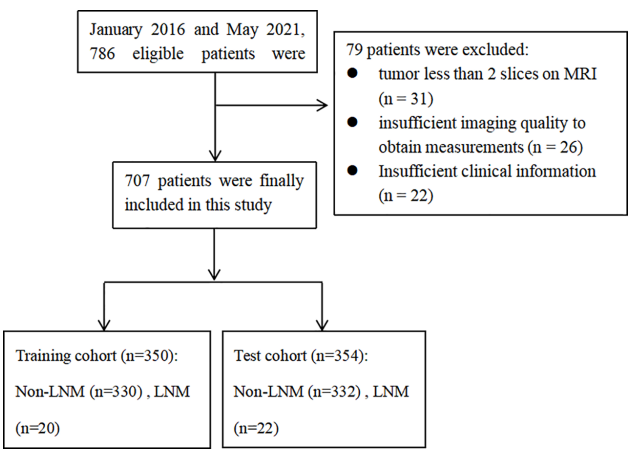

FIGURE 1 | The workflow of this study.

with repetition time (TR)/echo time (TE) = 761/10 ms; turbo axial SE T2-weighted imaging (T2WI) with fat saturation (TR/TE = 4000/98 ms); sagittal T2WI (TR/TE = 3849/83 ms) and coronal T2WI (TR/TE = 4490/83 ms); Axial echo planar imaging DWI was obtained with b values of 0 and 800 s/mm^2. Sagittal and coronal CE-T1WI with FS (TR/TE =439/10 ms, thickness = 4 mm) and axial CE-T1WI with FS (TR/TE = 196/2.9 ms, thickness = 4 mm) were performed at the arterial phase (30-40 sec), venous phase (75-90 sec), and delayed phase (120-180 sec) after the intravenous administration of gadopentetate dimeglumine (Magnevist, Bayer Schering, Berlin, Germany) at a dose of 0.2 mmol/kg of body weight and a rate of 2 to 3 mL/s.

Tumor segmentation was performed by manually delineating (by radiologist 1) the region of interest (ROI) along the tumor contour on each axial T2WI and then referred to the DWI (the tumor area showed as high signal in high b value sequences), ADC (the tumor area showed as low signal), and axial DCE images (delayed phase) using an open-source imaging platform (MITK, version 4.9.0; http://www.mitk.org). Thirty days later, 50 randomly chosen images were used to assess the reliability for each radiomics feature. The ROI delineation was performed separately by two radiologists (radiologist 1 and radiologist 2, with 3 and 11 years of experience in pelvic imaging, respectively). The radiologists were blinded to the clinical and histopathology information. The reliability was calculated using the intraclass/interclass correlation coefficient (ICC). The features with ICCs greater than 0.75 indicated satisfactory reproducibility of radiomics feature extraction and were retained.

Radiomics Features Extraction and Selection

All feature extractions were implemented in Pyradiomics package of Python (v.3.9; https://www.python.org) The radiomics features, including shape-based, first-order, and texture features were extracted. Pearson's correlation was used to identify redundant features. If two features had a Pearson correlation coefficient > 0.9, the one with larger mean absolute coefficient was eliminated. Synthetic minority oversampling technique (SMOTE) method was used because of the unbalance of positive/negative LNM samples in the training cohort. Positive LNM (minority class) were over-sampled and negative LNM (majority class) were under-sampled to balance the training cohort to improve the classification performance. Then, a least absolute shrinkage and selection operator (LASSO) regression with 10-fold cross-validation was used to obtain the most significant features (radiomics signatures) for predicting LNM in the training cohort.

Clinical Risk Factors Selection

A multivariate logistic regression analysis was used to identify the clinical independent risk factors (age, metabolic syndrome, tumor size, MRI-reported MI, and CA125) for LNM in the training cohort. Backward stepwise selection was applied. The stopping rule was that the likelihood ratio test achieved a least Akaike's information criterion.

Development and Validation of the Radiomics Model, the Radiomics Nomogram Model, and the Clinical Model

A radscore was calculated for each patient from the training cohort *via* a linear combination of radiomics signatures that were weighted by their respective coefficients.

A multivariate logistic regression was applied to build the radiomics nomogram, which can be used as a visualized and individual tool that integrated the radiomics signatures with independent clinical risk factors to predict the probability of LNM in the training cohort.

The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was calculated to assess the predictive performance of the three models. The sensitivity, specificity, and AUC of the nomogram were calculated in the training cohort and validated in the test cohort.

Clinical Usefulness Analysis

Decision curve analysis was conducted to determine the clinical usefulness of the radiomics nomogram and the clinical model by quantifying the net benefits at different threshold probabilities using the training and test cohorts.

The performances of the radiomics nomogram and the clinical model were compared using net reclassification index (NRI) and total integrated discrimination index (IDI) by using the entire dataset.

Statistical Analysis

Statistical analysis was performed using R software (version 4.05; http://www.Rproject.org). Independent sample t-test (if met normality and variance homogeneity) or Mann-Whitney U (if not met normality or variance homogeneity) were used to compare the differences in continuous variables (age, CA125, tumor size, and radscore) between the LNM and non-LNM patients; and the chi-squared test was used to compare the differences in categorical variables (metabolic syndrome, D&C-reported tumor grade, MRI-reported MI, and histopathology-reported tumor grade, MI, LVSI, and histological subtype) between the LNM and non-LNM patients in both the training and test cohorts. Association between the radiomics signatures and clinical risk factors was further assessed using Spearman's correlation. The "glmnet" package was used for LASSO and logistic regression, the "DMwR" package was used for SMOTE, the "rms" package was used for nomogram calculation, the "pROC" package was used for AUC, and the "dca.R" package was used for DCA. ROC curve analysis was performed to calculate the AUC and corresponding 95% confidence interval. A P value less than 0.05 was considered statistically significant.

RESULTS

Clinical Characteristics

Among the 707 patients (aged 55 ± 8.9, ranged 25-89) with early-stage EC, 42/665 patients had LNM/non-LNM. Sixty-five patients had total hysterectomy and bilateral salpingo-oophorectomy without lymphadenectomy, none of them were

found LNM within 2 years follow-up. These patients were considered as non-LNM. The clinicopathologic characteristics of all the patients are shown in **Table 1**.

The time interval between D&C and MR scanning is 5.8 ± 2.4 d, ranged 0-10d (0, on the same day). Tumor grade (D&C diagnosed G1 and G2) and MI status (MRI diagnosed MI status) were downgraded in 92 cases after surgery. Of these, 30 (4.2%) were downgraded from G1/G2 to AH/G1; 62 (8.7%) were downgraded from DMI to SMI/non-MI, respectively. On the contrary, 46 (6.5%) were upgraded from G1/G2 to G3/non-endometrioid adenocarcinoma; 102 (14.4%) were upgraded from non-MI/SMI to DMI according to the final pathology examination, respectively.

Development of the Clinical Model

According to the clinical model, 469 (66.3%) patients were identified as ineligible candidates for lymphadenectomy and 238 (33.7%) patients were identified as eligible candidates for lymphadenectomy (**Table 1**).

Radiomics Features Selection and Radiomics Signatures Construction

In the training cohort, 358 features were extracted from the T1WI, T2WI, DWI, CE, and ADC images. After removing features with either interobserver or intraobserver ICC < 0.75 and Pearson correlation coefficients > 0.9, 234 and 89 features were retained, respectively. LASSO analysis finally included 18 radiomics features, which were defined as the radiomics signatures (**Figure 2** and **Figure 3**). The association between radiomics signatures and clinical risk factors is shown in **Figure 3**. The radscore calculation is shown in the following: Radscore = 0.04994 + 0.0017×shape_M2DDS + -0.01212×T2WI_glszm_GLNUN + 0.01356×T2WI_glszm_SAE + -0.02575×T2WI_gldm_DNUN + 0.00589×DWI_glcm_DV + -0.00816×DWI_glszm_LAHGLE + -0.00132×DWI_glszm_ZoneEntropy + -0.00855×DWI_glszm_ZP + 0.02578×DWI_gldm_DependenceVariance + -0.01982×DWI_gldm_LDLGE + 0.01929×DWI_gldm_LGLEG + -0.00855×CE_firstorder_Minimum + -0.03477×CE_glcm_Contrast + -0.00393

TABLE 1 | The comparisons of clinicopathologic characteristics between LNM and non-LNM patients in training and test cohorts.

	Training cohort			Test cohort		
	non-LNM (N=333)	LNM (N=20)	P-value	non-LNM (N=332)	LNM (N=22)	P-value
Radscore	0.052 (0.062)	0.133 (0.071)	<0.001	0.057 (0.065)	0.137 (0.068)	<0.001
CA125	23.8 (20.1)	71.1 (83.7)	0.021	24.3 (23.3)	44.5 (45.8)	0.052
Age	55.9 (9.1)	54.9 (8.3)	0.580	55.3 (8.9)	56.8 (8.3)	0.424
Tumor size	17.1 (6.8)	24.1 (12.2)	0.019	16.4 (6.5)	21.7 (8.3)	0.008
Metabolic syndrome			0.450			0.117
(–)	171 (51.4%)	8 (40.0%)		171 (51.5%)	7 (31.8%)	
(+)	162 (48.6%)	12 (60.0%)		161 (48.5%)	15 (68.2%)	
D&C tumor grade			0.357			1
G1	284 (85.3%)	15 (75.0%)		288 (86.7%)	19 (86.4%)	
G2	49 (14.7%)	5 (25.0%)		44 (13.3%)	3 (13.6%)	
MRI MI			0.042			<0.001
(-)	294 (88.3%)	14 (70.0%)		297 (89.5%)	13 (59.1%)	
(+)	39 (11.7%)	6 (30.0%)		35 (10.5%)	9 (40.9%)	
MRI LM			1			0.477
(-)	323 (97.0%)	19 (95.0%)		320 (96.4%)	20 (90.9%)	
(+)	10 (3.0%)	1 (5.0%)		12 (3.6%)	2 (9.1%)	
Clinical decision lymphadenectomy			0.010			0.002
(-)	220 (66.1%)	7 (35.0%)		234 (70.5%)	8 (36.4%)	
(+)	113 (33.9%)	13 (65.0%)		98 (29.5%)	14 (63.6%)	
Histopathology tumor grade			0.059			<0.001
AH	1 (0.3%)	0 (0%)		0 (0%)	0 (0%)	
G1	232 (69.7%)	13 (65.0%)		228 (68.7%)	8 (36.4%)	
G2	85 (25.5%)	4 (20.0%)		81 (24.4%)	9 (40.9%)	
G3	11 (3.3%)	1 (5.0%)		15 (4.5%)	1 (4.5%)	
Non-endometrioid	4 (1.2%)	2 (10.0%)		8 (2.4%)	4 (18.2%)	
Histopathology MI			0.040			<0.001
Non-MI	83 (24.9%)	3 (15.0%)		97 (29.2%)	0 (0%)	
Superficial MI	192 (57.7%)	9 (45.0%)		184 (55.4%)	10 (45.5%)	
Deep MI	58 (17.4%)	8 (40.0%)		51 (15.4%)	12 (54.5%)	
LVSI			<0.001			<0.001
(-)	292 (87.7%)	7 (35.0%)		282 (84.9%)	5 (22.7%)	
(+)	41 (12.3%)	13 (65.0%)		50 (15.1%)	17 (77.3%)	
Histopathology tumor type			0.008			<0.001
Endometrioid Adenocarcinoma	328 (98.5%)	18 (90.0%)		324 (97.6%)	18 (81.8%)	
Mixed Adenocarcinoma	2 (0.6%)	1 (5.0%)		4 (1.2%)	2 (9.1%)	
Serous Adenocarcinoma	1 (0.3%)	1 (5.0%)		3 (0.9%)	2 (9.1%)	
Other	2 (0.6%)	0 (0%)		1 (0.3%)	0 (0%)	

AH, atypical hyperplasia; CA125, cancer antigen 125; D&C, dilatation and curettage; LVSI, lymphovascular space invasion; MI, myometrial invasion.

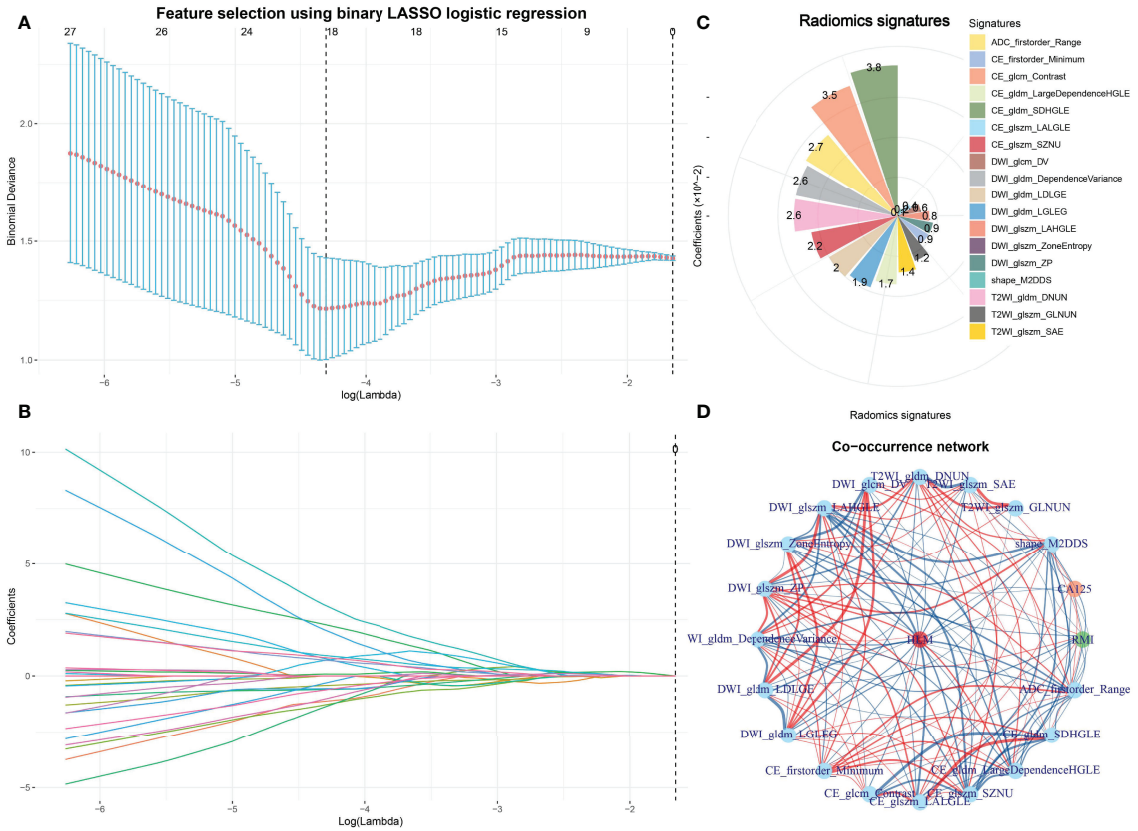

FIGURE 2 | Feature selection using LASSO and the selected radiomics signatures and co-occurrence of radiomics signatures and clinical features. The parameter lambda is chosen using 10-fold cross-validation *via* minimum criteria, which resulted in 10 features with nonzero coefficients **(A)**. LASSO coefficient profiles of the selected features **(B)**. The selected radiomics signatures of LNM by the LASSO method **(C)**. A co-occurrence map shows the correlations between radiomics features and clinical features of LNM in early-stage EC **(D)**.

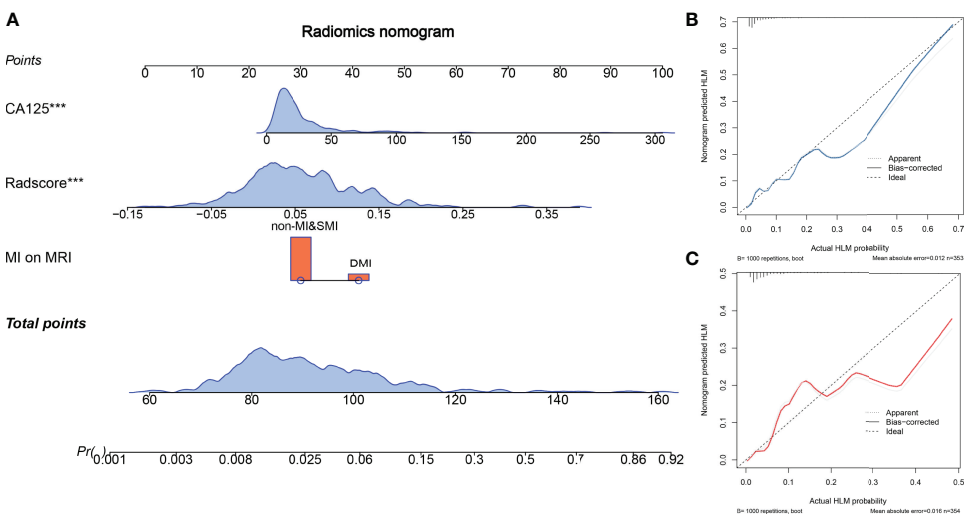

FIGURE 3 | The radiomics nomogram and calibration curves. The radiomics nomogram is constructed by integrating CA125, radscore, and myometrial invasion (MI) on MRI **(A)**. Calibration curve of the radiomics nomogram for predicting LNM in the training cohort **(B)** and the test cohort **(C)**.

×CE_glszm_LALGLE + 0.0224×CE_glszm_SZNU + 0.01677×CE_gldm_LargeDependenceHGLE + 0.03809×CE_gldm_SDHGLE + -0.027×ADC_firstorder_Range

Radiomics Nomogram Development and Validation

Multivariate logistic regression analysis showed that CA125 and tumor size were the risk factors for LNM in the early stage of EC. Considering that the selected feature "shape_M2DDC" reflects the tumor size, we did not include tumor size in the nomogram for avoiding over-fitting. Therefore, the radiomics nomogram was constructed by integrating the CA125, radscore, and MRI-reported MI status (**Figure 4**). The ROC curves of the three models in the training and test cohorts are shown in **Table 2**.

Clinical Usefulness

The decision curve analysis indicated that when the threshold probability was within a range from 10% to 90%, the net benefit of using the nomogram to predict LNM was greater than that of the treat-all or treat-none scheme in the training and test cohorts (**Figure 4**).

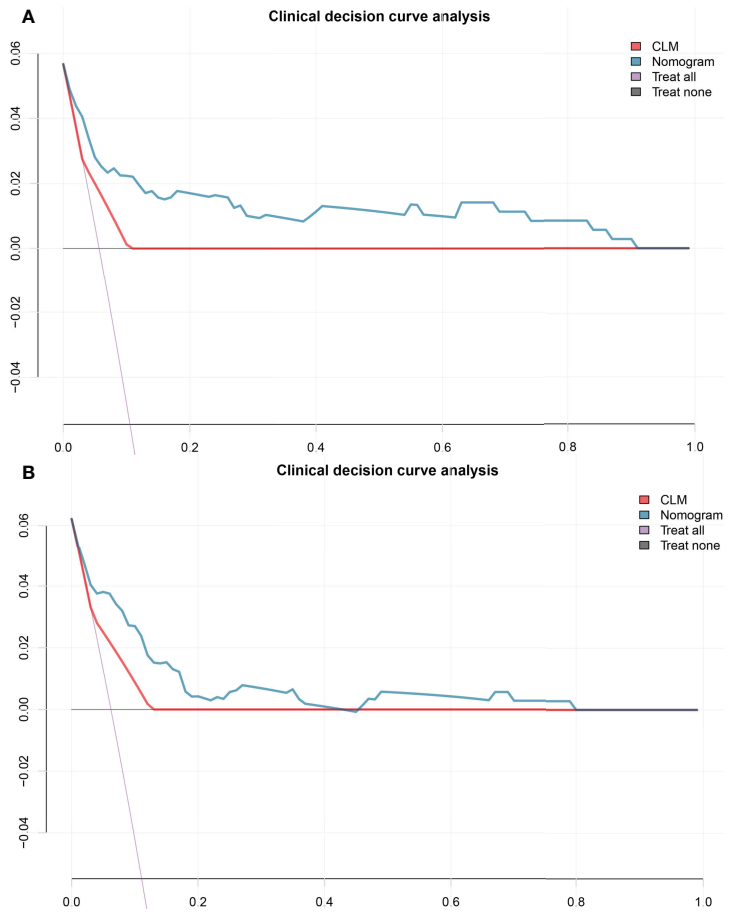

FIGURE 4 | The decision curve shows that when the threshold probability from 10% to 90%, the radiomics nomogram adds more net benefit than schemes of treat-all, treat-none and radscore in the training cohort **(A)**, and the decision curve of the test cohort **(B)**. CLM, clinical model.

TABLE 2 | Diagnostic performance of clinical model, radscore, and radiomics nomogram in the training and test cohorts.

Cohort	Index	AUC	95% CI	SPE	SEN	NPV	PPV	P*	P#
Training	Clinical model	0.66	0.55-0.77	0.66	0.65	0.97	0.10	0.004	–
	Radscore	0.82	0.74-0.90	0.80	0.75	0.98	0.18	–	0.004
	Nomogram	0.85	0.77-0.93	0.64	0.95	1.00	0.14	0.306	< 0.001
Test	Clinical model	0.67	0.56-0.78	0.70	0.64	0.97	0.13	0.005	–
	Radscore	0.81	0.72-0.90	0.56	0.95	0.99	0.13	–	0.005
	Nomogram	0.83	0.74-0.92	0.84	0.77	0.98	0.24	0.302	< 0.001

AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; SEN, sensitivity; SPE, specificity.
**Compared with Radscore; #Compared with clinical model by Delong test.*

The reclassification measures of discrimination confirmed that the radiomics nomogram performed better than the clinical model based on entire dataset with an NRI of 1.06 (95% confidence interval [CI]: 0.81-1.32) and an IDI of 0.05 (95% CI: 0.03-0.07) (both P < 0.001). Eighty-two patients were misclassified by the clinical model as candidates eligible for lymphadenectomy and 39 of them were corrected by the radiomics nomogram reclassification. Nine patients were misclassified by the clinical model as candidates ineligible for lymphadenectomy and three of them were corrected by the radiomics nomogram reclassification (**Figure 5**).

DISCUSSION

In this study, a preoperative individualized radiomics nomogram was developed and validated for predicting LNM in early-stage EC. The nomogram incorporated the radiomics signatures with two preoperative clinical risk factors (CA125 and MRI-reported MI status). This radiomics nomogram exhibited a good ability to predict LNM both in the training and test cohorts, which was easy to use and facilitated the preoperative individualized lymphadenectomy decision-making in early-stage EC.

Preoperatively assessing LNM status is crucial to guide the surgical management for EC patients. D&C and MRI are two recommended ways to preoperatively evaluate the tumor histological subtype, tumor grade, depth of MI, parametrial infiltration, and LNM (2). However, there is a relatively frequent discordance between the findings of D&C and final surgical pathology (12). Helpman et al. reported that 22% of G1 EC diagnosed by biopsy were upgraded to G2 or G3 in the final surgical pathology. As is shown in the result, 12.9% G1/G2 EC diagnosed by D&C were misdiagnosed. Furthermore, the approach of intra-operative frozen section is not readily available in most major cancer centers (13). However, a previous study showed that the radiomics features combining with ADC value could be used effectively to evaluate tumor grade with a AUC of 0.95 (14).

A previous study showed that conventional MRI is limited in detecting metastatic LN, even when the radiologists were informed of the radiomics prediction results of LN status (9). The reason for this disadvantage might be attributed to the metastatic LN having a normal size (< 0.8 cm), morphology, signal, or due to the MRI partial volume effects (9). However, the MRI-based radiomics model could be used to assess the LN status and help radiologists improve their performance in predicting LNM in EC (9). In accordance with the previous study, results showed a good ability to predict LNM both in the training and test cohorts in this study.

CA125 was found to be an independent risk factor for LNM in early-stage EC. CA125 is also a risk factor for high-risk EC (15). Several guidelines, including the European Society of Medical Oncology, European Society of Gynecological Oncology, and European Society for Radiotherapy and Oncology (ESMO-ESGO-ESTRO) consensus conference guideline, incorporate measurement of CA125 to assess LN status along with imaging as part of preoperative workup (3). The tumor size is also commonly used as a prognostic factor in EC, since it has been correlated with LN status and prognosis in EC patients (16).

A previous study reported a 5%-9% risk of LNM in G1 or G2 endometrioid endometrial carcinoma, which means when classifying patients based on preoperative histology, a substantial number of patients with LNM will be missed (17). In early-stage EC patients, 5.9% were found to have LNM in this study, which is in accordance with the previous report. Furthermore, it is estimated that 33% of patients with a preoperative histological diagnosis of non-LNM are upgraded to LNM on final postoperative histological examination, resulting in an incorrect risk estimation of LNM (18).

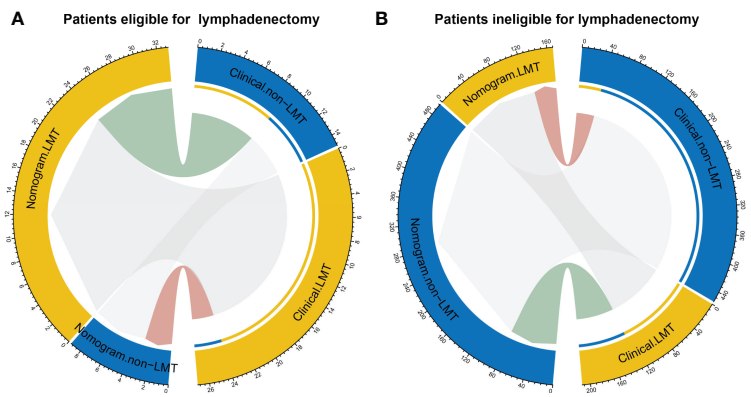

FIGURE 5 | Reclassification of patients for eligible for lymphadenectomy **(A)** and in eligible for lymphadenectomy (LMT) **(B)**. Groups are illustrated according to the radiomics nomogram and clinical model-determined lymphadenectomy eligibility basing on the entire dataset with the specific patient numbers are presented. The patients were pathological confirmed whether eligible for lymphadenectomy. In the circle plots, the patients who were classified both correctly by clinical and nomogram are represented as connections in light grey. The connections in light green indicate patients who were clinically diagnosed incorrectly but reclassified correctly by the nomogram, while connections in pink indicate patients who were clinically diagnosed correctly but reclassified incorrectly by the nomogram.

However, with the help of the radiomics nomogram, selective lymphadenectomy approaches might prevent unnecessary lymphadenectomy in low-risk (defined as G1 or G2 endometrioid endometrial carcinoma with MI less than 50% and primary tumor diameter less than 2 cm) patients.

The standard treatment of early-stage EC is hysterectomy and bilateral salpingo-oophorectomy (BSO), which may be performed *via* a laparotomy or by a laparoscopic approach (19). For patients with advanced stage EC (tumor spread beyond the womb), adjuvant radiotherapy (and increasingly chemotherapy) is administered to reduce the risk of recurrence. A previous study reported that no significant differences in 5-year survival rates were shown in patients with stage I and II disease who did or did not undergo lymphadenectomy (19). Furthermore, lymphadenectomy may not be routinely performed, and if it is, the extent of lymphadenectomy can range from taking a few LNs for sampling to performing complete dissection pelvic and para-aortic lymphadenectomy (19). In this study, LNM could be predicted by the radiomics nomogram, which would be used to reduce unnecessary morbidity caused by extensive LN dissection and improve staging by targeted removal of metastatic LNs missed by preoperative MRI scanning in early-stage EC. Studies assessing the diagnostic accuracy of sentinel LN biopsy have yielded promising results in the management of EC (20). Radiomics is expected to have the ability to predict and identify a metastatic LN, so as to further enhance the accuracy of sentinel LN biopsy.

Our study had several limitations. First, the current study included an inherent shortcoming by retrospective analysis of the patient records and the radiomics nomogram was established on the basis of single-center data. The robustness and reproducibility of the radiomics nomogram need to be further validated in prospective multi-center studies with larger participant pools. Second, the training cohort is re-sampled before constructing the radiomics nomogram. Bias might exist due to the imbalance of the samples. Third, all the enrolled patients received D&C before pelvic MRI scanning. D&C may result in decreased tumor volume, leading to some small tumors to be invisible on MRI. However, these cases were excluded from this study. Furthermore, the tumor size is positively correlated with lymph node metastasis in early-stage EC patients. D&C may lead to underestimates of lymph node metastasis. Further studies are warranted to investigate the effect on MRI after curettage by comparing MRI findings before and after curettage. Last, more studies that focus on comparing radiomics and prospective and randomized preoperative predictive techniques in systematic lymphadenectomy should be carried out.

In conclusion, we developed a convenient radiomics nomogram model that combines the D&C-reported tumor grade, MRI-reported MI status, clinical risk factors, and radiomics signatures (radscore) to preoperatively and non-invasively evaluate LN status in patients with early-stage EC. The application of the radiomics model could optimize clinical decision-making and potentially improve the selection of surgical scheme of early-stage EC patients.

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 below: The data is available at https://gitee.com/dr_yingli/EC_LM.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Jinshan Hospital, Fudan University (No. JIEC 2021-S55). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

B-CY and F-HM designed the research study. X-FL, B-CY, and YL performed the research. B-CY and F-HM provided help and advice on acquisition of data. YL and X-FL analyzed the data. YL and X-FL wrote the manuscript. YL and J-WQ were the supervisors of this study. All authors read and approved the final manuscript.

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REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654
2. Kim SI, Kim JW. Endometrial Cancer. *N Engl J Med* (2021) 384(6):586. doi: 10.1056/NEJMc2035378
3. Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-Up. *Ann Oncol* (2016) 27(1):16–41. doi: 10.1093/annonc/mdv484
4. Korkmaz V, Meydanli MM, Yalcin I, Sari ME, Sahin H, Kocaman E, et al. Comparison of Three Different Risk-Stratification Models for Predicting Lymph Node Involvement in Endometrioid Endometrial Cancer Clinically Confined to the Uterus. *J Gynecol Oncol* (2017) 28(6):e78. doi: 10.3802/jgo.2017.28.e78
5. Group SGOCPECW, Burke WM, Orr J, Leitao M, Salom E, Gehrig P, et al. Endometrial Cancer: A Review and Current Management Strategies: Part I. *Gynecol Oncol* (2014) 134(2):385–92. doi: 10.1016/j.ygyno.2014.05.018
6. Morrow CP, Bundy BN, Kurman RJ, Creasman WT, Heller P, Homesley HD, et al. Relationship Between Surgical-Pathological Risk Factors and Outcome

in Clinical Stage I and II Carcinoma of the Endometrium: A Gynecologic Oncology Group Study. *Gynecol Oncol* (1991) 40(1):55–65. doi: 10.1016/0090-8258(91)90086-K
7. Creutzberg CL. Lymphadenectomy in Apparent Early-Stage Endometrial Carcinoma: Do Numbers Count? *J Clin Oncol* (2005) 23(16):3653–5. doi: 10.1200/JCO.2005.11.947
8. Bi Q, Chen Y, Wu K, Wang J, Zhao Y, Wang B, et al. The Diagnostic Value of MRI for Preoperative Staging in Patients With Endometrial Cancer: A Meta-Analysis. *Acad Radiol* (2020) 27(7):960–8. doi: 10.1016/j.acra.2019.09.018
9. Yan BC, Li Y, Ma FH, Zhang GF, Feng F, Sun MH, et al. Radiologists With MRI-Based Radiomics Aids to Predict the Pelvic Lymph Node Metastasis in Endometrial Cancer: A Multicenter Study. *Eur Radiol* (2021) 31(1):411–22. doi: 10.1007/s00330-020-07099-8
10. Rizzo S, Botta F, Raimondi S, Origgi D, Fanciullo C, Morganti AG, et al. Radiomics: The Facts and the Challenges of Image Analysis. *Eur Radiol Exp* (2018) 2(1):36. doi: 10.1186/s41747-018-0068-z
11. Yan BC, Li Y, Ma FH, Feng F, Sun MH, Lin GW, et al. Preoperative Assessment for High-Risk Endometrial Cancer by Developing an MRI- and Clinical-Based Radiomics Nomogram: A Multicenter Study. *J Magn Reson Imaging* (2020) 52(6):1872–82. doi: 10.1002/jmri.27289
12. Meissnitzer M, Forstner R. MRI of Endometrium Cancer - How We do it. *Cancer Imaging* (2016) 16:11. doi: 10.1186/s40644-016-0069-1
13. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective Assessment of Lymphatic Dissemination in Endometrial Cancer: A Paradigm Shift in Surgical Staging. *Gynecol Oncol* (2008) 109(1):11–8. doi: 10.1016/j.ygyno.2008.01.023
14. Zhang K, Zhang Y, Fang X, Fang M, Shi B, Dong J, et al. Nomograms of Combining Apparent Diffusion Coefficient Value and Radiomics for Preoperative Risk Evaluation in Endometrial Carcinoma. *Front Oncol* (2021) 11:705456. doi: 10.3389/fonc.2021.705456
15. Saarelainen SK, Peltonen N, Lehtimaki T, Perheentupa A, Vuento MH, Maenpaa JU. Predictive Value of Serum Human Epididymis Protein 4 and Cancer Antigen 125 Concentrations in Endometrial Carcinoma. *Am J Obstet Gynecol* (2013) 209(2):142 e1–6. doi: 10.1016/j.ajog.2013.04.014
16. Ytre-Hauge S, Dybvik JA, Lundervold A, Salvesen OO, Krakstad C, Fasmer KE, et al. Preoperative Tumor Texture Analysis on MRI Predicts High-Risk Disease and Reduced Survival in Endometrial Cancer. *J Magn Reson Imaging* (2018) 48(6):1637–47. doi: 10.1002/jmri.26184
17. Trovik J, Wik E, Werner HM, Krakstad C, Helland H, Vandenput I, et al. Hormone Receptor Loss in Endometrial Carcinoma Curettage Predicts Lymph Node Metastasis and Poor Outcome in Prospective Multicentre Trial. *Eur J Cancer* (2013) 49(16):3431–41. doi: 10.1016/j.ejca.2013.06.016
18. Visser NCM, Reijnen C, Massuger L, Nagtegaal ID, Bulten J, Pijnenborg JMA. Accuracy of Endometrial Sampling in Endometrial Carcinoma: A Systematic Review and Meta-Analysis. *Obstet Gynecol* (2017) 130(4):803–13. doi: 10.1097/AOG.0000000000002261
19. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the Management of Endometrial Cancer. *Cochrane Database Syst Rev* (2017) 10:CD007585. doi: 10.1002/14651858.CD007585.pub4
20. Mehralivand S, van der Poel H, Winter A, Choyke PL, Pinto PA, Turkbey B. Sentinel Lymph Node Imaging in Urologic Oncology. *Transl Androl Urol* (2018) 7(5):887–902. doi: 10.21037/tau.2018.08.23

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Association of Tumor Size With Myometrial Invasion, Lymphovascular Space Invasion, Lymph Node Metastasis, and Recurrence in Endometrial Cancer: A Meta-Analysis of 40 Studies With 53,276 Patients

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Background: Myometrial invasion (MI), lymphovascular space invasion (LVSI), and lymph node metastasis (LNM) have been found to have independent prognostic factors in endometrial cancer. Tumor size has practical advantages in endometrial cancer. The cutoff values for tumor size conformed with current literature. More and more studies inferred that tumor size >20 mm showed a strong correlation. However, the relationship between tumor size >20 mm and MI, LVSI, LNM, recurrence, and overall survival (OS) remains controversial, and no meta-analysis has been conducted. Therefore, a systematic review and meta-analysis should be performed to discuss this issue later on.

Methods: Relevant articles were collected from PubMed, EMBASE, and Cochrane Library databases from January 1990 to June 2021. The predictive value of tumor size >20 mm in endometrial cancer was studied, and data were pooled for meta-analysis using Review Manager 5.1. Additionally, the odds ratio (OR) was analyzed, and cumulative analyses of hazard ratio (HR) and their corresponding 95% CI were conducted.

Results: A total of 40 articles with 53,276 endometrial cancer patients were included in the meta-analysis. It contained 7 articles for MI, 6 for LVSI, 21 for LNM, 7 for recurrence, and 3 for OS. Primary tumor size >20 mm was significantly associated with depth of MI (OR = 5.59, 95% CI [5.02, 6.23], $p < 0.001$), positive LVSI (OR = 3.35, 95% CI [2.34, 4.78], $p < 0.001$), positive LNM (OR = 4.11, 95% CI [3.63, 4.66], $p < 0.001$), and recurrence (OR = 3.52, 95% CI [2.39, 5.19], $p < 0.001$). Tumor size >20 mm was also related to OS *via* meta-synthesis of HR in univariate survival (HR 2.13, 95% CI [1.28, 3.53], $p = 0.003$). There was no significant publication bias in this study by funnel plot analysis.

Conclusion: Primary tumor size >20 mm was an independent predictive factor for the depth of MI, positive LVSI, positive LNM, recurrence, and poor OS. Therefore, it is more important to take into account the value of tumor size in the clinicopathological staging of endometrial carcinoma. Tumor size >20 mm should be integrated into the intraoperative algorithm for performing a full surgical staging. Well-designed and multicenter studies, with a larger sample size, are still required to verify the findings.

Keywords: endometrial cancer, tumor size, myometrial invasion, lymphovascular space invasion, lymph node metastasis, recurrence, overall survival

INTRODUCTION

Endometrial cancer is the sixth most common neoplasm in women worldwide, and the incidence rate is increasing rapidly (1). The International Federation of Gynecology and Obstetrics (FIGO) mandated that the treatment of endometrial cancer was surgical staging, which includes hysterectomy, bilateral salpingo-oophorectomy, or pelvic and para-aortic lymphadenectomy (2). A gynecologic oncology group study identified some risk factors, such as stage, histological subtype, depth of myometrial invasion (MI), lymphovascular space invasion (LVSI), grade, and lymph node metastasis (LNM), which could predict recurrence and survival (3).

A gynecologic oncology group study in 1987 proposed that primary tumor size was not considered a risk factor for lymphatic metastasis (4). Some published studies indicated tumor size was not a risk associated with recurrence in women with endometrial cancer (5, 6). However, other literature showed that tumor size seemed to be a significant risk factor for endometrial cancer (7, 8). Recent data suggested that primary tumor size was an important parameter in predicting the clinicopathological outcomes for endometrial cancer patients, but it seemed to be controversial. Gusberg et al. firstly implied that it came out to be a poor prognosis with a tumor size of >10 cm (9). Riggs et al. analyzed the optimal tumor diameter that can predict LNM and was noted to be 35 mm (10). The Mayo Criteria, which included the FIGO grade 1 or 2 endometrioid cancer, with tumor size <20 mm, MI < 50%, and no intraoperative evidence of macroscopic disease, was used to guide lymphadenectomy assessment (11). Milwaukee Model suggested that primary tumor size >50 mm and MI > 33% identifies possible lymphatic dissemination in low-risk endometrial cancer patients (12). The cutoff values for tumor size conformed with current literature, which varies from 20 to 50 mm (12, 13). Kilt et al. explored that cutoff of tumor size increasing from 20 to 30 and 50 mm had a lower at-risk rate of lymph node dissection but an unacceptably high false-negative rate (14). Tumor sizes <20 mm for low-risk endometrial cancer remained more sensitive than those with tumor sizes <30 mm for identifying lymphatic dissemination (14). Recently, more and more studies inferred that a tumor size of 20 mm remains clinically significant in relation to the risk of recurrence (7, 8). Therefore, we should focus on the relationship between the tumor size of 20 mm and MI, LVSI, LNM, recurrence, and OS.

There was no meta-analysis about the relationship between tumor size >20 mm and MI, LVSI, LNM, recurrence, and OS. The aim of our study was to investigate the relationship between primary tumor size of 20 mm and clinicopathological parameters, recurrence, and OS.

METHODS

Literature Search Strategy

A rigorous search of the PubMed, EMBASE, and Cochrane Library databases from January 1990 to June 2021 was undertaken to identify relevant articles. The key search terms were drafted as follows: "tumor size," "tumor diameter," "uterine cancer," "uterine carcinoma," "endometrial cancer," "endometrial carcinoma," "prognosis," "prognostic factor," "risk," "myometrial invasion," "lymphovascular space invasion," "lymph node metastasis," "recurrence," and "overall survival." The literature search was performed by two authors independently.

Criteria for Inclusion and Exclusion

The inclusion criteria included the following: 1) the patients were only diagnosed with endometrial cancer; 2) tumor size, which was defined as a cutoff of 20 mm; 3) one or more main clinicopathological factors included MI, LVSI, LNM, recurrence, and OS; and 4) article was published in English. The exclusion criteria included the following terms: 1) letters, editorials, expert opinions, reviews, and animal studies; 2) preoperative tumor size at MRI and PET/CT or ultrasound; and 3) studies of data were insufficient.

Data Extraction

The data from the selected trials were extracted and assessed by two authors independently. Any disagreements in data extraction were resolved by further discussion and consensus. Three categories of data extraction in each study are the following: baseline patient characteristics, clinicopathological outcomes, and survival outcomes. Baseline characteristics of the included studies need the first author's name, study publication year, country, and sample size. Clinicopathological outcomes included MI, LVSI, and LNM. Survival outcomes included recurrence and OS.

Data Analysis

All statistical analyses were performed by using the Cochrane Collaboration's Review Manager Software 5.1. Clinicopathological

outcomes and recurrence were pooled as odds ratio (OR) and 95% CI. Pooled hazard ratio (HR) and corresponding 95% CI were used to analyze the association between tumor size and OS. Fixed- or random-effects meta-analysis models were varied according to the existence of heterogeneity among the included studies. It appeared that heterogeneity with chi-square $p > 0.1$ and/or $I^2 > 50\%$, publication bias was evaluated by the shape of the funnel plot. The test for funnel plot asymmetry was applied only when at least 10 studies were included in a meta-analysis. A significant statistical difference was pointed out when a p-value was less than 0.05.

The quality of the included studies was assessed by the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2), which is essential to evaluate the risk of bias for included studies.

RESULTS

Study Characteristics

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram was shown in **Figure 1**. After titles and abstracts were screened, 225 records were excluded, including 97 that indicated that the cutoff tumor size was not 20 mm, 100 that indicated the preoperative tumor size, 21 without original data, and 7 without relevant outcome. A full text of 111 articles was assessed, 71 records were excluded, including studies with the same included patients, 2 that indicated HRs from univariate survival analyses not available, 25 that indicated preoperative tumor size, 36 that have no detailed results, and finally, forty studies with a total of 53,276 eligible patients. Baseline characteristics of the included studies are shown in **Table 1**. All of the included studies were retrospectively designed, including 7 for MI (20, 24, 27, 35, 44, 45, 48), 6 for LVSI (20, 35, 41–44), 27 for LNM (15–40, 45), 7 for recurrence (5–8, 20, 46, 47), and 3 for OS (16, 49, 50). Included studies consisted of 2 large-scale retrospective cohort studies (27, 38). The results of the meta-analysis are summarized in **Table 2**.

Literature Quality

The QUADAS-2 was used to evaluate the quality of the included studies. Two reviewers independently evaluated the quality of the included 40 studies. The outcome is shown in **Figure 2**.

Correlation Between Tumor Size and Myometrial Invasion in Endometrial Cancer

Seven studies (20, 24, 27, 35, 44, 45, 48) including 20,863 endometrial cancer patients were eligible to analyze the

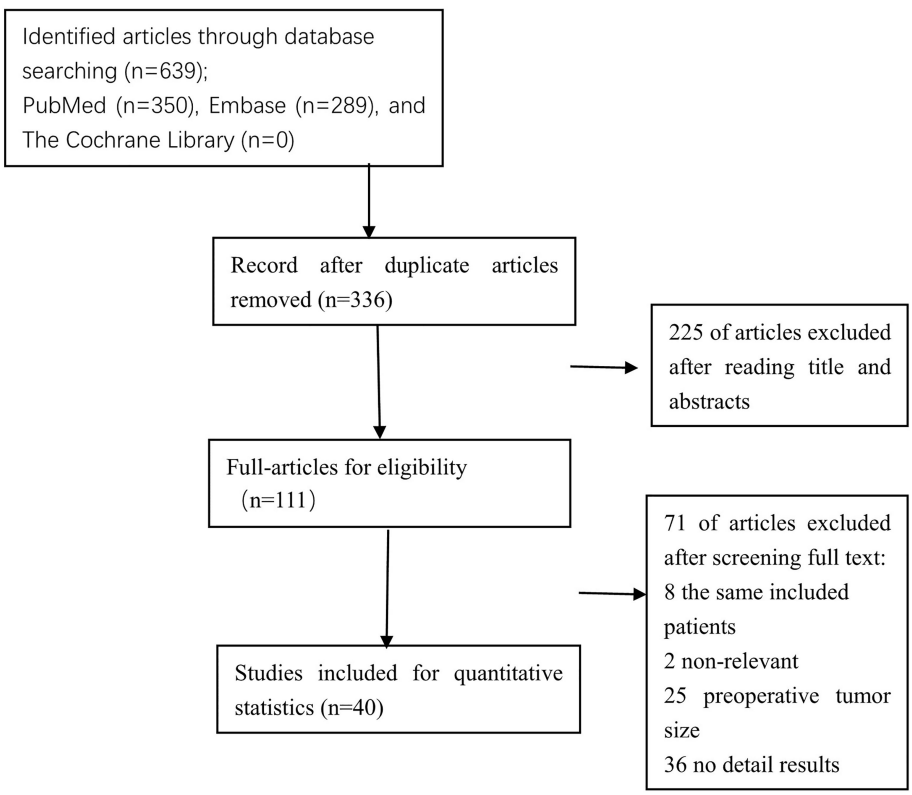

FIGURE 1 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

TABLE 1 | Baseline characteristics of the included studies.

First author	Year	Country	n	Stage	Tumor Grade	Histologic	Risk factors
Akıs (15)	2021	Turkey	146	I–III	I–III	Endometrioid	LNM
AlHilli (16)	2013	USA	883	I–IV	I–III	Endometrioid	LNM
							OS
Boyraz (17)	2017	Turkey	191	IA	I–II	Endometrioid	LNM
Boyraz (18)	2018	Turkey	307	NA	I–III	Endometrioid	LNM
Chang (19)	2011	Korea	203	I–IV	I–III	Mixed	Paraaortic LNM
Doll (20)	2014	USA	183	I–IV	High	Mixed	LNM
							LVSI
							Recurrence
							MI
Dali (21)	2019	USA	58	I	NA	Endometrioid	LNM
Gilani (22)	2014	USA	207	NA	I–III	Endometrioid	LNM
Günakan (23)	2019	Turkey	762	I–IV	I–III	Mixed	LNM
Karalok (24)	2017	Turkey	368	NA	I–III	Endometrioid	LNM
							MI
Lee (25)	2009	Korea	834	I–IV	I–III	Endometrioid	LNM
Li (26)	2019	China	874	I–III	I–III	Mixed	LNM
Mahdi (27)	2014	USA	19692	I	I–III	Endometrioid	LNM
							MI
Matsushita (28)	2019	Japan	185	I–IV	I–III	Endometrioid	LNM
Milam (29)	2012	USA	971	II–III	II–III	Endometrioid	LNM
Oz (30)	2017	Turkey	243	I	I	Endometrioid	LNM
Pavlakis (31)	2017	Greece	290	I–II	I	Endometrioid	LNM
Rathod (32)	2014	India	52	I–III	I–III	Mixed	LNM
Sari (33)	2017	Turkey	641	I–IV	I–III	Mixed	LNM
Shah (34)	2005	USA	194	I–IV	I–III	Mixed	LNM
Tecellioglu (35)	2021	Turkey	100	I–IV	I–III	Endometrioid	LVSI
							LNM
							MI
Turan (36)	2011	Turkey	198	I–IV	I–III	Mixed	LNM
Vaizoglu (37)	2013	Turkey	261	I	I–III	Endometrioid	Retroperitonea LNM
Vargas (38)	2014	USA	21011	NA	I–III	Endometrioid	LNM
Watanabe (39)	2003	Japan	107	I–III	I–II	Endometrioid	Pelvic LNM
Zanfagnin (40)	2019	USA	83	IIIC	I–III	Mixed	Pelvic LNM
Ilker (41)	2015	Turkey	47	I–III	II–III	Mixed	LVSI
Oliver-Perez (42)	2021	Spain	220	I–III	I–III	Mixed	LVSI
Ayhan (43)	2018	Turkey	912	I–IV	I–II	Endometrioid	LVSI
Laufer (44)	2013	Italy	181	I	I–III	Endometrioid	LVSI
							MI
Schink (45)	1991	Chicago	125	NA	I–III	Mixed	MI
							LNM
Gadducci (6)	2009	Italy	32	I–II	I–III	Endometrioid	Recurrence
Bendifallah (46)	2014	France	396	I–III	I–III	Mixed	Recurrence
Güngördük (7)	2018	Turkey	279	IA	I–II	Endometrioid	Recurrence
ÇAKIR (47)	2019	Turkey	550	I–II	I–III	Endometrioid	Recurrence
Nwachukwu (5)	2021	Japan	222	IA	I	Endometrioid	Recurrence
LiMingzhu (8)	2014	China	398	I–II	NA	Endometrioid	Recurrence
Marcickiewicz (48)	2010	Sweden	214	I–IV	I–III	Mixed	MI
Roma (49)	2015	USA	589	NA	I–II	Endometrioid	OS
Yamada (50)	2020	Japan	67	I–IV	I–III	Mixed	OS

OS, overall survival; LVSI, lymphovascular space invasion; MI, myometrial invasion.

association between tumor size and MI in endometrial cancer. Pooled analysis showed that tumor size >20 mm was significantly associated with incidences of depth of MI (>50%) (OR = 5.59, 95% CI [5.02, 6.23], $p < 0.001$, $I^2 = 45\%$, $p = 0.09$) (**Figure 3**).

Correlation Between Tumor Size and Lymphovascular Space Invasion in Endometrial Cancer

Six studies (20, 35, 41–44) with a total of 1,643 endometrial cancer patients were included for this analysis. The results of the pooled analysis revealed that tumor size >20 mm was significantly associated with positive LVSI (OR = 3.35, 95% CI [2.34, 4.78], $p < 0.001$, $I^2 = 0\%$, $p = 0.47$) (**Figure 4**).

Correlation Between Tumor Size and Lymph Node Metastasis in Endometrial Cancer

Twenty-seven studies with a total of 49,169 endometrial cancer patients were presented on the debate of association between tumor size and LNM (15–40, 45). The results of the pooled

TABLE 2 | The results of meta-analysis.

Analysis	Subgroup	Number of studies	Heterogeneity			Pooled result	
			χ^2	I^2	*P*	OR/HR(95% CI)	P
Tumor size and MI	In all FIGO stages	7	10.93	45%	0.09	5.59 (5.02–6.23)	<0.001
Tumor size and LVSI	In all FIGO stages	6	4.55	0%	0.47	3.35 (2.34–4.78)	<0.001
Tumor size and LNM	*In all FIGO stages*	27	20.28	0%	0.73	4.11 (3.63–4.66)	<0.001
	In FIGO stage I–II	6	1.38	0%	0.85	3.69 (2.97–4.60)	<0.001
	In all FIGO stages excluding I–II	21	18.12	0%	0.58	4.32 (3.71–5.03)	<0.001
Tumor size and recurrence	In all FIGO stages	7	4.16	0%	0.66	3.52 (2.39–5.19)	<0.001
	In FIGO stage IA	2	0.32	0%	0.57	5.94 (2.83–12.44)	<0.001
	In FIGO stage I–II	3	0.72	0%	0.70	3.15 (1.72–5.78)	<0.001
	In FIGO stage I–III	3	0.09	0%	0.77	2.37 (1.18–4.77)	<0.001
Tumor size and overall survival	In all FIGO stages	3	7.79	61%	0.05	2.13 (1.28–3.53)*	0.003

**HR (95% CI).*

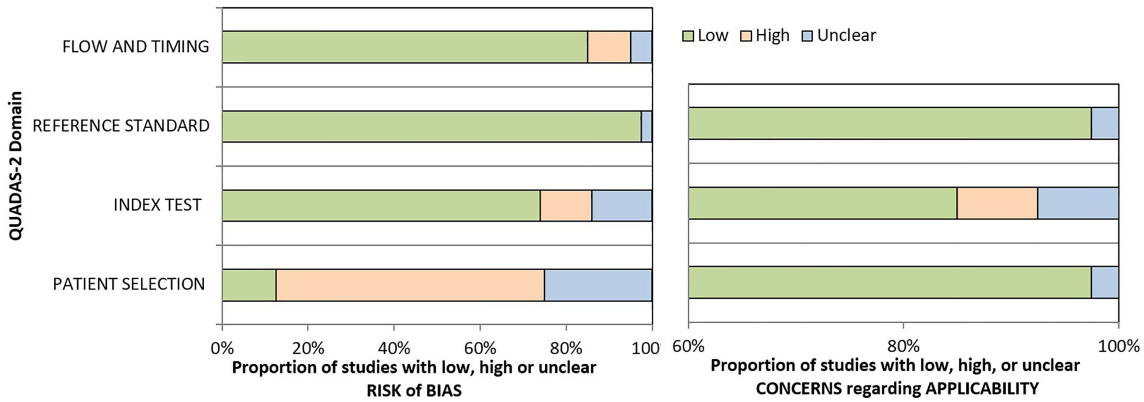

FIGURE 2 | Quality Assessment of Diagnostic Accuracy Studies-2.

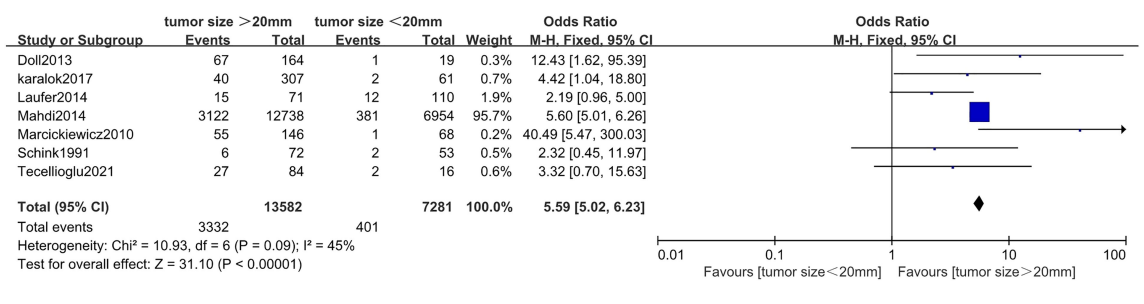

FIGURE 3 | Forest plots showing the correlation between tumor size and myometrial invasion (> 50%).

analysis revealed that tumor size >20 mm was significantly associated with LNM (OR = 4.11, 95% CI [3.63, 4.66], $p < 0.001$, $I^2 = 0\%$, $p = 0.73$). A total of 20,735 patients in FIGO stage I–II endometrial cancer that were based on 6 studies (17, 21, 27, 30, 31, 37) were enrolled in our meta-analysis. The pooled result showed that tumor size >20 mm was correlated with high LNM, and the pooled OR was 3.69 (95% CI [2.97, 4.60], $p < 0.001$), with heterogeneity ($I^2 = 0\%$, $p = 0.85$). A total of 28,434 patients had FIGO stage III–IV endometrial cancer, based on 21 studies that were enrolled in our meta-analysis (15, 16, 18–20, 22–26, 28, 29, 32–36, 38–40, 45). The pooled result showed that tumor size >20 mm was correlated with high LNM, and the pooled OR was 4.32 (95% CI [3.71, 5.03], $p < 0.001$), with heterogeneity ($I^2 = 0\%$, $p = 0.58$) (**Figure 5**).

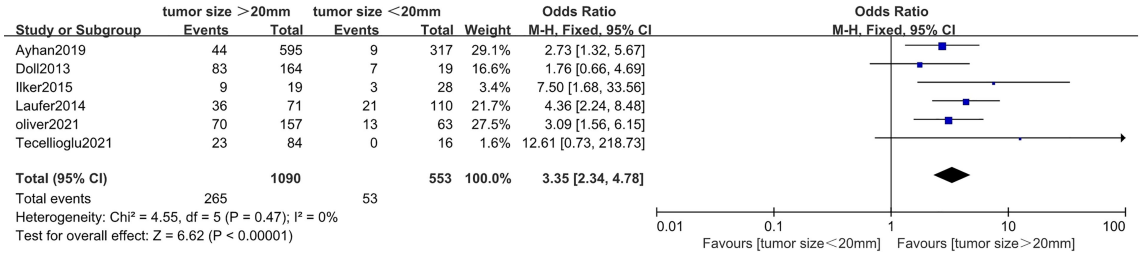

FIGURE 4 | Forest plots showing the correlation between tumor size and lymphovascular space invasion (LVSI).

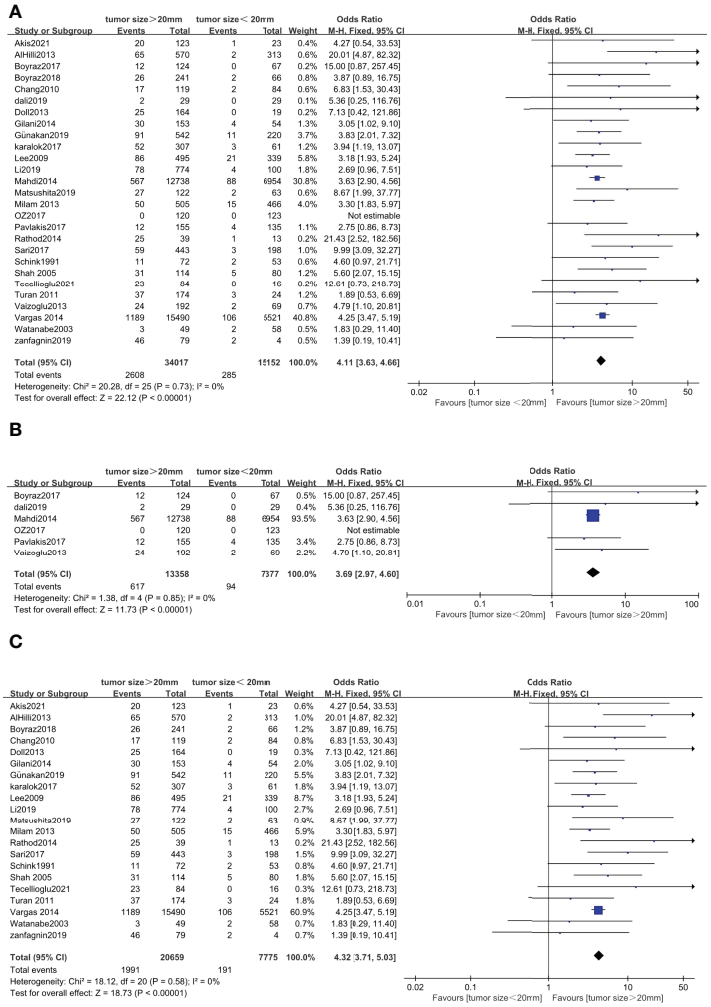

FIGURE 5 | Forest plots showing the correlation between tumor size and lymph node metastasis (LNM). **(A)** All International Federation of Gynecology and Obstetrics (FIGO) stages. **(B)** FIGO stage I–II. **(C)** FIGO stage I–IV excluding stage I–II.

Correlation Between Tumor Size and Recurrence in Endometrial Cancer

Seven studies (5–8, 20, 46, 47) with a total of 2,060 endometrial cancer patients were eligible for analysis of the association between tumor size and recurrence. The pooled analysis revealed that tumor size >20 mm was significantly associated with recurrence (OR = 3.52, 95% CI [2.39, 5.19], $p < 0.001$, $I^2 = 0\%$, $p = 0.66$). A total of 501 patients in FIGO stage IA endometrial cancer, based

on 2 studies, were enrolled in our meta-analysis (5, 7). The pooled result showed that tumor size >20 mm was correlated with high recurrence, and the pooled OR was 5.94 (95% CI [2.83, 12.44], $p < 0.001$), with heterogeneity ($I^2 = 0\%$, $p = 0.57$). A total of 980 patients in FIGO stage I–II endometrial cancer, based on 3 studies, were enrolled in our meta-analysis (6, 8, 47). The pooled result showed that tumor size >20 mm was also correlated with high recurrence, and OR was 3.15 (95% CI [1.72, 5.78], $p < 0.001$), with heterogeneity ($I^2 = 0\%$, $p = 0.70$). A total of 579 patients in FIGO stage I–III endometrial cancer, based on 2 studies, were enrolled in our meta-analysis (20, 46). The pooled result showed that tumor size >20 mm was also correlated with high recurrence, and OR was 2.37 (95% CI [1.18, 4.77], $p < 0.001$), with heterogeneity ($I^2 = 0\%$, $p = 0.77$) (**Figure 6**).

Correlation Between Tumor Size and Overall Survival in Endometrial Cancer

Three studies (16, 49, 50) with a total number of 1,937 endometrial cancer patients were presented on the debate of tumor size >20 mm and OS. The random-effects model was applied for the significant heterogeneity. The pooled HRs of OS for univariate analyses were 2.13 (95% CI [1.28, 3.53], $p = 0.003$), with heterogeneity ($I^2 = 61\%$, $p = 0.05$) (**Figure 7**).

Publication Bias of Included Studies

A funnel plot was applied for the assessment of publication bias in the literature. The funnel plot for the included 27 studies on the association between tumor size and LNM was relatively symmetrical. Thus, there was no significant publication bias risk in all included studies investigating the association between tumor size and LNM (**Figure 8**).

DISCUSSION

A few published studies indicated that tumor size >20 mm could provide important prognostic outcomes for endometrial cancer (27, 45, 51, 52), but others showed that tumor size of 20 mm was not a prognostic factor in endometrial cancer (20, 47). In the current study, we performed a meta-analysis to roundly evaluate the prognostic value of tumor size. Our conclusion showed tumor size >20 mm was characterized by the presence of MI, which has 50% of patients with all FIGO stages in endometrial cancer. MI is vitally important in the development of endometrial cancer and a well-recognized predictor of extra-uterine spread (4, 53). MI is quite an early action of cancer cells, which classifies patients with initial stages as low-risk or high-

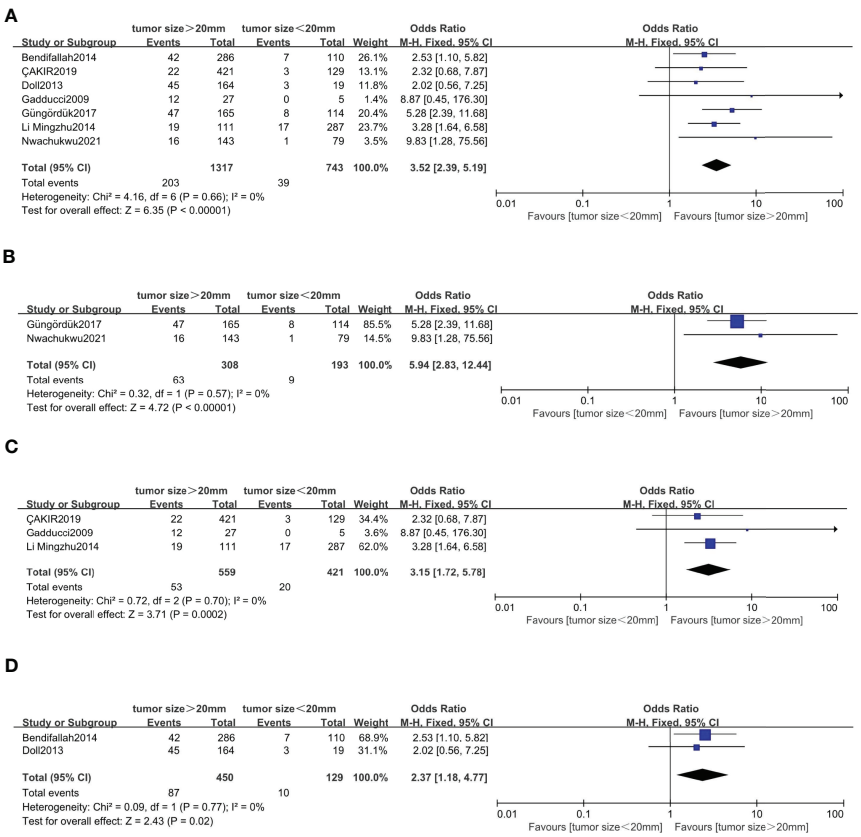

FIGURE 6 | Forest plots showing the correlation between tumor size and recurrence. **(A)** All International Federation of Gynecology and Obstetrics (FIGO) stage. **(B)** FIGO IA. **(C)** FIGO stage I–II. **(D)** FIGO stage I–III.

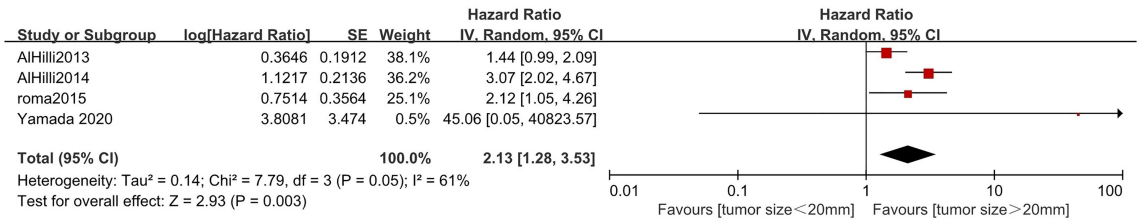

FIGURE 7 | Meta-analysis of the association between tumor size and overall survival in endometrial cancer patients according to hazard ratio (HR) from univariate survival analyses.

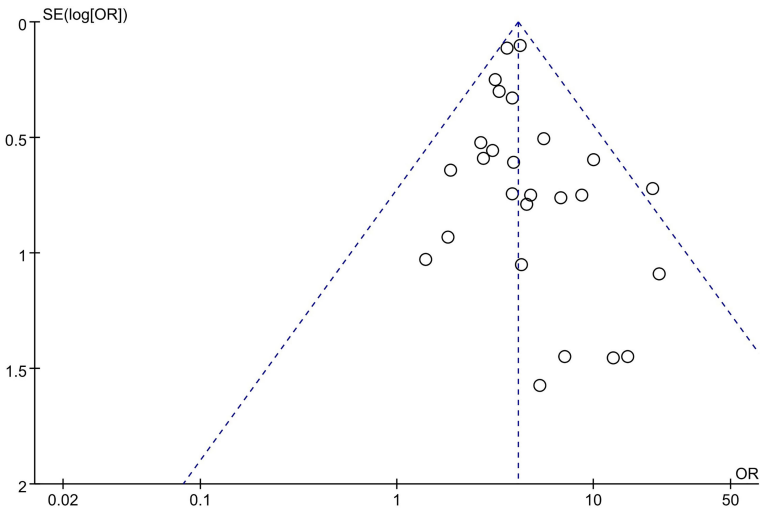

FIGURE 8 | Funnel plot analysis of tumor size and lymph node metastasis.

risk patients for surgical planning (53). Depth of MI (>50%) definitely correlated to LVSI, LNM, recurrence, and OS (53).

Six studies with a total of 1,643 endometrial cancer patients were eligible for analysis, and the results demonstrated that tumor size >20 mm has a significant prognostic implication for positive LVSI. A retrospective analysis reported the impact on positive LVSI was more relevant than MI > 50% for predicting survival in stage I endometrial cancer (43). Positive LVSI should be emphasized in early-stage endometrial cancer (54). Moreover, these as well as other studies substantiated the fact that positive LVSI patients had lower recurrence-free survival and OS rates (55). The European Society of Gynaecological Oncology (ESGO) guidelines introduced that positive LVSI should recommend lymphadenectomy (56). Unfortunately, it is usually not possible to diagnose LVSI status on the frozen section, until the final pathology report. So tumor size may be a useful tool for predicting markers of LVSI in a preoperative or intraoperative surgical stage.

We have reached an agreement that LNM was one of the most important prognostic factors. Lymphadenectomy is the most component of the surgical procedure, providing survival benefits in the early stages of endometrial cancer (57). However, it could increase morbidity and postoperative complications (58). Yet it is important to emphasize that there is usually a more difficult procedure to readily evaluate MI, LVSI, and LNM on a frozen diagnosis. Thus, it is liable to measure tumor diameter macroscopically. In addition, it is more feasible to measure the tumor size before surgery. Our pooling data have shown that tumor size >20 mm was significantly correlated with higher incidences of LNM, whether in surgically FIGO stage I or FIGO stage I–IV. Based on our results, tumor size from intraoperative and preoperative could plan the surgery strategy, which may minimize the risk of complications, lower the burden of operation, and decrease morbidity or mortality.

Han et al. investigated different prognostic factors for the recurrence in stage IA and IB endometrial cancer. MI was the prognostic factor in stage IA, whereas the grade was the prognostic factor in stage IB (59). Our findings disclosed that the prevalence of tumor size >20 mm increased the risk of recurrence in FIGO IA endometrial cancer. We also found out that tumor

size >20 mm significantly predicted higher recurrence in FIGO I–II/I–III endometrial cancer. Multivariate analysis showed that LVSI and depth of MI were independent risks for recurrence (49). Our pooled analysis also showed that tumor size >20 mm was a risk associated with LVSI and depth of MI, as well as higher recurrence. As it turned out, tumor size >20 mm was related to a greater risk of OS based on univariate survival analysis. Furthermore, we discovered that tumor size >20 mm could predict poorer OS in endometrial cancer.

Currently, gynecologists usually do not attach great importance to tumor size. In the evaluation criteria for the surgical–pathological staging, treatment, and prognosis of endometrial cancer, tumor size was rarely covered, and thereby its role may be underestimated. The relationship between tumor size and MI, LVSI, LNM, recurrence, and OS remains controversial. Therefore, we conducted this meta-analysis to investigate the relationship between primary tumor size of 20 mm and clinicopathological parameters, recurrence, and OS. The results showed that tumor size >20 mm was an independent predictive factor for the depth of MI, positive LVSI, positive LNM, recurrence, and poor OS, indicating the importance of tumor size. Tumor size >20 mm may provide additional information before surgery. Therefore, it is more important to take into account the value of tumor size in the clinicopathological staging of endometrial carcinoma.

The strength of the study was the first meta-analysis to discuss the value of tumor size >20 mm to predict clinicopathological outcomes and recurrence in patients with endometrial cancer. Nonetheless, the limitations of this meta-analysis included retrospective and non-randomized studies. In addition, the different cutoffs of tumor size will directly affect the association with the outcome. Other tumor sizes were not studied in the meta-analysis. A standardized cutoff of tumor size for future trials and studies should be highlighted.

CONCLUSION

The meta-analysis showed that tumor size >20 mm was an independent predictive factor for the depth of MI, positive LVSI, positive LNM, recurrence, and poor OS, indicating the importance of tumor size in endometrial cancer. Therefore, it is more important to take into account the value of tumor size in the clinicopathological staging of endometrial carcinoma. Tumor size >20 mm should be integrated into the intraoperative algorithm for performing a full surgical staging.

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 authors.

AUTHOR CONTRIBUTIONS

CS and XJ contributed equally to this work. CS and XJ: conceptualization, literature retrieval, data acquisition, and writing of the manuscript. XY and YY: statistical analysis. JW and XC: manuscript review and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International Patterns and Trends in Endometrial Cancer Incidence, 1978-2013. *J Natl Cancer Inst* (2018) 110 (4):354–61. doi: 10.1093/jnci/djx214
2. Barnes MN, Kilgore LC. Complete Surgical Staging of Early Endometrial Adenocarcinoma: Optimizing Patient Outcomes. *Semin Radiat Oncol* (2000) 10(1):3–7. doi: 10.1016/S1053-4296(00)80014-4
3. Morrow CP, Bundy BN, Kurman RJ, Creasman WT, Heller P, Homesley HD, et al. Relationship Between Surgical-Pathological Risk Factors and Outcome in Clinical Stage I and II Carcinoma of the Endometrium: A Gynecologic Oncology Group Study. *Gynecol Oncol* (1991) 40(1):55–65. doi: 10.1016/0090-8258(91)90086-K
4. Creasman WT, Morrow CP, Bundy BN, Homesley HD, Graham JE, Heller PB. Surgical Pathologic Spread Patterns of Endometrial Cancer. *A Gynecol Oncol Group Study Cancer* (1987) 60(8 Suppl):2035–41. doi: 10.1002/1097-0142(19901015)60:8+<2035::aid-cncr2820601515>3.0.co;2-8
5. Nwachukwu C, Baskovic M, Von Eyben R, Fujimoto D, Giaretta S, English D, et al. Recurrence Risk Factors in Stage IA Grade 1 Endometrial Cancer. *J Gynecol Oncol* (2021) 32(2):e22. doi: 10.3802/jgo.2021.32.e22
6. Gadducci A, Cavazzana A, Cosio S, DIC C, Tana R, Fanucchi A, et al. Lymph-Vascular Space Involvement and Outer One-Third Myometrial Invasion Are Strong Predictors of Distant Haematogeneous Failures in Patients With Stage I-II Endometrioid-Type Endometrial Cancer. *Anticancer Res* (2009) 29 (5):1715–20. doi: 10.31838/srp.2020.5.26
7. Güngördük K, Firat Cüylan Z, Kahramanoglu I, Oge T, Akbayir O, Dede M, et al. Risk Factors for Recurrence in Low-Risk Endometrial Cancer: A Case-Control Study. *Oncol Res Treat* (2018) 41(7-8):466–70. doi: 10.1159/000488112
8. Li M, Wang Z, Zhao L, Li X, Wang J, Zhang C, et al. Predictors of Recurrence and Prognosis in Patients With Stage I and II Endometrial Carcinoma. *Zhonghua Fu Chan Ke Za Zhi* (2014) 49(6):455–9. doi: 10.26226/morressier.599bdc79d462b80296ca1138
9. Gusberg SB, Jones HCJr., Tovell HM. Selection of Treatment for Corpus Cancer. *Am J Obstet Gynecol* (1960) 80:374–80. doi: 10.1016/0002-9378(60)90141-1
10. Riggs MJ, Cox Bauer CM, Miller CR, Aden JK, Kamelle SA. Validation of an Endometrial Tumor Diameter Model for Risk Assessment in the Absence of Lymph Node Mapping. *J Patient Cent Res Rev* (2020) 7(4):323–8. doi: 10.17294/2330-0698.1768
11. Mariani A, Webb MJ, Keeney GL, Haddock MG, Calori G, Podratz KC. Low-Risk Corpus Cancer: Is Lymphadenectomy or Radiotherapy Necessary? *Am J Obstet Gynecol* (2000) 182(6):1506–19. doi: 10.1067/mob.2000.107335
12. Cox Bauer CM, Greer DM, Kram JJF, Kamelle SA. Tumor Diameter as a Predictor of Lymphatic Dissemination in Endometrioid Endometrial Cancer. *Gynecol Oncol* (2016) 141(2):199–205. doi: 10.1016/j.ygyno.2016.02.017
13. Pollom EL, Conklin CM, von Eyben R, Folkins AK, Kidd EA. Nomogram to Predict Risk of Lymph Node Metastases in Patients With Endometrioid Endometrial Cancer. *Int J Gynecol Pathol* (2016) 35(5):395–401. doi: 10.1097/PGP.0000000000000246

14. Kilts TP, Glaser GE, Langstraat CL, Kumar A, Weaver AL, Mc Gree ME, et al. Comparing Risk Stratification Criteria for Predicting Lymphatic Dissemination in Endometrial Cancer. *Gynecol Oncol* (2019) 155(1):21–6. doi: 10.1016/j.ygyno.2019.08.005
15. Akar S, Çelik ZE, Fındık S, İlhan TT, Ercan F, Çelik Ç. Prognostic Significance of Solid Growth in Endometrioid Endometrial Adenocarcinoma. *Int J Clin Oncol* (2020) 25(1):195–202. doi: 10.1007/s10147-019-01529-4
16. AlHilli MM, Mariani A, Bakkum-Gamez JN, Dowdy SC, Weaver AL, Peethambaram PP, et al. Risk-Scoring Models for Individualized Prediction of Overall Survival in Low-Grade and High-Grade Endometrial Cancer. *Gynecol Oncol* (2014) 133(3):485–93. doi: 10.1016/j.ygyno.2014.03.567
17. Boyraz G, Salman MC, Gultekin M, Basaran D, Cagan M, Ozgul N, et al. Incidence of Lymph Node Metastasis in Surgically Staged FIGO IA G1/G2 Endometrial Cancer With a Tumor Size of More Than 2 Cm. *Int J Gynecol Cancer* (2017) 27(3):486–92. doi: 10.1097/IGC.0000000000000919
18. Boyraz G, Atalay FO, Salman MC, Usubutun A, Erturk A, Gultekin M, et al. Comparison of Mayo and Milwaukee Risk Stratification Models for Predicting Lymph Node Metastasis in Endometrial Cancer. *Int J Gynecol Cancer* (2018) 28(5):869–74. doi: 10.1097/IGC.0000000000001261
19. Chang SJ, Kong TW, Kim WY, Yoo SC, Yoon JH, Chang KH, et al. Lymph-Vascular Space Invasion as a Significant Risk Factor for Isolated Para-Aortic Lymph Node Metastasis in Endometrial Cancer: A Study of 203 Consecutive Patients. *Ann Surg Oncol* (2011) 18(1):58–64. doi: 10.1245/s10434-010-1206-x
20. Doll KM, Tseng J, Denslow SA, Fader AN, Gehrig PA. High-Grade Endometrial Cancer: Revisiting the Impact of Tumor Size and Location on Outcomes. *Gynecol Oncol* (2014) 132(1):44–9. doi: 10.1016/j.ygyno.2013.10.023
21. Al-Dali F, Puig M, Lopez C, Fernandez M, Salanas G, Ojeda F. Tumor Size as Prognostic Factor of Lymph Node Involvement in Endometrial Cancer. *Int J Gynecol Cancer* (2019) 29:A297. doi: 10.1136/ijgc-2019-ESGO.527
22. Gilani S, Anderson I, Fathallah L, Mazzara P. Factors Predicting Nodal Metastasis in Endometrial Cancer. *Arch Gynecol Obstet* (2014) 290(6):1187–93. doi: 10.1007/s00404-014-3330-5
23. Günakan E, Atan S, Haberal AN, Küçükyıldız İA, Gökçe E, Ayhan A. A Novel Prediction Method for Lymph Node Involvement in Endometrial Cancer: Machine Learning. *Int J Gynecol Cancer* (2019) 29(2):320–4. doi: 10.1136/ijgc-2018-000033
24. Karalok A, Turan T, Basaran D, Turkmen O, Comert Kimyon G, Tulunay G, et al. Lymph Node Metastasis in Patients With Endometrioid Endometrial Cancer: Overtreatment Is the Main Issue. *Int J Gynecol Cancer* (2017) 27(4):748–53. doi: 10.1097/IGC.0000000000000937
25. Lee KB, Ki KD, Lee JM, Lee JK, Kim JW, Cho CH, et al. The Risk of Lymph Node Metastasis Based on Myometrial Invasion and Tumor Grade in Endometrioid Uterine Cancers: A Multicenter, Retrospective Korean Study. *Ann Surg Oncol* (2009) 16(10):2882–7. doi: 10.1245/s10434-009-0535-0
26. Li M, Wu S, Xie Y, Zhang X, Wang Z, Zhu Y, et al. Cervical Invasion, Lymphovascular Space Invasion, and Ovarian Metastasis as Predictors of Lymph Node Metastasis and Poor Outcome on Stages I to III Endometrial Cancers: A Single-Center Retrospective Study. *World J Surg Oncol* (2019) 17(1):193. doi: 10.1186/s12957-019-1733-2
27. Mahdi H, Munkarah AR, Ali-Fehmi R, Woessner J, Shah SN, Moslemi-Kebria M. Tumor Size is an Independent Predictor of Lymph Node Metastasis and Survival in Early Stage Endometrioid Endometrial Cancer. *Arch Gynecol Obstet* (2015) 292(1):183–90. doi: 10.1007/s00404-014-3609-6
28. Matsushita C, Fujiwara H, Takei Y, Saga Y, Machida S, Taneichi A, et al. New Criteria for the Omission of Lymphadenectomy in Endometrioid Carcinoma. *Int J Gynecol Cancer* (2019) 29(3):541–6. doi: 10.1136/ijgc-2018-000044
29. Milam MR, Java J, Walker JL, Metzinger DS, Parker LP, Coleman RL. Nodal Metastasis Risk in Endometrioid Endometrial Cancer. *Obstet Gynecol* (2012) 119(2 Pt 1):286–92. doi: 10.1097/AOG.0b013e318240de51
30. Oz M, Korkmaz V, Meydanli MM, Sari ME, Cuylan ZF, Gungor T. Is Tumor Size Really Important for Prediction of Lymphatic Dissemination in Grade 1 Endometrial Carcinoma With Superficial Myometrial Invasion? *Int J Gynecol Cancer* (2017) 27(7):1393–8. doi: 10.1097/IGC.0000000000001025
31. Pavlakis K, Rodolakis A, Vagios S, Voulgaris Z, Messini I, Yiannou P, et al. Identifiable Risk Factors for Lymph Node Metastases in Grade 1 Endometrial Carcinoma. *Int J Gynecol Cancer* (2017) 27(8):1694–700. doi: 10.1097/IGC.0000000000001070
32. Rathod PS, Shakuntala PN, Pallavi VR, Kundaragi R, Shankaranand B, Vijay CR, et al. The Risk and Pattern of Pelvic and Para Aortic Lymph Nodal Metastasis in Patients With Intermediate and High Risk Endometrial Cancer. *Indian J Surg Oncol* (2014) 5(2):109–14. doi: 10.1007/s13193-014-0303-x
33. Sari ME, Yalcin İ, Sahin H, Meydanli MM, Gungor T. Risk Factors for Paraaortic Lymph Node Metastasis in Endometrial Cancer. *Int J Clin Oncol* (2017) 22(5):937–44. doi: 10.1007/s10147-017-1139-5
34. Shah C, Johnson EB, Everett E, Tamimi H, Greer B, Swisher E, et al. Does Size Matter? Tumor Size and Morphology as Predictors of Nodal Status and Recurrence in Endometrial Cancer. *Gynecol Oncol* (2005) 99(3):564–70. doi: 10.1016/j.ygyno.2005.06.011
35. Tecellioglu FS, Akpolat N, Sahin N. Mmp-9 and Fascin-1 Expression in Endometrioid-Type Endometrial Carcinoma and Their Prognostic Value. *Indian J Gynecol Oncol* (2021) 19(1):24–30. doi: 10.1007/s40944-020-00492-7
36. Turan T, Hizli D, Sarici S, Boran N, Gundogdu B, Karadag B, et al. Is it Possible to Predict Para-Aortic Lymph Node Metastasis in Endometrial Cancer? *Eur J Obstet Gynecol Reprod Biol* (2011) 158(2):274–9. doi: 10.1016/j.ejogrb.2011.04.031
37. Vaizoglu F, Yuce K, Salman MC, Basaran D, Calis P, Ozgul N, et al. Lymphovascular Space Involvement is the Sole Independent Predictor of Lymph Node Metastasis in Clinical Early Stage Endometrial Cancer. *Arch Gynecol Obstet* (2013) 288(6):1391–7. doi: 10.1007/s00404-013-2913-x
38. Vargas R, Rauh-Hain JA, Clemmer J, Clark RM, Goodman A, Growdon WB, et al. Tumor Size, Depth of Invasion, and Histologic Grade as Prognostic Factors of Lymph Node Involvement in Endometrial Cancer: A SEER Analysis. *Gynecol Oncol* (2014) 133(2):216–20. doi: 10.1016/j.ygyno.2014.02.011
39. Watanabe M, Aoki Y, Kase H, Fujita K, Tanaka K. Low Risk Endometrial Cancer: A Study of Pelvic Lymph Node Metastasis. *Int J Gynecol Cancer* (2003) 13(1):38–41. doi: 10.1136/ijgc-00009577-200301000-00007
40. Zanfagnin V, Huang Y, Mc Gree ME, Weaver AL, Casarin J, Multinu F, et al. Predictors of Extensive Lymphatic Dissemination and Recurrences in Node-Positive Endometrial Cancer. *Gynecol Oncol* (2019) 154(3):480–6. doi: 10.1016/j.ygyno.2019.07.006
41. Ilker S, Nilufer C, Firat CZ, Bulent O, Hatice B, Tayfun G. Predicting Lympho-Vascular Space Invasion in Endometrial Cancers With Mucinous Carcinomatous Components. *Asian Pac J Cancer Prev* (2015) 16(10):4247–50. doi: 10.7314/APJCP.2015.16.10.4247
42. Oliver-Perez MR, Magriña J, Villalain-Gonzalez C, Jimenez-Lopez JS, Lopez-Gonzalez G, Barcena C, et al. Lymphovascular Space Invasion in Endometrial Carcinoma: Tumor Size and Location Matter. *Surg Oncol* (2021) 37:101541. doi: 10.1016/j.suronc.2021.101541
43. Ayhan A, Şahin H, Sari ME, Yalçin I, Haberal A, Meydanli MM. Prognostic Significance of Lymphovascular Space Invasion in Low-Risk Endometrial Cancer. *Int J Gynecol Cancer* (2019) 29(3):505–12. doi: 10.1136/ijgc-2018-000069
44. Laufer J, Scasso S, Papadia A, Sosa C, Cirillo F, Raspagliesi F. Association Between Tumor Diameter and Lymphovascular Space Invasion Among Women With Early-Stage Endometrial Cancer. *Int J Gynaecol Obstet* (2013) 123(2):142–5. doi: 10.1016/j.ijgo.2013.05.012
45. Schink JC, Rademaker AW, Miller DS, Lurain JR. Tumor Size in Endometrial Cancer. *Cancer* (1991) 67(11):2791–4. doi: 10.1002/1097-0142(19910601)67:11<2791::AID-CNCR2820671113>3.0.CO;2-S
46. Bendifallah S, Canlorbe G, Huguet F, Coutant C, Hudry D, Graesslin O, et al. A Risk Scoring System to Determine Recurrence in Early-Stage Type 1 Endometrial Cancer: A French Multicentre Study. *Ann Surg Oncol* (2014) 21(13):4239–45. doi: 10.1245/s10434-014-3864-6
47. Çakır C, Kılıç İ, Yüksel D, Karyal YA, Üreyen I, Boyraz G, et al. Does Tumor Size Have Prognostic Value in Patients Undergoing Lymphadenectomy in Endometrioid-Type Endometrial Cancer Confined to the Uterine Corpus? *Turk J Med Sci* (2019) 49(5):1403–10. doi: 10.3906/sag-1902-224
48. Marcickiewicz J, Sundfeldt K. Accuracy of Intraoperative Gross Visual Assessment of Myometrial Invasion in Endometrial Cancer. *Acta Obstet Gynecol Scand* (2011) 90(8):846–51. doi: 10.1111/j.1600-0412.2011.01166.x
49. Roma AA, Rybicki LA, Barbuto D, Euscher E, Djordjevic B, Frauenhoffer E, et al. Risk Factor Analysis of Recurrence in Low-Grade Endometrial Adenocarcinoma. *Hum Pathol* (2015) 46(10):1529–39. doi: 10.1016/j.humpath.2015.06.015

50. Yamada S, Tsuyoshi H, Yamamoto M, Tsujikawa T, Kiyono Y, Okazawa H, et al. Prognostic Value of 16α-(18)F-Fluoro-17β-Estradiol PET as a Predictor of Disease Outcome in Endometrial Cancer: A Prospective Study. *J Nucl Med* (2021) 62(5):636–42. doi: 10.2967/jnumed.120.244319
51. Numazaki R, Miyagi E, Konnai K, Ikeda M, Yamamoto A, Onose R, et al. Analysis of Stage IVB Endometrial Carcinoma Patients With Distant Metastasis: A Review of Prognoses in 55 Patients. *Int J Clin Oncol* (2009) 14(4):344–50. doi: 10.1007/s10147-009-0878-3
52. AlHilli MM, Podratz KC, Dowdy SC, Bakkum-Gamez JN, Weaver AL, McGree ME, et al. Preoperative Biopsy and Intraoperative Tumor Diameter Predict Lymph Node Dissemination in Endometrial Cancer. *Gynecol Oncol* (2013) 128(2):294–9. doi: 10.1016/j.ygyno.2012.10.009
53. Wang J, Xu P, Yang X, Yu Q, Xu X, Zou G, et al. Association of Myometrial Invasion With Lymphovascular Space Invasion, Lymph Node Metastasis, Recurrence, and Overall Survival in Endometrial Cancer: A Meta-Analysis of 79 Studies With 68,870 Patients. *Front Oncol* (2021) 11:762329. doi: 10.3389/fonc.2021.762329
54. Keys HM, Roberts JA, Brunetto VL, Zaino RJ, Spirtos NM, Bloss JD, et al. A Phase III Trial of Surgery With or Without Adjunctive External Pelvic Radiation Therapy in Intermediate Risk Endometrial Adenocarcinoma: A Gynecologic Oncology Group Study. *Gynecol Oncol* (2004) 92(3):744–51. doi: 10.1016/j.ygyno.2003.11.048
55. dos Reis R, Burzawa JK, Tsunoda AT, Hosaka M, Frumovitz M, Westin SN, et al. Lymphovascular Space Invasion Portends Poor Prognosis in Low-Risk Endometrial Cancer. *Int J Gynecol Cancer* (2015) 25(7):1292–9. doi: 10.1097/IGC.0000000000000490
56. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-Up. *Radiother Oncol* (2015) 117(3):559–81. doi: 10.1016/j.radonc.2015.11.013
57. Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. Efficacy of Systematic Pelvic Lymphadenectomy in Endometrial Cancer (MRC ASTEC Trial): A Randomised Study. *Lancet* (2009) 373(9658):125–36. doi: 10.1016/s0140-6736(08)61766-3
58. Todo Y, Sakuragi N. Systematic Lymphadenectomy in Endometrial Cancer. *J Obstet Gynaecol Res* (2013) 39(2):471–7. doi: 10.1111/j.1447-0756.2012.02062.x
59. Han KH, Kim HS, Lee M, Chung HH, Song YS. Prognostic Factors for Tumor Recurrence in Endometrioid Endometrial Cancer Stages IA and IB. *Med (Baltimore)* (2017) 96(21):e6976. doi: 10.1097/MD.0000000000006976

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A Model to Identify Candidates for Lymph Node Dissection Among Patients With High-Risk Endometrial Endometrioid Carcinoma According to Mayo Criteria

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Background: The Mayo criteria are the most widely accepted algorithm for predicting the risk of lymph node metastasis in endometrial endometrioid carcinoma (EEC). However, the clinical value of these criteria in high-risk patients is limited and inconclusive.

Methods: A total of 240 patients with EEC meeting the Mayo high-risk criteria between January 1, 2015, and December 31, 2018 were included in our study. We retrospectively collected the laboratory reports, basic clinical information, clinicopathological and immunohistochemistry (IHC) findings, and the sequences of molecular pathological markers of these patients. A nomogram for predicting the likelihood of positive lymph node status was established based on these parameters.

Results: Among the 240 patients, 17 were diagnosed with lymph node metastasis. The univariable analyses identified myometrial invasion >50%, aberrant p53 expression, microsatellite instable (MSI), and cancer antigen 125 (CA125) ≥35 U/ml as potential risk factors for lymph node metastasis. The multivariable analyses showed that aberrant p53 expression, MSI, and CA125 ≥35 U/ml were independent predictors of lymph node metastasis. The area under the curve (AUC) for the nomogram was 0.870, as compared to 0.665 for the Mayo criteria.

Conclusions: Our novel prediction model effectively identifies patients at high risk for lymphatic metastasis. This model is a promising strategy for personalized surgery in patients with high risk according to the Mayo criteria.

Keywords: endometrial, endometrioid carcinoma, lymph node dissection, Mayo criterion, molecular pathological markers, serum CA125

INTRODUCTION

Endometrial endometrioid carcinoma (EEC) is a surgically staged disease (1). Regional lymph node metastasis is the most important factor for determining prognosis and recommending treatment. Traditionally, primary surgical treatment includes total hysterectomy, bilateral salpingo-oophorectomy (TH/BSO), and standard lymph-node dissection (LND) (2). EEC is typically hormone sensitive and is often accompanied by moderate malignancy, along with obvious symptoms exhibited in early-stage disease. Thus, most patients are diagnosed at early stages without lymph node metastasis; the potential morbidity of routine LND may outweigh clinical benefits. Nowadays, whether and to what extent LND is necessary remain controversial. The acceptance and indications of LND vary among countries and facilities (3, 4).

The Mayo criteria are the most widely accepted algorithm for predicting the risk of lymph node metastasis in EEC (5, 6). They are largely based on specific preoperative and intraoperative clinicopathological findings (7) and categorize low-risk patients with EEC as those meeting the following characteristics: tumor diameter ≤2 cm, grade 1 or 2, and myometrial invasion (MI) ≤50%. In contrast, high-risk patients have tumors with >50% myometrial invasion, grade 3 histology, or tumor size >2 cm.

The reported lymph node involvement risk for patients classified as low and high risk according to the Mayo criteria were 1.4% and 6.4%, respectively (8). Most institutions in China today omit systematic LND in patients with EEC meeting the Mayo criteria for low risk. The Mayo criteria help avoid unnecessary systematic lymphadenectomy in patients with features of low-risk EEC. However, the clinical value of these criteria in high-risk patients is limited and inconclusive. Surgical staging with lymphadenectomy is routinely performed in most clinics in patients with high-risk EEC according to Mayo criteria; however, considerable overtreatment remains.

The molecular-based classification introduced by The Cancer Genome Atlas (TCGA) has initiated a new era and tremendous infusion of hope for individualized surgical treatment in endometrial cancer. A novel pragmatic molecular classifier using immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded (FFPE) tissues has recently been validated (9). The Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) identifies four molecular subtypes (10), which are analogous—but not identical—to the four genomic subtypes described in the Cancer Genome Atlas (11, 12). The ProMisE is reported to be a pragmatic molecular classifier to category endometrial cancers with different prognosis (10).

We retrospectively collected the laboratory reports, basic clinical information, clinicopathological and IHC findings, and the sequences of molecular pathological markers of patients with EEC meeting the Mayo high-risk criteria. We found that the combination of preoperative serum cancer antigen 125 (CA125) level and molecular parameters with Mayo criteria improved the prognostic accuracy of lymph node metastasis risk in patients with high-risk EEC per Mayo criteria. This investigation aimed to develop a modified model based on the Mayo criteria, with adequate accuracy to predict negative nodes and could be used to provide precise guidance on the scope of surgery in patients with high-risk EEC per Mayo criteria. To our knowledge, no similar research has yet been published.

MATERIAL AND METHODS

Study Population and Surgical Procedure

Data of patients with EEC who underwent surgical treatments at the Department of Gynecology at Shanghai First Maternity and Infant Hospital between January 2015 and December 2018 were retrospectively evaluated. The inclusion criteria were (1) EEC diagnosed by two gynecological pathologists, (2) complete clinical and pathological data, and (3) high risk according to the Mayo criteria. The exclusion criteria were (1) lymphadenectomy not performed during the primary surgery, (2) multiple primary tumors, and (3) patients administered neoadjuvant chemotherapy. Overall, 240 patients met the inclusion criteria. Written informed consent for the use of their biospecimens for research purposes was obtained from all patients before treatment. Research ethics approval for the tissue/biospecimen analysis and this project was granted by the review board of Shanghai First Maternity and Infant Hospital of Tongji University School of Medicine.

All patients underwent preoperative magnetic resonance imaging (MRI) to evaluate cervical invasion. Patients indicative of having gross cervical involvement received radical TH/BSO. Patients diagnosed with grade 3 disease underwent simultaneous paraaortic lymphadenectomy according to National Comprehensive Cancer Network (NCCN) guidelines. All operations were performed by the same gynecologic oncologist. The extent of the LND was the same regardless of the surgical technique (open or laparoscopic). Systematic pelvic lymphadenectomy included resection of the internal and external iliac, medial and lateral deep inguinal, obturator, sacral, and common iliac nodes. Para-aortic lymphadenectomy included the systematic resection of all nodes from the precaval, laterocaval, interaortocaval, preaortic, and lateroaortic areas up to the inferior mesenteric vein. Each specimen was collected separately according to its anatomical location for selective histopathological examination.

Variables and Definitions

The patients' tumors were staged according to the 2009 International Federation of Gynecology and Obstetrics staging system. Histological type was determined according to the World Health Organization classification.

The uterus was bisected to inspect the endometrial surface during frozen section. The tumor diameter was defined as the largest dimension of the lesion. In cases with more than one lesion, only the lesion with the largest diameter was considered. The extent of MI was categorized as ≤50% or >50%. For the frozen examination of MI, the uterus is bisected along the longitudinal axis and then serially sectioned from lower uterine segment to the fundus. Gross assessment is performed to figure

out the lesion and to identify the area concerning for deepest invasion. Then, cancer tissues were biopsied carefully to ensure all tumor sites were included. Full-thickness representative sections are submitted for frozen section examination to assess the maximum depth of myometrial invasion.

Pretreatment serum CA125 level was determined by radioimmunoassay (RIA) (Abbott Diagnostics, Abbott Park, IL). The concentration was considered increased for values ≥35 U/ml.

The formalin-fixed and paraffin-embedded tissues from the hysterectomy specimens were collected for IHC analyses. Immunostaining was performed at the Pathology Department of Shanghai First Maternity and Infant Hospital. The immunostaining results were assessed independently by two pathologists blinded to the patient characteristics and outcome. Tissue sections were incubated overnight with primary antibodies against p53 (clone DO-7, 1:2,000, Neomarkers), MLH1 (clone ES05, 1:100, DAKO), MSH2 (clone FE11,1:100, DAKO), and MSH6 (clone EPR3945, 1:800, GENE TEX) at room temperature or with primary antibody PMS2 (clone EP51, 1:50, DAKO), anti-SPOP (ab81163, Abcam), ER (clone SP1, Denmark), and PR (clone IE2, Denmark) at 4°C. A linker (mouse linker, SM804, DAKO; rabbitlinker, SM805, DAKO) was used afterwards. A 30-min incubation with a secondary antibody (Poly-HRP-GAM/R/R; DPV0110HRP; ImmunoLogic) was then performed. DAB+ (K3468, DAKO) was used as chromogen, and sections were counterstained with hematoxylin. Immunostaining for p53 was considered aberrant if a completely negative or strongly positive staining was observed in >75% of tumor cells (nuclear or cytoplasmic). Mismatch repair protein status was also investigated. Tumors were considered microsatellite instable (MSI) if the tumor cells showed a loss of nuclear staining of at least one mismatch repair protein among MLH1, MSH2, MSH6, and PMS. Tumor cells exhibiting nuclear positivity for all mismatch repair proteins were categorized as mismatch-repair (MSS) positive. Estrogen receptor (ER) and progesterone receptor (PR) were scored as positive when at least 10% of tumor cells showed nuclear expression. For the identification of DNA polymerase epsilon, catalytic subunit (POLE) exonuclease domain hotspot mutations, Sanger sequencing was used to analyze exons 9, 12, 13, and 14 (13).

Statistical Analysis

Descriptive statistics of the demographic and clinical–pathological characteristics are reported as frequencies and proportions for categorical variables and medians and interquartile ranges for continuous variables. Univariable logistic regression analyses were conducted to assess the potential predictors for lymph node metastasis. Next, all parameters significantly associated with lymph node metastasis in univariate analyses and variables that might be related to lymph node metastasis according to clinical relevance were included in the full multivariable model and were selected to develop the final nomogram.

The nomogram performance was assessed by discrimination and calibration. Discrimination in the current context was the ability to differentiate between women with and without lymph node metastasis. This assessment was performed using the receiver operating characteristic (ROC)-derived area under the curve (AUC). A calibration plot with 2,000 bootstrap replications was used to assess the agreement between the observed incidence and the nomogram-predicted probability. The optimal cutoff point of the nomogram was estimated by Youden's J index. A decision-curve analysis (DCA) was used to determine the clinical net benefit associated with the use of the model.

All statistical tests were performed using IBM SPSS Statistics for Windows, version 22.0, and R statistical package v.3.4.4 (R Project for Statistical Computing, www.r-project.org). All tests were two-sided, with a significance level set at $p < 0.05$.

RESULTS

Clinical Patient Characteristics

We identified 467 women who were eligible for the study between January 1, 2015, and December 31, 2018. Among these, 240 patients met the inclusion criteria. The demographic and clinical data of these 240 patients are presented in **Table 1**. The median age of the cohort was 55 years [interquartile range (IQR), 49.00–60.25 years]. Most patients were overweight, with a median body mass index (BMI) of 24.60 kg/m^2 (IQR, 22.70–26.60 kg/m^2). In this population, 223 patients (92.9%) were staged as between IA and IIIB, whereas 17 patients (7.1%) were diagnosed with advanced disease (IIIC–IV). All patients underwent pelvic lymphadenectomy, among whom 25 patients with G3 differentiation also underwent para-aortic lymphadenectomy. Among all patients, 17 (7.08%) were diagnosed with lymph node metastasis. Age at diagnosis, BMI, histology differentiation, and tumor diameter did not differ between patients with EEC with and without lymph node metastasis. MI, kg/m^2, and cervix involvement differed between the two groups (all p<0.03).

Univariable and Multivariable Models Predicting Lymph Node Metastasis

The univariable analyses identified MI >50% [odds ratio (OR), 4.160; 95% confidence interval (CI), 1.527–11.947), aberrant p53 expression (OR, 11.618; 95%CI, 3.442–37.778), MSI (OR, 4.577; 95%CI, 1.660–12.853), and CA125 ≥35 (OR, 6.865; 95% CI, 2.481–20.840) as potential risk factors for lymph node metastasis (**Table 2**, all p<0.01). All these variables, and histological grade (for clinical relevance consideration), were included in the multivariable logistic regression model. The multivariable analyses showed that aberrant p53 expression (OR, 12.661; 95%CI, 3.006–57.364), MSI (OR, 4.414; 95%CI, 1.331–15.326), and CA125 ≥35 (OR, 5.309; 95%CI, 1.563–20.013) were independent predictors of lymph node metastasis. The nomogram also included histological grade and MI because of their clinical relevance. The results of the univariate and multivariable analyses are presented in **Table 2**.

TABLE 1 | The demographics and pathological characteristics of the patients.

n	Overall 240	Negative Lymph Nodes 223	Positive Lymph Nodes 17	p
Age	55.00 [49.00, 60.25]	55.00 [50.00, 61.00]	50.00 [44.00, 58.00]	0.141
<60	167 (69.6)	154 (69.1)	13 (76.5)	0.714
≥60	73 (30.4)	69 (30.9)	4 (23.5)	
BMI (median [IQR])	24.60 [22.70, 26.60]	24.60 [22.70, 26.60]	25.50 [23.60, 26.60]	0.313
FIGO 2009 stage				
IA	152 (63.3)	152 (68.2)	0 (0.0)	<0.001
IB	42 (17.5)	42 (18.8)	0 (0.0)	
II	27 (11.2)	27 (12.1)	0 (0.0)	
IIIA	1 (0.4)	1 (0.4)	0 (0.0)	
IIIB	1 (0.4)	1 (0.4)	0 (0.0)	
IIIC1	15 (6.2)	0 (0.0)	15 (88.2)	
IIIC2	2 (0.8)	0 (0.0)	2 (11.8)	
Histology				
1–2	215 (89.6)	201 (90.1)	14 (82.4)	0.548
3	25 (10.4)	22 (9.9)	3 (17.6)	
Primary tumor size				
<20 mm	9 (3.8)	9 (4.0)	0 (0.0)	0.856
≥20 mm	231 (96.2)	214 (96.0)	17 (100.0)	
Myometrial invasion				
≤50%	173 (72.1)	166 (74.4)	7 (41.2)	0.008
>50%	67 (27.9)	57 (25.6)	10 (58.8)	
LVSI				
Negative	197 (82.1)	196 (87.9)	1 (5.9)	<0.001
Positive	43 (17.9)	27 (12.1)	16 (94.1)	
Involving cervix				
Negative	206 (85.8)	195 (87.4)	11 (64.7)	0.026
Positive	34 (14.2)	28 (12.6)	6 (35.3)	
p53				
Normal	224 (93.3)	213 (95.5)	11 (64.7)	<0.001
Aberrant	16 (6.7)	10 (4.5)	6 (35.3)	
dMMR				
MSS	187 (77.9)	179 (80.3)	8 (47.1)	0.004
MSI	53 (22.1)	44 (19.7)	9 (52.9)	
Ca125				
<35	182 (75.8)	176 (78.9)	6 (35.3)	<0.001
≥35	58 (24.2)	47 (21.1)	11 (64.7)	
POLE				
No mutation	212 (88.3)	197 (88.3)	15 (88.2)	1
Mutation	28 (11.7)	26 (11.7)	2 (11.8)	
PR				
<10%	34 (14.2)	32 (14.3)	2 (11.8)	1
≥10%	206 (85.8)	191 (85.7)	15 (88.2)	

N, number; BMI, body mass index; IQR, interquartile range; LVSI, lymph vascular space invasion.

Development and Validation of the Prediction Model

The final nomogram is as shown in **Figure 1A** and depicts the multivariable effect of each variable on lymph node metastasis. The calibration plot of the predicted probabilities against the observed probabilities of lymph node metastasis indicated excellent concordance (**Figure 1B**). The DCA demonstrated that our nomogram improved clinical risk prediction against the Mayo criteria by comparing the net benefit to a threshold probability of 0–20% (**Figure 1C**). The AUC for the nomogram was 0.870 (95% CI, 0.801–0.938), whereas the bootstrap optimism-corrected AUC was 0.827 as compared to 0.665 (95% CI, 0.528–0.802) for the Mayo criteria (**Figure 2**).

Table 3 lists the errors associated with the use of the novel model to predict lymph node metastasis. Using 3% as the best cutoff point (43 points in the nomogram), 133 unnecessary lymphadenectomies would have been spared, and all patients with lymph node metastasis were taken into account.

Histopathological and Molecular Concordance of Endometrial Tissues From Resected Uterus or Curettage Samples

Histology and p53, and deficient mismatch repair (dMMR) staining were analyzed in endometrial tissues from either the bisected uterus or preoperative curettage specimens. We observed a high consistency between these samples, as shown

TABLE 2 | Univariate and multivariate analysis of lymph node metastasis.

	Univariable analyses		Multivariable analyses	
	OR (95% CI)	P value	OR (95% CI)	p-value
Histological grade				
1–2	1.0		1.0	
3	1.958 (0.428, 6.590)	0.319	1.700 (0.320, 7.149)	0.491
Myometrial invasion				
<50%	1.0		1.0	
≥50%	4.160 (1.527, 11.947)	0.006*	2.067 (0.609, 7.051)	0.238
p53				
Normal	1.0		1.0	
Aberrant	11.618 (3.442, 37.778)	<0.001*	12.661 (3.006, 57.364)	0.001*
dMMR				
MSS	1.0		1.0	
MSI	4.577 (1.660, 12.853)	0.003*	4.414 (1.331, 15.326)	0.015*
CA125				
<35	1.0		1.0	
≥35	6.865 (2.481, 20.840)	<0.001*	5.309 (1.563, 20.013)	0.009*
POLE				
No mutation	1.0			
Mutation	1.010 (0.154, 3.860)	0.99		
PR				
<10%	1.0			
≥10%	1.257 (0.333, 8.212)	0.769		
LVSI				
Negative	1.0			
Positive	116.148 (22.359, 2,138.550)	<0.001		

*$P < 0.05$.

in **Table 4**. The concordance rates for histology, p53 expression, and dMMR were 94.3%, 92.9%, and 84.5%, respectively.

DISCUSSION

The diagnostic accuracy of lymph node status in endometrial cancer is an important issue. Although surgical staging is the golden standard, LND is controversial in EEC because of its long-term morbidity, uncertain treatment value, and high negative lymph node metastasis rate in histology. The accuracy of the existing lymph node metastasis risk models is not satisfactory. Even according to the most accepted Mayo risk-adopted algorithm, more than 70% of patients without lymph node metastasis were overtreated with unnecessary LND. This investigation is the only study of patients with high-risk EEC according to Mayo criteria to structure a model for the assessment of the risk of lymph node metastasis. We developed a novel model by retrospectively analyzing the relationship of lymph node metastasis with preoperative CA125 levels, traditional histology findings, and molecular indicators. Our study further divided high-risk patients per Mayo criteria into two subgroups: those less likely to experience lymph node metastasis and those more likely to have positive lymph nodes in lymphadenectomy. Our novel model helped 55.42% of patients with high-risk EEC according to Mayo criteria avoid LND, and all patients with lymph node metastasis were taken into account.

The Mayo criteria comprise three indicators: tumor size, MI depth, and differentiation. However, in the current study, tumor size was not associated with the lymph node status. This may be partly attributed to the fact that the sizes of most cancers in our study were ≥20 mm (231/240, 96.2%). Tumor size in our study was defined as the diameter of the largest dimension of the lesion in the bisected uterus, which was more accurate than radiological modalities. The signal of the inner zone of endometrial carcinoma is often higher than that at the margin; thus, evaluating tumor size on MRI might ignore the lesion periphery, leading to a smaller measured tumor size than the actual size (14). Similarly, the MI depth was estimated intraoperatively, which was reportedly significantly better than MRI in determining deep MI (15–17). Although the tumor grades did not differ between the lymph nodes metastasis or non-metastasis groups, it was included in our prediction model for clinical relevance consideration.

In contrast to the Mayo criteria, our prediction model included dMMR and p53 expression. The factors were surrogate markers of microsatellite instability and low copy number subgroups of endometrial cancer, as defined by the TCGA, which were associated with intermediate and unfavorable prognoses, respectively (18). The results of our study showed that dMMR and p53 expression were associated with lymph node metastasis in both univariable and multivariable analysis, a finding consistent with those reported previously (19–21). Moreover, elevated serum CA125 level was also associated with lymphatic metastasis in EEC (22, 23), which was also verified by our study. Incorporation of these parameters into the prediction model could improve its performance for the discrimination of low-risk patients among those with Mayo

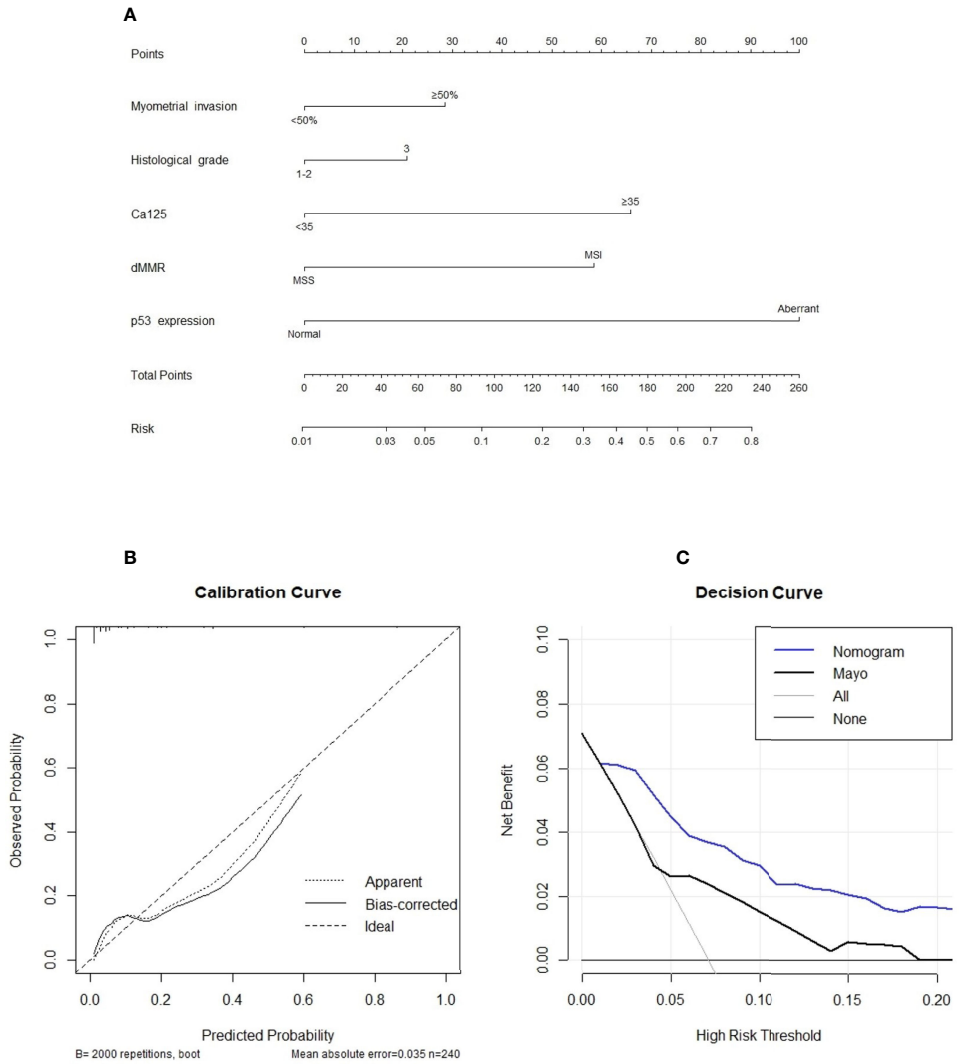
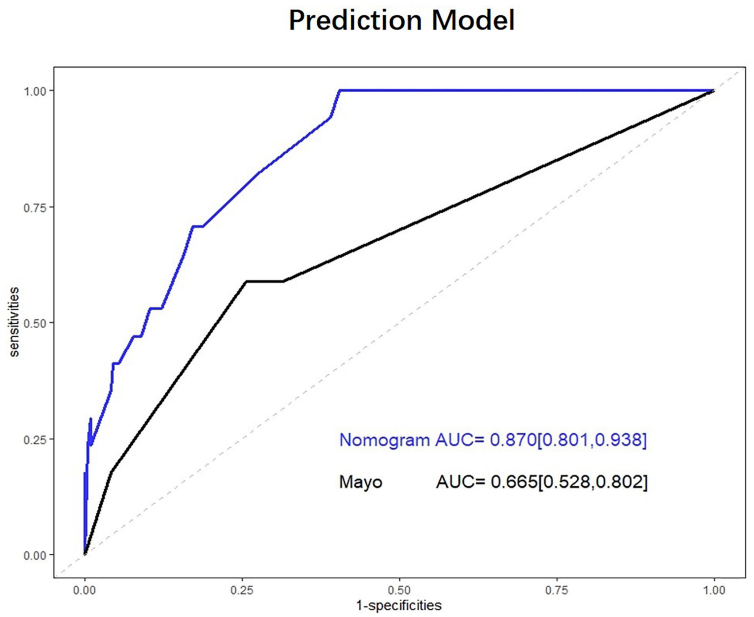


FIGURE 1 | **(A)** A nomogram for predicting the likelihood of positive lymph node status. To use the nomogram, the value for each predictor is determined by first drawing a line upward to the point reference line. The points are then summed and a line is drawn downward from the total points line to determine the predicted probability of node positivity. **(B)** Calibration plot of the observed proportions and predicted probabilities of lymph node metastasis based on the novel nomogram. The predicted probability of pathological lymph node invasion aligns closely with the actual probability. **(C)**, decision curve analyses demonstrating the net benefit associated with the use of the novel nomogram for the detection of lymph node metastasis.

high-risk factors. ROC curve analysis showed that the prediction accuracy of our novel algorithm was 0.870 (95%CI, 0.801–0.938), which was superior to that of the Mayo criteria (AUC=0.665, 95% CI, 0.528–0.802).

Oncologists have attempted to tailor lymphadenectomy according to the combinations of multiple clinical indicators in EEC. For instance, American researchers have established a risk-scoring system for the individualized prediction of lymphatic dissemination. A set of pathological variables, namely, MI, grade, primary tumor diameter, cervical stromal invasion, and lymph-vascular space invasion (metastasis) were incorporated into the nomogram. The internal validation of the nomogram showed good discrimination (AUC=0.88) (24). French oncologists have further provided external validation of this nomogram (25). The predictive accuracy according to the discrimination of the AUC criteria was 0.64 for the nomogram. Recently, several studies have managed to combine clinicopathological parameters and molecular indicators to predict lymph node metastasis in EEC (20, 26–29). It was showed that incorporating molecular indicators can predict lymph node metastasis more accurately (20). However, POLE and MMR are important parameters in the molecular-based classification introduced by TCGA. No published nomogram included these molecular markers. This study addressed this gap based on the integration of traditional pathological parameters with genomic findings to aid doctors in determining treatment.



Bootstrap optimism-corrected AUC		
corrected AUC	optimism	apparent AUC
0.827	0.043	0.870

FIGURE 2 | Receiver operating characteristic (ROC) curve showing the performance of the Mayo criteria and novel model.

Our model did not include lymph-vascular space invasion (LVSI) for several reasons. First, to be utilized as a prediction model, LVSI should be diagnosed by frozen section. However, time constraints, limited sampling, and technical artifacts might lead to erroneous interpretation. A relatively low agreement (68.3%) has been observed for the comparison of LVSI diagnosed by frozen section with that diagnosed by permanent section (30). Second, although LVSI has gained a prominent position in most risk stratification systems for EC (31, 32), the reproducibility among pathologists in the presence (or absence) of LVSI is the Achilles heel of histology diagnosis, with poor reported reproducibility of LVSI assessment and grading in EEC (33). The high variability of LVSI suggests that it cannot be used as a reliable component of the prediction model. Finally, based on the current model, we hope to screen for suitable factors in curettage samples to establish a feasible prediction model through the current model. It is impossible to obtain LVSI information from curettage specimens.

Sentinel lymph node (SLN) mapping is another proposed research path to identify patients at risk for lymph node metastasis (34, 35). However, the requirement for special dyes and imaging systems has impeded its widespread implementation

TABLE 3 | Systematic analyses of the nomogram-derived cutoffs used to discriminate between patients with or without histologically confirmed lymph node metastasis.

Probability of LNM, cutoff(%)	Patients above cutoff with histologically approved LNM	Patients below cutoff without histologically approved LNM	Patients above cutoff without histologically approved LNM	Patients below cutoff with histologically approved LNM	Sensitivity	Specificity	Positive predicted value	Negative predicted value
1	17	0	223	0	1	0	0.07083	
2	17	106	117	0	1	0.475336	0.12687	1
3	17	133	90	0	1	0.596413	0.15888	1
4	16	136	87	1	0.941176	0.609865	0.15534	0.9927
5	14	161	62	3	0.823529	0.721973	0.18421	0.98171
6	12	181	42	5	0.705882	0.811659	0.22222	0.97312
7	12	181	42	5	0.705882	0.811659	0.22222	0.97312
8	12	183	40	5	0.705882	0.820628	0.23077	0.9734
9	11	188	35	6	0.647059	0.843049	0.23913	0.96907
10	11	188	35	6	0.647059	0.843049	0.23913	0.96907

TABLE 4 | Concordance of histopathological features and molecular alterations in endometrial hysterectomy and pre-operative specimens.

	Hysterectomy *n* = 142 (%)	Curettage *n* = 142 (%)	Total discordant cases	Concordance rate
Histology				
Endometrioid grade 1–2	134 (94.3)	132 (92.9)	8	94.3
Endometrioid grade 3	8 (5.7)	10 (7.1)		
p53				
Normal	125(88.1)	126 (88.7)	10	92.9
Aberrant	17(11.9)	16 (11.3)		
dMMR				
MSS	107 (75.6)	107 (75.6)	22	84.5
MSI	35 (24.4)	35 (24.4)		

(36). Moreover, mapping failure is not rare and can be caused by lymphatic obstruction, obesity, surgeon expertise, or the depth of cervical injection (37). Most importantly, the accuracy of this technique remains controversial since its first mention in the NCCN in 2014 (38). In our previous study, the overall sensitivity of the SLN to identify nodal metastatic disease was 50% (95% CI, 17.4–82.5), whereas the negative predictive value (NPV) and false negative (FN) rate were 96.6% (95%CI 91.0–98.9) and 50%, respectively. We concluded that SLN mapping was not sensitive and had a high FN rate for node metastasis in endometrial cancer with high-risk histology (39). Our novel model is superior to SLN in both cost reduction and accessibility. Most importantly, no patients below the cutoff had been histologically confirmed for lymph node metastasis according to our novel model.

This study has several limitations. First, this was a single-center retrospective study; thus, the model requires validation in other cohorts in different centers. Second, only 17 lymph node metastases occurred in our cohort. In addition, external validation was not performed. This was due to the reason that lymph node metastasis rate is low in EEC and the relatively small sample size of our trial. Further assessment in prospective studies were needed. Third, the detection of MSI and p53 expression was performed in postoperative resection specimens rather than curettage specimens. The ideal prediction model would be based on the genomic findings of curettage specimens to accurately discriminate patients at high risk for lymph node metastasis. However, we instead compared the molecular alterations in endometrial specimens obtained from the resected uterus or curettage and observed a high concordance between these specimens (93.5% for p53, 84.5% for dMMR). Our finding highlighted the potential use of curettage specimens to predict lymphatic metastasis.

CONCLUSION

In conclusion, our novel prediction model effectively identified patients at high risk for lymphatic metastasis. This model is a promising strategy for personalized surgery in patients with high risk according to the Mayo criteria. Further studies are needed to assess the feasibility of this prediction model in preoperative curettage specimens.

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 authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Shanghai First Maternity and Infant Hospital (Approval No. 2017026). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW has full access to all data and takes responsibility for the study accuracy and originality. Study concept and design: WL and TS. Manuscript drafting: XC and SL. Statistical analysis: JN and ZL. Critical revision of the manuscript for important intellectual content: XW and SL. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.895834/full#supplementary-material

REFERENCES

1. Braun MM, Overbeek-Wager EA, Grumbo RJ. Diagnosis and Management of Endometrial Cancer. *Am Fam Physician* (2016) 93(6):468–74.
2. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial Cancer. *Lancet* (2005) 366(9484):491–505. doi: 10.1016/S0140-6736(05)67063-8
3. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the Management of Endometrial Cancer. *Cochrane Database Syst Rev* (2017) 10: CD007585. doi: 10.1002/14651858.CD007585.pub4
4. ASTEC study group, Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. Efficacy of Systematic Pelvic Lymphadenectomy in Endometrial Cancer (MRC ASTEC Trial): A Randomised Study. *Lancet* (2009) 373(9658):125–36. doi: 10.1016/S0140-6736(08)61766-3
5. Li X, Cheng Y, Dong Y, Zhou J, Wang Z, Li X, et al. Development and Validation of Predictive Model for Lymph Node Metastasis in Endometrial Cancer: A SEER Analysis. *Ann Transl Med* (2021) 9(7):538. doi: 10.21037/atm-20-5034
6. Yang LY, Siow TY, Lin YC, Wu RC, Lu HY, Chiang HJ, et al. Computer-Aided Segmentation and Machine Learning of Integrated Clinical and Diffusion-Weighted Imaging Parameters for Predicting Lymph Node Metastasis in Endometrial Cancer. *Cancers (Basel)* (2021) 13(6):1406. doi: 10.3390/cancers13061406
7. Mariani A, Webb MJ, Keeney GL, Haddock MG, Calori G, Podratz KC. Low-Risk Corpus Cancer: Is Lymphadenectomy or Radiotherapy Necessary? *Am J Obstet Gynecol* (2000) 182(6):1506–19. doi: 10.1067/mob.2000.107335
8. Vargas R, Rauh-Hain JA, Clemmer J, Clark RM, Goodman A, Growdon WB, et al. Tumor Size, Depth of Invasion, and Histologic Grade as Prognostic Factors of Lymph Node Involvement in Endometrial Cancer: A SEER Analysis. *Gynecol Oncol* (2014) 133(2):216–20. doi: 10.1016/j.ygyno.2014.02.011
9. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A Simple, Genomics-Based Clinical Classifier for Endometrial Cancer. *Cancer* (2017) 123(5):802–13. doi: 10.1002/cncr.30496
10. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series. *Ann Oncol* (2018) 29 (5):1180–8. doi: 10.1093/annonc/mdy058
11. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated Genomic Characterization of Endometrial Carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12325
12. Raffone A, Travaglino A, Mascolo M, Carbone L, Guida M, Insabato L, et al. TCGA Molecular Groups of Endometrial Cancer: Pooled Data About Prognosis. *Gynecol Oncol* (2019) 155(2):374–83. doi: 10.1016/j.ygyno.2019.08.019
13. Imboden S, Nastic D, Ghaderi M, Rydberg F, Rau TT, Mueller MD, et al. Phenotype of POLE-Mutated Endometrial Cancer. *PLoS One* (2019) 14(3): e0214318. doi: 10.1371/journal.pone.0214318
14. Song Y, Shang H, Ma Y, Li X, Jiang J, Geng Z, et al. Can Conventional DWI Accurately Assess the Size of Endometrial Cancer? *Abdom Radiol* (2020) 45 (4):1132–40. doi: 10.1007/s00261-019-02220-y
15. Nagar R, Peters T, Nagar H, McCluggage WG, Gupta M. Diagnostic Accuracy in Assessment of Depth of Myometrial Invasion in Low-Grade Endometrioid Carcinoma: A 2 Center Comparative Study by MRI and Intraoperative Assessment. *Int J Gynecol Pathol* (2021) 40(5):495–500. doi: 10.1097/PGP.0000000000000703
16. Tanaka T, Terai Y, Ono YJ, Fujiwara S, Tanaka Y, Sasaki H, et al. Preoperative MRI and Intraoperative Frozen Section Diagnosis of Myometrial Invasion in Patients With Endometrial Cancer. *Int J Gynecol Cancer* (2015) 25(5):879–83. doi: 10.1097/IGC.0000000000000470
17. Gallego JC, Porta A, Pardo MC, Fernandez C. Evaluation of Myometrial Invasion in Endometrial Cancer: Comparison of Diffusion-Weighted Magnetic Resonance and Intraoperative Frozen Sections. *Abdom Imaging* (2014) 39(5):1021–6. doi: 10.1007/s00261-014-0134-9
18. Nero C, Ciccarone F, Pietragalla A, Duranti S, Daniele G, Scambia G, et al. Adjuvant Treatment Recommendations in Early-Stage Endometrial Cancer: What Changes With the Introduction of The Integrated Molecular-Based Risk Assessment. *Front Oncol* (2021) 3224. doi: 10.3389/fonc.2021.612450
19. Ohwada M, Suzuki M, Kohno T, Saga Y, Takei Y, Jobo T, et al. Involvement of Microsatellite Instability in Lymph Node Metastasis of Endometrial Carcinoma. *Cancer Genet cytogenetics* (2002) 132(2):152–5. doi: 10.1016/S0165-4608(01)00540-4
20. Jiang P, Huang Y, Tu Y, Li N, Kong W, Di F, et al. Combining Clinicopathological Parameters and Molecular Indicators to Predict Lymph Node Metastasis in Endometrioid Type Endometrial Adenocarcinoma. *Front Oncol* (2021) 11:682925. doi: 10.3389/fonc.2021.682925
21. Chu MM, Liu SS, Tam KF, Ip PP, Cheung AN, Ngan HY. The Significance of Mismatch Repair Deficiency in Young Patients With Endometrial Cancer. *Int J Gynecol Pathol* (2015) 34(5):403–10. doi: 10.1097/PGP.0000000000000174
22. O'Toole SA, Huang Y, Norris L, Foley MP, Shireen R, McDonald S, et al. HE4 and CA125 as Preoperative Risk Stratifiers for Lymph Node Metastasis in Endometrioid Carcinoma of the Endometrium: A Retrospective Study in a Cohort With Histological Proof of Lymph Node Status. *Gynecologic Oncol* (2021) 160(2):514–9. doi: 10.1016/j.ygyno.2020.11.004
23. Li Y, Cong P, Wang P, Peng C, Liu M, Sun G. Risk Factors for Pelvic Lymph Node Metastasis in Endometrial Cancer. *Arch Gynecol Obstet* (2019) 300 (4):1007–13. doi: 10.1007/s00404-019-05276-9
24. AlHilli MM, Podratz KC, Dowdy SC, Bakkum-Gamez JN, Weaver AL, McGree ME, et al. Risk-Scoring System for the Individualized Prediction of Lymphatic Dissemination in Patients With Endometrioid Endometrial Cancer. *Gynecol Oncol* (2013) 131(1):103–8. doi: 10.1016/j.ygyno.2013.06.037
25. Bendifallah S, Canlorbe G, Raimond E, Hudry D, Coutant C, Graesslin O, et al. External Validation of Nomograms Designed to Predict Lymphatic Dissemination in Patients With Early-Stage Endometrioid Endometrial Cancer: A Multicenter Study. *Am J Obstet Gynecol* (2015) 212(1):56.e1–7. doi: 10.1016/j.ajog.2014.06.058
26. Reijnen C, Gogou E, Visser NCM, Engerud H, Ramjith J, van der Putten LJM, et al. Preoperative Risk Stratification in Endometrial Cancer (ENDORISK) by a Bayesian Network Model: A Development and Validation Study. *PLoS Med* (2020) 17(5):e1003111. doi: 10.1371/journal.pmed.1003111
27. Lee J, Kong TW, Paek J, Chang SJ, Ryu HS. Predicting Model of Lymph Node Metastasis Using Preoperative Tumor Grade, Transvaginal Ultrasound, and Serum CA-125 Level in Patients With Endometrial Cancer. *Int J Gynecol Cancer* (2016) 26(9):1630–5. doi: 10.1097/IGC.0000000000000820
28. Gülseren V, Kocaer M, Özdemir İA, Çakır İ, Sancı M, Güngördük K. Do Estrogen, Progesterone, P53 and Ki67 Receptor Ratios Determined From Curettage Materials in Endometrioid-Type Endometrial Carcinoma Predict Lymph Node Metastasis? *Curr problems Cancer* (2020) 44(1):100498. doi: 10.1016/j.currproblcancer.2019.07.003
29. Yang B, Shan B, Xue X, Wang H, Shan W, Ning C, et al. Predicting Lymph Node Metastasis in Endometrial Cancer Using Serum CA125 Combined With Immunohistochemical Markers PR and Ki67, and a Comparison With Other Prediction Models. *PLoS One* (2016) 11(5):e0155145. doi: 10.1371/journal.pone.0155145
30. Tsuruchi N, Kaku T, Kamura T, Tsukamoto N, Tsuneyoshi M, Akazawa K, et al. The Prognostic Significance of Lymphovascular Space Invasion in Endometrial Cancer When Conventional Hemotoxylin and Eosin Staining is Compared to Immunohistochemical Staining. *Gynecol Oncol* (1995) 57 (3):307–12. doi: 10.1006/gyno.1995.1148
31. Boothe D, Wolfson A, Christensen M, Francis S, Werner TL, Gaffney DK. Lymphovascular Invasion in Endometrial Cancer: Prognostic Value and Implications on Adjuvant Radiation Therapy Use. *Am J Clin Oncol* (2019) 42(7):549–54. doi: 10.1097/COC.0000000000000559
32. Bosse T, Peters EE, Creutzberg CL, Jurgenliemk-Schulz IM, Jobsen JJ, Mens JW, et al. Substantial Lymph-Vascular Space Invasion (LVSI) Is a Significant Risk Factor for Recurrence in Endometrial Cancer–A Pooled Analysis of PORTEC 1 and 2 Trials. *Eur J Cancer* (2015) 51(13):1742–50. doi: 10.1016/j.ejca.2015.05.015
33. Peters EEM, Bartosch C, McCluggage WG, Genestie C, Lax SF, Nout R, et al. Reproducibility of Lymphovascular Space Invasion (LVSI) Assessment in Endometrial Cancer. *Histopathology* (2019) 75(1):128–36. doi: 10.1111/his.13871
34. Holloway RW, Abu-Rustum NR, Backes FJ, Boggess JF, Gotlieb WH, Jeffrey Lowery W, et al. Sentinel Lymph Node Mapping and Staging in Endometrial Cancer: A Society of Gynecologic Oncology Literature Review With

Consensus Recommendations. *Gynecol Oncol* (2017) 146(2):405–15. doi: 10.1016/j.ygyno.2017.05.027
35. Abu-Rustum NR. Sentinel Lymph Node Mapping for Endometrial Cancer: A Modern Approach to Surgical Staging. *J Natl Compr Canc Netw* (2014) 12 (2):288–97. doi: 10.6004/jnccn.2014.0026
36. Farzaneh F, Moridi A, Azizmohammadi Z, Ansari JM, Hosseini MS, Arab M, et al. Value of Sentinel Lymph Node (SLN) Mapping and Biopsy Using Combined Intracervical Radiotracers and Blue Dye Injections for Endometrial Cancer. *Asian Pac J Cancer Prev* (2017) 18(2):431–5. doi: 10.22034/APJCP.2017.18.2.431
37. Stephens AJ, Kennard JA, Fitzsimmons CK, Manyam M, Kendrick JE, Singh C, et al. Robotic Sentinel Lymph Node (SLN) Mapping in Endometrial Cancer: SLN Symmetry and Implications of Mapping Failure. *Int J Gynecol Cancer* (2020) 30(3):305–10. doi: 10.1136/ijgc-2019-000915
38. Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Chan J, et al. Uterine Neoplasms, Version 1.2014. *J Natl Compr Canc Netw* (2014) 12 (2):248–80. doi: 10.6004/jnccn.2014.0025
39. Ye L, Li S, Lu W, He Q, Li Y, Li B, et al. A Prospective Study of Sentinel Lymph Node Mapping for Endometrial Cancer: Is It Effective in High-Risk Subtypes? *Oncologist* (2019) 24(12):e1381–e7. doi: 10.1634/theoncologist.2019-0113

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REVIEW
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The Clinicopathological Significance and Prognostic Value of Androgen Receptor in Endometrial Carcinoma: A Meta-Analysis

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Background: The role of androgen receptor (AR) in evaluating the prognosis of patients with endometrial cancer (EC) remains controversial. Here, we performed a meta-analysis to assess whether AR expression improves EC survival outcomes.

Methods: We searched related articles published before August 2021 in PubMed, EMBASE, and Web of Science. The association between AR expression and patient prognosis was estimated with hazard ratios (HRs) and odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs). The review is registered on PROSPERO, registration number: CRD42021268591.

Results: Ten studies including 1,485 patients were enrolled in the meta-analysis. The results showed that AR expression in EC tissues was associated with a better survival in crude analyses (HR = 1.63, 95% CI = 1.32–2.02, P < 0.001). However, no significant relation was found after the adjustment of the confounding factors (HR = 1.68, 95% CI = 0.75–3.75, P = 0.205). In subgroup analyses, grade 1–2 disease, stage I–II disease, negative lymph node status, and lack of the lymphovascular invasion were more common in AR-positive groups (OR = 0.47, 0.48, 0.37, and 0.57; 95% CI = 0.45–0.62, 0.35–0.65, 0.24–0.56, and 0.37–0.89). Furthermore, AR expression was more common in endometrioid cancers (OR = 2.39, 95% CI = 1.79–3.20).

Conclusions: AR expression is significantly associated favorable characteristics including low-grade disease, early-stage disease, negative lymph node status, and lack of the lymphovascular invasion and a specific histology—endometrioid cancer. However, AR is not an independent prognostic factor.

Keywords: androgen receptor, clinicopathological, prognosis, endometrial cancer, meta-analysis

INTRODUCTION

Endometrial cancer (EC) is the most common gynecologic malignancy and continues to increase by about 1% per year (1). During 2021, almost 66,570 new cases of uterine corpus cancer and 12,940 deaths are projected to occur due to this cancer in the United States (2).

An excess-estrogen environment is linked with EC development, especially type I cancer (3). As the main source of estrogen especially in postmenopausal women, the importance of androgens in EC has been recognized for the last decades. In addition, androgen receptor (AR) also has been evaluated for its prognostic power in EC. In some studies, AR expression has been reported to be associated with better survival in patients with EC (4–8), whereas the better prognosis was not noted in other studies (9, 10). For explaining better prognosis in patients with EC, some investigators thought that the heterogeneity of histology resulted in the different patient survival of EC. However, the identical findings were not identified (5, 8–10).

With the aim of disentangling these controversial issues, we present a systematic review and meta-analysis to evaluate the association between the AR expression and the prognosis of patients with EC.

MATERIALS AND METHODS

This research was conducted according to Preferred Reporting for Systematic Reviews and Meta-Analyses (PRISMA) principles.

Literature Search

We performed a comprehensive search in PubMed, EMBASE, and Web of Science. The search terms included "endometrial cancer" or "endometrial carcinoma" or "endometrial neoplasms" in combination with "androgen receptors". Titles and abstracts were checked to identify potential eligible articles by two researchers, who then reviewed full texts. In addition, the references of included articles were checked manually for more related studies.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) studies published in English; (2) studies on EC that confirmed by histopathological examination; (3) studies assessing AR expression with positive or negative labels; and (4) studies comparing the relationship between AR and clinic-pathological characteristics or prognosis. However, we excluded studies as follows: (1) studies based on animals or *in vitro* experiments; (2) review articles, meta-analyses, letters, or case reports; and (3) non-English literature.

Data Extraction

For included articles, two investigators independently extracted the related data using a fixed form. The form included the name of the first author, the year of publication, age, the expression level of AR, clinic-pathological characteristics, hazard ratios (HRs), and 95% confidence intervals (CIs) for survival analysis. If the HRs and 95% CIs could not be acquired directly, then they were estimated from Kaplan–Meier curves using the method described by Parmar et al. (11). Two studies (6, 7) were excluded because of the significant difference between the estimated and actual HR. Any disagreements were resolved by discussion and consultation with the third author.

Quality Assessment

The guidelines from the Newcastle-Ottawa Scale (NOS) criteria were used to evaluated the quality of studies (12). The NOS criteria included three domains: (1) selection: 0–4; (2) comparability: 0–2; and (3) exposure or outcomes: 0–3. Good quality was considered when the NOS scores ≥6.

Statistical Analysis

Dichotomous data eligible in each research were shown as a odds ratio (OR) with its 95% CI.

Moreover, the pooled HRs and 95% CIs were calculated to evaluate the associations between AR and prognosis of patients with EC. Heterogeneity between studies was assessed using I^2 (13). If I^2 >50%, substantial heterogeneity was considered and the random effects model was implemented. When I^2 ≤50%, the fixed effect model was used in this meta-analysis.

Publication and selection bias was investigated by funnel plots and the Egger and Begg test. All analyses were performed in STATA software, and $P < 0.05$ was considered statistically significant.

RESULTS

Study Search

A total of 660 studies were identified. After removal of 298 duplicates, 362 records were checked based on title and/or abstract and 17 studies remained. The full texts of remaining articles were further assessed for more details, and seven articles were excluded for the lack of data on prognosis or clinicopathological characteristics. Finally, 10 studies including 1,485 patients were enrolled in the meta-analysis (**Figure 1**). The main characteristics of included studies are shown in **Table 1**. Briefly, all of the articles investigated the association between AR and various clinicopathologic factors (4–10, 14–16), among which five of them further performed survival analysis (4–8).

Impact of AR on EC Prognosis

Given the effect of the confounding factors, a stratified analysis was conducted on the subsets of survival analysis. The two available studies on univariate survival analysis suggest that AR overexpression predicted a favorable survival (HR = 1.63, 95% CI = 1.32–2.02, P < 0.001; **Figure 2A**) (5, 8). However, in two studies using multivariate survival analysis (4, 8), no significant relation was observed after adjustment for potential confounding factors (HR = 1.68, 95% CI = 0.75–3.74, P = 0.205; **Figure 2B**).

Clinicopathologic Characteristics of AR Expression in EC

Finally, we evaluated clinicopathologic characteristics between AR-positive and AR-negative groups. In crude analyses, low grade (OR = 0.466, 95% CI = 0.352–0.618, P < 0.001; **Figure 3B**), negative lymph nodes (OR = 0.367, 95% CI = 0.239–0.564, P < 0.001; **Figure 3C**), FIGO stage I–II disease (OR = 0.480,

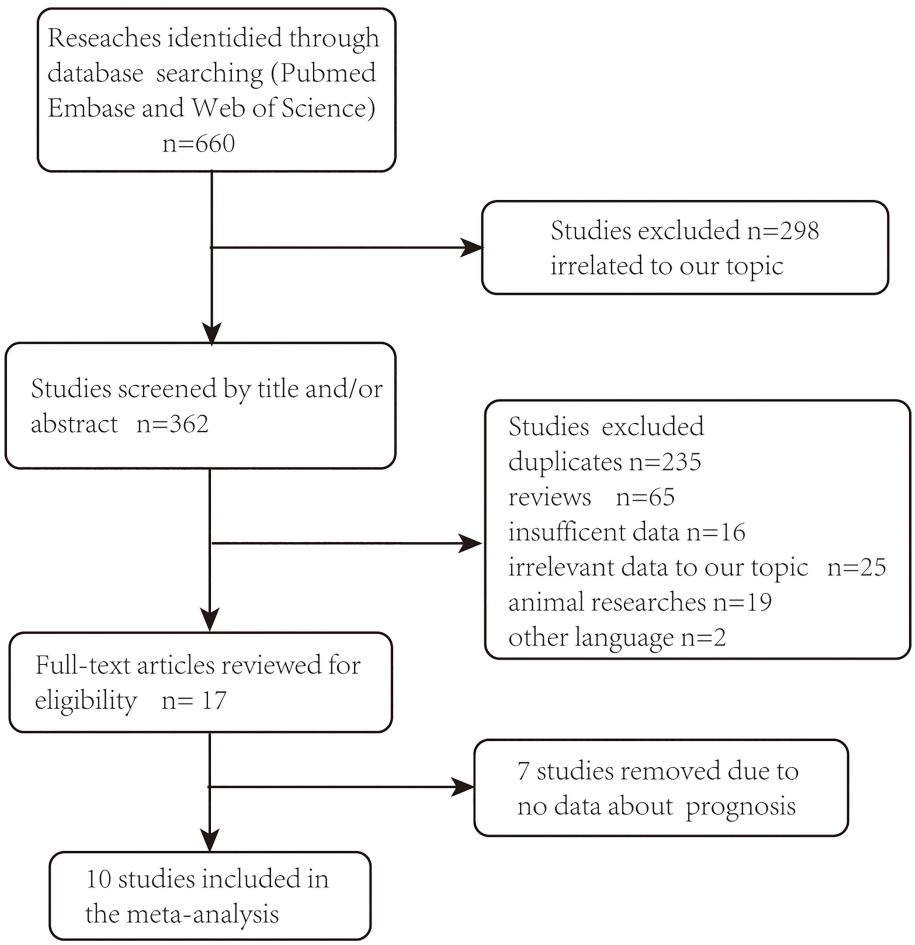

FIGURE 1 | PRISMA flowchart of eligible studies selection process.

95% CI = 0.353–0653, P < 0.001; **Figure 3F**), and negative lymphovascular invasion (OR = 0.572, 95% CI = 0.368–0.890, P = 0.013; **Figure 3G**) were more common in AR-positive group. However, the associations between AR expression and age, myometrial invasion and cervical invasion were not statistically significant (**Figures 3A, D, E**; P=0.941, P=0.063, and P=0.317, respectively).

In terms of histology, crude analysis showed type I cancers were more frequent in AR-positive group (OR = 2.393, 95% CI = 1.789–3.202, P < 0.001; **Figure 3H**).

Publication Bias Assessment

Begg's funnel plot was conducted to assess the publication bias of included studies and no evidence of publication bias was seen (**Supplementary Figure 1**).

DISCUSSION

The role of AR in EC has been widely discussed for decades. However, the prognostic usefulness of AR is still controversial. This is the first systematic review with meta-analysis to examine the effect of AR on survival outcomes in patients with EC. We found that AR expression imparts a better survival outcome. The effect on better prognosis was consistently observed in subgroup analyses according to clinicopathologic characteristics. EC is a biologically and histologically diverse group of neoplasms characterized by a dualistic model of pathogenesis. Unlike type II EC, type I endometrial tumors usually portend a less aggressive clinical course (17). Our meta-analytic results showed that AR may have favorable characteristics of type I EC including early-stage disease, low-grade disease, negative lymph node status, and lack of the lymphovascular invasion. Indeed, we found that the expression of AR significantly increased in type I cancers. These findings mean that AR plays a crucial role in type I rather than type II cancers.

Notably, numerous studies have also examined the potential role of androgens as risk factors for EC. In addition, most of them claimed to have found that elevated serum testosterone level increased EC risk (18–21). It is tempting to speculate that AR is one of negative prognostic factors in EC. However, our meta-analysis reports that AR expression is a favorable prognostic

TABLE 1 | Characteristics of the included studies.

Study	Year	Country	No. of Cases	Examination Methods	Clinic-Pathological Characteristics			
					AR Positive (%)	Histological Type	Disease-Progressive Indicators	Survival Analyses
Abu Shahin et al.	2021	Jordan	52	IHC	28/52 (53.8%)	Endometrioid Serous Clear-cell	FIGO stage Grade Lymph node status	NA
Nisar et al.	2020	Pakistan	54	IHC	29/54 (53.7%)	Endometrioid Serous Clear-cell Carcinosarcoma	Grade Lymphovascular invasion Myometrial invasion	NA
Hashmi et al.	2018	Pakistan	103	IHC	18/89	Endometrioid Serous Clear-cell Carcinosarcoma	FIGO stage Grade Lymphovascular invasion Myometrial invasion Cervical invasion Lymph node status	NA
Park et al.	2018	Korea	51	IHC	30/51 (58.8%)	NA	Grade Myometrial invasion	DFS+OS
Roy et al.	2017	India	25	IHC	14/25 (56.0%)	Stromal sarcoma	Grade	NA
Mahdi et al.	2017	USA	261	IHC	135/261 (51.7%)	Endometrioid Mucinous Serous Clear-cell Carcinosarcoma	FIGO stage Grade Lymphovascular invasion Lymph node status	OS
Zadeh et al.	2017	USA	50	IHC	27/50 (54%)	Endometrioid Serous Clear-cell Carcinosarcoma	Grade	NA
Kamal et al.	2016	UK	85	IHC	54/86 (62.8%)	Endometrioid Serous Clear-cell Carcinosarcoma	FIGO stage Grade Lymphovascular invasion Myometrial invasion Cervical invasion	DFS
Tangen et al.	2016	Norway	718	IHC	447/718 (62.3%)	Endometrioid Serous Clear-cell Carcinosarcoma Adeosquamous Undifferentiated/other	FIGO stage Grade Lymph node status	DSS
Tanaka et al.	2015	Japan	86	IHC	65/86 (75.6%)	NA	FIGO stage Grade Lymphovascular invasion Myometrial invasion Lymph node status	PFS

IHC, immunohistochemistry; DFS, disease-free survival; DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival; NA, not applicable.

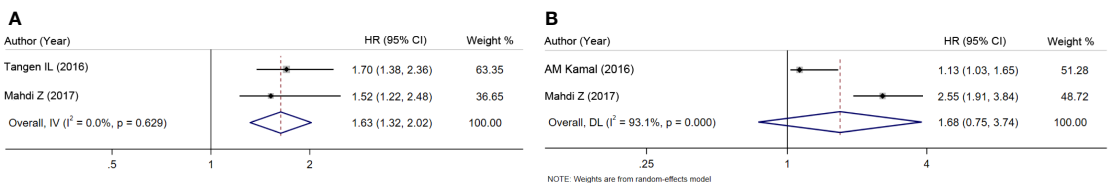

FIGURE 2 | Meta-analysis of the association between AR and patient survival. **(A)** Univariate survival analysis. **(B)** Multivariate survival analysis.

indicator. It is well known that testosterone can be metabolized by aromatase and 5α-reductase to estradiol and dihydrotestosterone (DHT), respectively (22). An excess-estrogen environment can trigger the development and progression of EC, especially for type I. It is reported that the inhibition of aromatase activity has been applied to the treatment of EC. A retrospective cohort study recently reported longer PFS (HR = 0.23; 95% CI = 0.04–1.27) and OS (HR = 0.11; 95% CI = 0.01–1.36) in patients receiving aromatase inhibitors

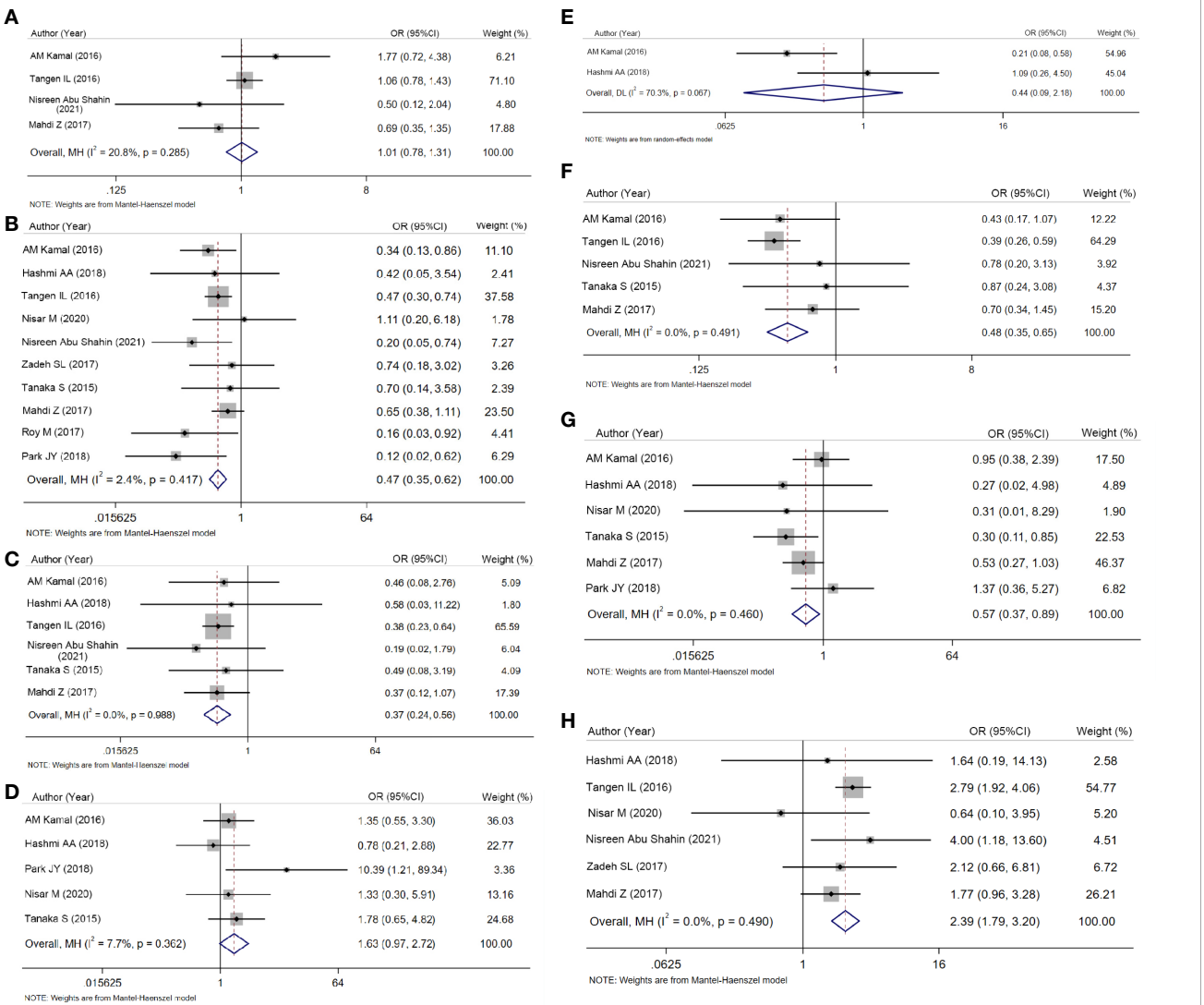

FIGURE 3 | Forest plots for ORs and 95% CIs to compare clinicopathologic characteristics. **(A)** Age. **(B)** Grade. **(C)** Lymph node status. **(D)** Myometrial invasion. **(E)** Cervical invasion. **(F)** Stage (I + II vs. III + IV). **(G)** Lymphovascular invasion. **(H)** Histological type (I vs. II).

(AIs) (23). On the other hand, Hashimoto et al. have reported that DHT could inhibit the proliferation of EC cells (24). Consistent with these findings, the results in our study indirectly show that the conversion of testosterone to DHT and further activation of AR by DHT inhibit the continuum of EC progression.

Two of the included articles performed multivariate Cox survival analysis including tumor stage, myometrial invasion, race, BMI, diabetes, and AR, ER, and PR expression (4, 8). This meta-analysis integrated these disparate results, and the data in these studies were not always consistent. This might be ascribable to the following factors. First, AR signaling may have both oncogenic and tumor suppressive roles. In mouse models of type I EC, short-term enzalutamide treatment, an inhibitor of AR signaling, reduced endometrial tumor burden and increased cancer cell apoptosis in a dose-dependent way. However, enzalutamide increased the incidence of invasive and metastatic tumor (25). Oncogenic role of AR may be more involved in EC initiation. Later stages of invasion and metastasis in EC maybe partly due to inactivation of cancer suppressive AR signaling. Second, the histological structures and the carcinogenesis are different in type I and II cancers. Type I cancers are hormone-dependent. Our meta-analytic results showed AR expression was more likely to be observed in type I cancers. This might indicate that the impact of AR may be more inclined to type I EC. Further studies should also focus on the evaluation of the role of AR in type I cancers. Third, studies in the analysis employed different antibodies and cutoff values that led to variations of the results. Fourth, the numbers of patients and outcome events were small that implied poor statistical precision.

This is the first meta-analysis to uncover the prognostic value of AR in patients in EC. However, some limitations in our study

should be mentioned. First, some of the studies in the meta-analyses did not mention any preoperative and/or postoperative therapies. Radiotherapy and/or chemotherapy are usually offered for those in advanced stage (26, 27). Such variations in treatment modalities must have an impact on the prognosis and prognostic analyses. Second, the numbers of patients and outcome events were mostly small implying poor statistical precision. Third, heterogeneity was evident among the included studies with respect to the specifics of staining methods, cutoff values, and so on.

In summary, the results from this meta-analysis suggested that AR may be useful prognostic biomarkers for EC. Further well-designed, multi-center, and larger-scale trials are needed to confirm our findings.

AUTHOR CONTRIBUTIONS

XW conceived and designed the study, interpreted the data, and drafted the manuscript. XY and YZ designed and revised the manuscript. XZ and JZ selected the articles. XZ and XH retrieved the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.905809/full#supplementary-material

REFERENCES

1. Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International Patterns and Trends in Endometrial Cancer Incidence, 1978-2013. *J Natl Cancer Institute* (2018) 110(4):354–61. doi: 10.1093/jnci/djx214
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA: Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654
3. Kaaks R, Lukanova A, Kurzer MS. Obesity, Endogenous Hormones, and Endometrial Cancer Risk: A Synthetic Review. *Cancer epidemiology Biomarkers Prev Publ Am Assoc Cancer Research cosponsored by Am Soc Prev Oncol* (2002) 11(12):1531–43.
4. Kamal AM, Bulmer JN, DeCruze SB, Stringfellow HF, Martin-Hirsch P, Hapangama DK. Androgen Receptors Are Acquired by Healthy Postmenopausal Endometrial Epithelium and Their Subsequent Loss in Endometrial Cancer Is Associated With Poor Survival. *Br J cancer* (2016) 114(6):688–96. doi: 10.1038/bjc.2016.16
5. Tangen IL, Onyango TB, Kopperud R, Berg A, Halle MK, Øyan AM, et al. Androgen Receptor as Potential Therapeutic Target in Metastatic Endometrial Cancer. *Oncotarget* (2016) 7(31):49289–98. doi: 10.18632/oncotarget.10334
6. Park JY, Baek MH, Park Y, Kim YT, Nam JH. Investigation of Hormone Receptor Expression and Its Prognostic Value in Endometrial Stromal Sarcoma. *Virchows Archiv an Int J pathol* (2018) 473(1):61–9. doi: 10.1007/s00428-018-2358-5
7. Tanaka S, Miki Y, Hashimoto C, Takagi K, Doe Z, Li B, et al. The Role of 5α-Reductase Type 1 Associated With Intratumoral Dihydrotestosterone Concentrations in Human Endometrial Carcinoma. *Mol Cell endocrinol* (2015) 401:56–64. doi: 10.1016/j.mce.2014.11.022
8. Mahdi Z, Abdulfatah E, Pardeshi V, Hassan O, Schultz D, Morris R, et al. The Impact of Androgen Receptor Expression on Endometrial Carcinoma Recurrence and Survival. *Int J gynecol Pathol Off J Int Soc Gynecol Pathologists* (2017) 36(5):405–11. doi: 10.1097/PGP.0000000000000355
9. Hashmi AA, Hussain ZF, Qadri A, Irfan M, Ramzan S, Faridi N, et al. Androgen Receptor Expression in Endometrial Carcinoma and Its Correlation With Clinicopathologic Features. *BMC Res notes* (2018) 11 (1):289. doi: 10.1186/s13104-018-3403-9
10. Nisar M, Mushtaq S, Hassan U, Akhtar N, Azma M. Androgen Receptor Expression In Endometrial Carcinoma And Its Correlation With Estrogen Receptor And Progesterone Receptor And Clinicopathological Findings. *J Ayub Med College Abbottabad JAMC* (2020) 32(2):160–4.
11. Parmar MK, Torri V, Stewart L. Extracting Summary Statistics to Perform Meta-Analyses of the Published Literature for Survival Endpoints. *Stat Med* (1998) 17(24):2815–34. doi: 10.1002/(SICI)1097-0258(19981230)17:24<2815::AID-SIM110>3.0.CO;2-8
12. Stang A. Critical Evaluation of the Newcastle-Ottawa Scale for the Assessment of the Quality of Nonrandomized Studies in Meta-Analyses. *Eur J Epidemiol* (2010) 25(9):603–5. doi: 10.1007/s10654-010-9491-z
13. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring Inconsistency in Meta-Analyses. *BMJ (Clinical Res ed)* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557
14. Abu Shahin N, Aladily T, Abu Alhaj N, Al-Khader A, Alqaqa S, Aljaberi R, et al. Differential Expression of Androgen Receptor in Type I and Type II Endometrial Carcinomas: A Clinicopathological Analysis and Correlation With Outcome. *Oman Med J* (2021) 36(2):e245. doi: 10.5001/omj.2021.53
15. Zadeh SL, Duska LR, Mills AM. Androgen Receptor Expression in Endometrial Carcinoma. *Int J gynecol Pathol Off J Int Soc Gynecol Pathologists* (2018) 37(2):167–73. doi: 10.1097/PGP.0000000000000401
16. Roy M, Kumar S, Bhatla N, Ray MD, Kumar L, Jain D, et al. Androgen Receptor Expression in Endometrial Stromal Sarcoma: Correlation With Clinicopathologic Features. *Int J gynecol Pathol Off J Int Soc Gynecol Pathologists* (2017) 36(5):420–7. doi: 10.1097/PGP.0000000000000353
17. Buchanan EM, Weinstein LC, Hillson C. Endometrial Cancer. *Am Family physician* (2009) 80(10):1075–80.
18. Teng F, Ma X, Yu X, Yan Y, Zhao J, Gao J, et al. High Serum Androgen and Insulin Concentrations Increase the Tendency of Endometrial Carcinoma. *J Cancer* (2020) 11(19):5656–64. doi: 10.7150/jca.46391
19. Michels KA, Brinton LA, Wentzensen N, Pan K, Chen C, Anderson GL, et al. Postmenopausal Androgen Metabolism and Endometrial Cancer Risk in the Women's Health Initiative Observational Study. . *JNCI Cancer Spectr* (2019) 3 (3):pkz029. doi: 10.1093/jncics/pkz029
20. Audet-Walsh E, Lépine J, Grégoire J, Plante M, Caron P, Têtu B, et al. Profiling of Endogenous Estrogens, Their Precursors, and Metabolites in Endometrial Cancer Patients: Association With Risk and Relationship to Clinical Characteristics. *J Clin Endocrinol Metab* (2011) 96(2):E330–9. doi: 10.1210/jc.2010-2050
21. Lukanova A, Lundin E, Micheli A, Arslan A, Ferrari P, Rinaldi S, et al. Circulating Levels of Sex Steroid Hormones and Risk of Endometrial Cancer in Postmenopausal Women. *Int J cancer* (2004) 108(3):425–32. doi: 10.1002/ijc.11529
22. McNamara KM, Moore NL, Hickey TE, Sasano H, Tilley WD. Complexities of Androgen Receptor Signalling in Breast Cancer. *Endocrine-related Cancer* (2014) 21(4):T161–81. doi: 10.1530/ERC-14-0243
23. Paleari L, Rutigliani M, Siri G, Provinciali N, Colombo N, Decensi A. Aromatase Inhibitors as Adjuvant Treatment for ER/PgR Positive Stage I

Endometrial Carcinoma: A Retrospective Cohort Study. *Int J Mol Sci* (2020) 21(6):2227–33. doi: 10.3390/ijms21062227
24. Hashimoto C, Miki Y, Tanaka S, Takagi K, Fue M, Doe Z, et al. 17β-Hydroxysteroid Dehydrogenase Type 2 Expression Is Induced by Androgen Signaling in Endometrial Cancer. *Int J Mol Sci* (2018) 19(4):1139–52. doi: 10.3390/ijms19041139
25. Koivisto CS, Parrish M, Bonala SB, Ngoi S, Torres A, Gallagher J, et al. Evaluating the Efficacy of Enzalutamide and the Development of Resistance in a Preclinical Mouse Model of Type-I Endometrial Carcinoma. *Neoplasia (New York NY)* (2020) 22(10):484–96. doi: 10.1016/j.neo.2020.07.003
26. Hoskins PJ, Swenerton KD, Pike JA, Wong F, Lim P, Acquino-Parsons C, et al. Paclitaxel and Carboplatin, Alone or With Irradiation, in Advanced or Recurrent Endometrial Cancer: A Phase II Study. *J Clin Oncol Off J Am Soc Clin Oncol* (2001) 19(20):4048–53. doi: 10.1200/JCO.2001.19.20.4048
27. Aalders J, Abeler V, Kolstad P, Onsrud M. Postoperative External Irradiation and Prognostic Parameters in Stage I Endometrial Carcinoma: Clinical and Histopathologic Study of 540 Patients. *Obstetrics gynecology* (1980) 56(4):419–27.

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Primary Neuroendocrine Tumors of the Endometrium: Management and Outcomes

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Objective: To analyze clinical behavior of, optimal treatment regimens for, outcomes, and prognosis of 170 patients with neuroendocrine tumors (NETs) of the endometrium.

Methods: The Surveillance, Epidemiology, and End Results database was used to identify patients with endometrial NETs diagnosed between 2004 and 2015. Clinical features and treatment regimens were analyzed, and 5-year overall survival (OS) and cancer-specific survival (CSS) were compared among different stages and treatment regimens. Univariate and multivariate analyses were performed to identify independent prognostic factors associated with endometrial NETs. Finally, prognosis was compared between small- and large-cell neuroendocrine carcinoma (SCNEC and LCNEC, respectively) of the endometrium.

Results: There were 20, 8, 47, and 95 patients with stage I, II, III, and IV NET, respectively. The 5-year OS rates of patients in each stage were 59.86%, 42.86%, 32.75%, and 6.04%, respectively. The 5-year CSS survival rates were 59.86%, 50.0%, 38.33%, and 6.39%, respectively. In the multivariate analysis, American Joint Committee on Cancer (AJCC) stage and treatment were associated with poor OS, while AJCC stage, nodal metastasis, and treatment were associated with poor CSS. Neither pathological type nor distant metastasis was associated with prognosis. The rate of distant metastasis was significantly higher for LCNEC than for SCNEC, while 5-year OS and CSS rates were significantly lower.

Conclusion: Complete surgical treatment should be selected regardless of staging for patients with endometrial NETs. For early-stage disease, individualized postoperative treatment with single chemotherapy or radiotherapy may improve OS and CSS. For advanced-stage disease, comprehensive postoperative adjuvant therapy may improve OS and CSS.

Keywords: SEER, prognostic factors, overall survival, cancer-specific survival, neuroendocrine tumors of the endometrium

Abbreviations: ACT, atypical carcinoid; AJCC, American Joint Committee on Cancer; CCRT, concurrent chemoradiotherapy; CI, confidence interval; CSS, cancer-specific survival; CT, chemotherapy; EBRT, external beam radiation therapy; HR, hazard ratio; LCNEC, large-cell neuroendocrine carcinoma; NETs, neuroendocrine tumors; OS, overall survival; RT, radiotherapy; SEER, Surveillance, Epidemiology, and End Results; SCNEC, small-cell neuroendocrine carcinoma; WHO, World Health Organization.

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INTRODUCTION

Neuroendocrine tumors (NETs) are malignant tumors with neuroendocrine function. NETs occur mainly in the lungs, although they are occasionally observed in the gastrointestinal and genitourinary tracts. Cases of tumors involving the female reproductive tract are rare, with primary NETs of the endometrium accounting for less than 1% of all endometrial cancers (1). In addition to the characteristic histological and immunohistochemical features of NETs, hematogenous and lymphatic metastasis may occur early during the disease in patients with endometrial NETs (2). Furthermore, several studies have reported that endometrial NETs are usually identified in the advanced stage and have a poor prognosis (3–6).

In 2014, the World Health Organization (WHO) classified endometrial NETs as either low-grade or high-grade (3). Low-grade NETs are rarely reported in existing literature (7–9). Nonetheless, low-grade endometrial NETs can be further categorized as either carcinoid or atypical carcinoid (ACT), while high-grade endometrial NETs can be categorized as either small- or large-cell neuroendocrine carcinoma (SCNEC or LCNEC, respectively).

The National Comprehensive Cancer Network (NCCN) has published guidelines concerning treatment strategies for cervical neuroendocrine cancer (10). However, owing to the rarity of endometrial NETs, relevant clinical data from large samples are limited, and standardized treatment options need to be established. To aid in the development of standardized treatment guidelines, the present study aimed to clarify the clinical characteristics, prognosis/prognostic indicators, and outcomes of endometrial NETs.

MATERIALS AND METHODS

Data Collection

Patients histologically diagnosed with NETs of the endometrium from 2004 to 2015 were identified using the SEER database (http://www.seer.cancer.gov; SEER*Stat database: Version 8.3.8) based on the following codes for primary malignant tumors in the endometrium (ICD-O-3/WHO 2008): small-cell carcinoma (8041/3), non-small-cell carcinoma (8046/3), large-cell carcinoma (8012/3), LCNEC (8013/3), atypical carcinoid (8249/3), and carcinoid (8240/3). The exclusion criteria included diagnosis of carcinoma in situ, unknown treatment, unknown survival time, non-endometrial NETs not being the first tumor. Cases were screened for patient-related information, including and clinical characteristics and treatment modality (surgery, chemotherapy, and radiotherapy). Staging was determined in accordance with the American Joint Committee on Cancer (AJCC) staging system. The SEER database is publicly available and contains de-identified data; thus, there was no need to obtain local ethics committee approval for data access.

Clinical Characteristics

Demographic data including age at diagnosis (<60 years, ≥60 years), year at diagnosis (2004–2009, 2010–2015), AJCC stage (I, IA, IB, IC, INOS; II, IIA, IIB, IINOS; III, IIIA, IIIB, IIIC, IIINOS; IV, IVA, IVB), grade (well/moderately differentiated, poorly/undifferentiated differentiated), lymph node metastasis (negative, positive, not examined, and unknown), sampled pelvic nodes (1–9,10–19, ≥20, not examined, unknown), distant metastases (lung, brain, bone, liver, no, unknown), treatment (surgery alone, chemotherapy [CT] + surgery, radiotherapy [RT] + surgery; concurrent chemoradiotherapy [CCRT] + surgery, CT only, CCRT only; RT only), and surgical approach (curettage, subtotal hysterectomy + adnexectomy, total hysterectomy + adnexectomy + lymphadenectomy, extended radical hysterectomy + adnexectomy + lymphadenectomy, extended radical hysterectomy + adnexectomy + lymphadenectomy + rectal resection, none) were extracted. Data on duration of post-diagnosis follow-up, living status, and cause of death were also extracted from the database to assess OS and CSS, which represented the primary endpoints of the study. For the analysis of OS, death from any cause was considered an event. In the CSS analysis, among the cancer-related deaths, only deaths due to endometrial NETs were considered events. Survival and death from other causes were considered as alive.

Statistical Analysis

Categorical data are expressed as numbers and percentages (N, %). Pearson's chi-square analysis was used to analyze the clinical and demographic characteristics of patients with NETs of the endometrium. Kaplan–Meier curves were used to estimate OS and CSS in different groups, and log-rank tests were used to analyze the differences between curves. Univariate and multivariate Cox regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for determining the independent prognostic factors associated with OS and CSS. Statistical analysis was performed using SPSS version 25.0 (IBM Corp, Armonk, NY, USA). Kaplan–Meier survival curves were drawn using GraphPad Prism (9.2.0 GraphPad Software, San Diego, CA, USA). P-values < 0.05 were considered statistically significant.

RESULTS

Patient Characteristics and Treatment

A total of 170 patients with NETs of the endometrium in the SEER registry met our inclusion criteria, including 56 (32.9%) patients with SCNEC, 60 (35.3%) patients with LCNEC, 2 (1.2%) patients with carcinoid NETs, 1 (0.6%) patient with atypical carcinoid (ACT) NEC, and 51 (30.0%) patients with NETs not otherwise classified. AJCC stage I, II, III, and IV disease was observed in 20 (11.8%), 8 (4.7%), 47 (27.6%), and 95 (55.9%) patients, respectively. **Table 1** presents a more detailed summary of patient characteristics.

Table 2 summarizes the treatments used for each stage of endometrial NETs. Among patients with stage I, II, III, and IV disease, surgery was the main treatment in 15(8.8%), 6(3.5%), 37 (21.8%), and 31(18.2%) cases, respectively. Other main treatments included RT only (n=2; beam radiation therapy [EBRT] in 1 case and EBRT with implants in 1 case),combination of EBRT with implants+CT(n=1) for stage I; CT + RT for stage II (n=2;EBRT in 1 case and EBRT with implants in 1 case); CT only (n=2)and EBRT +

TABLE 1 | Patient characteristicso of neuroendocrine tumors (NETs)of the endometrium.

Subject	N=170	N(%)
Hystological type		
SCNEC	56	32.9
LCNEC	60	35.3
Carcinoid	2	1.2
Atypical carcinoid	1	0.6
NEC(not elsewhere classified)	51	30
Age(y)		
<60	52	30.5
≥60	118	69.5
Year at diagnosis		
2004-2009	58	34.1
2010-2015	112	65.8
AJCC stage		
I	**20**	
IA	7	4.1
IB	5	2.9
IC	4	2.4
INOS	4	2.4
II	**8**	
IIA	2	1.2
IIB	4	2.3
IINOS	2	1.2
III	**47**	
IIIA	9	5.3
IIIB	5	2.9
IIIC	32	18.8
IIINOS	1	0.6
IV	**95**	
IVA	4	2.3
IVB	91	53.6
Grade		
Well/Moderately differentiated	1	0.6
Poorly/undifferentiateddifferentiated	128	75.3
Unknown	41	24.1
Lymph nodal metastasis		
Negative	29	17.1
Positive	33	19.4
Not examined	105	61.8
Unknown	3	1.7
Sampled pelvic nodes		
1–9	24	14.1
10–19	19	11.2
≥20	20	11.8
Not examined	105	61.8
Unknown	2	1.1
Distant metastasis		
bone	13	7.6
brain	8	4.7
liver	16	9.4
lung	23	13.6
No	68	40
Unknown	42	24.7
Treatment		
Surgery alone	24	14.1
Surgery + CT	43	25.2
Surgery + CCRT	19	11.1
Surgery + RT	3	1.8
CT alone	25	14.7
CCRT	19	11.2
RT alone	5	3.0
No treatment	32	18.9
Surgical approach		
Curettage	1	0.5
Subtotal hysterectomy +Ad	1	0.5
Total hysterectomy+Ad+LN	69	40.6
Extended radical hysterectomy+Ad+LN	18	10.7
No Surgical	81	47.7

RT, radiation; CT, chemotherapy; CCRT, concurrent chemoradiation; N, Number (%); y, years; AJCC, American Joint Commission on Cancer; NOS, not otherwise specified; SCNEC, small cell neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma; Ad, adnexectomy; LN, lymph node resection.
Bold means p < 0.05.

CT (n=5) for stage III, EBRT only (n=3), EBRT + CT (n=11) and CT only (n=23) for stage IV. Among surgically treated patients with stage I, II, III, and IV disease, treatments included CT in 3, 1, 21, and 18 cases and EBRT + CT in 4, 2, 6, and 4 cases and EBRT with implants in 1, 0, 2, and 0 cases, respectively. Additionally, 1 and 2 patients who underwent surgery for stage I and IV disease received EBRT.

Survival Outcomes

We discussed the survival results of patients with different stages. The OS and CCS curves of patients with different stages are shown in **Figure 1**. The 5-year OS rates for patients with stage I, II, III, and IV disease were 59.86%, 42.86%, 32.75%, and 6.04%, respectively. When stage I was used as the reference, HR for death at stage II, III, and IV were 1.370 (95% CI: 0.3815–4.919), 1.714 (95% CI: 0.845–3.48), and 3.174 (95% CI: 1.875–5.37), respectively. The 5-year CSS rates among patients with stage I, II, III, and IV disease were 59.86%, 50.0%, 38.33%, and 6.39%, respectively. When stage I was used as the reference, the HRs for death at stage II, III, and IV were 1.193 (95% CI: 0.298–4.769), 1.422 (95% CI: 0.663–3.047), and 3.819 (95% CI: 2.335–6.245), respectively.

Since only 1 ACT case and 2 carcinoid cases were identified, comparisons between histological subtypes were restricted to SCNEC and LCNEC. **Figure 2** shows the OS and CSS curves of patients with SCNEC and LCNEC. The median OS time among patients with SCNEC was 25 months, while that among patients with LCNEC was only 8 months. The 5-year OS rates for SCNEC and LCNEC were 33.16% and 16.94%, respectively. Relative to SCNEC, the HR for LCNEC was 1.623 (95% CI: 1.008–2.614, P=0.0373). The 5-year CSS rates for SCNEC and LCNEC were 41.02% and 25.22%, respectively. Relative to SCNEC, the HR for LCNEC was 1.708 (95% CI: 1.011–2.887; P=0.0375). The 5-year OS and CSS rates were thus significantly lower for LCNEC than for SCNEC.

We discussed the survival outcomes of patients who underwent different surgeries. The OS and CCS curves of the patients according to surgery type are shown in **Figure 3**. The 5-year OS rates for patients who underwent curettage, subtotal hysterectomy + adnexectomy, total hysterectomy + adnexectomy + lymphadenectomy, extended radical hysterectomy + adnexectomy + lymphadenectomy, and no surgery were 0%, 100%, 32.02%, 50.15%, and 5.80%, respectively. The 5-year CSS rates were 0%, 100.0%, 38.52%, 60.19%, and 7.32%, respectively.

TABLE 2 | Treatment at each stage for neuroendocrine tumors (NETs) of the endometrium.

	StageI n=20	StageII n=8	StageIII n=47	StageIV n=95
Surgery alone	6	3	8	7
Surgery+CT	3	1	21	18
Surgery+EBRT+CT	4	2	6	4
surgery+combination of EBRT with implants+CT	1	0	2	0
surgery+EBRT	1	0	0	2
EBRT+CT	0	1	5	11
Combination of EBRT with implants+CT	1	1	0	0
Combination of EBRT with implants	1	0	0	0
Implants radiation	0	0	0	0
EBRT	1	0	0	3
CT	0	0	2	23
Not treatment	2	0	3	27

CT, chemotherapy; EBRT, external beam radiation therapy.

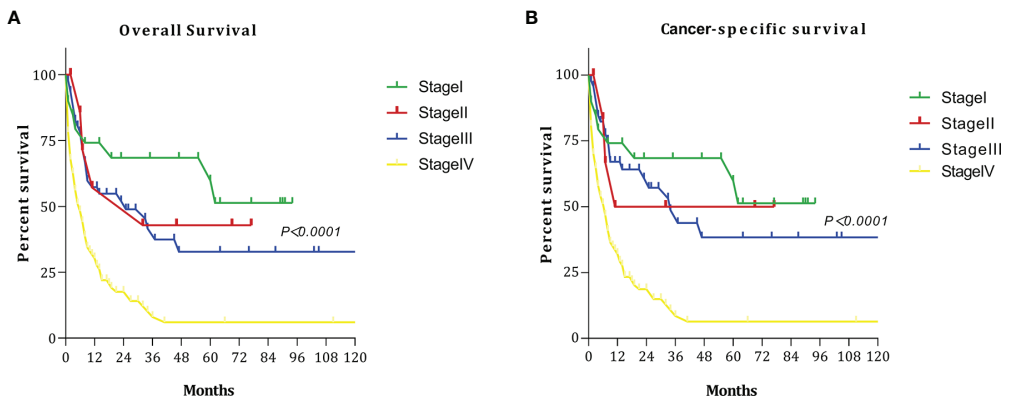

FIGURE 1 | Survival curves at each stage: **(A)** overall survival (OS); **(B)** cancer-specific survival (CSS).

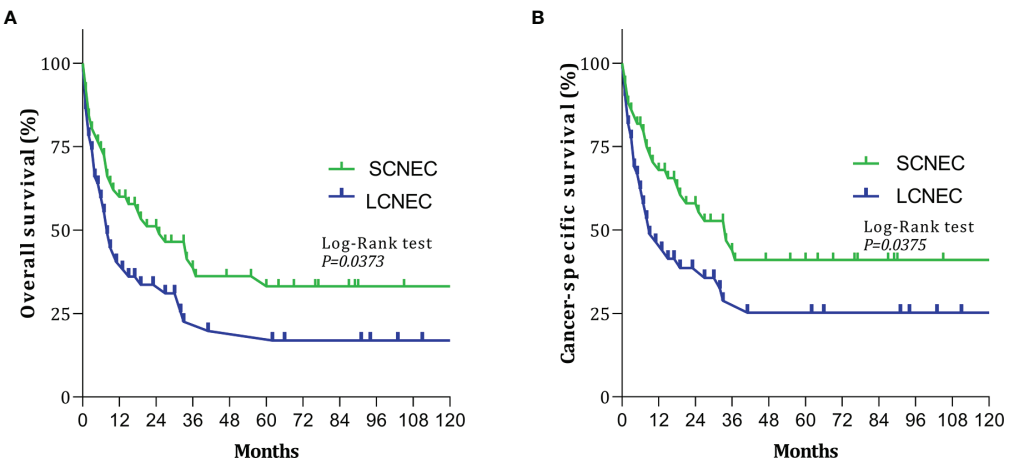

FIGURE 2 | Survival curves for patients with small-cell neuroendocrine carcinoma (SCNEC) and large-cell neuroendocrine carcinoma (LCNEC): **(A)** overall survival (OS); **(B)** cancer-specific survival (CSS).

Prognostic Factors for OS and CSS

To identify factors influencing prognosis among patients with NETs of the endometrium, we selected age, year at diagnosis, AJCC stage, number of lymph nodes sampled, lymph node metastasis, distant metastasis, histological type, and treatment as variables for the univariate and multivariate analyses (**Table 3**). The multivariate analysis showed that AJCC stage and treatment were independent predictors of OS. When stage I was used as the reference, the HR for death in stage III and IV were 3.368 (95% CI: 0.956–11.860) (p=0.039) and 6.750 (95% CI: 1.872–24.345, p=0.004), respectively. When surgery only was used as the reference, the HR for death among the patients who underwent surgery + CT and surgery + CCRT were 0.280 (95% CI: 0.142–0.553) (p <0.001), 0.157 (95% CI: 0.056–0.440) (p <0.001). Meanwhile, AJCC stage, lymph node metastasis, and treatment were independent predictors of CSS. When stage I was used as the reference, the HR for death in stage III and IV were 11.500 (95% CI: 1.259–25.069, p=0.030) and 35.096 (95% CI: 3.673–55.307, p=0.002), respectively. When the lymph node-negative patients were used as the reference, the HR for death in the non-examined lymph node-positive patients were 4.722 (95% CI: 1.552–14.369, p=0.006) and 3.632 (95% CI: 1.027–12.845, p=0.045), respectively. When surgery only was used as the reference, the HR for death in the surgery + CT and surgery + CCRT groups were 0.269 (95% CI: 0.127–0.570, p=0.001) and 0.154 (95% CI: 0.049–0.448, p=0.001), respectively.

Treatment

The main treatment for NETs of the endometrium was surgery, and the most common procedure was hysterectomy + bilateral adnexectomy + pelvic lymphadenectomy in 69 (40.6%) patients, followed by radical total hysterectomy + bilateral adnexectomy + pelvic lymphadenectomy in 18 (10.7%) patients, subtotal hysterectomy + bilateral adnexectomy in 1 (0.5%) patient, and curettage only in 1 (0.5%) patients (**Table 1**). Adjuvant therapy included CT and RT. RT included EBRT, radioactive implants, and EBRT with implants. The SEER database does not provide comprehensive information on CT; it only specifies whether CT was performed, without any specific information. Therefore, in this study, we were unable to determine the CT regimens used or the number of treatments performed.

Among the 28 patients with early-stage disease (I and II), the 5-year OS and CSS rates for surgery + CT and surgery + RT were both 100%, which were significantly better than those for other treatment regimens. Among the 142 patients with advanced-stage disease (III and IV), the 5-year OS and CSS rates for surgery + CCRT were both 65.27%. Thus, the survival rates were significantly higher with these treatments than with other treatments (**Figure 4**). The 5-year OS rates and CSS rates for CT only, RT only, and CCRT only were 4.16%, 0%, and 0% and 4.55%, 0%, and 0%, respectively (**Table 4**).

DISCUSSION

Endometrial NETs is a rare disease with poor prognosis. Given its extremely low incidence, the most effective methods for treating endometrial NETs and the most important factors for determining prognosis remain unknown, making clinical management difficult. In addition, due to its rarity, there are no evidence-based standards or international guidelines for the diagnosis and treatment of endometrial NETs. Therefore, we utilized the large sample size of the SEER database to investigate clinical features, prognosis, and treatment options for NETs of the endometrium.

Because NETs of the endometrium are very rare, the existing reports include case reports and small case series (4, 5, 7–16). The largest previous study analyzed data for 42 cases of endometrial NETs occurring in Japan over a 19-year period (17). This multicenter study suggested that stage III–IV disease and pure SCNEC are associated with significantly poorer prognosis than other disease stages and histological types. However, some studies have reported long-term survival in patients with advanced disease (10, 12, 16). Sawada et al. (17) reported a rare case of advanced SCNEC with liver and brain metastases in a patient who underwent pelvic tumor reduction surgery + metastatic resection and postoperative treatment with CT (irinotecan + cisplatin) + RT, following which the patient survived for 12 years. Viau et al. (18) reported a case of stage IV

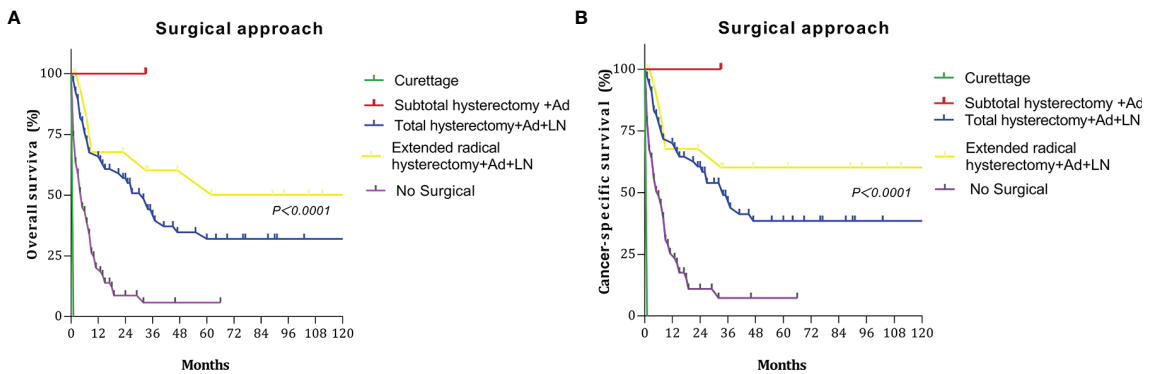

FIGURE 3 | Survival curves at different surgery type: **(A)** overall survival (OS); **(B)** cancer-specific survival (CSS).

TABLE 3 | Prognostic factors for neuroendocrine tumors (NETs) of the endometrium.

Subject characteristics	n	Overall survival				Cancer-specific survival			
		Univariate HR (95%CI)	p value	Multivariate HR (95%CI)	p value	Univariate HR (95%CI)	p value	Multivariate HR (95%CI)	p value
Age									
<60	52	Ref	0.071	–	–	Ref	0.201	–	–
≥60	118	1.451 (0.968-2.176)				1.318 (0.863-2.013)			
Year at diagnosis									
2004-2009	58	Ref	0.094	–	–	Ref	0.149	–	–
2010-2015	112	1.389 (0.946-2.04)		–	–	1.351 (0.898-2.031)		–	–
AJCC stage									
I	20	Ref	**<0.001**	Ref	**0.008**	Ref	**<0.001**	Ref	**0.001**
II	8	1.396 (0.420-4.640)	0.586	2.18 (0.457-10.395)	0.328	2.100 (0.470-9.389)	0.332	9.064 (0.769-16.813)	0.080
III	47	1.745 (0.787-3.871)	0.17	3.368 (0.956-11.860)	0.039	2.808 (0.961-8.208)	0.059	11.500 (1.259-25.069)	0.030
IV	95	5.030 (2.400-10.542)	<0.001	6.750 (1.872-24.345)	0.004	9.482 (3.431-26.204)	<0.001	35.096 (3.673-55.307)	0.002
Sampled pelvic nodes									
Negative	29	Ref	**<0.001**	Ref	0.099	Ref	**<0.001**	Ref	**0.024**
Positive	33	2.778 (1.264-6.102)	0.011	2.803 (1.078-7.289)	0.035	4.623 (1.728-12.369)	**0.002**	4.722 (1.552-14.369)	0.006
Not examined	105	5.918 (2.945-11.893)	<0.001	1.941 (0.675-5.580)	0.218	9.249 (3.714-23.037)	<0.001	3.632 (1.027-12.845)	0.045
Lymph node sampling									
1–9	24	Ref	**<0.001**	Ref	0.594	Ref	**<0.001**	Ref	0.166
10–19	19	0.588 (0.228-1.515)	0.271	1.030 (0.341-3.113)	0.958	0.811 (0.300-2.194)	0.680	2.640 (0.880-7.920)	0.083
≥20	20	0.594 (0.252-1.401)	0.234	1.017 (0.402-2.577)	0.971	0.720 (0.279-1.857)	0.496	1.797 (0.643-5.020)	0.263
Not examined	105	2.378 (1.366-4.138)	0.002	1.921 (0.672-5.582)	0.212	2.866 (1.515-5.421)	0.001	0.769 (0.331-1.790)	0.543
Distant metastasis									
Yes	41	Ref	**0.001**	Ref	0.345	Ref	**<0.001**	Ref	0.485
No	68	0.480 (0.305-0.756)	0.002	0.891 (0.476-1.670)	0.720	0.383 (0.236-0.623)	<0.001	0.785 (0.399-1.542)	0.482
Unknown	61	0.469 (0.297-0.742)	0.001	0.645 (0.339-1.228)	0.182	0.434 (0.270-0.696)	0.001	0.670 (0.349-1.287)	0.229
Hystological type									
SCNEC	56	Ref	0.055	–	–	Ref	0.059	–	–
LCNEC	60	1.544 (0.991-2.405)		–	–	1.669 (1.026-2.716)		–	–
Treatment									
Surgery alone	24	Ref	**<0.001**	Ref	**<0.001**	Ref	**<0.001**	Ref	**0.001**
Surgery + CT	43	0.585 (0.312-1.097)	0.095	0.280 (0.142-0.553)	<0.001	0.643 (0.327-1.266)	0.202	0.269 (0.127-0.570)	0.001
Surgery + CCRT	19	0.206 (0.076-0.560)	0.002	0.157 (0.056-0.440)	<0.001	0.202 (0.066-0.614)	0.005	0.154 (0.049-0.448)	0.001
Surgery + RT	3	0.985 (0.227-4.280)	0.984	1.496 (0.309-7.251)	0.617	1.244 (0.282-5.491)	0.773	2.219 (0.429-11.489)	0.342
CT alone	25	2.066 (1.094-3.904)	0.025	0.664 (0.303-1.457)	0.307	2.371 (1.201-4.679)	0.013	0.627 (0.271-1.451)	0.276
CCRT	19	1.399 (0.691-2.832)	0.351	0.673 (0.300-1.513)	0.339	1.441 (0.671-3.095)	0.349	0.642 (0.267-1.546)	0.323
RT alone	5	2.293 (0.834-6.301)	0.108	1.608 (0.525-4.921)	0.406	2.157 (0.701-6.635)	0.18	1.326 (0.384-4.574)	0.655

AJCC, American Joint Commission on Cancer; SCNEC, small cell neuroendocrine; LCNEC, large cell neuroendocrine carcinoma; HR, hazard ratio; CI, confidence interval. Bold means p < 0.05.

SCNEC treated with surgery + CT (cisplatin + etoposide) + RT, and their patient remained alive 5 years later.

The current study included the largest cohort of patients with NETs of the endometrium to date. Given its large sample size relative to previous reports (170 cases), our study provides stronger evidence that surgery should be the main treatment strategy regardless of the endometrial NET stage. In addition, our results suggest that for early-stage disease, individualized postoperative treatment *via* single CT or radiotherapy may improve OS and CSS. For advanced-stage disease, comprehensive postoperative adjuvant therapy may improve OS and CSS. Since only one patient underwent subtotal hysterectomy + adnexectomy, it is necessary to continue accumulating cases for further analyses. From our analysis, the 5-year OS and CSS of patients who underwent total hysterectomy + adnexectomy + lymphadenectomy and extended radical hysterectomy + adnexectomy + lymphadenectomy are higher than those of patients who underwent other treatment methods. Therefore, complete surgical treatment may improve outcomes in patients with the disease.

Nonetheless, comprehensive treatment may not enable long-term survival in all patients with NETs of the endometrium, especially those with LCNEC. Tu (19) reported a case of stage IVB LCNEC treated with adjuvant CT (cisplatin + etoposide) postoperatively, following which a cisplatin + ifosfamide regimen was used to treat disease progression. Two months later, obstructive ileus was observed, and the patient underwent second surgery. However, she died of infection 8 days after surgery. Kobayashi (20) reported a case of stage IIIC2 LCNEC in which CCRT was initiated 1 month after surgery. The patient developed rapidly progressing metastases in the upper abdominal and cervical regions subsequently and died eventually of the disease 309 days after surgery.

Based on the treatment plan for pulmonary NETs, platinum-based CT is often used for adjuvant treatment in patients with NETs of the endometrium. Currently, the most common regimen is paclitaxel + carboplatin, followed by EP (cisplatin + etoposide) and other treatment options. EBRT, implants, or a combination of EBRT and implants is recommended for RT. Some researchers have suggested that CT is also required in the early stage given the

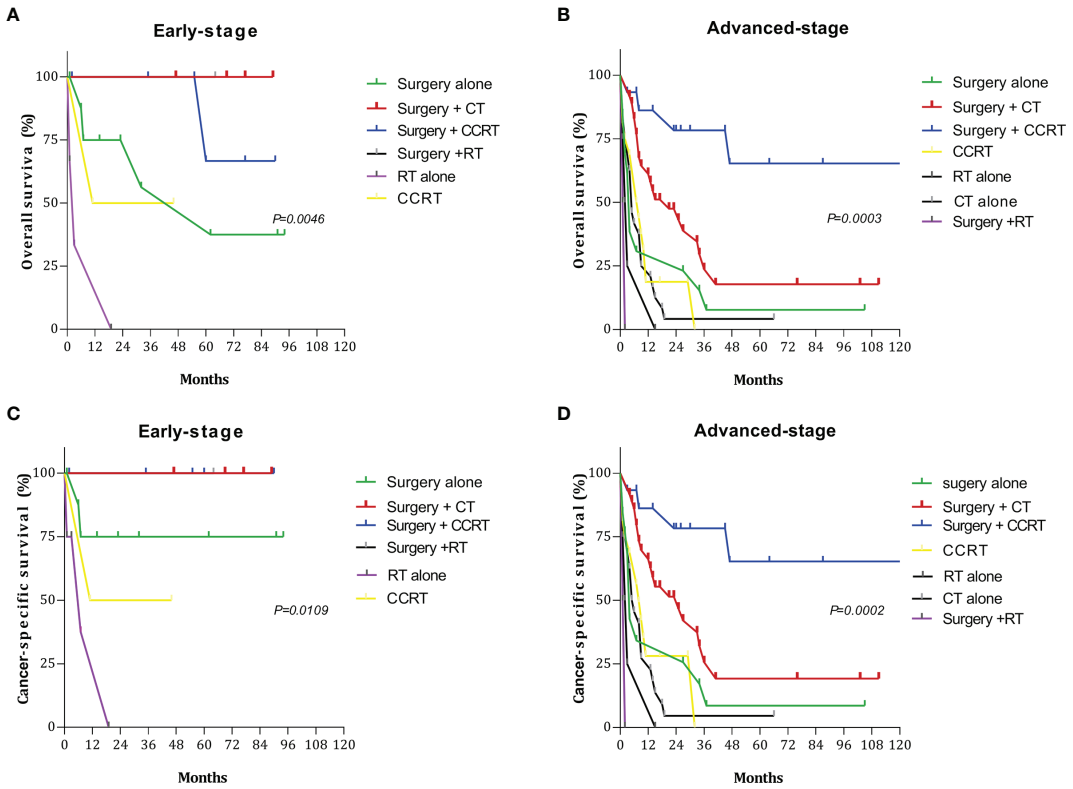

FIGURE 4 | Survival curves for patients with early- and advanced-stage disease for different treatment regimens: **(A)** overall survival (OS) in the early stage; **(B)** overall survival (OS) in the advanced stage; **(C)** cancer-specific survival (CSS) in the early stage; **(D)** cancer-specific survival (CSS) in the advanced stage.

TABLE 4 | Five-year OS and CSS according to stage and treatment in patients with neuroendocrine tumors (NETs) of the endometrium.

Treatments	N	5-year OS	*P*	5-year CSS	P
Stage I-II			0.0046		0.0109
Surgery alone	9	37.50%		75.00%	
Surgery + CT	4	100.00%		100.00%	
Surgery + CCRT	6	66.67%		100.00%	
Surgery + RT	1	100.00%		100.00%	
CCRT	3	50.00%		50.00%	
RT alone	2	0.00%		0.00%	
CT alone	0	0		0	
Stage III-IV			0.0003		0.0002
Surgery alone	15	7.69%		8.54%	
Surgery + CT	39	17.76%		19.23%	
Surgery + CCRT	13	65.27%		65.27%	
Surgery + RT	2	0.00%		0.00%	
CCRT	16	0.00%		0.00%	
RT alone	3	0.00%		0.00%	
CT alone	25	4.16%		4.55%	

RT, radiotherapy; CT, chemotherapy; CCRT, concurrent chemoradiotherapy; OS, overall survival; CSS, cancer-specific survival.

aggressive nature of NETs of the endometrium (4, 21). Korcum et al. (22) argued that brachytherapy may be sufficient when performed in conjunction with cisplatin treatment to prevent systemic micrometastases. NETs of the endometrium often presents with disseminated disease, indicating that radical surgery with CT would be appropriate for both early and advanced cases (1). Combined treatment with CT and somatostatin-like octreotide has also been reported in patients with NETs of the endometrium. The inhibitory effect of somatostatin analogs on tumor growth has been demonstrated (23).

To date, no studies have characterized the specific imaging findings associated with endometrial NETs. Makihara et al. (24)

reported that MRI findings for LCNEC were similar to those for other poorly differentiated endometrial carcinomas and sarcomas, and preoperative diagnosis of endometrial NETs based on MRI or PET/CT remains difficult (25).

Previous studies analyzing the relationship between prognosis and histological subtypes of endometrial NETs have yielded contradictory conclusions. In this study, we compared the prognoses of SCNEC and LCNEC. Several studies have indicated that SCNEC is the most common histological subtype of endometrial NETs (1, 5, 6, 16, 26–28). While some authors have reported worse prognosis for SCNEC than for LCNEC (8), others have reported that LCNEC tends to be more aggressive and has a worse prognosis than SCNEC (1, 6, 29). Furthermore, Mulvany et al. (27) reported very poor prognosis among patients with LCNEC regardless of stage. These discrepancies are likely due to the small sample size. In this study, we compared data for 56 cases of SCNEC and 60 cases of LCNEC. The median survival time for SCNEC was 25 months, while that for LCNEC was only 8 months. The prognosis of LCNEC is significantly lower than that of SCNEC. These findings may help to clarify the influence of histological subtype on prognosis in patients with endometrial NETs.

Common metastasis sites in patients with NETs of the endometrium include the brain, lungs, liver, kidney, and bone; and NETs of the endometrium often has rapid metastasis and recurrence (27, 30, 31). Our study found that distant metastasis sites of NETs of the endometrium were the brain, lungs, liver, and bone, accounting for 35.3% of all cases, and there was no information regarding recurrence in the SEER database. To improve the prognosis of recurrent endometrial NETs, future studies focusing on early detection techniques and optimal strategies for managing recurrence are warranted.

This article has certain limitations. First, while the SEER database informs whether patients received CT, it does not specify the type of CT or the number of CT/RT cycles, highlighting the need for further studies to determine which regimens are most effective at each disease stage. The SEER database has other limitations, as it does not provide details related to the time of treatment, the treatment location, or the treatments used in cases of recurrence. Additional clinical cases must be accumulated to address these issues. Moreover, there are currently no standard treatment options for recurrent NETs of the endometrium. Although molecular typing focuses on endometrial non-neuroendocrine carcinomas, novel drug treatments based on molecular targeting represent a key area of research. Nonetheless, there is currently no method for molecular typing that can aid in identifying prognostic subgroups among patients with NETs of the endometrium (32), and only one study has demonstrated the role of mismatch repair proteins in endometrial NETs (6).

CONCLUSION

Our findings indicate that AJCC stage and treatment are independent prognostic factors for OS, while AJCC stage, nodal metastasis, and treatment are independent prognostic factors for CSS. Complete surgical treatment may improve outcomes in patients with the disease. For patients with early NETs of the endometrium, treatment regimens including surgery and postoperative adjuvant RT or CT can significantly improve OS and CSS. For patients with advanced NETs of the endometrium, surgery should be selected as the primary treatment method when feasible, and postoperative adjuvant comprehensive therapy (surgery + CT + RT) may help to improve OS and CSS. Further studies are required to determine the most appropriate treatment regimens and prognostic factors for recurrent endometrial NETs.

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.

AUTHOR CONTRIBUTIONS

JZ collected clinical data and wrote the manuscript; Conceptualization; Data curation; Formal analysis; Writing - original draft. LP Data curation; Writing - review and editing. Both authors contributed to the article and approved the submitted version.

REFERENCES

1. Albores-Saavedra J, Martinez-Benitez B, Luevano E. Small Cell Carcinomas and Large Cell Neuroendocrine Carcinomas of the Endometrium and Cervix: Polypoid Tumors and Those Arising in Polyps may Have a Favorable Prognosis. *Int J Gynecol Pathol* (2008) 27:333–9. doi: 10.1097/PGP.0b013e31815de006
2. Crowder S, Tuller E. Small Cell Carcinoma of the Female Genital Tract. *Semin Oncol* (2007) 34:57–63. doi: 10.1053/j.seminoncol.2006.10.028
3. Kurman RJ, Carcangiu ML, Herrington CS Young RH. WHO Classification of Tumours of Female Reproductive Organs. *Lyon: IARC* (2014) 122.
4. Huntsman DG, Clement PB, Gilks CB, Scully RE. Small-Cell Carcinoma of the Endometrium. A Clinicopathological Study of Sixteen Cases. *Am J Surg Pathol* (1994) 18:364–75. doi: 10.1097/00000478-199404000-00005
5. van Hoeven KH, Hudock JA, Woodruff JM, Suhrland MJ. Small Cell Neuroendocrine Carcinoma of the Endometrium. *Int J Gynecol Pathol* (1995) 14:21–9. doi: 10.1097/00004347-199501000-00005
6. Pocrnich CE, Ramalingam P, Euscher ED, Malpica A. Neuroendocrine Carcinoma of the Endometrium: A Clinicopathologic Study of 25 Cases. *Am J Surg Pathol* (2016) 40:577–86. doi: 10.1097/PAS.0000000000000633
7. Bahig H, Portelance L, Legler C, Gilbert L, Souhami L. Small Cell Carcinoma of the Endometrium: Report of a Case and Review of the Literature. *Minerva Ginecol* (2009) 61:365–9.
8. Chetty R, Clark SP, Bhathal PS. Carcinoid Tumour of the Uterine Corpus. *Virchows Arch A Pathol Anat Histopathol* (1993) 422:93–5. doi: 10.1007/BF01605139
9. González-Bosquet E, González-Bosquet J, García Jiménez A, Gil A, Xercavins J. Carcinoid Tumor of the Uterine Corpus. *A Case Rep J Reprod Med* (1998) 43:844–6.

10. Abu-Rustum NR, Yashar CM, Bean S, Bradley K, Campos SM, Chon HS, et al. NCCN Guidelines Insights: Cervical Cancer, Version 1.2020. *J Natl Compr Canc Netw* (2020) 18:660–6. doi: 10.6004/jnccn.2020.0027
11. *National Cancer Institute Surveillance, Epidemiology and End Results Program* . Available at: https://seer.cancer.gov/2019 (Accessed 8 Nov 2019).
12. Shopov ST, Anavi BL, Krastev DK. Large-Cell Neuroendocrine Carcinoma of the Endometrium in Myomatous Uterus. *Folia Med (Plovdiv)* (2020) 62:412–7. doi: 10.3897/folmed.62.e49815
13. Jenny C, Kimball K, Kilgore L, Boone J. Large Cell Neuroendocrine Carcinoma of the Endometrium: A Report and Review of the Literature. *Gynecol Oncol Rep* (2019) 28:96–100. doi: 10.1016/j.gore.2019.03.006
14. Du R, Jiang F, Wang ZY, Kang YQ, Wang XY, Du Y. Pure Large Cell Neuroendocrine Carcinoma Originating From the Endometrium: A Case Report. *World J Clin cases* (2021) 9:3449–57. doi: 10.12998/wjcc.v9.i14.3449
15. Akgor U, Kuru O, Sakinci M, Boyraz G, Sen S, Cakır I, et al. Neuroendocrine Carcinoma of the Endometrium: A Very Rare Gynecologic Malignancy. *J Gynecol Obstet Hum Reprod* (2021) 50:101897. doi: 10.1016/j.jogoh.2020.101897
16. Bige O, Saatli B, Secil M, Koyuncuoglu M, Saygili U. Small Cell Neuroendocrine Carcinoma of the Endometrium and Laparoscopic Staging: A Clinicopathologic Study of a Case and a Brief Review of the Literature. *Int J Gynecol Cancer* (2008) 18:838–43. doi: 10.1111/j.1525-1438.2007.01059.x
17. Matsumoto H, Shimokawa M, Nasu K, Shikama A, Shiozaki T, Futagami M, et al. Clinicopathologic Features, Treatment, Prognosis and Prognostic Factors of Neuroendocrine Carcinoma of the Endometrium: A Retrospective Analysis of 42 Cases From the Kansai Clinical Oncology Group/Intergroup Study in Japan. *J Gynecol Oncol* (2019) 30:e103. doi: 10.3802/jgo.2019.30.e103
18. Sawada M, Matsuzaki S, Yoshino K, Ueda Y, Yoshida S, Kimura T, et al. Long-Term Survival in Small-Cell Carcinoma of the Endometrium With Liver and Brain Metastases. *Anticancer Drugs* (2016) 27:138–43. doi: 10.1097/CAD.0000000000000289
19. Tu YA, Chen YL, Lin MC, Chen CA, Cheng WF. Large Cell Neuroendocrine Carcinoma of the Endometrium: A Case Report and Literature Review. *Taiwan J Obstet Gynecol* (2018) 57:144–9. doi: 10.1016/j.tjog.2017.12.025
20. Kobayashi A, Yahata T, Nanjo S, Mizoguchi M, Yamamoto M, Mabuchi Y, et al. Rapidly Progressing Large-Cell Neuroendocrine Carcinoma Arising From the Uterine Corpus: A Case Report and Review of the Literature. *Mol Clin Oncol* (2017) 6:881–5. doi: 10.3892/mco.2017.1229
21. Varras M, Ch A, Demou A, Hadjopoulos G, Stefanaki S, Antoniou N. Primary Small-Cell Carcinoma of the Endometrium: Clinicopathological Study of a Case and Review of the Literature. *Eur J Gynaecol Oncol* (2002) 23:577–81.
22. Korcum AF, Aksu G, Ozdogan M, Erdogan G, Taskin O. Stage I Small Cell Carcinoma of the Endometrium: Survival and Management Options. *Acta Obstet Gynecol Scand* (2008) 87:122.e6. doi: 10.1080/00016340701709307
23. Shahabi S, Pellicciotta I, Hou J, Graceffa S, Huang GS, Samuelson RN, et al. Clinical Utility of Chromogranin A and Octreotide in Large Cell Neuroendocrine Carcinoma of the Uterine Corpus. *Rare Tumors* (2011) 3: e41. doi: 10.4081/rt.2011.e41
24. Makihara N, Maeda T, Nishimura M, Deguchi M, Sonoyama A, Nakabayashi K, et al. Large Cell Neuroendocrine Carcinoma Originating From the Uterine Endometrium: A Report on Magnetic Resonance Features of 2 Cases With Very Rare and Aggressive Tumor. *Rare Tumors* (2012) 4:e37. doi: 10.4081/rt.2012.e37
25. Kitajima K, Kihara T, Kawanaka Y, Takahama J, Ueno Y, Murakami T, et al. Characteristics of MR Imaging for Staging and Survival Analysis of Neuroendocrine Carcinoma of the Endometrium: A Multicenter Study in Japan. *Magn Reson Med Sci* (2021) 20:236–44. doi: 10.2463/mrms.mp.2020-0056
26. Katahira A, Akahira J, Niikura H, Ito K, Moriya T, Matsuzawa S, et al. Small Cell Carcinoma of the Endometrium: Report of Three Cases and Literature Review. *Int J Gynecol Cancer* (2004) 14:1018–23. doi: 10.1111/j.1048-891X.2004.14545.x
27. Mulvany NJ, Allen DG. Combined Large Cell Neuroendocrine and Endometrioid Carcinoma of the Endometrium. *Int J Gynecol Pathol* (2008) 27:49–57. doi: 10.1097/pgp.0b013e31806219c5
28. Ureyen I, Karalok A, Turan T, Boran N, Tapısız OL, Okten H, et al. Small Cell Carcinoma of the Endometrium: A Report of Three Cases. *J Turk Ger Gynecol Assoc* (2013) 14:113–5. doi: 10.5152/jtgga.2013.33396
29. Viau M, Baragar I, Altman AD. Long-Term Survival in a Stage IV Small Cell Carcinoma of the Endometrium. *Gynecol Oncol Rep* (2020) 32:100580. doi: 10.1016/j.gore.2020.100580
30. Erhan Y, Dikmen Y, Yucebilgin MS, Zekioglu O, Mgoyi L, Terek MC. Large Cell Neuroendocrine Carcinoma of the Uterine Corpus Metastatic to Brain and Lung: Case Report and Review of the Literature. *Eur J Gynaecol Oncol* (2004) 25:109–12.
31. Rouzbahman M, Clarke B. Neuroendocrine Tumors of the Gynecologic Tract: Select Topics. *Semin Diagn Pathol* (2013) 30:224–33. doi: 10.1053/j.semdp.2013.06.007
32. Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng A, et al. Molecular Classification of Endometrial Carcinoma on Diagnostic Specimens is Highly Concordant With Final Hysterectomy: Earlier Prognostic Information to Guide Treatment. *Gynecol Oncol* (2016) 143:46–53. doi: 10.1016/j.ygyno.2016.07.090

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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Oncological Safety of Diagnostic Hysteroscopy for Apparent Early-Stage Type II Endometrial Cancer: A Multicenter Retrospective Cohort Study

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Objective: To study the oncological safety of diagnostic hysteroscopy for women with apparent early-stage type II endometrial cancer.

Patients and Methods: A total of 429 women with presumed early-stage type II endometrial cancer were included. The 5-year disease-free survival (DFS) and overall survival (OS) were estimated and compared using the Kaplan-Meier method and the log-rank test among patients diagnosed by Dilation & Curettage (D&C) or diagnostic hysteroscopy. The Cox proportional hazards regression model was employed to adjust for potential confounding factors.

Results: 160 cases underwent D&C and 269 cases were diagnosed by diagnostic hysteroscopy. The 5-year DFS rate was 72.17% in the diagnostic hysteroscopy group and 76.16% in the D&C group, diagnostic hysteroscopy was not associated with deteriorated 5-year DFS rate (HR 1.25, 95% CI 0.84-1.86, *P*=0.281). The 5-year OS rate was 67.23% in the diagnostic hysteroscopy group and 70.71% in the D&C group, diagnostic hysteroscopy did not increase the risk of all-cause death (HR 1.11, 95% CI 0.78-1.57, *P*=0.573). Multivariable analysis showed that the method of endometrial sampling was not independently associated with DFS (aHR 1.38, 95% CI 0.92-2.07, *P*=0.122) and OS (aHR 1.23, 95% CI 0.85-1.77, *P*=0.272).

Conclusion: For apparent early-stage type II endometrial cancer, endometrial sampling by diagnostic hysteroscopy was as safe as D&C.

Keywords: uterine serous carcinoma, uterine clear cell carcinoma, diagnostic hysteroscopy, overall survival, disease-free survival

INTRODUCTION

In developed countries, endometrial cancer ranks first in common gynecological malignancies (1, 2). In 2020, endometrial cancer is diagnosed in about 420,000 women worldwide, and an estimated 98,000 women die from this cancer (3). To make matters worse, the incidence of endometrial cancer and the associated mortality are increasing among women of all backgrounds (2, 3).

In 1983, to reflect the disparate biologic behaviors and to refine the different prognoses, Bokhman classified endometrial cancer to type I cancers and type II cancers (4). Since then, this categorization system of endometrial cancer was universally adopted (2). Unlike type I endometrial cancer, type II endometrial cancer usually develops in nonobese women and is not related to hyperestrogenemia, endometrial hyperplasia, or metabolic syndrome (2, 5). Histologically, type II endometrial cancer is poorly differentiated or undifferentiated, including uterine serous carcinoma (USC), uterine clear cell carcinoma (UCCC), and uterine carcinosarcoma (2, 6, 7). Generally, type II endometrial cancer is clinically aggressive, usually presenting at advanced stages, having high rates of extrauterine involvement, and having a high risk of recurrence (2, 5, 6).

For women with endometrial cancer, the most common manifestation is abnormal uterine bleeding (2, 8). In women with abnormal uterine bleeding, to rule out malignant diseases, ultrasound and endometrial sampling are often required (8). Dilation & Curettage (D&C) and diagnostic hysteroscopy are the two most common methods for endometrial evaluation (8). Compared with D&C, by providing physicians with a visualization of the uterine cavity and facilitating the directed biopsy of suspicious lesions, diagnostic hysteroscopy is considered more accurate (9, 10). However, some researchers present their concerns. They think that in the process of diagnostic hysteroscopy, the elevated pressure in the uterine cavity may increase the risk of dissemination of cancer cells (11, 12). To date, however, many published studies have agreed that diagnostic hysteroscopy, although it can increase the spread of tumor cells into the peritoneal cavity, does not deteriorate the prognosis of endometrial cancer (13–16). But, it is worth noting that in these studies, almost all the included cases were low-risk endometrial cancer (14–16). Due to its rarity, the oncological safety of diagnostic hysteroscopy for type II endometrial cancers is always under-researched. Given the large biological and clinical heterogeneity between type I endometrial cancer and type II endometrial cancer, it is unknown whether diagnostic hysteroscopy is oncological safe for type II endometrial cancer.

Taken together, to explore the oncological safety of diagnostic hysteroscopy for apparent early-stage type II endometrial cancer, we conducted this multicenter retrospective cohort study.

PATIENTS AND METHODS

Study Design

This was a multicenter retrospective cohort study, which was based on six Chinese teaching hospitals. This study was approved by the Institutional Review Board (IRB) of each participating institution. In consideration of the retrospective nature of the study design and this study did not report any identifiable private data, the written informed consent to participate was exempted by the IRBs of the participating centers.

Patients

In this study, women with apparent early-stage type II endometrial cancer who had received a diagnosis during the 2011-2016 period and had been managed with surgical staging were included.

Patients would be eligible for this study if they met the following criteria: were between 18 and 80 years old, diagnosed with USC and UCCC by pathological examination, had no signs of a suspicious advanced disease, managed with surgical staging (at least including total hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy) within one month after the definite diagnosis, and were consecutively followed up at the participating institutions.

In this study, the signs of suspected advanced diseases were defined as follows: suspicious involvement of the vagina, suspicious metastases of fallopian tubes and/or ovaries, enlarged regional lymph nodes (pelvic and/or para-aortic), or suspicious extrauterine metastases identified by pelvic examination or/and preoperative imaging (including ultrasound, computed tomography, and magnetic resonance imaging). All included cases were staged postoperatively based on the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system for endometrial cancer.

We excluded patients from this study for whom the method of endometrial sampling was unknown, those who lost to follow-up after initial management, those who were managed nonsurgically, those who had undergone neoadjuvant therapy, those who had a history of other malignancies, and those whose postoperative stage of disease was unknown. In this study, patients with a preoperative American Society of Anesthesiologists (ASA) physical status score of IV or larger were also considered not qualified for inclusion.

Data Collection

Demographic, clinical, and pathological data of the included cases were extracted from the medical record management systems of the participating institutions. The data of interest were as follows: year of diagnosis, age at diagnosis, marital status at diagnosis, body mass index (BMI) at diagnosis, the preoperative ASA physical status score, the histological type of the tumor, the grade of tumor differentiation, tumor size, the FIGO stage of disease, the status of lymphovascular space invasion (LVSI), the result of peritoneal cytology, the approach of surgical staging, the scope of lymphadenectomy, the method of endometrial sampling, and the protocol of postoperative adjuvant therapy. Given the retrospective nature of this study, we accepted the clinical heterogeneity in the method of performing diagnostic hysteroscopy among the participating institutions, such as the pressure value of the solution jet, the number of biopsies, and the place of diagnostic hysteroscopy (an office setting or operative room setting), etc.

Outcomes of Interest

In this study, disease-free survival (DFS) and overall survival (OS) were the primary outcomes of interest. DFS was defined as the time from diagnosis to disease recurrence or death from endometrial cancer. OS was defined as the time from diagnosis to death from any cause.

All included patients were followed up to death or until January 1, 2022. Data regarding patients with no evidence of recurrence or death were censored at the date of the last follow-up. Data on survival outcomes were collected as follows: vital status, time of disease recurrence, site of disease recurrence, time of death, and cause of death.

Statistical Analysis

Based on the method of endometrial sampling, the included patients were divided into the D&C group and the diagnostic hysteroscopy group. The baseline characteristics were compared between the two groups. When assumptions of normal distribution were confirmed, comparisons of continuous variables would be performed by parametric methods. While the comparisons of non-normally distributed variables and categorical data were performed using nonparametric tests.

The Kaplan–Meier method was employed to generate the survival curves. The comparisons of the survival outcomes between the D&C group and the diagnostic hysteroscopy group were carried out by using the Log-rank test. To adjust the unbalanced confounding factors between the two groups, the Cox proportional hazards regression model was employed to estimate the adjusted hazard ratios (aHR) and 95% confidence intervals (95% CI) for the effect of diagnosis methods on DFS and OS in women with apparent early-stage type II endometrial cancer. To ensure parsimony of the final model, the following variables would be included in the Cox proportional hazards regression model: that was considered clinically relevant to prognosis or that showed a univariate relationship (P-value < 0.2) with outcomes of interest.

Statistical analyses were performed using STATA software, version 17 (StataCorp). Unless otherwise stated, all analyses were carried out with a two-sided significance level of 0.05.

RESULTS

Study Cohort

Between January 2011 and January 2016, a total of 11,759 women with endometrial cancer were managed at these participating institutions. After excluding 11,330 patients who were not qualified for the current study, a total of 429 women with apparent early-stage type II endometrial cancer were included in this study. The process of case selection is presented in **Figure 1**. Among the included patients, 160 patients (37.3%) got diagnosed by diagnostic hysteroscopy, the remaining patients were diagnosed by D&C.

According to the methods of endometrial sampling, the included patients were divided into the D&C group (N=269) and the diagnostic hysteroscopy group (N=160). The comparisons of patient demographics, clinicopathologic characteristics, and treatment variables between the D&C group and the diagnostic hysteroscopy group are summarized in **Table 1**.

For the entire cohort, the mean age was 66.5 years (standard deviation: 7.62), and the median duration of follow-up was 50 months (range: 4 months to 107 months). In terms of the age at diagnosis and the duration of follow-up, there was no statistical difference between the two groups (P=0.171 and P=0.071, respectively). There was also no statistical difference in the mean BMI between the two groups, 22.5 kg/m^2 and 22.7 kg/m^2, respectively. At diagnosis, the proportion of patients being single (including divorced, widowed, separated, and never married) in the hysteroscopy group was significantly higher than that in the D&C group (P=0.003).

In terms of the clinicopathological features of the tumors, 72% of cases were serous carcinomas, about 64% of tumors were poorly differentiated and less than 4 cm, about 20% of cases were found to be advanced (FIGO stage III or IV), 20.5% of patients were identified with positive peritoneal cytology, and 25.6% of the included patients had LVSI. Generally, the histologic type, the grade of tumor differentiation, the size of the tumor, the stage of the disease, and the incidence of LVSI were statistically similar between the D&C group and the diagnostic hysteroscopy group. However, the proportion of patients with positive peritoneal cytology in the diagnostic hysteroscopy group was significantly higher than that in the D&C group, at 31.9% and 13.8%, respectively. The difference in the incidence of positive peritoneal cytology between the two groups was statistically significant (P< 0.001).

Of the included patients, 62.2% got surgical staged by laparoscopy, 36.6% underwent complete regional lymph node removal (combined pelvic and para-aortic lymphadenectomy), and about 75% had postoperative adjuvant therapy (chemotherapy or/and radiotherapy). The protocol of management (surgical approach of staging, extent of lymphadenectomy, and postoperative adjuvant therapy) between the D&C group and the hysteroscopy group was not statistically different.

Survival Outcomes

A total of 55 patients experienced disease recurrence, 18 from the diagnostic hysteroscopy group and the rest from the D&C group, rates of disease recurrence were not statistically different between the two groups (P=0.551). In terms of the pattern of disease recurrence in the two groups, the three most common sites of recurrence are the abdomen (3.0%), lungs (2.6%), and pelvis (1.9%). There was no statistical difference in the pattern of disease recurrence between the two groups (P>0.999). **Table 2** shows the pattern and rate of disease recurrence by diagnostic hysteroscopy vs. D&C.

With a median follow-up of 50 months, a total of 106 cases of recurrence or/and death from endometrial cancer were identified. **Supplementary Material 1A** shows the DFS curve of the entire cohort. Among them, 63 cases were from the D&C group, and the remaining 43 cases were in the diagnostic hysteroscopy group. The 5-year DFS rate by the Kaplan-Meier method was 72.17% (95% CI 63.68%–79.00%) in the diagnostic hysteroscopy group and 76.16% (95% CI 69.91%–81.29%) in the

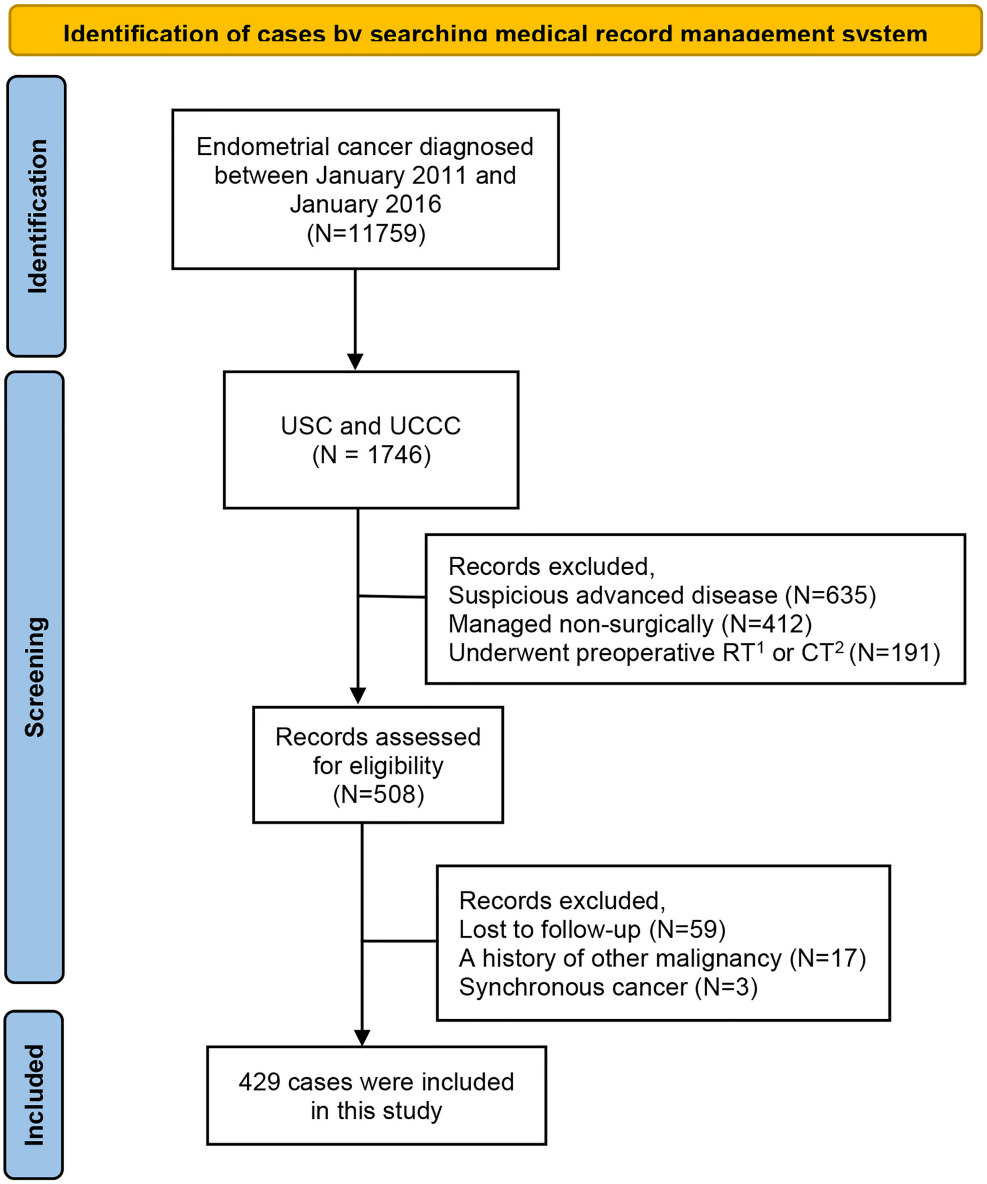

FIGURE 1 | Flow chart of cases selection.

D&C group. The Log-rank test indicated that for patients with apparent early-stage type II endometrial cancer, diagnostic hysteroscopy was not associated with deteriorated 5-year DFS (HR 1.25, 95% CI 0.84-1.86, *P*=0.281). **Figure 2A** shows the Kaplan-Maier curve of DFS (diagnostic hysteroscopy *VS.* D&C).

As of January 1, 2022, a total of 135 all-cause deaths have been confirmed. **Supplementary Material 1B** shows the OS curve of the entire cohort. Among them, 84 cases were from the D&C group, and the remaining 51 cases were in the diagnostic hysteroscopy group. The 5-year OS rate by the Kaplan-Meier method was 67.23% (95% CI 58.60%–74.45%) in the diagnostic hysteroscopy group and 70.71% (95% CI 64.30%–76.18%) in the D&C group. For women with apparent early-stage type II endometrial cancer, diagnostic hysteroscopy did not increase the risk of all-cause death (HR 1.11, 95% CI 0.78-1.57, *P*=0.573). **Figure 2B** shows the Kaplan-Maier curve of OS (diagnostic hysteroscopy *VS.* D&C).

Theoretically, diagnostic hysteroscopy can increase the risk of tumor cells spreading into the peritoneal cavity, this was consistent with the finding of our study (**Table 1**). However, the Kaplan-Meier method and the Log-rank test showed that for women with apparent early-stage type II endometrial cancer, the positive peritoneal cytology was not associated with the deterioration of DFS (HR 1.03, 95% CI 0.65-1.64, *P*=0.901)

TABLE 1 | Characteristics of the study cohort[a].

	Overall	Dilation & Curettage group	Hysteroscopy group	*P*
Year of diagnosis				0.172
2011-2013	147 (34.3%)	99 (36.8%)	48 (30.0%)	
2014-2016	282 (65.7%)	170 (63.2%)	112 (70.0%)	
Age at diagnosis (year)	66.5 ± 7.62	66.9 ± 7.72	65.8 ± 7.41	0.171
Duration of follow-up (month)	50 (4-107)	52 (4-107)	44.5 (4-107)	0.071
Marital status at diagnosis				0.003
Married	223 (52.0%)	155 (57.6%)	68 (42.5%)	
Single[b]	206 (48.0%)	114 (42.4%)	92 (57.5%)	
Body mass index	22.6 ± 4.08	22.5 ± 3.97	22.7 ± 4.27	0.654
ASA[c] score				0.236
I	265 (61.8%)	173 (64.3%)	92 (57.5%)	
II	87 (20.3%)	48 (17.8%)	39 (24.4%)	
III	77 (17.9%)	48 (17.8%)	29 (18.1%)	
Histology				0.059
Clear cell carcinoma	120 (28.0%)	84 (31.2%)	36 (22.5%)	
Serous carcinoma	309 (72.0%)	185 (68.8%)	124 (77.5%)	
Grade				>0.999
Poorly differentiated	274 (63.9%)	172 (63.9%)	102 (63.8%)	
Undifferentiated	155 (36.1%)	97 (36.1%)	58 (36.2%)	
Tumor size				0.023
Less than 4cm	272 (63.4%)	182 (67.7%)	90 (56.2%)	
At least 4cm	157 (36.6%)	87 (32.3%)	70 (43.8%)	
Postoperative stage[d]				0.091
I/II	344 (80.2%)	215 (79.9%)	129 (80.6%)	
III/IV	85 (19.8%)	54 (20.1%)	31 (19.4%)	
LVSI[e]				0.363
Negative	319 (74.4%)	204 (75.8%)	115 (71.9%)	
Positive	110 (25.6%)	65 (24.2%)	45 (28.1%)	
Peritoneal cytology				<0.001
Negative	341 (79.5%)	232 (86.2%)	109 (68.1%)	
Positive	88 (20.5%)	37 (13.8%)	51 (31.9%)	
Approach of staging				0.537
Laparoscopy	267 (62.2%)	164 (61.0%)	103 (64.4%)	
Laparotomy	162 (37.8%)	105 (39.0%)	57 (35.6%)	
Lymphadenectomy				0.606
Pelvic	272 (63.4%)	168 (62.5%)	104 (65.0%)	
Pelvic and para-aortic	157 (36.6%)	101 (37.5%)	56 (35.0%)	
Adjuvant therapy				0.760
No	109 (25.4%)	66 (24.5%)	43 (26.9%)	
RT[f] or CT[g]	191 (44.5%)	119 (44.2%)	72 (45.0%)	
Combined RT and CT	129 (30.1%)	84 (31.2%)	45 (28.1%)	

[a]*Values are presented as mean ± standard deviation, median (minimum–maximum), or as number (percentage).*
[b]*Includes divorced, widowed, separated, and never married.*
[c]*The American Society of Anesthesiologists.*
[d]*Based on the 2009 staging system of the International Federation of Gynecology and Obstetrics.*
[e]*Lymphovascular space invasion.*
[f]*Radiotherapy.*
[g]*Chemotherapy.*

and OS (HR 1.06, 95% CI 0.70-1.60, *P*=0.797). **Figure 3** shows the Kaplan-Maier curves of DFS and OS (positive peritoneal cytology *VS.* negative peritoneal cytology).

The Cox Proportional Hazards Regression Analysis of Survival in Patients With Apparent Early-Stage Type II Endometrial Cancer

Based on the results of univariate analysis (**Supplementary Material 2**) and considering the clinical relevance of the candidate variables, the following variables were included in the Cox proportional hazards regression model: age at diagnosis, BMI at diagnosis, the preoperative ASA physical status score, tumor size, the postoperative FIGO stage of the disease, the status of LVSI, adjuvant therapy, and the method of endometrial sampling. The results of the Cox proportional hazards regression analysis demonstrated that for women with apparent early-stage type II endometrial cancer, the methods of preoperative endometrial sampling did not affect the oncological survival (for DFS: diagnostic hysteroscopy *VS.* D&C, aHR 1.38, 95% CI 0.92-2.07, *P*=0.122; for OS: diagnostic hysteroscopy *VS.* D&C, aHR 1.23, 95% CI 0.85-1.77, *P*=0.272).

TABLE 2 | Patterns and rates of disease recurrence by diagnostic hysteroscopy vs. Dilation & Curettage.

	Diagnostic hysteroscopy group (N=160)	Dilation & Curettage group (N=269)	*P*
Disease recurrence			0.551
No	142 (88.8%)	232 (86.2%)	
Yes	18 (11.2%)	37 (13.8%)	
Site of recurrence			> 0.999
Vagina	2 (1.3%)	3 (1.1%)	
Pelvis	2 (1.3%)	6 (2.2%)	
Abdomen	4 (2.5%)	9 (3.3%)	
Nodal	2 (1.3%)	3 (1.1%)	
Liver	2 (1.3%)	4 (1.5%)	
Lung	4 (2.5%)	7 (2.6%)	
Bone	1 (0.6%)	2 (0.7%)	
Multiple	1 (0.6%)	3 (1.1%)	

The Cox proportional hazards regression model also indicated that for apparent early-stage type II endometrial cancer, having a preoperative ASA physical status score of III (III *VS.* I: aHR 2.11, 95% CI 1.01-4.43, *P*=0.048), having an advanced disease (III/IV *VS.* I/II: aHR 2.68, 95% CI 1.68-4.28, *P*=0.000), and having LVSI (Yes *VS.* No: aHR 2.71, 95% CI 1.49-4.95, *P*=0.001) could worsen the DFS of patients; while postoperative adjuvant therapy was beneficial to the DFS of patients (radiotherapy or chemotherapy *VS.* without adjuvant therapy: aHR 0.54, 95% CI 0.34-0.87, *P*=0.011; combined radiotherapy and chemotherapy *VS.* without adjuvant therapy: aHR 0.39, 95% CI 0.23-0.67, *P*=0.001). In terms of the risk of all-cause death in patients with apparent early-stage type II endometrial cancer, age at diagnosis (*P*=0.039), the preoperative ASA physical status score (*P*=0.029), the stage of disease (*P*=0.000), the status of LVSI (*P*=0.000), and postoperative adjuvant therapy (*P*=0.000) were all independent predictors. **Table 3** shows the Cox proportional hazards regression model for survival in patients with apparent early-stage type II endometrial cancer.

DISCUSSION

Based on six Chinese tertiary hospitals, this multicenter retrospective cohort study finds that for women with apparent early-stage type II endometrial cancer, diagnostic hysteroscopy was as safe as traditional D&C.

Postmenopausal bleeding, unscheduled bleeding, and menorrhagia are very common gynecologic complaints (17, 18). The main purpose of the management for these women is to rule out malignant lesions or diseases with malignant potentials, such as cancer of the endometrium and endometrial hyperplasia (19). For the elderly with abnormal uterine bleeding, all kinds of evaluations are justified by the common acceptance that postmenopausal bleeding is "cancer until proven otherwise" (20). Thus, for women with abnormal uterine bleeding, the necessity of endometrial sampling is mainly based on the risk of endometrial cancer (20, 21).

The sensitivity of endometrial sampling is high for the identification of endometrial lesions (endometrial cancer included), and D&C has been the standard procedure for

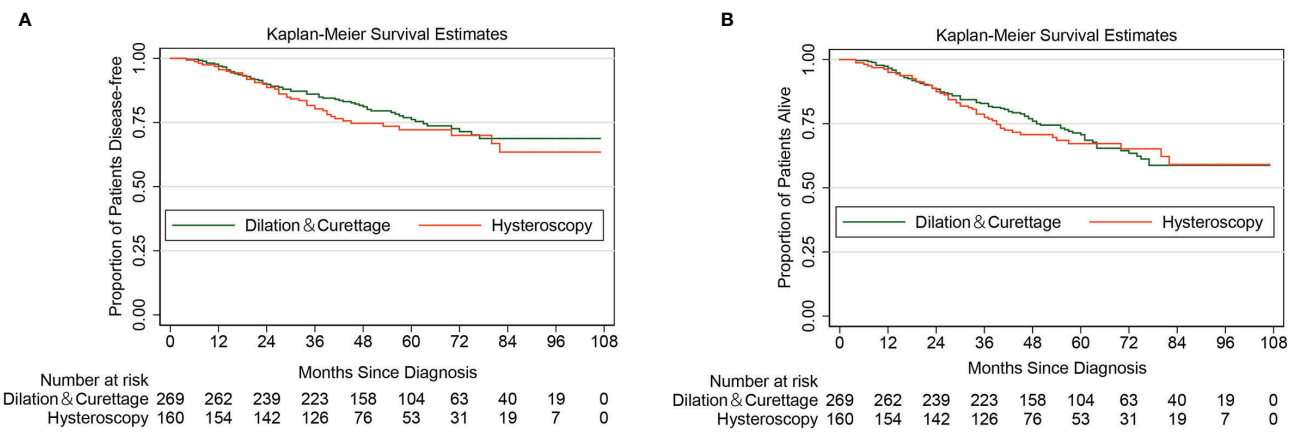

FIGURE 2 | Kaplan-Meier curves of disease-free survival and overall survival for patients with apparent early-stage type II endometrial cancer, by the methods of endometrial sampling. (**A** for disease-free survival; **B** for overall survival).

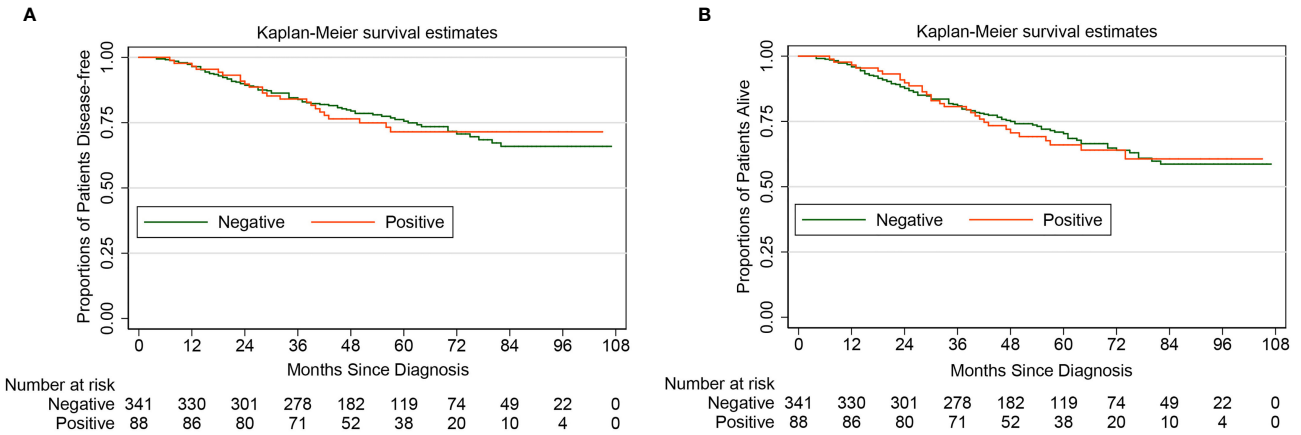

FIGURE 3 | Kaplan-Meier curves of disease-free survival and overall survival for patients with apparent early-stage type II endometrial cancer, by peritoneal cytology. (**A** for disease-free survival; **B** for overall survival).

diagnosing cancer of the endometrium for years (22). However, with the advances in instrumentation, hysteroscopy plays an increasingly important role in the diagnosis of endometrial cancer, even in an ambulatory setting (23, 24). With endoscopic visualization of the endometrial cavity and the directed biopsy, diagnostic hysteroscopy is considered more accurate and reliable than traditional D&C in diagnosing endometrial lesions (9, 25, 26). A meta-analysis conducted by Bourdel et al. found that for patients with atypical endometrial hyperplasia, compared with D&C, diagnostic hysteroscopy results in a lower underestimation of endometrial cancer (27). However, the high pressure of the uterine cavity during the process of hysteroscopy may facilitate the spreading of tumor cells into the abdominal cavity. Having 1015 women with endometrial cancer included, the study by Polyzos et al. reported that compared with patients who did not undergo diagnostic hysteroscopy, those who underwent diagnostic hysteroscopy had a significantly higher rate of malignant peritoneal cytology (odds ratio 1.78, 95% CI 1.13-2.79, P=0.013) (28). This finding was consistent with that of many other studies (11, 29, 30). In our study, the rate of positive peritoneal cytology in the diagnostic hysteroscopy group was also significantly higher than that in the D&C group, 31.9% and 13.8%, respectively.

But, the negative effects of tumor cells disseminated into the peritoneal cavity during diagnostic hysteroscopy on the prognosis of women with endometrial cancer are not well established. Although the result of peritoneal cytology is no longer a factor to consider in the 2009 FIGO staging system for endometrial cancer, numerous studies still find that malignant peritoneal cytology is strongly associated with the deterioration of long-term prognosis in patients with endometrial cancer (31–34). However, some facts deserve our attention. Almost all of the included cases in the mentioned studies were endometrioid adenocarcinoma of the endometrium (31–34). Few studies have reported the prognostic significance of malignant peritoneal cytology in type II endometrial cancer. What is more, all the malignant peritoneal cytology in the mentioned studies was not associated with diagnostic hysteroscopy (31–34). Whether the malignant cells disseminated into the peritoneal cavity during diagnostic hysteroscopy can survive, colonize, invade the normal tissue, and worsen the prognosis of patients is unknown. A systematic review and meta-analysis by Du et al. showed that for endometrial cancer, although can increase the risk of spreading of malignant cells, diagnostic hysteroscopy did not worsen the prognosis (13). With 127 type II endometrial cancer cases included, the study conducted by Ribeiro et al. also reported that compared with traditional D&C, diagnostic hysteroscopy did not increase the risk of recurrence and all-cause death (35). This result is consistent with ours. But, large and adequately powered prospective studies with long-term follow-up are still needed to testify the safety of diagnostic hysteroscopy for type II endometrial cancer. Until such studies become available, we still need to be careful about the employment of diagnostic hysteroscopy in type II endometrial cancer.

Based on six centers, our study has a sample size of 429 patients. Considering the rarity of type II endometrial cancer, the sample size of the current study is relatively large. Also, the entire cohort underwent a long-term follow-up. However, there are some limitations to our study. First, due to the limited resources, the pathological diagnoses of UCCC and USC were not reviewed again by experts in pathology. We extracted postoperative pathological diagnoses from patients' electronic medical records. Second, the pressure of the uterine cavity during diagnostic hysteroscopy was not reported in patients' electronic medical records. Therefore, we could not explore the effect of intrauterine pressure during diagnostic hysteroscopy on the long-term survival of type II endometrial cancer patients who underwent diagnostic hysteroscopy. Third, there was possible confounding by indications of diagnostic hysteroscopy due to our study design. In clinical practice, there is currently no widely accepted indication for diagnostic hysteroscopy in the diagnosis of endometrial cancer. Gynecologists of the participating centers of this study chose the method of endometrial sampling mainly based on their preference and judgment. The last, considering

TABLE 3 | Multivariate analysis of prognosis for women with apparent early-stage type II endometrial cancer.

	DFS[a]			OS[b]		
	aHR[c]	95% CI[d]	*P*	aHR	95% CI	*P*
Age						
< 65 years	Reference			Reference		
≥ 65 years	1.34	0.82-2.17	0.239	1.58	1.02-2.43	0.039
Body mass index						
< 24 kg/m^2	Reference			Reference		
≥ 24 kg/m^2	1.19	0.74-1.91	0.470	1.21	0.79-1.85	0.391
ASA[e] score			0.035			0.029
I	Reference			Reference		
II	1.18	0.544-2.56	0.676	1.18	0.46-1.88	0.138
III	2.11	1.01-4.43	0.048	2.33	1.16-3.65	0.025
Tumor size						
< 4 cm	Reference			Reference		
≥ 4 cm	1.36	0.82-2.28	0.237	1.17	0.740-1.85	0.502
Stage (FIGO[f] 2009)						
I/II	Reference			Reference		
III/IV	2.68	1.68-4.28	0.000	3.08	2.01-4.71	0.000
LVSI[g]						
Negative	Reference			Reference		
Positive	2.71	1.49-4.95	0.001	2.80	1.60-4.88	0.000
Adjuvant therapy			0.002			0.000
No	Reference			Reference		
RT[h] or CT[i]	0.54	0.34-0.87	0.011	0.47	0.31-0.71	0.000
RT and CT	0.39	0.23-0.67	0.001	0.34	0.21-0.55	0.000
Method of diagnosis						
Dilation&Curettage	Reference			Reference		
Hysteroscopy	1.38	0.92-2.07	0.122	1.23	0.85-1.77	0.272

[a]*Disease-free survival.*
[b]*Overall survival.*
[c]*adjusted hazard ratio.*
[d]*Confidence interval.*
[e]*American Society of Anesthesiologists.*
[f]*The International Federation of Gynecology and Obstetrics.*
[g]*Lymphovascular space invasion.*
[h]*Radiotherapy.*
[i]*Chemotherapy.*

the retrospective nature of the current study, there were some inevitable biases, such as recall bias, selection bias, etc. To reduce these biases as much as possible, we screened cases strictly according to established inclusion and exclusion criteria and excluded those with incomplete data.

CONCLUSION

For apparent early-stage type II endometrial cancer, endometrial sampling by diagnostic hysteroscopy is as safe as traditional D&C. This finding needs further large and adequately powered prospective studies to verify.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HZ: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, and Supervision. YX: Methodology, Investigation, Data analysis, Writing – original draft, Writing – review & editing, and Project administration. K-FL, QX, Q-WZ, CH, X-GM, CC, WH, G-SM, JS, YT, and F-MK: Methodology, Investigation, Data analysis, and Writing – review & editing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.918693/full#supplementary-material

Supplementary Material 1 | Kaplan-Meier curves of disease-free survival and overall survival for patients with apparent early-stage type II endometrial cancer. (**A** for disease-free survival; **B** for overall survival).

Supplementary Material 2 | Univariate analysis of prognosis for women with apparent early-stage type II endometrial cancer.

REFERENCES

1. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-Up. *Ann Oncol* (2016) 27(1):16–41. doi: 10.1097/IGC.0000000000000609
2. Lu KH, Broaddus RR. Endometrial Cancer. *N Engl J Med* (2020) 383 (21):2053–64. doi: 10.1056/NEJMra1514010
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA* (2021) 71 (3):209–49. doi: 10.3322/caac.21660
4. Bokhman JV. Two Pathogenetic Types of Endometrial Carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7
5. Bogani G, Ray-Coquard I, Concin N, Ngoi NYL, Morice P, Enomoto T, et al. Uterine Serous Carcinoma. *Gynecol Oncol* (2021) 162(1):226–34. doi: 10.1016/j.ygyno.2021.04.029
6. Faber MT, Sperling CD, Bennetsen AKK, Aalborg GL, Kjaer SK. A Danish Nationwide Study of Risk Factors Associated With Type I and Type II Endometrial Cancer. *Gynecol Oncol* (2021) 161(2):553–8. doi: 10.1016/j.ygyno.2021.02.010
7. Murali R, Soslow RA, Weigelt B. Classification of Endometrial Carcinoma: More Than Two Types. *Lancet Oncol* (2014) 15(7):e268–278. doi: 10.1016/S1470-2045(13)70591-6
8. Clarke MA, Long BJ, Sherman ME, Lemens MA, Podratz KC, Hopkins MR, et al. Risk Assessment of Endometrial Cancer and Endometrial Intraepithelial Neoplasia in Women With Abnormal Bleeding and Implications for Clinical Management Algorithms. *Am J Obstet Gynecol* (2020) 223(4):549.e541–549.e513. doi: 10.1016/j.ajog.2020.03.032
9. van Dongen H, de Kroon CD, Jacobi CE, Trimbos JB, Jansen FW. Diagnostic Hysteroscopy in Abnormal Uterine Bleeding: A Systematic Review and Meta-Analysis. *BJOG* (2007) 114(6):664–75. doi: 10.1111/j.1471-0528.2007.01326.x
10. Gkrozou F, Dimakopoulos G, Vrekoussis T, Lavasidis L, Koutlas A, Navrozoglou I, et al. Hysteroscopy in Women With Abnormal Uterine Bleeding: A Meta-Analysis on Four Major Endometrial Pathologies. *Arch Gynecol Obstet* (2015) 291(6):1347–54. doi: 10.1007/s00404-014-3585-x
11. Dong H, Wang Y, Zhang M, Sun M, Yue Y. Whether Preoperative Hysteroscopy Increases the Dissemination of Endometrial Cancer Cells: A Systematic Review and Meta-Analysis. *J Obstet Gynaecol Res* (2021) 47 (9):2969–77. doi: 10.1111/jog.14897
12. Obermair A, Geramou M, Gucer F, Denison U, Graf AH, Kapshammer E, et al. Does Hysteroscopy Facilitate Tumor Cell Dissemination? Incidence of Peritoneal Cytology From Patients With Early Stage Endometrial Carcinoma Following Dilatation and Curettage (D & C) Versus Hysteroscopy and D & C. *Cancer* (2000) 88(1):139–43. doi: 10.1002/(SICI)1097-0142(20000101)88:1<139::AID-CNCR19>3.0.CO;2-U
13. Du Y, Xu Y, Qin Z, Sun L, Chen Y, Han L, et al. The Oncology Safety of Diagnostic Hysteroscopy in Early-Stage Endometrial Cancer: A Systematic Review and Meta-Analysis. *Front Oncol* (2021) 11:742761. doi: 10.3389/fonc.2021.742761
14. Namazov A, Gemer O, Helpman L, Hag-Yahia N, Eitan R, Raban O, et al. The Oncological Safety of Hysteroscopy in the Diagnosis of Early-Stage Endometrial Cancer: An Israel Gynecologic Oncology Group Study. *Eur J Obstet Gynecol Reprod Biol* (2019) 243:120–4. doi: 10.1016/j.ejogrb.2019.10.042
15. Cicinelli E, Tinelli R, Colafiglio G, Fortunato F, Fusco A, Mastrolia S, et al. Risk of Long-Term Pelvic Recurrences After Fluid Minihysteroscopy in Women With Endometrial Carcinoma: A Controlled Randomized Study. *Menopause* (2010) 17(3):511–5. doi: 10.1097/gme.0b013e3181c8534d
16. de la Cuesta RS, Espinosa JA, Crespo E, Granizo JJ, Rivas F. Does Fluid Hysteroscopy Increase the Stage or Worsen the Prognosis in Patients With Endometrial Cancer? A Randomized Controlled Trial. *Eur J Obstet Gynecol Reprod Biol* (2004) 115(2):211–5. doi: 10.1016/j.ejogrb.2004.01.029
17. Goldstein SR, Lumsden MA. Abnormal Uterine Bleeding in Perimenopause. *Climacteric* (2017) 20(5):414–20. doi: 10.1080/13697137.2017.1358921
18. Kaunitz AM. Abnormal Uterine Bleeding in Reproductive-Age Women. *Jama* (2019) 321(21):2126–7. doi: 10.1001/jama.2019.5248
19. Pennant ME, Mehta R, Moody P, Hackett G, Prentice A, Sharp SJ, et al. Premenopausal Abnormal Uterine Bleeding and Risk of Endometrial Cancer. *BJOG* (2017) 124(3):404–11. doi: 10.1111/1471-0528.14385
20. Izetbegovic S, Stojkanovic G, Ribic N, Mehmedbasic E. Features of Postmenopausal Uterine Haemorrhage. *Med Arch* (2013) 67(6):431–4. doi: 10.5455/medarh.2013.67.431-434
21. Papakonstantinou E, Adonakis G. Management of Pre-, Peri-, and Post-Menopausal Abnormal Uterine Bleeding: When to Perform Endometrial Sampling? *Int J Gynaecol Obstet* (2021). doi: 10.1002/ijgo.13988
22. Ben-Yehuda OM, Kim YB, Leuchter RS. Does Hysteroscopy Improve Upon the Sensitivity of Dilatation and Curettage in the Diagnosis of Endometrial Hyperplasia or Carcinoma? *Gynecol Oncol* (1998) 68(1):4–7. doi: 10.1006/gyno.1997.4891
23. Giampaolino P, Della Corte L, Di Filippo C, Mercorio A, Vitale SG, Bifulco G. Office Hysteroscopy in the Management of Women With Postmenopausal Bleeding. *Climacteric* (2020) 23(4):369–75. doi: 10.1080/13697137.2020.1754389
24. Valle RF. Office Hysteroscopy. *Clin Obstet Gynecol* (1999) 42(2):276–89. doi: 10.1097/00003081-199906000-00011
25. Clark TJ, Voit D, Gupta JK, Hyde C, Song F, Khan KS. Accuracy of Hysteroscopy in the Diagnosis of Endometrial Cancer and Hyperplasia: A Systematic Quantitative Review. *Jama* (2002) 288(13):1610–21. doi: 10.1001/jama.288.13.1610
26. van Hanegem N, Prins MM, Bongers MY, Opmeer BC, Sahota DS, Mol BW, et al. The Accuracy of Endometrial Sampling in Women With Postmenopausal Bleeding: A Systematic Review and Meta-Analysis. *Eur J Obstet Gynecol Reprod Biol* (2016) 197:147–55. doi: 10.1016/j.ejogrb.2015.12.008
27. Bourdel N, Chauvet P, Tognazza E, Pereira B, Botchorishvili R, Canis M. Sampling in Atypical Endometrial Hyperplasia: Which Method Results in the Lowest Underestimation of Endometrial Cancer? A Systematic Review and Meta-Analysis. *J Minimally Invasive Gynecol* (2016) 23(5):692–701. doi: 10.1016/j.jmig.2016.03.017
28. Polyzos NP, Mauri D, Tsioras S, Messini CI, Valachis A, Messinis IE. Intraperitoneal Dissemination of Endometrial Cancer Cells After Hysteroscopy: A Systematic Review and Meta-Analysis. *Int J Gynecol Cancer* (2010) 20(2):261–7. doi: 10.1111/IGC.0b013e3181ca2290
29. Chang YN, Zhang Y, Wang YJ, Wang LP, Duan H. Effect of Hysteroscopy on the Peritoneal Dissemination of Endometrial Cancer Cells: A Meta-Analysis. *Fertil Steril* (2011) 96(4):957–61. doi: 10.1016/j.fertnstert.2011.07.1146
30. Revel A, Tsafrir A, Anteby SO, Shushan A. Does Hysteroscopy Produce Intraperitoneal Spread of Endometrial Cancer Cells? *Obstet Gynecol Survey* (2004) 59(4):280–4. doi: 10.1097/01.OGX.0000120173.09136.4A
31. Shiozaki T, Tabata T, Yamada T, Yamamoto Y, Yamawaki T, Ikeda T. Does Positive Peritoneal Cytology Not Affect the Prognosis for Stage I Uterine Endometrial Cancer?: The Remaining Controversy and Review of the Literature. *Int J Gynecol Cancer* (2014) 24(3):549–55. doi: 10.1097/IGC.0000000000000072
32. Matsuo K, Yabuno A, Hom MS, Shida M, Kakuda M, Adachi S, et al. Significance of Abnormal Peritoneal Cytology on Survival of Women With Stage I-II Endometrioid Endometrial Cancer. *Gynecol Oncol* (2018) 149 (2):301–9. doi: 10.1016/j.ygyno.2018.02.012
33. Matsuo K, Matsuzaki S, Nusbaum DJ, Machida H, Nagase Y, Grubbs BH, et al. Malignant Peritoneal Cytology and Decreased Survival of Women With Stage I Endometrioid Endometrial Cancer. *Eur J Cancer* (2020) 133:33–46. doi: 10.1016/j.ejca.2020.03.031

34. Seagle BL, Alexander AL, Lantsman T, Shahabi S. Prognosis and Treatment of Positive Peritoneal Cytology in Early Endometrial Cancer: Matched Cohort Analyses From the National Cancer Database. *Am J Obstet Gynecol* (2018) 218 (3):329.e321–329.e315. doi: 10.1016/j.ajog.2017.11.601
35. Ribeiro CM, Brito LGO, Benetti-Pinto CL, Teixeira JC, Yela DA. Is Diagnostic Hysteroscopy Safe for the Investigation of Type II Endometrial Cancer? A Retrospective Cohort Analysis. *J Minimally Invasive Gynecol* (2021) 28 (8):1536–43. doi: 10.1016/j.jmig.2021.01.002

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Association of Tumor Size With Prognosis in Patients With Resectable Endometrial Cancer: A SEER Database Analysis

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This study aimed to explore the relationship between tumor size (Ts) and prognosis in endometrial cancer (EC). A total of 52,208 patients with EC who underwent total hysterectomy were selected from the Surveillance, Epidemiology, and End Results Program database. Overall survival (OS) and endometrial cancer-specific survival (ESS) were chosen as survival outcomes. The Cox proportional hazards model was used to explore the effect of Ts on prognosis. The restricted cubic splines based on the Cox regression model were used to determine the nonlinear relationship between Ts and survival. When Ts was analyzed as a categorical variable, the risk of death increased with Ts, with the highest risk in patients with Ts > 9 cm with regard to all-cause death (ACD) (hazard ratio [HR] 1.317; 95% confidence interval [CI], 1.196-1.450; $P < 0.001$) and endometrial cancer-specific death (ESD) (HR, 1.378; 95% CI, 1.226-1.549; $P < 0.001$). As a continuous variable, Ts showed a nonlinear relationship with ACD (HR, 1.061; 95% CI, 1.053-1.069; $P < 0.001$) and ESD (HR, 1.062; 95% CI, 1.052-1.073; $P < 0.001$). The risk of mortality increased quickly with Ts when Ts was less than 7.5 cm and then leveled off when Ts was larger than 7.5 cm in all patients. Among patients with lymph node metastasis, the risk of poor prognosis decreased rapidly with Ts when Ts was less than 3.5 cm, and subsequently increased sharply with Ts when Ts ranged from 3.5 cm to 7.5 cm, and then increased slowly when Ts was larger than 7.5 cm ($P < 0.001$ for nonlinearity). There was a nonlinear relationship between Ts and prognosis in patients with EC. Clinicians should not ignore the impact of small tumors on prognosis in EC patients with lymph node metastasis.

Keywords: endometrial cancer, tumor size, prognosis, SEER database, death risk

INTRODUCTION

According to the latest statistics from the Global Cancer Observatory, endometrial cancer (EC) was ranked third in gynecological tumors, with an estimated 417,367 new cases and 97,370 deaths around the world in 2020 with an increase of 9.2% and 8.3%, respectively, compared to those in 2018. (1, 2) Reducing the recurrence rate and prolonging survival time were the goals for clinician to improve the prognosis of patients with EC, as current medical methods cannot completely cure this disease. (3–5)

Currently, the American Joint Committee on Cancer (AJCC) and the International Federation of Gynecology and Obstetrics (FIGO) staging system have been widely used for prognostic prediction and treatment selection in patients with EC. However, the prognosis of patients with the same stage varies dramatically; thus, management according to the tumor staging system may lead to undertreatment, as Marcos et al. (6) found that 10% of women with low-risk EC (type 1, stage IA grade 1 or 2) and 15% of women with intermediate-risk EC (type 1, stage IA grade 3, or stage IB grade 1 or 2) suffer from lymph node metastasis (LNM) according to FIGO staging system. Therefore, additional tools are needed to improve the management of patients with EC to accommodate surgical staging and adjuvant therapy.

Tumor size (Ts) was first reported as a prognostic indicator of EC in the 1980s. (3) Since then, many investigators have examined the prognostic significance of Ts. Thus far, studies have observed that Ts is an independent predictive factor for LNM, recurrence, and prognosis of EC. (4, 7–9) Mariam et al. (10) revealed that the combination of preoperative biopsy and intraoperative Ts could improve the accuracy of surgical staging. They suggested that among patients with preoperative histological grade 1 or 2, lymphadenectomy was recommended for those with Ts > 2 cm if an accurate frozen section was lacking, but not for those with Ts ≤ 2 cm. Although evidence has shown that Ts can be used as a prognostic indicator in EC, it has not yet been included in the tumor-nodes-metastasis staging system, possibly because the relationship between Ts and prognosis of EC is still controversial. Ozgul et al. (11) conducted a retrospective study based on 250 patients with stage II EC and found that Ts was not associated with five-year disease-free survival and overall survival (OS). Moreover, Shah et al. (12) had the same results in a study involving 345 surgically treated EC patients. Doll et al. (13) observed no association between Ts > 2 cm and recurrence in high-grade EC.

To date, studies on the association between Ts and the prognosis of EC have mainly focused on the survival differences among different Ts categories. (14–16) However, this method cannot reflect the effect of Ts on prognosis in detail. Some evidence has shown that the relationship between Ts and prognosis is nonlinear in a variety of cancers. (17) Based on the available evidence, we hypothesized that Ts and prognosis of EC may have a complex rather than a simple linear relationship, and the effects of different Ts on the risk of mortality might be distinct in these patients. Therefore, this study aims to better characterize the relationship between Ts and prognosis based on a large sample of EC patients who underwent surgery and to provide evidence for revising the tumor staging system.

MATERIALS AND METHODS

Study Population

The data for the study were extracted from the Surveillance, Epidemiology, and End Results Program (SEER) database by using the SEER*Stat software (version 8.3.9.2, National Cancer Institute, Bethesda, MD), the cases we chosen were registered in SEER between 2004 and 2018. The SEER database covers 28% of the US population from 18 cancer registries and is one of the largest population-based cancer registries in the world. Institutional ethical approval and informed consent are not required for this study because the SEER database is anonymous and freely available to the public.

In the study, we utilized the Incidence-SEER Research Data, 18 Registries, Nov 2020 Sub (2000–2018) registry as the data source. All patients diagnosed with EC (site recode ICD-O-3/WHO 2008 of corpus uteri, behavior recode ICD-O-3 of "malignant," histology type ICD-O-3 of "8140-8389 and 8440-8499") who underwent surgery were included in this study. Inclusion criteria for patients were as follows: (1) diagnosis with EC as the first and only cancer; (2) age at diagnosis ≥ 18 years; (3) patients underwent total hysterectomy; (4) patients had complete postoperative follow-up data.

Variable Selection

Information including age, race, histological type, grade, stage, Ts, number of nodes examined, lymph node (LN) status, follow-up time and tumor number were extracted from the SEER database. Age was divided into four groups (18–56, 57–61, 62–69, and 70+ years) according to the X-tile software. Race was classified as white, black, and others. The histological type included endometrial endometrioid adenocarcinoma (EEA, codes: 8140–8389) and serous endometrioid adenocarcinoma (SEA, codes: 8440–8499) by using the ICD-O-3 codes. The eighth edition of the AJCC staging system was applied to patients in this study. Data recorded using the sixth and seventh editions were converted to the eighth edition system. The tumor grades were grouped as Grade I (well-differentiated), Grade II (moderately differentiated), Grade III (poorly differentiated), and Grade IV (undifferentiated or anaplastic), and the TNM stages consisted of stage I to stage IV. Ts was divided into 10 subgroups: Group 1 (≤1 cm), Group 2 (1.1–2 cm), Group 3 (2.1–3 cm), Group 4 (3.1–4 cm), Group 5 (4.1–5 cm), Group 6 (5.1–6 cm), Group 7 (6.1–7 cm), Group 8 (7.1–8 cm), Group 9 (8.1–9 cm), and Group 10 (> 9 cm). Overall survival (OS) and endometrial cancer-specific survival (ESS) were chosen as survival outcomes. OS was defined as the period from diagnosis until death from any cause, and ESS was defined as the period from diagnosis until death from EC. The process of variable selection was showed in **Figure 1**.

Statistical Analysis

The distribution of the variables was evaluated with the Shapiro-Wilk test. Normally distributed variables were expressed as mean and standard deviation (SD), non-normally distributed variables were reported as medians with interquartile ranges (IQRs) and compared using the Wilcoxon rank-sum test, while categorical

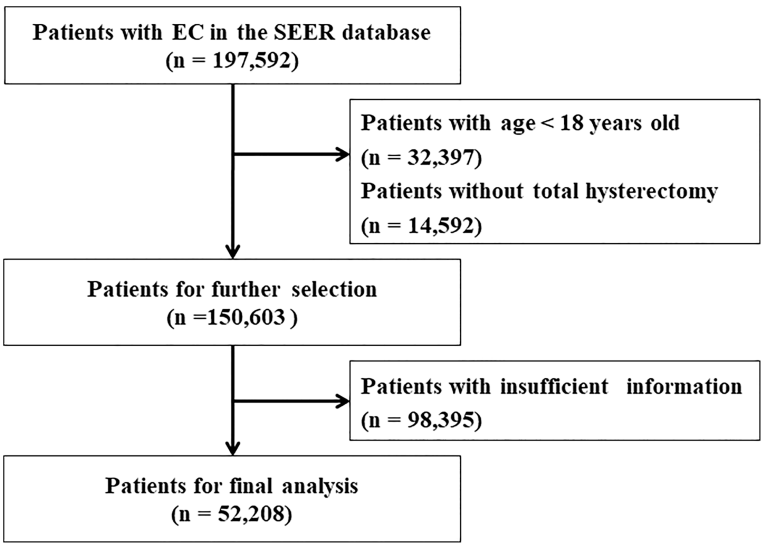

FIGURE 1 | Flow chart for screening eligible patients. EC, endometrial cancer. SEER, the Surveillance, Epidemiology, and End Results Program database.

variables were expressed as number and percentage and compared using the chi-squared test. The time-dependent ROC curve was used to calculate optimal cut-offs of tumor size according to final survival status. The Kaplan-Meier method was used to calculate OS and ESS. The univariate and multivariate Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI). The restricted cubic spline analysis (RCS) for the Cox model was used to flexibly model and visualize the association between Ts and prognosis (18). Subgroup analyses for patients with and without LNM were conducted to further examine the effects of Ts on survival outcomes. All statistical analyses were performed using *R* software version 4.0.5, and a two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

Characteristics of Patients

Table 1 shows the details of the patient characteristics. A total of 52,208 patients were involved in this study, with an average age of 62.9 ± 10.7 years and a median Ts of 3.5 cm. The number of nodes examined and the follow-up median times were 13 (6–21) and 56 (23–100) months, respectively. There were 13,715 (26.3%), 9719 (18.6%), 14,891 (28.5%), and 13,888 (26.6%) patients in the age groups of 18–56, 57–61, 62–59, and 70+ years, respectively. Most patients were white (n = 42,265, 81.0%), had a histological type of EEA (n = 47,127, 90.3%), with stage I cancer (n = 36,108, 69.2%). The numbers of patients with grade 1, grade 2, grade 3, and grade 4 tumors were 18,780 (36.0%), 17,047 (32.7%), 12,556 (24.0%), and 3825 (7.3%), respectively. More than half of the patients were LN negative (n = 44,982, 86.2%) and had one tumor (n = 41,342, 79.2%).

Association Between Ts and Prognosis

The optimal cut-offs of tumor size were 3.9 cm in OS and 4.0 cm in ESS, which was calculated by the time-dependent ROC curve (**Supplementary Figure 1**). So, we defined group (3.1-4 cm) as a reference when tumor size was analyzed as a categorical variable.

When Ts was analyzed as a categorical variable, the univariate Cox regression models showed that the risk of all-cause death (ACD) and endometrial cancer-specific death (ESD) gradually increased as the tumor grew (**Table 2**, Model 1 and Model 2). As compared with patients with Ts of 3.1–4 cm (the reference group), the highest risk of ACD and ESD was observed in patients with Ts > 9 cm with HRs of 2.29 (95% CI, 2.10–2.49; $P < 0.001$) and 3.17 (95% CI, 2.87–3.51; $P < 0.001$), respectively, whereas the lowest risk was observed in patients with Ts ≤ 1cm with HRs of 0.56 (95% CI, 0.51–0.62; $P < 0.001$) and 0.52 (95% CI, 0.45–0.60; $P < 0.001$), respectively. After adjustment for confounding factors of which $P < 0.05$ in univariate analysis, the multivariate Cox regression analysis indicated that patients with large Ts were prone to suffer a high risk of death, with the highest HRs of 1.61 (95% CI, 1.48–1.76; *P)* for ACD and 1.61 (95% CI, 1.46–1.79; $P < 0.05$) for ESD in patients with Ts > 9 cm, compared with patients with Ts of 3.1–4 cm (the reference group) (**Table 2**, Model 3 and Model 4). The results of multivariate analyses for Ts as categorical variables in all patients are listed in **Supplementary Table 1**.

When Ts was analyzed as a continuous variable, an increased Ts was also significantly associated with a high risk of ACD (HR, 1.092; 95% CI, 1.049–1.066; $P < 0.001$) and ESD (HR, 1.101; 95% CI, 1.095–1.108; $P < 0.001$). In the fully adjusted model (**Table 2**, Model 3 and Model 4), a larger Ts also indicated a higher risk of ACD (HR, 1.06; 95% CI, 1.05–1.07; $P < 0.001$) and ESD(HR, 1.06; 95% CI, 1.05–1.07; $P < 0.001$). In the RCS model, there is a nonlinear relationship between Ts and prognosis ($P < 0.001$ for

TABLE 1 | Baseline characteristics of patients with endometrial cancer according to tumor size categories.

Variable	Overall	Ts categories (cm)									
		≤ 1	1.1–2	2.1–3	3.1–4	4.1–5	5.1–6	6.1–7	7.1–8	8.1–9	> 9
Age (years), n (%)											
18–56	13715 (26.3)	1158 (8.4)	1822 (13.3)	2304 (16.8)	2434 (17.7)	1915 (14.0)	1359 (9.9)	908 (6.6)	658 (4.8)	360 (2.6)	797 (5.8)
57–61	9719 (18.6)	703 (7.2)	1405 (14.5)	2010 (20.7)	1865 (19.2)	1419 (14.6)	860 (8.8)	547 (5.6)	330 (3.4)	202 (2.1)	378 (3.9)
62–69	14891 (28.5)	1095 (7.4)	2087 (14.0)	3066 (20.6)	3055 (20.5)	2257 (15.2)	1344 (9.0)	727 (4.9)	467 (3.1)	279 (1.9)	514 (3.5)
70+	13883 (26.6)	771 (5.6)	1856 (13.4)	2811 (20.2)	2995 (21.6)	2262 (16.3)	1307 (9.4)	749 (5.4)	444 (3.2)	264 (1.9)	424 (3.1)
Histological type, n (%)											
EEA	47127 (90.3)	3256 (6.9)	9281 (19.7)	9475 (20.1)	7121 (15.1)	4389 (9.3)	2644 (5.6)	1696 (3.6)	966 (2.0)	1866 (4.0)	6433 (13.7)
SEA	5081 (9.7)	471 (9.3)	910 (14.5)	874 (17.9)	732 (17.2)	481 (14.4)	287 (9.5)	203 (5.6)	139 (4.0)	247 (2.7)	737 (4.9)
Race, n (%)											
White	42265 (81.0)	3045 (7.2)	5896 (14.0)	8531 (20.2)	8554 (20.2)	6437 (15.2)	3852 (9.1)	2208 (5.2)	1405 (3.3)	825 (2.0)	1512 (3.6)
Black	4452 (8.5)	298 (6.7)	476 (10.7)	614 (13.8)	762 (17.1)	675 (15.2)	522 (11.7)	374 (8.4)	270 (6.1)	142 (3.2)	319 (7.2)
Others	5491 (10.5)	384 (7.0)	798 (14.5)	1046 (19.0)	1033 (18.8)	741 (13.5)	496 (9.0)	349 (6.4)	224 (4.1)	138 (2.5)	282 (5.1)
Grade, n (%)											
G1	18780 (36.0)	1849 (9.8)	3188 (17.0)	4011 (21.4)	3764 (20.0)	2559 (13.6)	1426 (7.6)	764 (4.1)	486 (2.6)	278 (1.5)	455 (2.4)
G2	17047 (32.7)	937 (5.5)	2145 (12.6)	3461 (20.3)	3587 (21.0)	2699 (15.8)	1683 (9.9)	987 (5.8)	604 (3.5)	323 (1.9)	621 (3.6)
G3	12556 (24.0)	661 (5.3)	1412 (11.2)	2076 (16.5)	2324 (18.5)	1998 (15.9)	1366 (10.9)	925 (7.4)	633 (5.0)	381 (3.0)	780 (6.2)
G4	3825 (7.3)	280 (7.3)	425 (11.1)	643 (16.8)	674 (17.6)	597 (15.6)	395 (10.3)	255 (6.7)	176 (4.6)	123 (3.2)	257 (6.7)
Stage, n (%)											
I	36108 (69.2)	3243 (9.0)	5938 (16.4)	8026 (22.2)	7549 (20.9)	5123 (14.2)	2845 (7.9)	1494 (4.1)	837 (2.3)	425 (1.2)	628 (1.7)
II	4356 (8.3)	157 (3.6)	406 (9.3)	675 (15.5)	815 (18.7)	714 (16.4)	515 (11.8)	356 (8.2)	259 (5.9)	167 (3.8)	292 (6.7)
III	9861 (18.9)	276 (2.8)	712 (7.2)	1294 (13.1)	1728 (17.5)	1755 (17.8)	1265 (12.8)	893 (9.1)	640 (6.5)	392 (4.0)	906 (9.2)
IV	1883 (3.6)	51 (2.7)	114 (6.1)	196 (10.4)	257 (13.6)	261 (13.9)	245 (13.0)	188 (10.0)	163 (8.7)	121 (6.4)	287 (15.2)
Lymph node status, n (%)											
Negative	44982 (86.2)	3567 (7.9)	6760 (15.0)	9397 (20.9)	9166 (20.4)	6597 (14.7)	3903 (8.7)	2198 (4.9)	1365 (3.0)	754 (1.7)	1275 (2.8)
Positive	7226 (13.8)	1600 (2.2)	410 (5.7)	794 (11.0)	1183 (16.4)	1256 (17.4)	967 (13.4)	733 (10.1)	534 (7.4)	351 (4.9)	838 (11.6)
Tumor number, n (%)											
Single	41342 (79.2)	2879 (7.0)	5514 (13.3)	7997 (19.3)	8210 (19.9)	6231 (15.1)	3892 (9.4)	2388 (5.8)	1561 (3.8)	909 (2.2)	1761 (4.3)
Multiple	10866 (20.8)	848 (7.8)	1656 (15.2)	2194 (20.2)	2139 (19.7)	1622 (14.9)	978 (9.0)	543 (5.0)	338 (3.1)	196 (1.8)	352 (3.2)
Number of nodes examined [Median (IQR)]	13 (6-21)										

SD, standard deviation; IQR, interquartile range; EEA, endometrial endometrioid adenocarcinoma; SEA, serous endometrioid adenocarcinoma.

TABLE 2 | Univariate and multivariate Cox regression analyses of ACD and CSD according to Ts in patients with endometrial cancer.

Ts	HR (95% CI)							
	Model 1 (ACD)	*P*	Model 2 (ESD)	*P*	Model 3 (ACD)	*P*	Model 4 (ESD)	*P*
≤ 1 cm	0.561 (0.506–0.622)	< 0.001	0.518 (0.448–0.599)	< 0.001	0.731 (0.659–0.811)	< 0.001	0.744 (0.642–0.861)	< 0.001
1.1–2 cm	0.709 (0.658–0.763)	< 0.001	0.650 (0.585–0.721)	< 0.001	0.826 (0.767–0.891)	0.024	0.829 (0.747–0.921)	0.024
2.1–3 cm	0.806 (0.755–0.860)	< 0.001	0.745 (0.680–0.815)	< 0.001	0.880 (0.824–0.939)	< 0.001	0.864 (0.789–0.946)	< 0.001
3.1–4 cm	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
4.1–5 cm	1.274 (1.196–1.358)	< 0.001	1.359 (1.249–1.479)	< 0.001	1.151 (1.080–1.227)	< 0.001	1.170 (1.075–1.273)	< 0.001
5.1–6 cm	1.442 (1.343–1.549)	< 0.001	1.622 (1.479–1.779)	< 0.001	1.230 (1.145–1.321)	< 0.001	1.232 (1.123–1.352)	< 0.001
6.1–7 cm	1.637 (1.510–1.776)	< 0.001	2.051 (1.854–2.268)	< 0.001	1.335 (1.230–1.449)	< 0.001	1.406 (1.270–1.557)	< 0.001
7.1–8 cm	1.669 (1.517–1.836)	< 0.001	2.171 (1.934–2.438)	< 0.001	1.317 (1.196–1.450)	< 0.001	1.378 (1.226–1.549)	< 0.001
8.1–9 cm	1.908 (1.702–2.139)	< 0.001	2.617 (2.289–2.991)	< 0.001	1.416 (1.262–1.589)	< 0.001	1.514 (1.323–1.733)	< 0.001
> 9 cm	2.291 (2.104–2.494)	< 0.001	3.169 (2.865–3.506)	< 0.001	1.613 (1.478–1.760)	< 0.001	1.614 (1.455–1.790)	< 0.001
Ts+	1.092 (1.086–1.099)	< 0.001	1.101 (1.095–1.108)	< 0.001	1.061 (1.053–1.069)	< 0.001	1.062 (1.052–1.073)	< 0.001

Model 1: Results of univariate Cox proportional hazards models for ACD. Model 2: Results of univariate Cox proportional hazards models for ESD. Model 3: Results of multivariate Cox proportional hazards models for ACD after adjustment for age, histological type, race, grade, stage, lymph node status, number of lymph node examined, and tumor number. Mode 4: Results of multivariate Cox proportional hazards models for ESD after adjustment for age, histological type, race, grade, stage, lymph node status, number of lymph node examined, and tumor number. Ts+: Ts was analyzed as a continuous variable. Ts, tumor size; HR, hazard ratio; CI, confidence interval; ACD, all-cause death; ESD, endometrial cancer-specific death.

nonlinearity), with a trend toward rising rapidly and then gradually (**Figure 2**). Taking the value of 7.5 cm as a turning point, the slope of the low Ts part (< 7.5 cm) was steeper than that of the high part (≥ 7.5 cm). The results of multivariate analyses for Ts as continuous variables in all patients are listed in **Supplementary Table 2**.

Subgroup Analyses

To further explore the relationship between Ts and prognosis in different LN statuses, all patients were divided into two groups, namely, LNM (N=7,226) and non-LNM (N=44,982). For patients without LNM, the fully-adjusted Cox regression models showed that the highest risk of ACD (HR, 1.457; 95% CI, 1.284–1.653; $P < 0.001$) and ESD (HR, 1.702; 95% CI, 1.471–1.970; $P < 0.001$) was observed in patients with Ts > 9 cm as compared to the risk in patients with Ts of 3.1–4 cm (the reference group), when Ts was analyzed as a categorical variable (**Table 3**). The results of multivariate analyses for Ts as categorical variables in patients without LNM are listed in **Supplementary Tables 3**. When Ts was analyzed as a continuous variable, Ts was independently associated with ACD (HR, 1.067; 95% CI, 1.057–1.077; $P < 0.001$) and ESD (HR, 1.075; 95% CI, 1.061–1.088; $P < 0.001$) in the fully adjusted models (**Table 3**). Ts also showed a nonlinear relationship with OS ($P < 0.001$ for nonlinearity) and ESS ($P < 0.001$ for nonlinearity) (**Figure 3**). The results of multivariate analyses for Ts as continuous variables in patients without LNM are listed in **Supplementary Tables 4**.

For patients with LNM, the highest HR of Ts was 1.359 (95% CI, 1.138–1.624; $P < 0.05$) for ACD and 1.702 (95% CI, 1.471–1.970; $P < 0.05$) for ESD in patients with Ts > 9 cm as compared with those in patients with Ts of 3.1–4 cm (the reference group) when Ts was analyzed as a categorical variable. The results of multivariate analyses for Ts as categorical variables in patients with LNM are listed in **Supplementary Tables 5**. When Ts was analyzed as a continuous variable, Ts was independently associated with ACD (HR, 1.047; 95% CI, 1.032–1.062; $P < 0.05$) and ESD (HR, 1.047; 95% CI, 1.032–1.063; $P < 0.05$) in the fully adjusted models (**Table 3**). A nonlinear relationship was also found between Ts and prognosis of EC ($P < 0.05$ for nonlinearity), with the risk of poor prognosis decreasing quickly with Ts when Ts was less than 3.5 cm, subsequently increasing rapidly with Ts when Ts ranged from 3.5 cm to 7.5 cm, and then increasing slowly when Ts was larger than 7.5 cm ($P < 0.05$ for nonlinearity) (**Figure 4**). The results of multivariate analyses for Ts as continuous variables in patients with LNM are listed in **Supplementary Tables 6**.

DISCUSSION

Our results indicated that large Ts was significantly associated with poor survival outcomes in patients with resectable EC. Among all patients with EC, we observed a nonlinear relationship between Ts and prognosis ($P < 0.05$ for nonlinearity), with a trend toward rising rapidly and then gradually. Among patients with LNM, the risk of poor prognosis decreased quickly with Ts when Ts was less than 3.5 cm, subsequently increasing rapidly with Ts when Ts ranged from 3.5 cm to 7.5 cm, and then increasing slowly when Ts was larger than 7.5 cm.

The T staging of EC in AJCC and FIGO systems is classified by the degree of tumor invasion and whether it is confined to the uterus. Ts has not been adopted in the staging system in EC but has been used in other cancers, such as cervical cancer, liver cancer, and pancreatic cancer. (19, 20) In cervical cancer, patients with the deepest invasion of ≥ 5 mm and lesion limited to the cervix uteri were grouped as stage IB. In the more detailed division, patients with a depth of stromal invasion ≥ 5 cm and Ts < 2 cm can be classified as IB1, patients with Ts of 2 to 4 cm can be grouped into IB2, and patients with Ts ≥ 4 cm can be categorized as IB3. (21) Similarly, Ts has also been used in the staging system of vaginal cancer. The patients with vaginal cancer only in the vagina were grouped into two stages: T1a (Ts ≤ 2.0 cm) and T1b (Ts > 2.0 cm). Patients with vaginal cancer whose tumor grew through the vaginal wall but did not reach the pelvic wall were divided into two stages: T2a (Ts ≤ 2.0 cm) and T2b (Ts >2.0 cm). (22) In the previous study, some scholars had proposed adopting Ts in the staging system of EC. Roberto et al. (23) suggested that Ts should be a useful marker

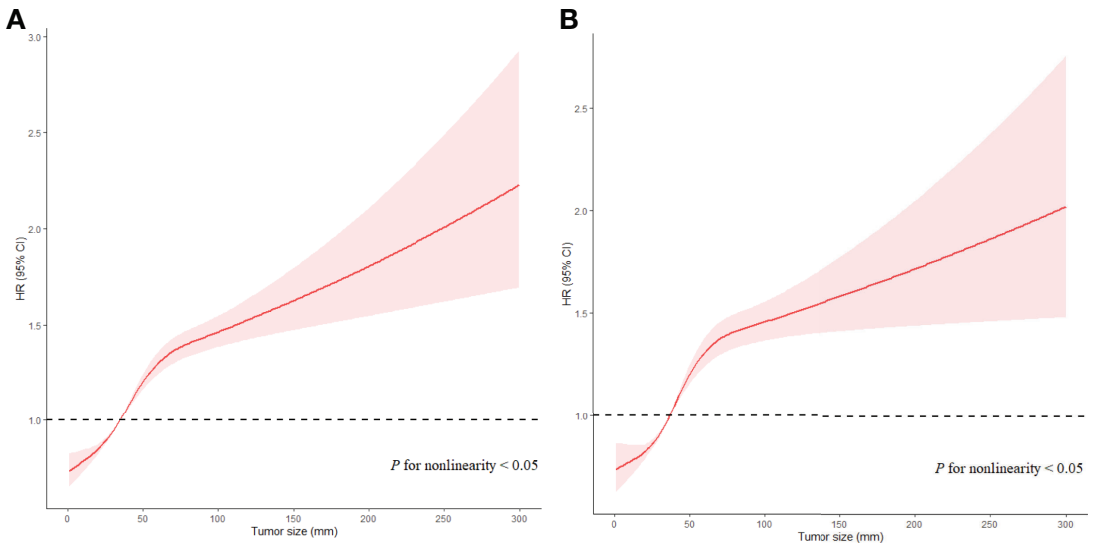

FIGURE 2 | Associations of Ts with prognosis in EC patients in Cox models with RCS after adjustment. Red lines estimated HR of Ts; shadow area 95% CI. **(A)** Adjusted RCS model for ACD. **(B)** Adjusted RCS model for ESD.

TABLE 3 | Multivariate Cox regression analyses of ACD and ESD according to Ts in patients with endometrial cancer according to LNM.

Ts	HR (95% CI)							
	Without LNM				With LNM			
	Model 1 (ACD)	*P*	Model 2 (ESD)	*P*	Model 1 (ACD)	*P*	Model 2 (ESD)	*P*
≤ 1 cm	0.658 (0.587–0.738)	< 0.001	0.732 (0.646–0.829)	< 0.001	1.254 (0.982–1.601)	0.336	1.156 (0.953–1.401)	0.272
1.1–2 cm	0.771 (0.710–0.838)	0.009	0.811 (0.727–0.905)	0.066	1.097 (0.922–1.306)	0.087	0.978 (0.830–1.153)	0.055
2.1–3 cm	0.840 (0.781–0.904)	< 0.001	1.211 (1.090–1.347)	0.002	1.004 (0.869–1.161)	0.070	1.108 (0.962–1.275)	0.066
3.1–4 cm	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
4.1–5 cm	1.175 (1.090–1.266)	< 0.001	1.267 (1.123–1.429)	< 0.001	1.112 (0.981–1.259)	0.331	1.195 (1.031–1.385)	0.593
5.1–6 cm	1.262 (1.159–1.375)	< 0.001	1.468 (1.278–1.685)	< 0.001	1.175 (1.029–1.341)	0.604	1.360 (1.166–1.586)	0.687
6.1–7 cm	1.382 (1.246–1.533)	< 0.001	1.491 (1.273–1.747)	< 0.001	1.320 (1.149–1.516)	0.687	1.304 (1.094–1.554)	0.924
7.1–8 cm	1.350 (1.192–1.528)	< 0.001	1.680 (1.386–2.037)	< 0.001	1.279 (1.092–1.499)	0.879	1.395 (1.150–1.693)	0.588
8.1–9 cm	1.454 (1.245–1.698)	< 0.001	1.443 (1.228–1.696)	< 0.001	1.359 (1.138–1.624)	0.562	1.702 (1.471–1.970)	0.039
9.1–10 cm	1.457 (1.284–1.653)	< 0.001	1.702 (1.471–1.970)	< 0.001	1.286 (0.983–1.681)	0.030	1.156 (0.953–1.401)	< 0.001
Ts+	1.067 (1.057-1.077)	< 0.001	1.075 (1.061-1.088)	< 0.001	1.047 (1.032-1.062)	< 0.001	1.047 (1.032-1.063)	< 0.001

Model 1: Results of multivariate Cox proportional hazards models for ACD after adjustment for age, histological type, race, grade, stage, lymph node status, number of lymph node examined, and tumor number. Mode 2: Results of multivariate Cox proportional hazards models for ESD after adjustment for age, histological type, race, grade, stage, lymph node status, number of lymph node examined, and tumor number. Ts+: Ts was analyzed as a continuous variable. Ts, tumor size; HR, hazard ratio; CI, confidence interval; ACD, all-cause death; ESD, endometrial cancer-specific death; LNM, lymph node metastasis.

for the surgical staging of EC. Therefore, incorporating Ts into the classification of EC may help to improve the accuracy of tumor staging and provide a basis for doctors to select a better treatment.

In the entire cohort, we observed that the risk of mortality gradually rose as the tumor grew, and larger Ts indicated poorer prognosis in patients with EC. Similarly, Julian et al. (9) demonstrated that the five-year survival rate progressively decreased when the tumor volume grew. As Maraelys et al (24). used three mathematical models (Gompertz, Logistic and Kolmogorov-Johnson-Mehl-Avrami) to imitate unperturbed fibrosarcoma Sa-37 tumor growth, and those models showed the same results that tumor exhibits a sigmoidal kinetics characteristic. Moreover, Laird et al (25). analyzed 19 examples of 12 different tumors in mice, rats, and rabbits and concluded that the growth of a transplanted, or primary, tumor can be well described by the Gompertz equation, that is, the tumor grows at an exponential rate in the early stage, but with the increase of Ts, the growth rate slows down and leveled off. According to the results of RCS, the risk of mortality increased rapidly with the expansion of the Ts (≤ 7.5 cm) and then increased slowly (Ts > 7.5 cm). So, we hypothesized that the tumor cells proliferate rapidly at this stage (Ts ≤ 7.5 cm), and as the Ts increases, the tumor progresses more aggressively, leading to a rapid increase in the risk of mortality. After Ts increases to a certain extent, the tumor proliferation slows down due to the influence of external environmental factors, such as the

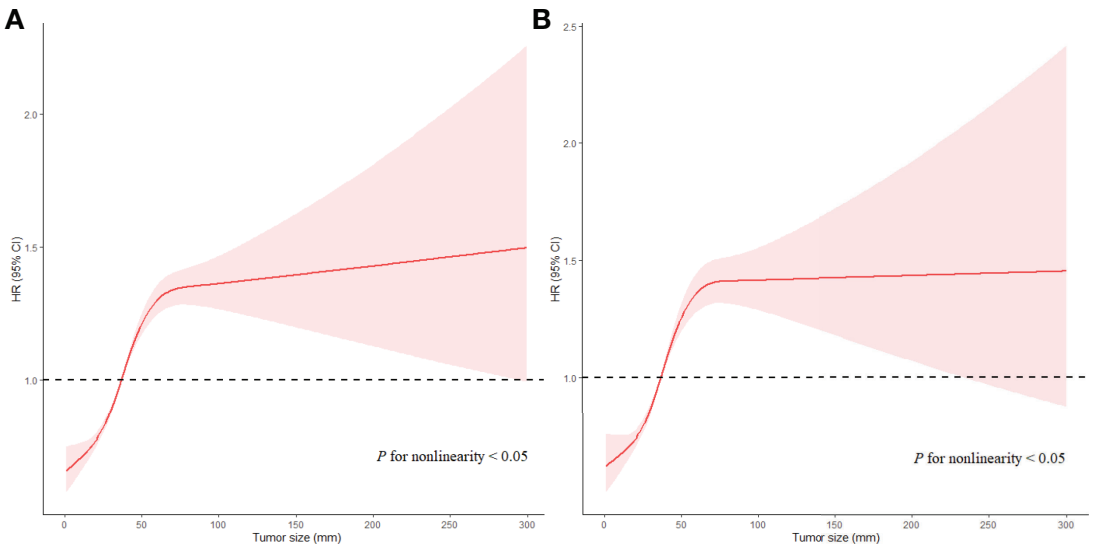

FIGURE 3 | Associations of Ts with prognosis in EC patients with LNM in RCS with Cox models after adjustment. Red lines estimated hazard ratio of tumor size; shadow area 95% CI. **(A)** Adjusted RCS model for ACD. **(B)** Adjusted RCS model for ESD.

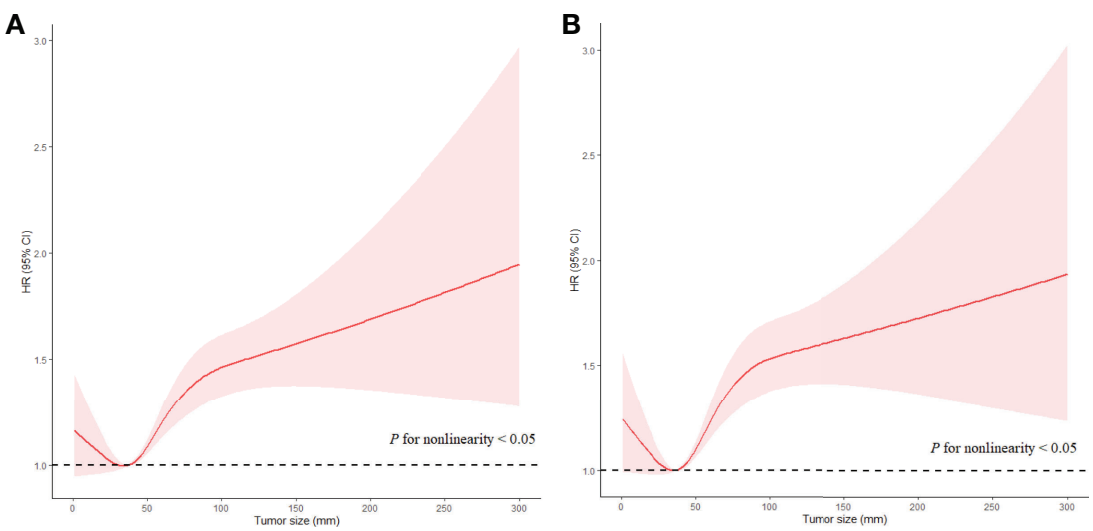

FIGURE 4 | Associations of Ts with prognosis in EC patients without LNM in RCS with Cox models after adjustment. Red lines estimated HR of Ts; shadow area 95%CI. **(A)** Adjusted RCS model for ACD. **(B)** Adjusted RCS model for ESD.

formation of microenvironments and microvessels, resulting in a slower rate of tumor progression and a slow increase in the risk of death as the curve showed in Ts > 7.5 cm. Moreover, if the tumor is smaller than 7.5 cm, the drug of treatment may choose tumor growth blockers, and if the tumor is larger than 7.5 cm, surgical resection may be better. Based on this study, we only explored the relationship between tumor size and the risk of death, the process of tumor growth is complex and the biological mechanism is not entirely clear, further research is needed on whether the above-mentioned treatment options are feasible.

The effect of Ts on prognosis was significantly different in patients with LNM and those without LNM. It was acknowledged that large Ts is associated with lymph node involvement and poor survival outcomes. (7, 14, 26) However, the risk of mortality decreased rapidly with Ts when the Ts was less than 3.5 cm, indicating that smaller Ts predicted a worse prognosis within this range of Ts in EC patients with LNM. Until now, few studies have examined the effects of small tumors on poor survival in EC. However, some evidence could be found for other cancers. Muralidhar et al (27). observed that patients with

small Ts (< 0.1 cm) in prostate cancer suffered from a poorer long-term prognosis than did patients with larger Ts, and small Ts might be associated with LN involvement. Similarly, Wo et al (28). demonstrated that patients with Ts less than 0.5 cm in breast cancer had a lower survival rate compared with patients with Ts larger than 0.5 cm. These studies may support our hypothesis that smaller tumors in EC patients with LNM may represent greater biological aggressiveness and earlier acquisition of genetic changes that promote tumor cell spread to regional or distant sites. As vinayak et al. (27) had same view, they found patients who had LNM in very small prostate cancers presented a particularly aggressive disease variant compared with larger tumors. These small tumors may represent higher mutation rates and thus evade the body's immune surveillance and anti-tumor immune response. Haffner et al (29). used whole-genome sequencing and molecular to analyze and trace the lineage of cell clones from node-positive patients who eventually died of prostate cancer. They found that lethal clones tended to arise from small tumor and low-grade disease rather than from larger and higher-grade diseases. The reason why these small tumors are more migratory may be that the deregulation of miRNAs, likes miR-142 targetes CCND1 to activate cyclin-dependent kinase (CDK)4/6 for stimulating proliferation, migration, and invasion of cells (30). Mahecha et al. (31) observed that the overexpressed gene of vascular endothelial growth can lead to an increase of the number of new blood vessels in tumor tissues, and the newer blood vessels, the deeper the tumor invasion into myometrium, resulting in vascular metastasis, poor grade, and poor prognosis. Ray et al. (32) found that the overexpression of pro-inflammatory adipocytokines, such as leptin, can also promote the transformation of epithelial mesenchymal to stimulate endometrial cancer growth, proliferation, invasion, and metastasis. Moreover, a larger Ts usually leads to more aggressive treatment, such as a more complete lymphadenectomy and surgical evaluation, resulting in a better prognosis. Therefore, further research on the biological basis of small tumors associated with LNM in EC may discover novel genomic changes, new drug targets, or prognostic markers, thus providing new approaches to guide the selection of treatment options and improve prognosis.

The study also had several limitations. First, due to its retrospective nature, selection bias was inevitable, as the variable of treatment history (radiation, chemotherapy, hormonal therapy) not been included, so the results should be interpreted with caution. Second, in this study, we only selected the variables (EEA, SEA) with large sample sizes, but other pathological types also are worthy of study. And because of the limited classification of races in the SEER database, we could not get detailed information about it. Third, the lack of standardization in pathological classification may result in some patients being misclassified. Fourth, we only extracted prognostic information on OS and ESS, as more information such as recurrence and metastasis cannot be obtained from the database. Finally, the factors affecting tumor growth were complex, but we are unable to simulate the real environment of tumor growth, so there may be a certain gap between the model and the real situation of diagnosis and treatment.

CONCLUSION

In this study, we revealed a nonlinear relationship between Ts and prognosis in patients with EC, and the risk of mortality increased monotonically with increasing Ts. However, the effect pattern of Ts on prognosis in patients with LNM was significantly different from that in patients without LNM. Among patients with LNM, a smaller Ts indicated a worse survival outcome when Ts was less than 3.5 cm, suggesting that clinicians should not ignore the impact of small tumor size on prognosis in these patients.

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 authors.

ETHICS STATEMENT

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XFH and SY wrote the draft and revised it. JW and LH designed and supervised the study. JL, ZQ, and LX extracted and cleaned the data. XYH and SL designed the figures and tables. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.887157/full#supplementary-material

Supplementary Figure 1 | The ROC curve in all EC patients. **(A)** The ROC curve for ACD. **(B)** The ROC curve for ESD.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2018) 68:394–424. doi: 10.3322/caac.21492
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660
3. Berman ML, Ballon SC, Lagasse LD, Watring WG. Prognosis and Treatment of Endometrial Cancer. *Am J Obstet Gynecol* (1980) 136:679–88. doi: 10.1016/0002-9378(80)91024-8
4. Mahdi H, Munkarah AR, Ali-Fehmi R, Woessner J, Shah SN, Moslemi-Kebria M. Tumor Size is an Independent Predictor of Lymph Node Metastasis and Survival in Early Stage Endometrioid Endometrial Cancer. *Arch Gynecol Obstet* (2015) 292:183–90. doi: 10.1007/s00404-014-3609-6
5. Senol T, Polat M, Ozkaya E, Karateke A. Tumor Diameter for Prediction of Recurrence, Disease Free and Overall Survival in Endometrial Cancer Cases. *Asian Pac J Cancer Prev* (2015) 16:7463–6. doi: 10.7314/apjcp.2015.16.17.7463
6. Ballester M, Dubernard G, Lécuru F, Heitz D, Mathevet P, Marret H, et al. Detection Rate and Diagnostic Accuracy of Sentinel-Node Biopsy in Early Stage Endometrial Cancer: A Prospective Multicentre Study (SENTI-ENDO). *Lancet Oncol* (2011) 12:469–76. doi: 10.1016/s1470-2045(11)70070-5
7. Canlorbe G, Bendifallah S, Laas E, Raimond E, Graesslin O, Hudry D, et al. Tumor Size, an Additional Prognostic Factor to Include in Low-Risk Endometrial Cancer: Results of a French Multicenter Study. *Ann Surg Oncol* (2016) 23:171–7. doi: 10.1245/s10434-015-4583-3
8. Ytre-Hauge S, Husby JA, Magnussen IJ, Werner HM, Salvesen ØO, Bjørge L, et al. Preoperative Tumor Size at MRTumor Size, an Additional Prognostic Factor to Include in Low-Risk Endometrial Cancer: Results of a French Multicenter Study I Predicts Deep Myometrial Invasion, Lymph Node Metastases, and Patient Outcome in Endometrial Carcinomas. *Int J Gynecol Cancer* (2015) 25:459–66. doi: 10.1097/igc.0000000000000367
9. Chattopadhyay S, Cross P, Nayar A, Galaal K, Naik R. Tumor Size: A Better Independent Predictor of Distant Failure and Death Than Depth of Myometrial Invasion in International Federation of Gynecology and Obstetrics Stage I Endometrioid Endometrial Cancer. *Int J Gynecol Cancer* (2013) 23:690–7. doi: 10.1097/IGC.0b013e31828c85c6
10. AlHilli MM, Podratz KC, Dowdy SC, Bakkum-Gamez JN, Weaver AL, McGree ME, et al. Preoperative Biopsy and Intraoperative Tumor Diameter Predict Lymph Node Dissemination in Endometrial Cancer. *Gynecol Oncol* (2013) 128:294–9. doi: 10.1016/j.ygyno.2012.10.009
11. Ozgul N, Boyraz G, Salman MC, Gultekin M, Yuce K, Ibrahimov A, et al. Oncological Outcomes of Stage II Endometrial Cancer: A Retrospective Analysis of 250 Cases. *Int J Gynecol Cancer* (2018) 28:161–7. doi: 10.1097/igc.0000000000001133
12. Shah C, Johnson EB, Everett E, Tamimi H, Greer B, Swisher E, et al. Does Size Matter? Tumor Size and Morphology as Predictors of Nodal Status and Recurrence in Endometrial Cancer. *Gynecol Oncol* (2005) 99:564–70. doi: 10.1016/j.ygyno.2005.06.011
13. Doll KM, Tseng J, Denslow SA, Fader AN, Gehrig PA. High-Grade Endometrial Cancer: Revisiting the Impact of Tumor Size and Location on Outcomes. *Gynecol Oncol* (2014) 132:44–9. doi: 10.1016/j.ygyno.2013.10.023
14. Winer I, Mahdi H, Bandyopadhyay S, Semaan A, Van de Vijver KK, Nucci MR, et al. Correlation of Tumor Size With Other Prognostic Factors in Uterine Serous Carcinoma: A Large Multi-Institutional Study. *Gynecol Oncol* (2013) 128:316–21. doi: 10.1016/j.ygyno.2012.10.027
15. Vargas R, Rauh-Hain JA, Clemmer J, Clark RM, Goodman A, Growdon WB, et al. Tumor Size, Depth of Invasion, and Histologic Grade as Prognostic Factors of Lymph Node Involvement in Endometrial Cancer: A SEER Analysis. *Gynecol Oncol* (2014) 133:216–20. doi: 10.1016/j.ygyno.2014.02.011
16. Vargas R, Rauh-Hain JA, Clemmer J, Clark RM, Goodman A, Growdon WB, et al. Pathologic and Clinical Tumor Size Discordance in Early-Stage Cervical Cancer: Does it Matter? *Gynecol Oncol* (2020) 159:354–8. doi: 10.1016/j.ygyno.2020.08.004
17. Vargas R, Rauh-Hain JA, Clemmer J, Clark RM, Goodman A, Growdon WB, et al. Association of Tumor Size With Prognosis in Colon Cancer: A Surveillance, Epidemiology, and End Results (SEER) Database Analysis. *Surgery* (2021) 169:1116–23. doi: 10.1016/j.surg.2020.11.011
18. Wu J, Huang L, He H, Zhao Y, Niu D, Lyu J. Red Cell Distribution Width to Platelet Ratio Is Associated With Increasing In-Hospital Mortality in Critically Ill Patients With Acute Kidney Injury. *Dis Markers* (2022) 2022:4802702. doi: 10.1155/2022/4802702
19. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to Build a Bridge From a Population-Based to a More "Personalized" Approach to Cancer Staging. *CA: Cancer J Clin* (2017) 67:93–9. doi: 10.3322/caac.21388
20. Koskas M, Amant F, Mirza MR, Creutzberg CL. Cancer of the Corpus Uteri: 2021 Update. *Int J gynaecol ob: Off Organ Int Fed Gyn Ob* (2021) 155 Suppl 1:45–60. doi: 10.1002/ijgo.13866
21. Koskas M, Amant F, Mirza MR, Creutzberg CL. Cancer of the Cervix Uteri. *Int J Gynaecol Obstet* (2018) 143 Suppl 2:22–36. doi: 10.1002/ijgo.12611
22. Adams TS, Cuello MA. Cancer of the Vagina. *Int J gynaecol Ob: Off Organ Int Fed Gyn Ob* (2018) 143 Suppl 2:14–21. doi: 10.1002/ijgo.12610
23. Berretta R, Patrelli TS, Migliavacca C, Rolla M, Franchi L, Monica M, et al. Assessment of Tumor Size as a Useful Marker for the Surgical Staging of Endometrial Cancer. *Oncol Rep* (2014) 31:2407–12. doi: 10.3892/or.2014.3108
24. González MM, Joa JA, Cabrales LE, Pupo AE, Schneider B, Kondakci S, et al. Is Cancer a Pure Growth Curve or Does it Follow a Kinetics of Dynamical Structural Transformation? *BMC Cancer* (2017) 17(1):174. doi: 10.1186/s12885-017-3159-y
25. Vaidya VG, Alexandro FJJr. Evaluation of Some Mathematical Models for Tumor Growth. *Int J BioMed Comput* (1982) 13(1):19–36. doi: 10.1016/0020-7101(82)90048-4
26. Çakır C, Kılıç İÇ, Yüksel D, Karyal YA, Üreyen I, Boyraz G, et al. Does Tumor Size Have Prognostic Value in Patients Undergoing Lymphadenectomy in Endometrioid-Type Endometrial Cancer Confined to the Uterine Corpus? *Turk J Med Sci* (2019) 49:1403–10. doi: 10.3906/sag-1902-224
27. Muralidhar V, Mahal BA, Nezolosky MD, Beard CJ, Feng FY, Martin NE, et al. Association Between Very Small Tumour Size and Increased Cancer-Specific Mortality After Radical Prostatectomy in Lymph Node-Positive Prostate Cancer. *BJU Int* (2016) 118:279–85. doi: 10.1111/bju.13248
28. Wo JY, Chen K, Neville BA, Lin NU, Punglia RS. Effect of Very Small Tumor Size on Cancer-Specific Mortality in Node-Positive Breast Cancer. *J Clin Oncol* (2011) 29:2619–27. doi: 10.1200/jco.2010.29.5907
29. Haffner MC, Mosbruger T, Esopi DM, Fedor H, Heaphy CM, Walker DA, et al. Tracking the Clonal Origin of Lethal Prostate Cancer. *J Clin Invest* (2013) 123(11):4918–22. doi: 10.1172/JCI70354
30. Klicka K, Grzywa TM, Klinke A, Mielniczuk A, Włodarski PK. The Role of miRNAs in the Regulation of Endometrial Cancer Invasiveness and Metastasis-A Systematic Review. *Cancers* (2021) 13(14):3393. doi: 10.3390/cancers13143393
31. Mahecha AM, Wang H. The Influence of Vascular Endothelial Growth Factor-A and Matrix Metalloproteinase-2 and -9 in Angiogenesis, Metastasis, and Prognosis of Endometrial Cancer. *Onco Targets Ther* (2017) 10:4617–24. doi: 10.2147/OTT.S132558
32. Ray I, Meira LB, Michael A, Ellis PE. Adipocytokines and Disease Progression in Endometrial Cancer: A Systematic Review. *Cancer Metastasis Rev* (2022) 41(1):211–42. doi: 10.1007/s10555-021-10002-6

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Analysis of Factors Associated With Recurrence of Early-Stage Endometrial Carcinoma and Atypical Endometrial Hyperplasia in Infertile Women After *In Vitro* Fertilization Treatment

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Purpose: To explore the relationship between different artificial reproductive treatment (ART) strategies and tumor outcomes, by analyzing clinical data of patients with endometrial carcinoma (EC) and atypical endometrial hyperplasia (AEH).

Methods: This retrospective study was performed in a tertiary hospital. Patients (n=131) with EC or AEH, who underwent *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment between June 2010 and June 2021, were divided into a recurrence group and a non-recurrence group. Clinical characteristics and tumor outcomes were assessed.

Results: 131 patients were followed up for 4-132 months; 33 patients had recurrence, the recurrence rate was 25.2%, 3-year recurrence-free survival (RFS) rate was 83.2 ± 3.4%, and the 5-year RFS rate was 72.9 ± 4.4%. Factors including the frequency of controlled ovarian stimulation (COS) and the total days of ovarian stimulation had no significant effect on the recurrence of tumor lesions (p=0.368 and 0.969, respectively). Histology type (HR: 4.94, 95%CI: 2.41-10.15, p <0.001) and successful/un successful live birth (HR: 0.30, 95%CI: 0.14-0.65, p=0.003) were independent factors of recurrence. Twenty-two of the 82 patients who received a single COS had recurrence. Different COS protocols, the total dose of gonadotropin (Gn), and the serum E_2 level on the trigger day had no significant effect on recurrence (p=0.326, 0.889 and 0.468, respectively).

Conclusions: The degree at which an endometrial lesion progresses into carcinoma is a key factor affecting the recurrence of EC/AEH in patients after IVF/ICSI treatment, and successful live birth is a protective factor for the recurrence of endometrial lesions. Different COS protocols and COS frequencies, as well as the dosage and duration of Gn used during IVF did not affect the recurrence of endometrial lesions.

Keywords: endometrial carcinoma, atypical endometrial hyperplasia, assisted reproductive technology, recurrence, *in vitro* fertilization

INTRODUCTION

Endometrial cancer (EC) is one of the most common gynecological malignancies worldwide, with more than 410,000 new cases in 2020 (1). Atypical endometrial hyperplasia (AEH) is a precancerous lesion of endometrial carcinoma whose malignant transformation rate is 29%-52% (2). Although EC is often seen in postmenopausal women, approximately 5% of patients are diagnosed before age of 40 years, which includes 70% of childless women (3). The standard management for EC/AEH is hysterectomy and bilateral salpingo-oophorectomy which is not suitable for young patients with fertility desire (4). The effectiveness of conservative treatment in young patients with early-stage endometrial carcinoma (EEC) and AEH has been confirmed with a high complete remission (CR) rate (75-96.5%). However, the rate of recurrence is as high as 26.0-40.6%, and the median recurrence time was 12-28 months (5–7). Patients who underwent standard management for EEC/AEH had better prognosis, with 5- and 10-year survival rates of 99.2% and 98%, respectively (5). The challenge for EEC/AEH patients is how to get a livebirth as early as possible and then to receive the standard management. However, repeated intrauterine operations will lead to increased incidence of thin endometrium and intrauterine adhesions. Ovarian reserve and patients' fertility decreased with the growth of age. In addition, most patients with EC/AEH may have combined factors, such diabetes or obesity that may lead to infertility. In order to successfully achieve a livebirth as soon as possible before the recurrence of the disease, the use of artificial reproductive treatment (ART) has become the first choice for most doctors and patients. Indeed, many reports have confirmed the effectiveness of EC/AEH patients using ART for pregnancy, and the live birth rate of ART was 6.9 times than that of natural pregnancy (8). Furthermore, Zhou (9) observed that the clinical pregnancy rate of ART was significantly higher than that of natural pregnancy (72.7% vs 10.0%, p=0.04). Thus, we can conclude that ART is meaningful for EC/AEH patients with fertility issues. However, there are still some controversies in terms of the safety for EC/AEH patients in making them achieve a livebirth by means of ART. Controlled ovarian stimulation (COS) during ART treatment can lead to a significant increase in estrogen level over a short period of time. Whether it will lead to an increase in the recurrence rate and selection of the best COS protocol is of concern to reproductive endocrinology and infertility (REI) doctors. In this study, we analyzed the clinical data of EC/AEH patients who received IVF to elaborate the safety of EC/AEH patients receiving ART and the factors affecting recurrence rate, in order to provide more treatment experiences for REI doctors as well as gynecologists.

METHODS

Study Design and Patients

In this single-center retrospective study, we reviewed the medical records of infertile patients with EEC or AEH who underwent IVF after achieving CR at the Reproductive Center of Peking University Third Hospital (PUTH) between June 2010 and June 2021. Follow-up ended on October 31, 2021. This study was approved by the Ethics Committee of the PUTH (No. IRB 00006761-M2021237).

The inclusion criteria were as follows: (1) histologically proven well-differentiated endometrioid EC or AEH, magnetic resonance imaging confirmed no infiltration of myometrium; (2) accepted standard conservative therapy and achieved CR; (3) age ≤40 years old; (4) hysteroscopic evaluation performed and histologically proven normal endometrium before COS; and (5) performed standard COS protocol cycles.

The selection process of the study population is illustrated in **Figure 1**. Between June 2010 and June 2021, 139 infertile patients with EEC or AEH were referred to our reproductive center after achieving CR. Eight patients were excluded from the study for the following reasons: age >40 years old (n=2), prior history of IVF before conservative treatment (n=2), incomplete medical records (n=4). A total of 131 patients were included in the analysis. Clinical and IVF/ICSI characteristic data were reviewed and extracted from both paper and electronic medical records.

Conservative Treatment

The endometrial lesion of each patient was comprehensively evaluated by the gynecologic oncologist and met the criteria for fertility-sparing treatment. All patients received oral progestins or intrauterine progesterone therapy including four different treatment regimens as follows: (1) MA at a dose of 160–320 mg per day (n=32); (2) MPA at a dose of 250–500 mg per day (n=95); (3) Gonadotropin-releasing hormone agonist (GnRHa) combined levonorgestrel intrauterine sustained release system (LNG-IUS) (n=2); (4) Intrauterine LNG-IUS alone (n=2). Hysteroscopy and endometrial biopsy were performed every three months to evaluate the treatment response.

Once patients achieved CR, some discontinued MA or MPA, and were referred to the REI specialists directly for ART. Some patients continued to receive the same regimen for another 3–6 months according to different doctors' opinions, which was defined as maintenance therapy, before referral to REI specialists.

IVF Treatment

A comprehensive evaluation of infertility was performed by a REI specialist for every patient who was referred to the reproduction center. Agonist, antagonist, or mild-stimulation protocols were used for ovarian stimulation in patients who received IVF/ICSI treatment. Gonadotropin (Gn) usage involves follicle stimulating hormone (FSH), human menopausal gonadotropin (HMG), and recombinant follitropin β injection. Agonist protocol includes three different dosage forms of GnRHa as follows: ultra-long protocol, long protocol, and short protocol.

Ultra-long protocol means intramuscular injection of 3.75 mg of long-acting GnRHa was performed on the 1st or 2nd day of the menstrual cycle, and Gn was started 30 days later until the trigger day. Long protocol means intramuscular injection of 1.25 mg long-acting GnRHa was given during the luteal phase of the previous menstrual cycle, and Gn was started 14 days later until the trigger day. Short protocol means intramuscular injection of short-acting GnRHa 0.1 mg/d was started on the 2nd day of the

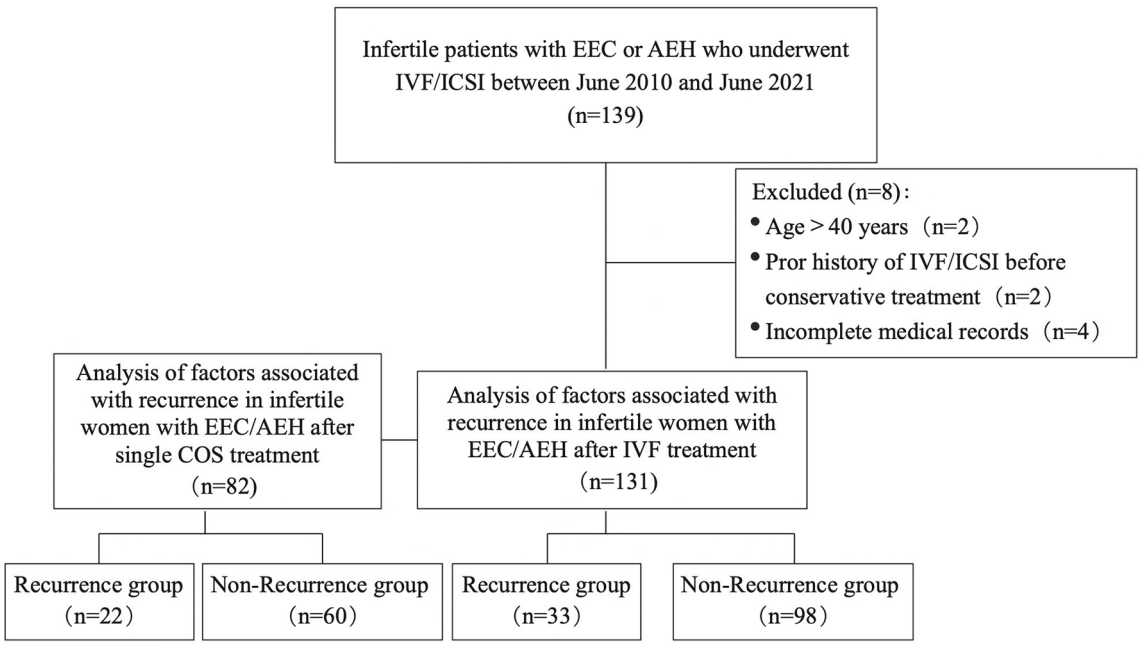

FIGURE 1 | Flow chart of the analysis cohort.

menstrual cycle, and Gn was started on the 3rd day of the menstrual cycle until the trigger day. Antagonist protocol means Gn was started on the 2nd day of the menstrual cycle, and 0.25 mg/d gonadotropin-releasing hormone antagonist (GnRH-A) was added when the dominant follicle diameter was 12-14 mm on the trigger day. Mild-stimulation protocol means that letrozole 2.5 or 5.0 mg/d was given orally from the 2nd to 6th day of the menstrual cycle for 5 days. Meanwhile, intramuscular injection of hMG 75-150 U/d was given from the 3rd day of the menstrual cycle. GnRH-A 0.25 mg/d was added when the diameter of the dominant follicle reached 12-14 mm, until the trigger day. The start dose of Gn is determined by the individual patient (150-300 U/d), and is adjusted according to follicular development in the process of COS.

Ovarian follicular development was monitored by TVS, and recombinant human chorionic gonadotropin (r-hCG) was administered to induce oocyte maturation when at least two leading follicles reached 18 mm in diameter. Oocyte retrieval was performed 34 and 38 h later. Oocytes were fertilized using conventional IVF or ICSI. The development and quality of embryos were assessed on day 3, as previously published, considering the percentage of fragmentation and quality of cytoplasm (10). Top-quality embryos on day 3 were defined as embryos that were derived from 2 PN embryos and could reach 5- to 8-cell stage with cytoplasmic fragmentation of <30% and even blastomeres. Non-top-quality embryos were extensively cultured to the blastocyst stage. Blastocyst morphology was evaluated on day 5 using the Gardner grading system (11). Two top-quality embryos on day 3 or one on day 5 were transferred in the fresh ET cycle. Some patients did not accept fresh ET because of the thin endometrium, a high risk of ovarian hyperstimulation syndrome, or some other reasons. Surplus viable embryos were cryopreserved according to a vitrification protocol and thawed as previously described (12). During frozen-thawed ET (FET) cycles, frozen embryos were transferred on day 3 or 5 throughout the natural or artificial cycles.

Regular luteal support was provided as oral dydrogesterone at 20 mg/d or vaginal administration of progesterone 60 mg/d from the day of ET to throughout the 10th week of gestation.

Definition of Observation Indicators

CR was defined as the absence of hyperplasia, cancerous lesions, or other abnormal histological findings. Recurrence was defined as endometrial carcinoma or atypical hyperplasia confirmed by endometrial biopsy that recurred during ART treatment or during follow-up after ART. Treatment duration was calculated as the interval from the start to the end of oral or intrauterine progesterone treatment. The time to CR was calculated as the interval from the start of progesterone treatment to CR. The duration of maintenance therapy was calculated from the date of CR to the end of progesterone treatment. The time to IVF was defined as the interval between the CR and the start of IVF cycle. Live birth was defined as any birth event beyond 28 weeks of gestation, in which at least one neonate was born alive. The cumulative live birth rate (CLBR) of the study cohort was defined as the number of women who achieved a live birth divided by the total study population.

Statistical Methods

Continuous data with normal distribution were represented as mean (standard deviation, SD), while continuous data with non-

normal distribution were represented as median (interquartile range, IQR). Continuous data were analyzed using T test and Mann-Whitney U test. Categorical data were expressed as percentages and analyzed using Chi-square test or Fisher exact test. The median recurrence interval and cumulative recurrence rate were calculated using the Kaplan-Meier method, and the difference in recurrence rate was tested with log-rank method. COX regression model was used for correlation analysis of tumor RFS time. All analyses were performed using SPSS software (version 25.0; IBM Corp, Armonk, NY, USA). Significance was defined as a two-sided p-value <0.05.

RESULTS

Baseline Characteristics

Up to October 31, 2021, 131 patients included in the study were followed up for 4.0-132.0 months, with a median follow-up of 50.0 months. As shown in **Table 1**, the average age of 131 patients was 33.6 ± 3.8 years and the average BMI was 26.0 ± 4.2 Kg/m^2 with a median infertility time of 4.0 (range: 2.0-6.0) years. Most of the study participants (80.9%) were diagnosed with primary infertility, and 25 patients (19.1%) with secondary infertility. Ovulatory dysfunction and fallopian tube factors were the main causes of infertility, accounting for 38.9% (51 cases) and 24.4% (32 cases), respectively.

One hundred thirty-one patients were assigned into the recurrence (33 cases) and non-recurrence (98 cases) groups. The number of patients combined with those with polycystic ovarian syndrome (PCOS), diabetes mellitus (DM) and hypertension were 33 (25.2%), 12 (9.2%) and 7 (5.3%), respectively. However, there were no significant differences in the incidence of these complications between the recurrence group and the non-recurrence group (PCOS, DM and hypertension, p=0.827,1.000 and 1.000, respectively). Meanwhile, there were no significant differences in basal sex hormone levels (LH, E_2 and FSH, p=0.419, 0.654 and 0.824, respectively) and basal Antral follicle count (AFC) (p=0.850) between the two groups.

In total, 131 patients underwent an average of 1.6 ± 0.9 COS cycles and 1.8 ± 1.2 embryo transfers (ETs), and there was no significant difference in the number of COS cycles between the recurrence group and the non-recurrence group (p=0.521). Each patient received 3600.0 (range: 2100.0-5268.8) IU of Gn in all COS cycles, and the total number of days of Gn injection was 14.0 (range:

TABLE 1 | Baseline characteristics of the analysis cohort.

Characteristics	Total (n=131)	Non-recurrence (n=98)	Recurrence (n=33)	*p* value
Age, mean (SD), years	33.6 (3.8)	33.7 (3.8)	33.6 (4.0)	0.420
BMI, mean (SD), Kg/m^2	26.0 (4.2)	26.1 (4.1)	25.7 (4.7)	0.861
Histology type, n (%)				0.001*
EC	30 (22.9)	15 (15.3)	15 (45.5)	
AEH	101 (77.1)	83 (84.7)	18 (54.5)	
Type of infertility, n (%)				0.614
Primary	106 (80.9)	78 (79.6)	28 (84.8)	
Secondary	25 (19.1)	20 (20.4)	5 (15.2)	
Duration of infertility, median (IQR), years	4.0 (2.0-6.0)	4.0 (2.0-6.0)	4.0 (2.0-6.5)	0.924
Causes of infertility, n (%)				0.192#
Male factors	15 (11.5)	9 (9.2)	6 (18.2)	
Tubal factors	32 (24.4)	24 (24.5)	8 (24.2)	
Ovarian factors	51 (38.9)	41 (41.8)	10 (30.3)	
Uterine factors	5 (3.8)	2 (2.0)	3 (9.1)	
Unknown factors	28 (21.4)	22 (22.4)	6 (18.2)	
Complications, n (%)				
PCOS	33 (25.3)	30 (26.1)	9 (23.1)	0.827
DM	12 (9.2)	9 (9.2)	3 (9.1)	1.000#
Hypertension	7 (5.3)	5 (5.1)	2(6.1)	1.000#
Ovarian reserve, median (IQR)				
AMH (ng/mL)	1.1 (0.4-2.1)	1.1 (0.5-1.7)	1.2 (0.3-2.5)	0.976
No. of basal AFC	7.0 (4.0-13.0)	7.0 (4.0-14.0)	7.0 (5.0-10.0)	0.850
Basal LH, median (IQR), mIU/mL	1.8 (0.6-4.0)	1.9 (0.8-4.0)	1.7 (0.5-3.2)	0.419
Basal FSH, median (IQR), mIU/mL	6.0 (4.4-8.1)	6.1 (4.5-8.1)	6.0 (4.4-8.1)	0.824
Basal E_2, median (IQR), pmol/L	131.0 (92.5-128.5)	128.5 (89.7-172.0)	132.0 (104.5-177.5)	0.654
No. of COS cycles, mean (SD)	1.6 (0.9)	1.6 (0.9)	1.5 (0.8)	0.521
No. of ET cycles, mean (SD)	1.8 (1.2)	1.8 (1.3)	1.9 (1.1)	0.352
Total dose of Gn, median (IQR), IU	3600.0 (2100.0-5268.8)	3550.0 (2306.3-5587.5)	3750.0 (2087.5-5025.0)	0.994
No. of days of ovarian stimulation, median (IQR)	14.0 (11.0-24.0)	14.0 (10.0-24.0)	14.0 (12.0-24.0)	0.493
With a livebirth, n (%)	66 (49.6)	57 (58.2)	9 (27.3)	0.003*

BMI, body mass index; EC, endometrial carcinoma; AEH, atypical endometrial hyperplasia; AMH, anti-mullerian hormone; AFC, antral follicle count; E_2, estradiol; CR, complete remission; IVF, in vitro fertilization; SD, standard deviation; Gn, gonadotropin; IQR, interquartile range; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOS, Polycystic ovary syndrome; DM, Diabetes mellitus; COS, controlled ovarian stimulation; ET, embryo transfer.

**p<0.05.*

#Fisher's exact test.

11.0-24.0) days, and there was no significant difference between the two groups (p=0.493 and 0.352, respectively).

Recurrence occurred in 15 of 30 EC patients and 18 of 101 AEH patients, with a significantly higher recurrence rate in the EC group than in the AEH group (50.0% vs 17.8%, p=0.001). The proportion of patients who achieved a live birth was significantly different between the two groups (p=0.003).

Characteristics of Conservative Treatment

According to **Table 2**, there were four conservative treatment regimens including 95 patients (72.5%) using MPA and 32 patients (24.4%) using MA. Both GnRHa combined with LNG-IUS and LNG-IUS alone were reported in 2 patients (1.5%), and there was a significant difference between the different regimens used in the recurrence and non-recurrence groups (p=0.021). The mean treatment duration was 7.2 months, and the treatment duration in the recurrence group was significantly longer than that in the non-recurrence group (8.6 vs 6.7 months, p=0.023). The number of hysteroscopic operations in the recurrence group was also significantly higher than that in the non-recurrence group (4.0 vs 3.0 times, p<0.001). The mean CR time and the median time to IVF in the recurrence group was not significantly different from that in the non-recurrence group.

Among 33 patients with recurrence, 12 patients with EC pathology after recurrence received comprehensive staging operation, 3 patients with AEH underwent hysterectomy, and 18 patients received conservative treatment again (12 with MPA, 3 with MA, 2 with MPA+LNG-IUS, and 1 with GnRHa+LNG-IUS). Fifteen patients achieved CR again and three were still on treatment. Three patients in the non-recurrence group underwent hysterectomy after delivery.

Factors Associated With Recurrence

Up to October 31, 2021, 33 of 131 patients had recurrence during follow-up, with a 3-year RFS rate of 83.2 ± 3.4% and a 5-year RFS rate of 72.9 ± 4.4%. Four of 131 patients with less than 12 months of follow-up were excluded, and 127 patients were finally included in the COX regression analysis.

As shown in **Table 3**, continuous variables were converted to categorical variables based on clinical experience and related literature reports. Univariate COX regression analysis was conducted and showed that the type of histology (HR: 5.56, 95%CI: 2.73-11.33, p<0.001), maintenance therapy before IVF (HR: 2.03, 95%CI: 1.01-4.09, p=0.047) were associated with a higher recurrence rate. Patients who successfully achieved a live birth had a significantly lower recurrence rate (HR: 0.27, 95%CI: 0.12-0.58, p=0.001). There were no significant differences in recurrence rates among patients receiving different conservative treatments (p=0.080).

The number of COS cycles, basal E_2 level, total dose of Gn, and total days of ovarian stimulation had no significant effect on the recurrence rate of EC/AEH (p=0.521, 0.785, 0.711, and 0.586, respectively).

As shown in **Table 3**, we included 9 variables (age, BMI, histology type, number of COS cycles, maintenance treatment before IVF, total days of ovarian stimulation, time to IVF, with livebirth, and clinical intervention after IVF and delivery) based on the COX univariate regression analysis, clinical experience, and published literature into COX regression model for multivariate analysis, and found that histology type (HR: 4.94, 95%CI: 2.41-10.15, p<0.001) and livebirth or not (HR: 0.30, 95% CI: 0.14-0.65, p=0.003) were independent influencing factors of recurrence. The influence on RFS of EC or AEH, for livebirth or not are shown in **Figure 2**.

Different COS Protocols and Tumor Recurrence

As shown in **Figure 1**, 82 of 131 patients received a single COS cycle, they included 64 (78.0%) AEH patients and 18 (22.0%) EC patients. These 82 patients were summarized and analyzed using different COS protocols.

82 patients were followed up for 13.0-128.0 months. By the time of follow-up, 22 of the 82 patients had recurrence, and the 3-year RFS rate was 81.0 ± 4.6%, and the 5-year RFS rate was 73.6 ± 5.5%. As

TABLE 2 | Characteristics of conservative treatment of the analysis cohort.

Characteristics	Total (n=131)	Non-recurrence (n=98)	Recurrence (n=33)	*p* value
Conservative treatment schedule				0.021*#
MPA	95 (72.5)	76 (77.6)	19 (57.6)	
MA	32 (24.4)	21 (21.4)	11 (33.3)	
GnRHa+LNG-IUS	2 (1.5)	1(1.0)	1 (3.0)	
LNG-IUS	2 (1.5)	0 (0.0)	2 (6.1)	
Treatment duration, mean (SD), months	7.2 (4.6)	6.7 (4.3)	8.6 (5.1)	0.023*
Time to CR, mean (SD), months	4.9 (2.2)	4.6 (1.8)	5.7 (3.1)	0.162
Recurrence before IVF, n (%)	19 (14.5)	11 (11.2)	8 (24.2)	0.086
Maintenance therapy before IVF, n (%)	63 (48.1)	43 (43.9)	20 (60.6)	0.110
No. of hysteroscope, median (IQR)	3.0 (2.0-4.0)	3.0 (2.0-4.0)	4.0 (3.0-5.5)	0.000*
Time to IVF, median (IQR), months	9.0 (5.0-16.0)	8.5 (4.0-16.0)	11.0 (6.0-18.0)	0.234
Clinical intervention after IVF or delivery, n (%)	18 (13.7)	14 (14.3)	4 (12.1)	1.000#
Time of follow-up, median (IQR), months	50.0 (31.0-80.0)	58 (37.8-86.5)	31.0 (22.5-46.0)	0.000*

EC, endometrial carcinoma; AEH, atypical endometrial hyperplasia; MPA, medroxyprogesterone acetate; MA, megestrol acetate; GnRHa, gonadotropin-releasing hormone agonist; LNG-IUS, levonorgestrel-releasing intrauterine system;IVF, in vitro fertilization; CR, complete remission; IQR, interquartile range; SD, standard deviation.

**p<0.05.*

#Fisher's exact test

TABLE 3 | Univariate and multiple COX regression analysis of factors associated with recurrence.

Variables	Univariate analysis		Multiple analysis	
	HR (95%CI)	*p* value	HR (95%CI)	*p* value
Age (years)		0.181		0.310
≤35	1		1	
>35	0.59 (0.28-1.28)		0.66 (0.26-1.65)	
BMI (Kg/m^2)		0.758		0.693
≤25.0	1		1	
>25.0	1.12 (0.56-2.22)		1.06 (0.48-2.36)	
Histological type		<0.001*		<0.001*
AEH	1		1	
EC	5.56 (2.73-11.33)		4.94 (2.41-10.15)	
Basal E_2 (pmol/L)		0.785		
≤165.0	1			
>165.0	1.11 (0.53-2.33)			
Treatment duration (months)		0.192		
≤6.0	1			
>6.0	1.60 (0.79-3.21)			
Time to CR (months)		0.382		
≤3.0	1			
>3.0	1.37 (0.68-2.75)			
Maintenance therapy before IVF		0.047*		0.209
No	1		1	
Yes	2.03 (1.01-4.09)		1.63 (0.58-4.63)	
Recurrence before IVF		0.168		
No	1			
Yes	1.75 (0.79-3.88)			
Time to IVF (months)		0.637		0.530
≤3.0	1		1	
3.0-6.0	1.02 (0.28-3.81)		0.74 (0.16-3.41)	
6.0-9.0	1.74 (0.47-6.48)		1.70 (0.39-7.35)	
>9.0	1.63 (0.56-4.81)		0.85 (0.24-3.06)	
Conservative treatment		0.080		
MPA	1			
MA	1.92 (0.91-4.05)			
GnRHa+LNG-IUS	7.50 (1.71-32.86)			
LNG-IUS	2.15 (0.29-16.11)			
Total dose of Gn (IU)		0.711		
≤3600.0	1			
>3600.0	1.14 (0.57-2.26)			
Total days of ovarian stimulation		0.586		0.969
≤14.0	1		1	
>14.0	1.21 (0.61-2.39)		2.03 (0.55-7.49)	
No. of COS cycles		0.521		0.368
≤1	1		1	
>1	0.79 (0.38-1.63)		0.32 (0.08-1.23)	
With livebirth		0.001*		0.003*
No	1		1	
Yes	0.27 (0.12-0.57)		0.30 (0.14-0.65)	
Clinical intervention after IVF or delivery		0.582		0.646
No	1		1	
Yes	0.75 (0.26-2.13)		1.30 (0.38-4.45)	

BMI, body mass index; EC, endometrial carcinoma; AEH, atypical endometrial hyperplasia; E_2, estradiol; AMH, anti-mullerian hormone; CR, complete remission; IVF, in vitro fertilization; Gn, gonadotropin; COS, controlled ovarian stimulation; IVF, in-vitro fertilization; CI, confidence interval; HR, hazard ratio; MPA, medroxyprogesterone acetate; MA, megestrol acetate; GnRHa, gonadotropin-releasing hormone agonist; LNG-IUS, levonorgestrel-releasing intrauterine system.
**p<0.05.*

shown in **Table 4**, all 82 patients received conventional COS protocols, among which more patients adopted agonist protocol and antagonist protocol, accounting for 47.6% and 40.2%, respectively. Different protocols had no significant effect on recurrence (p=0.683). The start dose and total dose of Gn in the recurrence group were slightly higher than those in the non-recurrence group; however, the difference was not significant (212.5 vs 200.0 IU, p=0.797; 2650.0 vs 2550.0 IU, p=0.802). In addition, there was no significant difference in serum E_2 level on the trigger day between the two groups (3880.5 vs 4678.5 pmol/L, p=0.530).

As shown in **Table 5**, we included 8 variables (age, BMI, histology type, protocols of COS, total dosage of Gn, E_2 level on trigger day, maintenance treatment before IVF, and with livebirth or not) into the COX regression model for

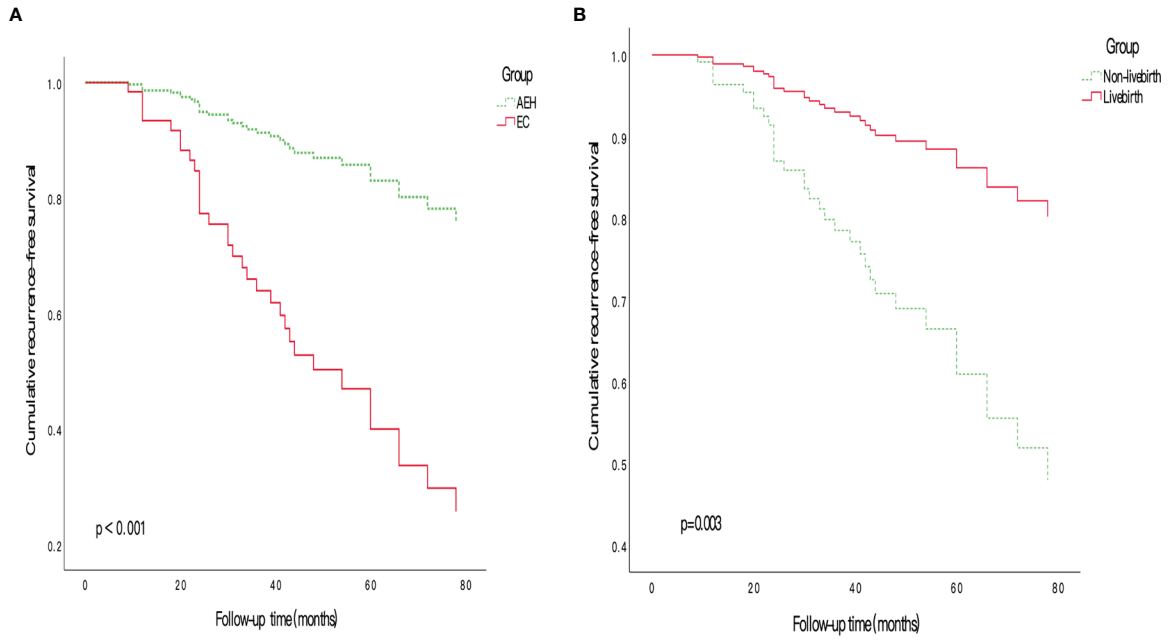

FIGURE 2 | Cumulative RFS curves in fertility-sparing EEC/AEH patients after ART **(A)** The cumulative RFS in patients of AEH group and EC group. Patients had longer RFS with histology of AEH than patients with EC. **(B)** The cumulative RFS in patients of livebirth group and non-livebirth group. The cumulative RFS in patients who got a child successfully was longer than patients failed to get a child. AEH, atypical endometrial hyperplasia; ART, assisted reproductive technology; EEC, early stage endometrial cancer; RFS, recurrence-free survival.

TABLE 4 | Protocols of COS and data of IVF of 82 EC/AEH patients treated with single COS cycle.

Characteristics	Total (n=82)	Non-recurrence (n=60)	Recurrence (n=22)	*p* value
Protocols of COS, n (%)				0.683
GnRH agonist	39 (47.6)	27 (45.0)	12 (54.5)	
GnRH antagonist	33 (40.2)	26 (43.3)	7 (31.8)	
Mild stimulation	10 (12.2)	7 (11.7)	3 (13.6)	
Starting dose of Gn, median (IQR), IU	200.0 (150.0-300.0)	200.0 (150.0-300.0)	212.5 (150.0-300.0)	0.797
Total dose of Gn, median (IQR), IU	2587.5 (1751.9-3618.8)	2550.0 (1725.0-3600.0)	2650.0 (1856.3-3706.3)	0.802
Total days of ovarian stimulation, median (IQR)	11.0 (10.0-13.0)	11.0 (9.3-13.0)	12.0 (10.8-13.0)	0.194
E_2 on trigger day, median (IQR), pmol/L	4572.0 (2369.8-8789.0)	4678.5 (2406.0-8927.0)	3880.5 (2051.0-7498.0)	0.530
No. of retrieved oocytes, median (IQR)	10.0 (4.0-14.0)	10.0 (4.3-13.8)	10.0 (3.0-15.0)	0.937
Fertilization, n (%)				0.181[#]
IVF	57 (69.5)	39 (65.0)	18 (81.8)	
ICSI	25 (30.5)	21 (35.0)	4 (18.2)	
Rate of good-quality embryos per cycle, mean(SD), %	74.7 (28.0)	73.3 (28.3)	78.7 (27.5)	0.405
No. of ETs, median (IQR)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.131

COS,controlled ovarian stimulation; E_2, estradiol; IVF, in vitro fertilization; GnRH, gonadotropin-releasing hormone; IQR, interquartile range; ICSI, intracytoplasmic sperm injection; SD, standard deviation; ET, embryo transfer.

[#]*Fisher's exact test.*

multivariate analysis, and found that histology type (HR: 4.48, 95%CI: 1.74-11.57, p=0.002) and with live birth or not (HR: 0.33, 95%CI: 0.12-0.87, p=0.024) were independent influencing factors of recurrence. Different protocols had no significant effect on the recurrence of EC/AEH (p=0.326).

Pregnancy Outcomes

In total, 66 of the 131 patients achieved a livebirth, with a CLBR of 50.4% (66/131). Fifty-six cases achieved livebirths by IVF/ICSI method and 6 cases through natural pregnancy after ART termination, as well as 3 by preimplantation genetic diagnostic (PGD) cycle, and 1 by *in vitro* maturation (IVM) method. Five delivered twins and four delivered twice, giving birth to 75 live babies.

DISCUSSION

In this single-center retrospective study, we shared our experience of IVF treatment in patients with EEC or AEH

TABLE 5 | Analysis of factors associated with recurrence for patients treated with single COS cycle.

Variables	Univariate analysis		Multiple analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*p* value
Age (years)		0.995		0.942
≤35	1		1	
>35	1.00 (0.42-2.40)		1.09 (0.36-3.28)	
BMI (Kg/m^2)		0.699		0.889
≤25.0	1		1	
>25.0	1.18 (0.51-2.73)		1.87 (0.57-6.12)	
Histological type		<0.001*		0.002*
AEH	1		1	
EC	6.08 (2.37-15.61)		4.48 (1.74-11.57)	
Treatment duration (months)		0.248		
≤6.0	1			
>6.0	2.27 (0.97-5.34)			
Time to CR (months)		0.990		
≤3.0	1			
>3.0	1.01 (0.43-2.33)			
Maintenance therapy before IVF		0.004*		0.059
No	1		1	
Yes	3.79 (1.54-9.35)		2.17 (0.47-10.17)	
Recurrence before IVF		0.463		
No	1			
Yes	2.51(1.07-5.88)			
Time to IVF (months)		0.139		
≤3.0	1			
3.0-6.0	0.57 (0.10-3.43)			
6.0-9.0	1.37 (0.23-8.19)			
>9.0	2.28 (0.66-7.90)			
Protocols of COS, n(%)		0.738		0.326
GnRH agonist	1		1	
GnRH antagonist	0.73 (0.28-1.85)		0.31 (0.08-1.20)	
Mild stimulation	1.15 (0.32-4.08)		0.26 (0.04-1.70)	
Starting dose of Gn (IU)		0.924		
≤200.0	1			
>200.0	0.96(0.42-2.22)			
Total dose of Gn (IU)		0.986		0.889
≤2500.0	1		1	
>2500.0	0.99 (0.43-2.30)		0.89 (0.23-3.47)	
Total days of ovarian stimulation (days)		0.991		
≤12	1			
>12	1.00(0.41-2.45)			
E_2 on trigger day (pmol/L)		0.425		0.468
≤4500.0	1		1	
>4500.0	0.71 (0.31-1.65)		0.89 (0.23-3.47)	
With livebirth		0.004*		0.024*
No	1		1	
Yes	0.25 (0.10-0.65)		0.33 (0.12-0.87)	
Clinical intervention after IVF or delivery		0.634		
No	1			
Yes	0.74 (0.22-2.52)			

BMI, body mass index; EC, endometrial carcinoma; AEH, atypical endometrial hyperplasia; E_2, estradiol; CR, complete remission; IVF, in vitro fertilization; Gn, gonadotropin; COS, controlled ovarian stimulation; IVF, in-vitro fertilization; CI, confidence interval; HR, hazard ratio.
**p<0.05.*

after conservative treatment. To the best of our knowledge, this is one of the largest studies to focus on IVF treatment and recurrence outcomes of patients with EEC or AEH.

According to current reports, the overall recurrence rate of EC/AEH after conservative treatment is 35.0-62.2% (13, 14), and the recurrence rate of EC/AEH patients after ART treatment is 21.0-47.0% with median recurrence time of 12-28 months (15–19). In this study, the recurrence rate was 25.2% and the median recurrence time was 31.0 (range: 22.5-46.0) months, which is consistent with current reports on recurrence in EC/AEH patients after ART treatment. Also, the overall recurrence rate is not significantly higher than that of EC/AEH patients who received conservative treatment. This once again confirmed the safety and necessity of ART for EC/AEH patients. Also, this study reaffirmed that endometrial cancer is an independent risk factor for recurrence, which is consistent with studies have been reported. In general, the

higher the grade of the lesion, the longer the patient needs to receive conservative treatment, and the more frequent intrauterine operations are required to evaluate the endometrial lesion during this period. So it explains why our analysis found that the conservative regimen duration and the number of hysteroscopic operations were higher in the recurrence group.

Although many studies have confirmed the safety of ART (20, 21), there are still many opposing opinions that COS may increase the recurrence of EC/AEH lesions (22, 23). It is well known that the COS process involves the use of high dosage Gn, and the level of serum estrogen is supraphysiological which may lead to the recurrence of the tumor lesion. Therefore, there is no definite conclusion on the choice of COS protocols for EC/AEH patients. Most REI doctors tend to choose COS protocols which combine letrozole with Gn and can reduce the estrogen level during COS process for EC/AEH patients with reproductive needs (24). However, the mild stimulation protocol usually has lower oocyte retrieval rate and fewer available embryos, and the possibility of a satisfactory pregnancy outcome is relatively low (25). Kalogiannidis proposed that GnRH-a can be used for conservative treatment of AEH due to its inhibitory effect on the endometrium, and long-term down-regulation can reduce the large dose drug accumulation effect on progeny (26). Considering this opinion, GnRH-a protocol may be beneficial in preventing the recurrence of EC/AEH lesions. However, Ichinose reported that the high level of serum estrogen after ovulation induction in EC/AEH patients did not increase recurrence (17). In our study, 82 patients who received only one COS cycle were screened for correlation analysis between different COS protocols and recurrence and it was found that compared to the mild stimulation protocol recommended by most scholars, there was no significant difference in recurrence rate among the three protocols (p=0.326). It can be considered that in terms of COS protocols for EC/AEH patients with reproductive needs, REI doctors have more choices based on oocyte retrieval rate, available embryos rate, clinical pregnancy rate, and live birth rate.

Our study found that the recurrence rate for patients with multiple COS cycles was not higher than that for patients with single COS cycles. We can consider that increase in COS cycles will not lead to an increase in the recurrence rate of tumor lesions. Current studies suggest that the recurrence of endometrial lesions requires long-term stimulation of estrogen, while estrogen levels only show short-term increases during COS, thus not increasing the risk of recurrence (17, 27), which supports the conclusion of our study. Of course, the analysis of COS frequency in this paper still has some limitations. Tumor outcomes were not analyzed for specific different times, but were only done with the classification of single and multiple times due to the limitation of sample. Currently, the number of EC/AEH patients receiving ART in a single center is relatively small. In future, multi-center clinical studies should be carried out to expand the sample size and provide more reliable evidence for current research theories.

Gn plays an important role in the process of COS, which is a key step in ART, to promote the development of dominant follicles and increase the number of oocytes retrieved. No matter the difference in frequency of COS or different COS protocols, it can be reflected in the duration and dose of Gn treatment in the ART process. It has been proven that different usage of Gn will lead to a great difference in serum E2 level, which may affect the outcomes of IVF pregnancy as well as the recurrence of tumor lesion (28). But there was no research analyzed the correlation of the use of Gn and recurrence of tumors in EC/AEH patients. Our analysis showed that there was no significant correlation between the total dose of Gn, duration of Gn used, basal E_2 level on trigger day, and tumor recurrence. This result supports that the routine COS protocols in EC/AEH patients with reproductive needs does not increase the risk of tumor recurrence.

Many studies have reported the relationship between pregnancy and tumor recurrence, and found that the recurrence rate in patients who achieved live births was significantly lower than that in patients who did not, suggesting that pregnancy is a protective factor for tumor lesions (16, 21, 29). Similarly, among the 131 patients in this study, the recurrence rate of patients without live births is significantly higher than that of patients with live births (36.9% vs 13.6%, p=0.003). The result was consistent with literature reports. On one hand, the high level of progesterone during pregnancy as well as delivery and the complete decidual detachment from the uterus in the puerperium played a role similar to shaving the tumor lesions, which may prevent the recurrence of the lesion. On the other hand, pregnant women with obesity and PCOS can avoid exposure to estrogen alone for a certain period of time and delay the tumor recurrence and progression (29, 30). However, the above theory is our speculation. There was no study that confirmed that the mechanism of pregnancy can prevent the recurrence of EC/AEH lesions.

Patients were followed up by a gynecologic oncologist after childbearing. We suggest the patient undertake standard management including hysterectomy and bilateral salpinogo-oophorectomy. However, if the patients still have a strong desire to preserve their fertility, they can choose to regularly take short-acting contraceptives or intrauterine LNG-IUS. And these patients are supposed to be followed up every three months. In our study, only two patients in the non-recurrence group underwent surgery after childbirth. Therefore, we consider that most patients are unwilling to undergo the hysterectomy. Due to the limited sample size, although we found that there was no significant effect of clinical intervention after IVF or delivery on tumor recurrence, the optimal management of these patients remains to be explored.

This study had some limitations. First, this was a retrospective study conducted in a single center; therefore, selection bias may have occurred. Second, conservative treatment of EC is mostly limited to patients with lesions confined to the endometrium. It is unknown whether the moderately-differentiated tumor, invasion of the muscle layer, and tumor size will affect the prognosis of EC patients after IVF treatments. Prospective studies with larger sample sizes are needed to answer these questions in the future.

CONCLUSION

The degree of the progression of an endometrial lesion into carcinoma is a key factor affecting recurrence in EC/AEH patients after IVF/ICSI treatment; successful live birth is a protective factor against the recurrence of endometrial lesions. Different COS protocols and COS frequencies, as well as different doses and duration of Gn used during ART, did not affect the recurrence of endometrial lesions.

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 human participants were reviewed and approved by Ethics Committee of the Peking University Third Hospital (No: IRB 00006761-M2021237). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YG, XZ, HL, and JQ developed the design of the study. YG and XZ collected the clinical data. YG drafted the manuscript and contributed the data analysis. XZ, HL, and JQ proofread and revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
2. Kurman RJ, Kaminski PF, Norris HJ. The Behavior of Endometrial Hyperplasia. A Long-Term Study of "Untreated" Hyperplasia in 170 Patients. *Cancer* (1985) 56(2):403–12. doi: 10.1002/1097-0142(19850715)56:2<403::aid-cncr2820560233>3.0.co;2-x
3. Lucchini SM, Esteban A, Nigra MA, Palacios AT, Alzate-Granados JP, Borla HF. Updates on Conservative Management of Endometrial Cancer in Patients Younger Than 45 Years. *Gynecol Oncol* (2021) 161(3):802–9. doi: 10.1016/j.ygyno.2021.04.017
4. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP Guidelines for the Management of Patients With Endometrial Carcinoma. *Radiother Oncol* (2021) 154:327–53. doi: 10.1016/j.radonc.2020.11.018
5. Kalogera E, Dowdy SC, Bakkum-Gamez JN. Preserving Fertility in Young Patients With Endometrial Cancer: Current Perspectives. *Int J Womens Health* (2014) 6:691–701. doi: 10.2147/IJWH.S47232
6. Fan Z, Li H, Hu R, Liu Y, Liu X, Gu L. Fertility-Preserving Treatment in Young Women With Grade 1 Presumed Stage IA Endometrial Adenocarcinoma: A Meta-Analysis. *Int J Gynecol Cancer* (2018) 28(2):385–93. doi: 10.1097/IGC.0000000000001164
7. Masciullo V, Trivellizzi N, Zannoni G, Catena U, Moroni R, Fanfani F, et al. Prognostic Impact of Hysteroscopic Resection of Endometrial Atypical Hyperplasia-Endometrioid Intraepithelial Neoplasia and Early-Stage Cancer in Combination With Megestrol Acetate. *Am J Obstet Gynecol* (2021) 224(4):408–10. doi: 10.1016/j.ajog.2020.12.1210
8. Harrison RF, He W, Fu S, Zhao H, Sun CC, Suidan RS, et al. National Patterns of Care and Fertility Outcomes for Reproductive-Aged Women With Endometrial Cancer or Atypical Hyperplasia. *Am J Obstet Gynecol* (2019) 221(5):474 e1–474.e11. doi: 10.1016/j.ajog.2019.05.029
9. Zhou R, Yang Y, Lu Q, Wang J, Miao Y, Wang S, et al. Prognostic Factors of Oncological and Reproductive Outcomes in Fertility-Sparing Treatment of Complex Atypical Hyperplasia and Low-Grade Endometrial Cancer Using Oral Progestin in Chinese Patients. *Gynecol Oncol* (2015) 139(3):424–8. doi: 10.1016/j.ygyno.2015.09.078
10. Alpha Scientists in Reproductive, M and E.S.I.G.o. Embryology. The Istanbul Consensus Workshop on Embryo Assessment: Proceedings of an Expert Meeting. *Hum Reprod* (2011) 26(6):1270–83. doi: 10.1016/j.rbmo.2011.02.001
11. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst Score Affects Implantation and Pregnancy Outcome: Towards a Single Blastocyst Transfer. *Fertil Steril* (2000) 73(6):1155–8. doi: 10.1016/S0015-0282(00)00518-5
12. Zheng X, Chen Y, Yan J, Wu Y, Zhuang X, Lin S, et al. Effect of Repeated Cryopreservation on Human Embryo Developmental Potential. *Reprod BioMed Online* (2017) 35(6):627–32. doi: 10.1016/j.rbmo.2017.08.016
13. Inoue O, Hamatani T, Susumu N, Yamagami W, Ogawa S, Takemoto T, et al. Factors Affecting Pregnancy Outcomes in Young Women Treated With Fertility-Preserving Therapy for Well-Differentiated Endometrial Cancer or Atypical Endometrial Hyperplasia. *Reprod Biol Endocrinol* (2016) 14:2. doi: 10.1186/s12958-015-0136-7
14. Yamazawa K, Hirai M, Fujito A, Nishi H, Terauchi F, Ishikura H, et al. Fertility-Preserving Treatment With Progestin, and Pathological Criteria to Predict Responses, in Young Women With Endometrial Cancer. *Hum Reprod* (2007) 22(7):1953–8. doi: 10.1093/humrep/dem088
15. Fujimoto A, Ichinose M, Harada M, Hirata T, Osuga Y, Fujii T. The Outcome of Infertility Treatment in Patients Undergoing Assisted Reproductive Technology After Conservative Therapy for Endometrial Cancer. *J Assist Reprod Genet* (2014) 31(9):1189–94. doi: 10.1007/s10815-014-0297-x
16. Chae SH, Shim SH, Lee SJ, Lee JY, Kim SN, Kang SB. Pregnancy and Oncologic Outcomes After Fertility-Sparing Management for Early Stage Endometrioid Endometrial Cancer. *Int J Gynecol Cancer* (2019) 29(1):77–85. doi: 10.1136/ijgc-2018-000036
17. Ichinose M, Fujimoto A, Osuga Y, Minaguchi T, Kawana K, Yano T, et al. The Influence of Infertility Treatment on the Prognosis of Endometrial Cancer and Atypical Complex Endometrial Hyperplasia. *Int J Gynecol Cancer* (2013) 23(2):288–93. doi: 10.1097/IGC.0b013e31827c18a1
18. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer Statistics in China, 2015. *CA Cancer J Clin* (2016) 66(2):115–32. doi: 10.3322/caac.21338
19. Gallos ID, Yap J, Rajkhowa M, Luesley DM, Coomarasamy A, Gupta JK. Regression, Relapse, and Live Birth Rates With Fertility-Sparing Therapy for Endometrial Cancer and Atypical Complex Endometrial Hyperplasia: A Systematic Review and Metaanalysis. *Am J Obstet Gynecol* (2012) 207(4):266.e1–12. doi: 10.1016/j.ajog.2012.08.011

20. Chao AS, Chao A, Wang CJ, Lai CH, Wang HS. Obstetric Outcomes of Pregnancy After Conservative Treatment of Endometrial Cancer: Case Series and Literature Review. *Taiwan J Obstet Gynecol* (2011) 50(1):62–6. doi: 10.1016/j.tjog.2009.10.006
21. Fan Y, Li X, Wang J, Wang Y, Tian L, Wang J. Analysis of Pregnancy-Associated Factors After Fertility-Sparing Therapy in Young Women With Early Stage Endometrial Cancer or Atypical Endometrial Hyperplasia. *Reprod Biol Endocrinol* (2021) 19(1):118. doi: 10.1186/s12958-021-00808-y
22. Althuis MD, Moghissi KS, Westhoff CL, Scoccia B, Lamb EJ, Lubin JH, et al. Uterine Cancer After Use of Clomiphene Citrate to Induce Ovulation. *Am J Epidemiol* (2005) 161(7):607–15. doi: 10.1093/aje/kwi084
23. Silva Idos S, Wark PA, McCormack VA, Mayer D, Overton C, Little V, et al. Ovulation-Stimulation Drugs and Cancer Risks: A Long-Term Follow-Up of a British Cohort. *Br J Cancer* (2009) 100(11):1824–31. doi: 10.1038/sj.bjc.6605086
24. Azim A, Oktay K. Letrozole for Ovulation Induction and Fertility Preservation by Embryo Cryopreservation in Young Women With Endometrial Carcinoma. *Fertil Steril* (2007) 88(3):657–64. doi: 10.1016/j.fertnstert.2006.12.068
25. Liu X, Li T, Wang B, Xiao X, Liang X, Huang R. Mild Stimulation Protocol vs Conventional Controlled Ovarian Stimulation Protocol in Poor Ovarian Response Patients: A Prospective Randomized Controlled Trial. *Arch Gynecol Obstet* (2020) 301(5):1331–9. doi: 10.1007/s00404-020-05513-6
26. Kalogiannidis I, Agorastos T. Conservative Management of Young Patients With Endometrial Highly-Differentiated Adenocarcinoma. *J Obstet Gynaecol* (2011) 31(1):13–7. doi: 10.3109/01443615.2010.532249
27. Park JY, Seong SJ, Kim TJ, Kim JW, Kim SM, Bae DS, et al. Pregnancy Outcomes After Fertility-Sparing Management in Young Women With Early Endometrial Cancer. *Obstet Gynecol* (2013) 121(1):136–42. doi: 10.1097/AOG.0b013e31827a0643
28. Danforth DR. Endocrine and Paracrine Control of Oocyte Development. *Am J Obstet Gynecol* (1995) 172(2 Pt 2):747–52. doi: 10.1016/0002-9378(95)90148-5
29. Park JY, Kim DY, Kim JH, Kim YM, Kim KR, Kim YT, et al. Long-Term Oncologic Outcomes After Fertility-Sparing Management Using Oral Progestin for Young Women With Endometrial Cancer (KGOG 2002). *Eur J Cancer* (2013) 49(4):868–74. doi: 10.1016/j.ejca.2012.09.017
30. Signorelli M, Caspani G, Bonazzi C, Chiappa V, Perego P, Mangioni C. Fertility-Sparing Treatment in Young Women With Endometrial Cancer or Atypical Complex Hyperplasia: A Prospective Single-Institution Experience of 21 Cases. *BJOG* (2009) 116(1):114–8. doi: 10.1111/j.1471-0528.2008.02024.x

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Survival after laparoscopy versus laparotomy for apparent early-stage uterine clear cell carcinoma: Results of a large multicenter cohort study

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Objective: To compare the long-term survival between laparoscopic surgery and open surgery in patients with apparent early-stage uterine clear cell carcinoma (UCCC).

Patients and methods: 254 patients with apparent early-stage UCCC were reviewed. Comparisons were made between patients who underwent laparoscopic surgery versus those who underwent open surgery. Baseline data, clinicopathological data, and oncological outcomes were analyzed. 5-year disease-free survival (DFS) rate and 5-year overall survival (OS) rate were estimated and compared using the Kaplan-Meier method and the Log-rank test. The Cox proportional hazard regression model was employed to control the confounding factors.

Results: 147 patients underwent laparoscopic surgery, and 107 patients were managed by open surgery. No differences in terms of recurrence rate (laparoscopy versus laparotomy: 10.9% versus 12.9%, *P*=0.842) and recurrence pattern were observed. For patients who underwent open surgery and patients who underwent laparoscopic surgery, the 5-year DFS rates and 5-year OS rate were 75.8% (95% CI: 65.8%-83.2%) and 69.1% (95% CI: 58.8%-77.4%), 66.0% (95% CI: 57.1%-73.5%) and 60.8% (95% CI: 52.0%-68.5%), respectively. The Cox proportional hazards regression model shown that for apparent early-stage UCCC, the approach of surgical staging was not an independent predictor for survival (laparoscopy versus laparotomy: for DFS, aHR=1.06, 95% CI=0.64-1.75, *P*=0.826; for OS, aHR=1.10, 95% CI=0.72-1.68, *P*=0.671).

Conclusion: For apparent early-stage UCCC, in terms of oncological survival, laparoscopic surgery was as safe as open surgery.

KEYWORDS

uterine clear cell carcinoma, laparoscopy, surgical staging, overall survival, disease-free survival

Introduction

Generally, endometrial cancer (EC) can be broadly divided into type I tumors (approximately 80%) and type II tumors (approximately 20%) (1–3). Usually developing among the elderly, Type II EC has a hormone-independent pathogenesis and no identified precursor lesions (1, 3, 4). Including uterine serous carcinoma, uterine clear cell carcinoma (UCCC), and carcinosarcoma, type II EC typically has a worse prognosis when compared with type I EC (1–3). They are often present at advanced stages, have a high rate of extrauterine metastases, and are at high risk of recurrence after initial management (1, 2, 4).

For clinical early-stage EC, the primary management is surgical staging, at least including total hysterectomy, bilateral salpingo-oophorectomy, and the assessment of regional lymph nodes (1, 3, 4). Based on the results of two randomized prospective studies comparing minimally invasive surgery with traditional open surgery, the minimally invasive approach was recommended for early-stage EC by the European Society of Gynaecological Oncology, the European Society for Radiotherapy and Oncology, and the European Society of Pathology (5). Furthermore, pooled results of prospective studies and retrospective observational studies also support the employment of minimally invasive surgery for women with high-risk early-stage EC (including type II EC) (6–10). These studies concluded that when compared with those who were managed with open surgical staging, early-stage EC patients who were treated with minimally invasive surgery experienced similar survival, quicker recovery, and lower risk of perioperative complications (5–7, 9, 10). In 2018, however, two clinical studies reported that for women with early-stage cervical cancer, minimally invasive radical hysterectomy caused lower rates of disease-free survival (DFS) and overall survival (OS) than open radical hysterectomy (11, 12). Since then, the oncological safety of minimally invasive surgery for gynecologic malignancies has once again become a focus of attention in clinical studies.

UCCC accounts for less than 10% of all EC (1, 13, 14). Due to the rarity of UCCC, a large, powerful, and prospectively designed study regarding the management of UCCC is exceedingly difficult (14). Thus, the current data on the clinical practice of UCCC are usually from small and retrospective designed studies (5, 7, 13). In the aforementioned studies comparing minimally invasive surgery with traditional open surgery for high-risk endometrial cancer, the fraction of UCCC was fairly low (6, 7, 9, 10). Thus, the oncological safety of minimally invasive surgery for clinical early-stage UCCC needs further study.

Taken together, based on four Chinese high-volume teaching hospitals, we conducted this study to compare the risk of recurrence and death associated with minimally invasive surgery versus open surgery for clinical early-stage UCCC.

Patients and methods

Study design

With four Chinese high-volume centers involved, this was a retrospectively designed and multi-institutional cohort study. Due to the retrospective nature and it did not report any identifiable private data, ethical approval and written informed consent for participation were not required for this study in accordance with the local legislation and institutional requirements. This study was conducted following the Declaration of Helsinki (15).

Study cohort

Data of consecutive patients with histologically proven EC who underwent surgical staging at the four Chinese tertiary referral centers (Central Hospital of Enshi Tujia and Miao Autonomous Prefecture, West China Mianzhu Hospital, West China Second University Hospital, and the Second Affiliated Hospital of Chengdu Medical College) between January 1, 2011 and January 1, 2018 were reviewed. Patients were included in this study if they: (1) were between 18 and 75 years old, (2) had pathologically confirmed clear cell carcinoma, (3) had a clinical early-stage disease, (4) underwent comprehensive surgical

staging at the participating hospitals, at least including total hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy, and (5) were consecutively followed up at these hospitals. In the current study, the clinical early-stage disease was defined as follows: cancer clinically confined to the uterus, no clinical evidence of bulky lymph nodes, and no clinical evidence of extrauterine macroscopic lesions. After surgical staging, all included cases were staged using the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system for EC.

Patients were excluded from this study if they: (1) were non-surgically managed, (2) underwent neoadjuvant therapies, (3) had a suspected advanced disease, (4) had synchronous cancer (s), (5) had a history of malignancy of the female reproductive system, (6) had a preoperative American Society of Anesthesiologists (ASA) physical status score of larger than III, (7) underwent assessment for regional lymph nodes by sentinel lymph node mapping, or (8) were lost to follow-up.

Data collection

The collected data regarding clinicopathological characteristics were as follows: year of diagnosis, age at diagnosis, marital status at diagnosis, body mass index (BMI) at diagnosis, the preoperative ASA physical status score, the stage of disease (based on the 2009 FIGO staging system), the grade of tumor differentiation, the size of the primary tumor, whether there was lymphovascular space invasion (LVSI), and the result of peritoneal cytology.

The following data on treatment were collected: the approach of surgical staging (laparoscopy or laparotomy), the scope of regional lymphadenectomy (pelvic lymphadenectomy or combined pelvic and para-aortic lymphadenectomy), the protocol of postoperative adjuvant therapies (chemotherapy, radiation, or chemoradiation).

The following data regarding oncological outcomes were collected: the vital status of the patient, disease recurrence (site and date), date of death, and the cause of death. In this study, all included patients were followed up until death or January 1, 2022.

Outcomes of interest

In the current study, the 5-year DFS rate and the 5-year OS rate were the primary outcomes of interest. DFS was defined as the time between the date of surgical staging for UCCC and the date of documented disease recurrence or death contributed by UCCC. OS was defined as the time from the date of surgical staging for UCCC to the date of documented death caused by any cause.

In this study, the secondary outcomes of interest were the independent predictors for the long-term survival of women with clinical early-stage UCCC.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 25 (SPSS Inc., Chicago, IL, USA). The Kaplan-Meier survival curves were generated by Stata version 17 (Stata Corp., College Station, TX, USA).

Based on the type of surgical staging approach, the included cases were divided into the laparoscopy group and the laparotomy group. Data on the characteristics of the study cohort were reported using standard descriptive statistics. Comparisons were made between the two groups using the chi-squared test or Fisher exact test for categorical variables and the *t*-test or the Wilcoxon rank-sum test for continuous variables. The 5-year DFS rate and the 5-year OS rate of the two groups were estimated and compared using the Kaplan-Meier method and the log-rank test. Hazard ratio (HR) and 95% confidence interval (CI) were calculated. The Cox proportional hazard regression model was employed to control the confounding factors. Candidate variables that were with a *P* value of less than 0.05 on univariate analysis or that were considered clinically relevant were included in the Cox proportional hazard regression model.

In the study, A two-sided *P* value of less than 0.05 was considered statistically significant.

Results

Characteristics of the study cohort

A total of 7127 women with EC were diagnosed and managed at these four participating hospitals between January 1, 2011 and January 1, 2018. After excluding 6873 patients who were not eligible for this study, a total of 254 women with apparent early-stage UCCC were eventually included in the current study. Among them, 147 patients underwent surgical staging by laparoscopy and were included in the laparoscopy group, the remaining 107 women underwent surgical staging by open approach and were included in the laparotomy group. Figure 1 shows the process of case selection.

For the entire study cohort, the mean age at diagnosis was 65.5 years with a standard deviation of 6.57, and the median duration of follow-up was 52.0 months (range: 4.0-131). Among the entire study cohort, 76 (29.9%) patients were identified with advanced diseases after surgical staging, 122 (48.0%) patients had primary tumors of larger than 4 centimeters, 58 (22.8%) patients were identified with LVSI,

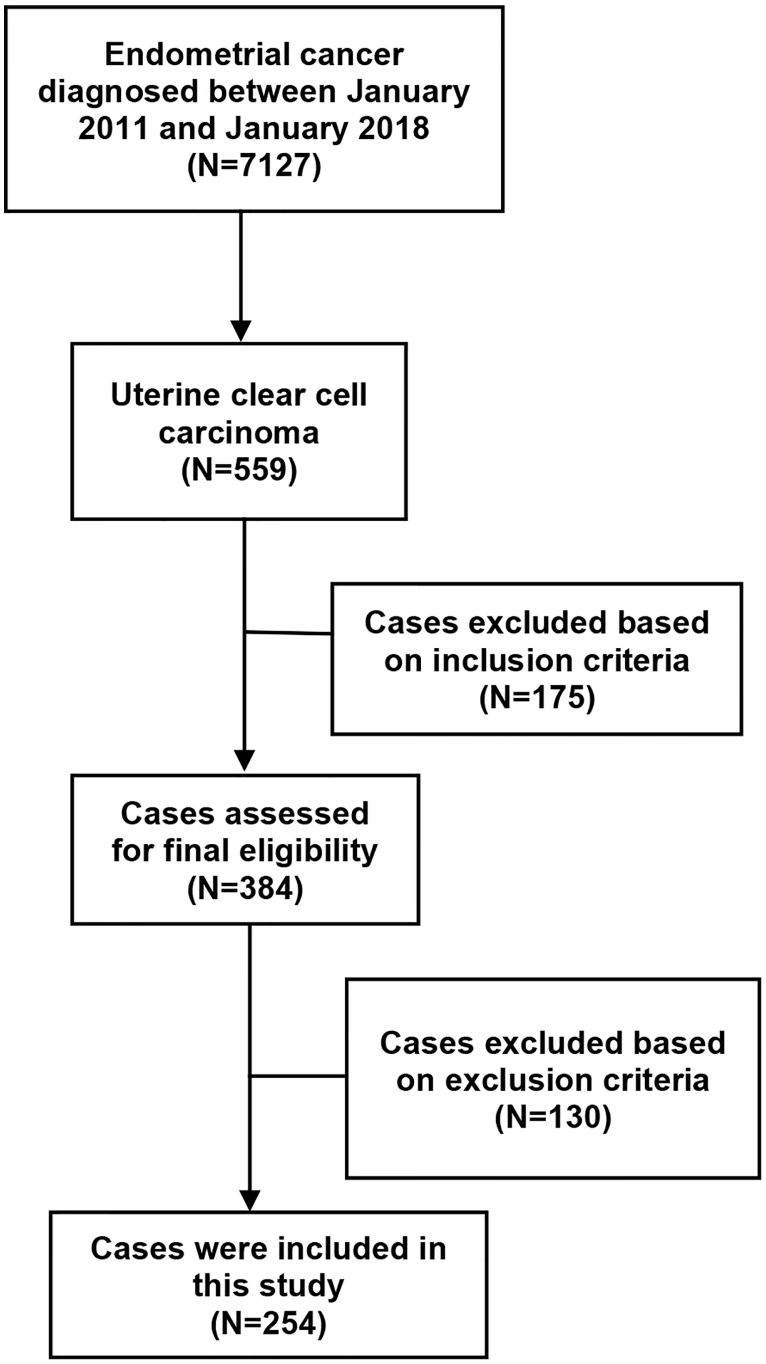

FIGURE 1
The flowchart of case selection.

36 (14.2%) patients had positive peritoneal cytology, and only 87 (34.3%) patients did not undergo any form of postoperative adjuvant therapy. In terms of the surgical-pathological stage of the disease, there was no statistical difference between the two groups (P=0.158).

Table 1 shows the comparisons of the characteristics of the two groups. Generally, there was good comparability between the laparoscopy group and the laparotomy group in terms of the baseline characteristics, the clinicopathologic data, and the treatment-related variables.

TABLE 1 Characteristics of the study cohort[a].

	Overall (N=254)	The laparoscopy group (N=147)	The laparotomy group (N=107)	*P*
Years of diagnosis				0.373
2011-2014	113 (44.5%)	69 (46.9%)	44 (41.1%)	
2015-2018	141 (55.5%)	78 (53.1%)	63 (58.9%)	
Age at diagnosis	65.5 ± 6.57	65.7 ± 6.46	65.3 ± 6.74	0.622
Duration of follow-up	52.0 (4.00, 131)	53.0 (4.00, 131)	49.0 (4.00, 127)	0.838
Marital status				0.612
Married	130 (51.2%)	73 (49.7%)	57 (53.3%)	
Single[b]	124 (48.8%)	74 (50.3%)	50 (46.7%)	
Body Mass Index[c]	21.2 ± 4.57	21.1 ± 4.30	21.3 ± 4.94	0.733
ASA physical status score				0.422
I/II	168 (66.1%)	94 (63.9%)	74 (69.2%)	
III	86 (33.9%)	53 (36.1%)	33 (30.8%)	
2009 FIGO stage				0.158
I	153 (60.2%)	82 (55.8%)	71 (66.4%)	
II	25 (9.8%)	13 (8.8%)	12 (11.2%)	
III	57 (22.4%)	38 (25.9%)	19 (17.8%)	
IV	19 (7.5%)	14 (9.5%)	5 (4.7%)	
Grade				0.687
Poorly differentiated	170 (66.9%)	100 (68.0%)	70 (65.4%)	
Undifferentiated	84 (33.1%)	47 (32.0%)	37 (34.6%)	
Tumor size				0.309
< 4 cm	132 (52.0%)	72 (49.0%)	60 (56.1%)	
≥ 4 cm	122 (48.0%)	75 (51.0%)	47 (43.9%)	
LVSI				0.762
No	196 (77.2%)	112 (76.2%)	84 (78.5%)	
Yes	58 (22.8%)	35 (23.8%)	23 (21.5%)	
Peritoneal cytology				0.147
Negative	218 (85.8%)	122 (83.0%)	96 (89.7%)	
Positive	36 (14.2%)	25 (17.0%)	11 (10.3%)	
Lymphadenectomy				0.429
Pelvic	162 (63.8%)	97 (66.0%)	65 (60.7%)	
Pelvic and para-aortic	92 (36.2%)	50 (34.0%)	42 (39.3%)	
Adjuvant therapy				0.961
CT or RT	96 (37.8%)	56 (38.1%)	40 (37.4%)	
CT plus RT	71 (28.0%)	40 (27.2%)	31 (29.0%)	
No	87 (34.3%)	51 (34.7%)	36 (33.6%)	

[a]Values are presented as mean ± standard deviation, median (minimum–maximum), or as number (percentage).
[b]Including never married, widowed, divorced, separated.
[c]Calculated as weight in kilograms divided by the square of height in meters.
ASA, American Society of Anesthesiologists; CT, Chemotherapy; FIGO, the International Federation of Gynecology and Obstetrics; LVSI, Lymphovascular Space Invasion; RT, Radiotherapy.

Rates and patterns of recurrence

By January 1, 2022, 29 recurrences of UCCC were identified, and the rate of recurrence was 11.4% among the entire study cohort.

13 of the 107 patients (12.1%) in the laparotomy group had disease recurrence, and 16 cases of UCCC recurrence (10.9%) were identified in the laparoscopy group. In terms of the rate of disease recurrence, there was no statistical difference observed between the two groups (*P*=0.842). As for the patterns of disease recurrence, the most four common sites of recurrence were the abdomen (2.8%), the pelvis (2.4%), the lung (2.4%), and the vagina (1.6%). Also, there was no statistical difference observed between the two groups in terms of the patterns of disease recurrence. Table 2 presents the rates and the patterns of recurrence by laparoscopic surgery versus laparotomy.

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TABLE 2 Rates and patterns of disease recurrence[a].

	Overall (N=254)	The laparoscopy group (N=147)	The laparotomy group (N=107)	*P*
Recurrence				0.842
Yes	29 (11.4%)	16 (10.9%)	13 (12.1%)	
No	225 (88.6%)	131 (89.1%)	94 (87.9%)	
Site of recurrence				
Vagina	4 (1.6%)	2 (1.4%)	2 (1.9%)	> 0.999
Pelvis	6 (2.4%)	3 (2.0%)	3 (2.8%)	0.699
Abdomen	7 (2.8%)	5 (3.4%)	2 (1.9%)	0.702
Nodal	2 (0.8%)	1 (0.7%)	1 (0.9%)	> 0.999
Lung	6 (2.4%)	3 (2.0%)	3 (2.8%)	0.699
Bone	2 (0.8%)	1 (0.7%)	1 (0.9%)	> 0.999
Multiple	2 (0.8%)	1 (0.7%)	1 (0.9%)	> 0.999

[a]Values are presented as number (percentage).

Survival outcomes

For the patients who underwent surgical staging by open surgery and the patients who underwent laparoscopic surgery, the 5-year DFS rates by the Kaplan-Meier method were 75.8% (95% CI: 65.8%-83.2%) and 66.0% (95% CI: 57.1%-73.5%), respectively. For patients of apparent early-stage UCCC, surgical staging by laparoscopy was not associated with worse DFS when compared with traditional laparotomy (HR: 1.34, 95% CI: 0.85-2.11, *P*=0.213).

For the laparotomy group, the 5-year OS rate by the Kaplan-Meier method was 69.1% (95% CI: 58.8%-77.4%). Similarly, the 5-year OS rate for patients in the laparoscopy group was 60.8% (95% CI: 52.0%-68.5%). The comparison made by the Log-rank test indicated that for women with clinical early-stage UCCC, compared with open surgery, surgical staging by laparoscopy did not increase the risk of all-cause death (HR: 1.19, 95% CI: 0.81-1.76, *P*=0.372).

Figure 2 shows the Kaplan-Meier survival curves of the study cohort by laparoscopy versus laparotomy, Figure 2A for disease-free survival and Figure 2B for overall survival.

Univariate analyses

Using the log-rank test, we found that for women with apparent early-stage UCCC, age at diagnosis (≥ 65 years versus < 65 years: for DFS, HR=1.66, 95% CI=1.07-2.77, *P*=0.031; for OS, HR=1.84, 95% CI=1.18-2.88, *P*=0.007), BMI at diagnosis (≥ 24 kg/m^2 versus < 24 kg/m^2: for DFS, HR=1.52, 95% CI=1.17-2.29, *P*=0.019; for OS, HR=1.46, 95% CI=1.09-2.14, *P*=0.037), the preoperative ASA physical status score (III versus I/II: for DFS, HR=3.51, 95% CI=2.14-4.99, *P*=0.000; for OS, HR=3.25, 95% CI=1.89-5.28, *P*=0.000), the 2009 FIGO stage of the disease (III/IV versus I/II: for DFS, HR=6.34, 95% CI=4.43-8.30, *P*=0.000; for OS, HR=5.95, 95% CI=3.38-10.01, *P*=0.007), the tumor size (≥ 4 cm versus < 4 cm: for DFS, HR=2.05, 95% CI=1.29-3.24, *P*=0.002; for OS, HR=1.80, 95% CI=1.22-2.64, *P*=0.003), LVSI (Yes versus No: for DFS, HR=1.54, 95% CI=1.04-2.70, *P*=0.013; for OS, HR=1.43, 95% CI=1.17-2.36, *P*=0.015), and postoperative adjuvant therapy (chemotherapy/radiotherapy versus No: for DFS, HR=0.68, 95% CI=0.27-0.89, *P*=0.007; for OS, HR=0.62, 95% CI=0.23-0.92, *P*=0.008; combined chemotherapy and radiotherapy versus No: for DFS, HR=0.45,

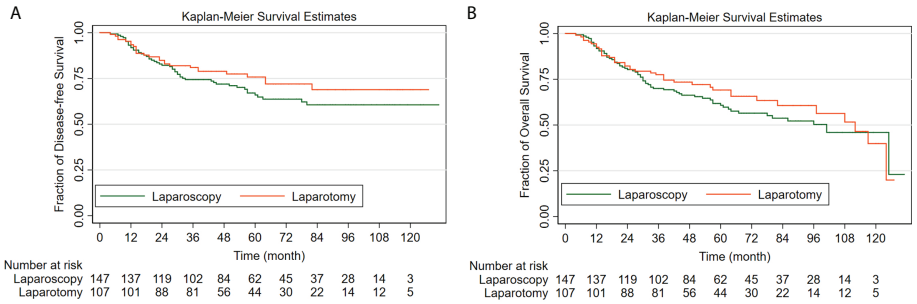

FIGURE 2
The Kaplan-Meier survival curves of the study cohort by laparoscopy versus laparotomy **(A)** for disease-free survival; **(B)** for overall survival.

95% CI=0.29-0.87, *P*=0.004; for OS, HR=0.37, 95% CI=0.25-0.73, *P*=0.000) were associated with the prognosis.

Table 3 shows the results of the univariate analyses.

Cox proportional hazards regression analyses

Variables that have potential clinical relevance or that showed a univariate relationship ($P < 0.05$) with survival were included in the multivariate Cox proportional hazards regression model, they were as follows: age at diagnosis, BMI at diagnosis, the preoperative ASA physical status score, the 2009 FIGO stage of the disease, the tumor size, the status of LVSI, the approach of surgical staging, and postoperative adjuvant therapy.

The Cox proportional hazards regression model showed that for apparent early-stage UCCC, the approach of surgical staging was not an independent predictor for long-term survival (laparoscopy versus laparotomy: for DFS, aHR=1.06, 95% CI=0.64-1.75, *P*=0.826; for OS, aHR=1.10, 95% CI=0.72-1.68, *P*=0.671).

TABLE 3 Univariate analyses of survival for apparent early-stage uterine clear cell carcinoma.

	OS			DFS		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age at diagnosis						
< 65 years	1			1		
≥ 65 years	1.84	1.18-2.88	0.007	1.66	1.07-2.77	0.031
Marital status						
Married	1			1		
Single	1.08	0.68-1.72	0.736	1.13	0.65-1.96	0.664
BMI at diagnosis						
< 24 kg/m^2	1			1		
≥ 24 kg/m^2	1.46	1.09-2.14	0.037	1.52	1.17-2.29	0.019
ASA physical status score						
I/II	1			1		
III	3.25	1.89-5.28	0.000	3.51	2.14-4.99	0.000
2009 FIGO stage						
I/II	1			1		
III/IV	5.95	3.38-10.01	0.007	6.34	4.43-8.30	0.000
Grade						
Poorly differentiated	1			1		
Undifferentiated	1.12	0.74-1.68	0.600	1.34	0.81-2.21	0.258
Tumor size						
< 4 cm	1			1		
≥ 4 cm	1.80	1.22-2.64	0.003	2.05	1.29-3.24	0.002
LVSI						
No	1			1		
Yes	1.43	1.17-2.36	0.015	1.54	1.04-2.70	0.013
Peritoneal cytology						
Negative	1			1		
Positive	1.12	0.71-1.77	0.622	1.11	0.66-1.89	0.692
Lymphadenectomy						
Pelvic	1			1		
Pelvic plus para-aortic	1.07	0.72-1.59	0.744	0.99	0.62-1.59	0.987
Adjuvant therapy						
No	1			1		
CT or RT	0.62	0.23-0.92	0.008	0.68	0.27-0.89	0.007
CT plus RT	0.37	0.25-0.73	0.000	0.45	0.29-0.87	0.004

ASA, American Society of Anesthesiologists; BMI, Body Mass Index; CI, Confidence Interval; CT, chemotherapy; DFS, Disease-free Survival; FIGO, the International Federation of Gynecology and Obstetrics; HR, Hazard Ratio; LVSI, Lymphovascular Space Invasion; OS, Overall Survival; RT, radiotherapy.

The Cox proportional hazards regression model also showed that for apparent early-stage UCCC, age at diagnosis (≥ 65 years versus < 65 years: for DFS, aHR=1.51, 95% CI=1.07-2.24, *P*=0.003; for OS, aHR=1.39, 95% CI=1.14-2.51, *P*=0.026), the preoperative ASA physical status score (III versus I/II: for DFS, aHR=1.98, 95% CI=1.14-3.27, *P*=0.021; for OS, aHR=2.02, 95% CI=1.10-3.09, *P*=0.016), the 2009 FIGO stage of the disease (III/IV versus I/II: for DFS, aHR=6.98, 95% CI=3.57-13.12, *P*=0.000; for OS, aHR=6.76, 95% CI=2.49-10.68, *P*=0.000), LVSI (Yes versus No: for DFS, aHR=2.14, 95% CI=1.11-2.57, *P*=0.010; for OS, aHR=2.09, 95% CI=1.27-2.92, *P*=0.001), and postoperative adjuvant therapy (chemotherapy/radiotherapy versus No: for DFS, aHR=0.64, 95% CI=0.28-0.97, *P*=0.012; for OS, aHR=0.67, 95% CI=0.32-0.89, *P*=0.033; combined chemotherapy and radiotherapy versus No: for DFS, aHR=0.49, 95% CI=0.23-0.78, *P*=0.018; for OS, aHR=0.55, 95% CI=0.27-0.90, *P*=0.025) were independently associated with the survival.

Table 4 shows the results of the multivariate Cox proportional hazards regression analyses.

Discussion

By reviewing the data of 254 patients from four Chinese high-volume centers, the current study showed that for apparent early-stage UCCC, when compared with patients who underwent open surgical staging, patients who underwent surgical staging by laparoscopy experienced similar oncological outcomes.

The research topic on the employment of minimally invasive surgery among women with EC is not new. The Gynecologic Oncology Group (GOG) LAP2 study was a prospective randomized controlled clinical study with the purpose to study the feasibility and safety of minimally invasive surgery for clinical early-stage uterine cancer (16, 17). With 2616 patients included, the GOG LAP2 study preliminarily concluded that laparoscopic surgery for clinical early-stage EC was feasible and safe in terms of short-term outcomes and resulted in a lower risk of perioperative complications (16). In 2012, the GOG LAP2 reported its findings regarding oncological outcomes (17). It reported that the 3-year recurrence rates among patients who underwent laparoscopy and patients who underwent open surgery were 11.4% and 10.2%,

TABLE 4 Multivariate analyses of survival for apparent early-stage uterine clear cell carcinoma.

	DFS			OS		
	aHR	**95% CI**	***P***	**aHR**	**95% CI**	***P***
Age at diagnosis						
< 65 years	1			1		
≥ 65 years	1.51	1.07-2.24	0.003	1.39	1.14-2.51	0.026
BMI at diagnosis						
< 24 kg/m^2	1			1		
≥ 24 kg/m^2	1.72	0.91-3.83	0.077	1.67	0.88-3.55	0.109
ASA physical status score						
I/II	1			1		
III	1.98	1.14-3.27	0.021	2.02	1.10-3.09	0.016
2009 FIGO stage						
I/II	1			1		
III/IV	6.98	3.57-13.12	0.000	6.76	2.49-10.68	0.000
Tumor size						
< 4 cm	1			1		
≥ 4 cm	1.40	0.82-2.39	0.218	1.20	0.76-1.91	0.442
LVSI						
No	1			1		
Yes	2.14	1.11-2.57	0.010	2.09	1.27-2.92	0.001
Surgical approach						
Laparotomy	1			1		
Laparoscopy	1.06	0.64-1.75	0.826	1.10	0.72-1.68	0.671
Adjuvant therapy						
No	1			1		
CT or RT	0.64	0.28-0.97	0.012	0.67	0.32-0.89	0.033
CT plus RT	0.49	0.23-0.78	0.018	0.55	0.27-0.90	0.025

aHR, adjusted Hazard Ratio; ASA, American Society of Anesthesiologists; BMI, Body Mass Index; CI, Confidence Interval; CT, chemotherapy; DFS, Disease-free Survival; FIGO, the International Federation of Gynecology and Obstetrics; LVSI, Lymphovascular Space Invasion; OS, Overall Survival; RT, radiotherapy.

respectively (17). The difference in recurrence rate by laparoscopy versus laparotomy was 1.14% (90% lower bound, -1.28; 95% upper bound, 4.0) (17). The Laparoscopic Approach to Cancer of the Endometrium (LACE) study, a multinational randomized equivalence study, also reported that for clinical early-stage uterine cancer, the employment of total laparoscopic hysterectomy compared with total open abdominal hysterectomy resulted in equivalent 4.5-year DFS rate (open surgery versus laparoscopic surgery: 81.6% versus 81.0%) and no difference in 4.5-year OS rate (open surgery versus laparoscopic surgery: 92.4% versus 92.0%) (18). Based on the evidence from the GOG LAP2 study, the LACE study, and other studies regarding this topic, minimally invasive surgery is recommended by many clinical practice guidelines as the preferred surgical approach for early-stage EC (5, 19–23).

However, one should note that in the aforementioned studies, the proportion of type II EC (including UCCC) was fairly low (16–18). Due to the rarity, prospectively designed clinical study regarding type II EC is difficult. So far, some retrospectively designed studies about the oncological safety of minimally invasive surgery for type II EC have been published. Including 295 patients from four Chinese teaching hospitals, the study conducted by Xu et al. found that for apparent early-stage uterine serous carcinoma, the approach of surgical staging was not an independent prognostic factor for oncological outcomes (laparoscopy versus open surgery: for DFS, aHR=1.16, 95% CI=0.63-2.12, *P*=0.636; for OS, aHR=1.11, 95% CI=0.52-2.38, *P*=0.794) (24). Comparing DFS between minimally invasive surgery and laparotomic surgery in patients with high-risk EC, the study conducted by Segarra-Vidal et al. included 626 patients (25). Among them, 468 women had type II EC (25). They found that there was no difference in 5-year DFS rate between the open surgery group (53.4%, 95% CI: 45.6%-60.5%) and the laparoscopy group (54.6%, 95% CI: 46.6%-61.8%) (25). They concluded that minimally invasive surgery was not associated with the deterioration of survival among patients with high-risk EC (25). Furthermore, the subgroup analysis showed that the employment of a uterine manipulator during laparoscopy surgery did not worsen the DFS (HR=1.01, 95% CI=0.65-1.58, *P*=0.960), the OS (HR=1.18, 95% CI=0.71-1.96, *P*=0.530), and the recurrence rate (HR=1.12, 95% CI=0.67-1.87, *P*=0.660) among patients with high-risk EC (25). To compare surgical and survival outcomes in patients with early-stage uterine carcinosarcoma managed by laparotomic surgery versus minimally invasive surgery, the study conducted by Corrado et al. included 170 patients and concluded that for women with early-stage uterine carcinosarcoma, there was no difference of oncologic outcome between the two approaches (26). The findings of our study were consistent with that of the aforementioned studies.

Our study also found that some of the classic risk factors that can be applied to predict the prognosis of type I EC were also useful for apparent early-stage UCCC. These risk factors were as follows: age at diagnosis, the preoperative ASA physical status score, the stage of cancer, and the status of LVSI (1, 3, 4, 27–31). However, unlike for low-risk early-stage type I EC, postoperative adjuvant therapy was beneficial to apparent early-stage UCCC (32–35). This was because when compared with patients of clinical early-stage type I EC, patients with clinical early-stage type II EC are at higher risk of extrauterine metastases and disease recurrence (1–4). In our study, nearly one-third of clinical early-stage UCCC patients were eventually confirmed to have extrauterine metastases after surgery. Among them, the most common site of extrauterine metastases was regional lymph nodes. These findings were consistent with that of previously published studies (34–37). The postoperative adjuvant therapy can reduce the risk of disease recurrence among patients of high-risk EC (including UCCC) (33–35).

Our study included 254 patients with apparent early-stage UCCC, this was a large sample in consideration of the rarity of UCCC. Also, almost all included patients in our study underwent guidelines-based management and a long-term follow-up, this can reduce the effect of confounding factors (such as protocol of treatment) on patients' prognosis as much as possible and enable us to identify the outcomes of interest. However, this study still suffers from some limitations. First, due to the retrospective nature of the study design, this study was at risk of inevitable biases, such as information bias, selection bias, et al. To reduce the possibility of these biases as much as possible, we pre-set inclusion and exclusion criteria and strictly followed them to screen eligible patients, and excluded those cases that lack relevant data. Second, because robotic-assisted minimally invasive surgery for EC is relatively new in these participating institutions, our study failed to explore its impact on oncological outcomes of apparent early-stage UCCC. However, according to the findings of the study conducted by Segarra-Vidal et al, the robotic-assisted minimally invasive surgery was oncological safe as open surgery for type II EC (25). Third, because of the limited resources, the pathological diagnoses of UCCC were not reviewed again by experts in pathology. The last, some variables of clinical significance, such as the protocol and the number of cycles of postoperative adjuvant therapy, comorbidities, etc. were not included in the statistical analysis, mainly due to the difficulty in obtaining these data. This was potentially representing a bias in our analysis.

Conclusion

In summary, there was no difference in recurrence rate, recurrence pattern, DFS, and the risk of all-cause death when comparing laparoscopic surgery and open surgical staging among women with apparent early-stage UCCC. Although our study has some limitations, the findings of our study support the assertion that surgical staging by laparoscopy did not compromise the survival of women with apparent early-stage UCCC.

Data availability statement

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

Ethics statement

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization: CS, LR, and YT. Methodology: LQ, XG, Hui Xu, CH, L-LZ, and YT. Data collection: all authors. Project administration: CS, LR, and YT. Supervision: YT. Writing - original draft: CS, LR, and YT. Writing - review and editing: all authors. 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.

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References

1. Lu KH, Broaddus RR. Endometrial cancer. *N Engl J Med* (2020) 383 (21):2053–64. doi: 10.1056/NEJMra1514010

2. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7

3. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. *Lancet (London England)* (2022) 399(10333):1412–28. doi: 10.1016/S0140-6736(22)00323-3

4. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. *Lancet (London England)* (2016) 387(10023):1094–108. doi: 10.1016/S0140-6736(15)00130-0

5. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J gynecol Cancer Off J Int Gynecol Cancer Soc* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

6. Vardar MA, Gulec UK, Guzel AB, Gumurdulu D, Khatib G, Seydaoglu G. Laparoscopic surgery for low, intermediate and high-risk endometrial cancer. *J gynecol Oncol* (2019) 30(2):e24. doi: 10.3802/jgo.2019.30.e24

7. Favero G, Anton C, Le X, Silva ESA, Dogan NU, Pfiffer T, et al. Oncologic safety of laparoscopy in the surgical treatment of type II endometrial cancer. *Int J gynecol Cancer Off J Int Gynecol Cancer Soc* (2016) 26(9):1673–8. doi: 10.1097/IGC.0000000000000803

8. Bennich G, Rudnicki M, Lassen PD. Laparoscopic surgery for early endometrial cancer. *Acta Obstet Gynecol Scand* (2016) 95(8):894–900. doi: 10.1111/aogs.12908

9. Monterossi G, Ghezzi F, Vizza E, Zannoni GF, Uccella S, Corrado G, et al. Minimally invasive approach in type II endometrial cancer: Is it wise and safe? *J Minim Invasive Gynecol* (2017) 24(3):438–45. doi: 10.1016/j.jmig.2016.12.022

10. Koskas M, Jozwiak M, Fournier M, Vergote I, Trum H, Lok C, et al. Long-term oncological safety of minimally invasive surgery in high-risk endometrial cancer. *Eur J Cancer (Oxford Engl 1990)* (2016) 65:185–91. doi: 10.1016/j.ejca.2016.07.001

11. Melamed A, Margul DJ, Chen L, Keating NL, Del Carmen MG, Yang J, et al. Survival after minimally invasive radical hysterectomy for early-stage cervical cancer. *N Engl J Med* (2018) 379(20):1905–14. doi: 10.1056/NEJMoa1804923

12. Ramirez PT, Frumovitz M, Pareja R, Lopez A, Vieira M, Ribeiro R, et al. Minimally invasive versus abdominal radical hysterectomy for cervical cancer. *N Engl J Med* (2018) 379(20):1895–904. doi: 10.1056/NEJMoa1806395

13. Abeler VM, Kjørstad KE. Clear cell carcinoma of the endometrium: A histopathological and clinical study of 97 cases. *Gynecol Oncol* (1991) 40(3):207–17. doi: 10.1016/0090-8258(90)90279-t

14. Olawaiye AB, Leath CA3rd. Contemporary management of uterine clear cell carcinoma: A society of gynecologic oncology (SGO) review and recommendation. *Gynecol Oncol* (2019) 155(2):365–73. doi: 10.1016/j.ygyno.2019.08.031

15. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* (2013) 310(20):2191–4. doi: 10.1001/jama.2013.281053

16. Walker JL, Piedmonte MR, Spirtos NM, Eisenkop SM, Schlaerth JB, Mannel RS, et al. Laparoscopy compared with laparotomy for comprehensive surgical staging of uterine cancer: Gynecologic oncology group study LAP2. *J Clin Oncol Off J Am Soc Clin Oncol* (2009) 27(32):5331–6. doi: 10.1200/JCO.2009.22.3248

17. Walker JL, Piedmonte MR, Spirtos NM, Eisenkop SM, Schlaerth JB, Mannel RS, et al. Recurrence and survival after random assignment to laparoscopy versus laparotomy for comprehensive surgical staging of uterine cancer: Gynecologic oncology group LAP2 study. *J Clin Oncol Off J Am Soc Clin Oncol* (2012) 30 (7):695–700. doi: 10.1200/JCO.2011.38.8645

18. Janda M, Gebski V, Davies LC, Forder P, Brand A, Hogg R, et al. Effect of total laparoscopic hysterectomy vs total abdominal hysterectomy on disease-free survival among women with stage I endometrial cancer: A randomized clinical trial. *Jama* (2017) 317(12):1224–33. doi: 10.1001/jama.2017.2068

19. Koh WJ, Abu-Rustum NR, Bean S, Bradley K, Campos SM, Cho KR, et al. Uterine neoplasms, version 1.2018, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Netw JNCCN* (2018) 16(2):170–99. doi: 10.6004/jnccn.2018.0006

20. Abu-Rustum NR, Yashar CM, Bradley K, Campos SM, Chino J, Chon HS, et al. NCCN guidelines® insights: Uterine neoplasms, version 3.2021. *J Natl Compr Cancer Netw JNCCN* (2021) 19(8):888–95. doi: 10.6004/jnccn.2021.0038

21. Santaballa A, Matías-Guiu X, Redondo A, Carballo N, Gil M, Gómez C, et al. SEOM clinical guidelines for endometrial cancer (2017). *Clin Trans Oncol Off Publ Fed Spa Oncol Soc Natl Cancer Inst Mex* (2018) 20(1):29–37. doi: 10.1007/s12094-017-1809-9

22. Ribeiro R, Fontes Cintra G, Barrozo A, Tieko Tsunoda A, Pupo Nogueira A, Andreazza Laporte G, et al. Brazilian Society of surgical oncology guidelines for surgical treatment of endometrial cancer in regions with limited resources. *J Surg Oncol* (2020) 121(5):730–42. doi: 10.1002/jso.25797

23. Barretina-Ginesta MP, Quindós M, Alarcón JD, Esteban C, Gaba L, Gómez C, et al. SEOM-GEICO clinical guidelines on endometrial cancer (2021). *Clin*

Trans Oncol Off Publ Fed Spa Oncol Soc Natl Cancer Inst Mex (2022) 24(4):625–34. doi: 10.1007/s12094-022-02799-7

24. Xu Y, Shen J, Zhang Q, He Y, Chen C, Tian Y. Oncologic safety of laparoscopic surgery for women with apparent early-stage uterine serous carcinoma: A multi-institutional retrospective cohort study. *Int J Gynaecol Obstet: Off Organ Int Fed Gynaecol Obstet* (2022) 158(1):162–71. doi: 10.1002/ijgo.13942

25. Segarra-Vidal B, Dinoi G, Zorrilla-Vaca A, Mariani A, Student V, Garcia NA, et al. Minimally invasive compared with open hysterectomy in high-risk endometrial cancer. *Obstet Gynecol* (2021) 138(6):828–37. doi: 10.1097/AOG.0000000000004606

26. Corrado G, Ciccarone F, Cosentino F, Legge F, Rosati A, Arcieri M, et al. Role of minimally invasive surgery versus open approach in patients with early-stage uterine carcinosarcomas: a retrospective multicentric study. *J Cancer Res Clin Oncol* (2021) 147(3):845–52. doi: 10.1007/s00432-020-03372-x

27. Koual M, Ngo C, Girault A, Lécuru F, Bats AS. Endometrial cancer in the elderly: does age influence surgical treatments, outcomes, and prognosis? *Menopause (New York NY)* (2018) 25(9):968–76. doi: 10.1097/GME.0000000000001119

28. Hag-Yahia N, Gemer O, Eitan R, Raban O, Vaknin Z, Levy T, et al. Age is an independent predictor of outcome in endometrial cancer patients: An Israeli gynecology oncology group cohort study. *Acta Obstet Gynecol Scand* (2021) 100 (3):444–52. doi: 10.1111/aogs.14015

29. Bosse T, Peters EE, Creutzberg CL, Jürgenliemk-Schulz IM, Jobsen JJ, Mens JW, et al. Substantial lymph-vascular space invasion (LVSI) is a significant risk factor for recurrence in endometrial cancer–a pooled analysis of PORTEC 1 and 2 trials. *Eur J Cancer (Oxford Engl 1990)* (2015) 51(13):1742–50. doi: 10.1016/j.ejca.2015.05.015

30. Ørtoft G, Lausten-Thomsen L, Høgdall C, Hansen ES, Dueholm M. Lymph-vascular space invasion (LVSI) as a strong and independent predictor for non-locoregional recurrences in endometrial cancer: a Danish gynecological cancer group study. *J Gynecol Oncol* (2019) 30(5):e84. doi: 10.3802/jgo.2019.30.e84

31. Tortorella L, Restaino S, Zannoni GF, Vizzielli G, Chiantera V, Cappuccio S, et al. Substantial lymph-vascular space invasion (LVSI) as predictor of distant relapse and poor prognosis in low-risk early-stage endometrial cancer. *J Gynecol Oncol* (2021) 32(2):e11. doi: 10.3802/jgo.2021.32.e11

32. Deleon MC, Ammakkanavar NR, Matei D. Adjuvant therapy for endometrial cancer. *J Gynecol Oncol* (2014) 25(2):136–47. doi: 10.3802/jgo.2014.25.2.136

33. Michalak M, Warenczak Florczak Z, Staszewska-Nowak A, Roszak A. Adjuvant therapy for early endometrial cancer - who benefits the most from a radiation therapy? *Ginekologia Polska* (2020) 91(1):6–12. doi: 10.5603/GP.2020.0003

34. Xu KM, Gill BS, Balasubramani GK, Sukumvanich P, Kelley JL, Beriwal S. Utilization and role of adjuvant radiotherapy and chemotherapy for uterine clear cell carcinoma: A national cancer data base analysis. *Int J Gynecol Cancer Off J Int Gynecol Cancer Soc* (2016) 26(3):472–82. doi: 10.1097/IGC.0000000000000640

35. Hong JC, Foote J, Broadwater G, Gaillard S, Havrilesky LJ, Chino JP. Impact of chemotherapy and radiotherapy on management of early stage clear cell and papillary serous carcinoma of the uterus. *Int J Gynecol Cancer Off J Int Gynecol Cancer Soc* (2017) 27(4):720–9. doi: 10.1097/IGC.0000000000000926

36. Sarı ME, Meydanlı MM, Türkmen O, Cömert GK, Turan AT, Karalök A, et al. Prognostic factors and treatment outcomes in surgically-staged non-invasive uterine clear cell carcinoma: a Turkish gynecologic oncology group study. *J Gynecol Oncol* (2017) 28(4):e49. doi: 10.3802/jgo.2017.28.e49

37. Hu C, Mao XG, Xu Y, Xu H, Liu Y. Oncological safety of laparoscopic surgery for women with apparent early-stage uterine clear cell carcinoma: A multicenter retrospective cohort study. *J Minim Invasive Gynecol* (2022) 29 (8):968–75. doi: 10.1016/j.jmig.2022.04.013

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Outcomes of "sandwich" chemoradiotherapy compared with chemotherapy alone for the adjuvant treatment of FIGO stage III endometrial cancer

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Objective: To analyze and compare outcomes of adjuvant chemoradiotherapy in patients with International Federation of Gynecology and Obstetrics (FIGO) stage III endometrial cancer (EC) patients using the "Sandwich" sequence and chemotherapy (CT) alone.

Methods: From, 2005 to, 2019, we retrospectively reviewed 80 patients with FIGO stage III EC who received treatment at our institute. We analyzed 66 patients who had undergone complete surgical staging followed by adjuvant treatment with sandwich chemoradiotherapy (39 patients) and CT alone (27 patients). The 5-year overall survival (OS), progression-free survival (PFS), and disease-specific survival (DSS) were calculated using the Kaplan–Meier method. Additional prognostic factors were analyzed using Cox proportional hazards regression.

Results: Herein, the analysis was conducted using 66 patients with a median follow-up period of 50 and 85 months in the sandwich and CT-alone arms. Comparing the sandwich sequence and CT-alone groups, the 5-year OS and PFS were 87% *vs*. 70% ($p = 0.097$) and 77% *vs*. 65% ($p = 0.209$), respectively. The sandwich therapy conferred an improved 5-year DSS (92% *vs*. 70%, $p = 0.041$) and a lower local recurrence rate (0% *vs*. 11%, $p = 0.031$). In multivariable analyses, grade 3 histology and deep myometrial invasion were independent risk factors for 5-year OS and DSS. The sandwich sequence was a positive predictor for 5-year DSS (hazard ratio [HR] = 0.23, $p = 0.029$). The sandwich arm demonstrated higher acute hematologic toxicity than the CT-alone arm. CT dose delay/reduction and treatment completion rates were similar in both groups.

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Conclusion: For patients with stage III EC, postoperative sandwich chemoradiotherapy appears to offer a superior 5-year DSS and local control with tolerable toxicity when compared with CT alone.

KEYWORDS

endometrial neoplasms, FIGO stage III, adjuvant therapy, chemotherapy, radiotherapy, chemoradiotherapy

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy with a steadily growing incidence (1). Although most ECs are diagnosed early with a favorable prognosis, approximately 21% of cases are presented as locally advanced diseases (2). A complete staging operation remains the cornerstone of EC management (3). However, the optimal adjuvant therapy for locally advanced ECs is yet to be established.

Following the Gynecologic Oncology Group study (GOG-122), chemotherapy has been established as the mainstay of adjuvant treatment for advanced EC. The study reported a superior PFS and OS when comparing doxorubicin plus cisplatin to whole abdominal radiation (4). However, chemotherapy alone was also associated with a higher local recurrence rate of 20% (5). Recently, two randomized control trials compared different therapies in patients with high-risk EC. The PORTEC-3 trial reported an improved OS and failure-free survival particularly in patients with stage III EC receiving chemoradiotherapy when compared with radiotherapy (RT) alone (6). In the GOG-258 trial, the addition of pelvic irradiation to CT failed to significantly benefit relapse-free survival, while presenting a trend toward improved local control and more distant metastasis (5). Given the increased adverse events following chemoradiotherapy and the lack of evidence supporting its benefit, the role of RT warrants further investigation.

Several studies with large retrospective cohorts from the National Cancer Database (NCDB) have addressed outcomes of different chemoradiotherapy sequences (7–10). As an initial adjuvant modality, potential benefits of systemic CT include early treatment of occult micro-metastatic disease, reduced likelihood of CT delay secondary to RT-related toxicities, and avoiding the potential for RT-induced tumor vascular bed alteration known to impair chemotherapeutic drug delivery to malignant cells (11). Conversely, initial treatment with CT prior to pelvic irradiation may delay local therapy, compromise tolerance to RT toxicity, and potentially induce a negative impact on local recurrence (12).

The sandwich sequence, comprising 2 to 4 cycles of CT followed by irradiation and subsequent CT, has shown promising results in several phase II studies and retrospective cohorts (11–22). However, its efficacy has been inconsistent and was further limited by small study samples, as well as heterogeneous compositions of histology and staging across studies. Herein, our primary objective was to determine the clinical outcomes of sandwich chemoradiotherapy as an optimal adjuvant treatment for locally advanced ECs.

Materials and methods

Patient selection

The present study was a single-centered, retrospective review of female patients with stage III ECs treated between, 2005 and, 2019. Following the approval of the institutional review board, we reviewed a tumor registry to identify all patients with pathologically confirmed stage III EC receiving adjuvant therapy at the Taichung Veterans General Hospital. Pathological reports were reviewed and categorized in accordance with the International Federation of Gynecology and Obstetrics (FIGO) 2009 classification.

All enrolled patients had undergone a primary complete staging surgery comprising total hysterectomy (TH; either open or minimally invasive approach), bilateral salpingo-oophorectomy (BSO), bilateral pelvic lymph node dissection (BPLND), with or without para-aortic lymph node dissection (PALND), and omentectomy. Following surgical intervention, adjuvant therapy with either a "sandwich" chemoradiotherapy sequence or CT alone was performed. The exclusion criteria were as follows: patients with gross residual disease >1 cm after primary staging surgery, patients receiving CT or RT prior to surgery, patients with stage III disease established only upon positive peritoneal washings or synchronous ovarian and endometrial cancer, patients treated with palliative intent, and patients concurrently diagnosed with other cancers within 5 years before and after diagnosis of EC. In addition, we excluded patients with a histological diagnosis of carcinosarcoma,

undifferentiated and dedifferentiated carcinoma, and any other type of sarcoma.

Treatment and monitoring protocol

Adjuvant treatments were initiated within 3 weeks postsurgery. All patients were treated according to the consensus of multidisciplinary tumor boards and clinicians' choice. The sandwich sequence included three consecutive cycles of platinum-based CT at an interval of 21 days, followed by RT and another 3 cycles of CT. In the CT-alone group, patients were treated with platinum-based CT, planned for 6 cycles. One week before initiating each CT cycle, all patients received blood tests including a complete blood count and differential count, along with liver and renal function assessments. Treatment-related toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0) (23). If a patient experienced grade ≥3 toxicity on blood test assessment, CT was postponed on a week-by-week basis. Delay of treatment was defined as a delay of ≧7 days from the scheduled date of therapy. Patients with treatment-related toxicity that required a delay for ≧4 consecutive weeks were excluded from our analysis.

At the end of adjuvant therapy, patients were followed up with clinical and physical examinations during the first 3 years, which were performed at 3-month intervals and thereafter at 6-to-12-month intervals. Abdominal computed tomography was performed during the first year at 3-to-6-month intervals and thereafter at 12 months. In the event of clinically suspected metastatic diseases, additional imaging was performed, including computed tomography of the chest, abdomen, and pelvis, as well as positron emission tomography. OS was estimated from the time of surgery to the time of death and censored at the date of the last contact. PFS was calculated from the time of surgery to the time of the first recurrence based on imaging evidence, censored at the date of the last outpatient visit. Recurrence at the vagina or pelvis was considered a local recurrence. Patients who had missed a scheduled follow-up were contacted by our gynecologic oncology managers.

Statistical analyses were conducted using the SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Baseline characteristics were compared using the chi-squared or Mann–Whitney U test. OS, PFS, and disease-specific survival (DSS) were estimated using the Kaplan–Meier method, and comparisons between the two treatment groups were performed using the log-rank test. Univariate analyses were used to identify independent risk factors associated with disease outcomes. Variables with a p-value <0.10 were first extracted. Subsequently, multivariable analysis was performed using the Cox proportional hazards model to estimate the hazard ratio of each variable and compare outcomes between treatment groups. Treatment-induced toxicity was compared using the chi-squared test.

Results

Patient characteristics

Between, 2005 and, 2019, we identified 138 patients diagnosed with FIGO stage III EC. After exclusion, 80 cases were eligible for study inclusion. In total, 10 patients underwent sequential chemoradiotherapy (six consecutive CTs followed by RT, or RT followed by CT) and four received RT alone; these two patient groups were excluded from the study, given their small numbers. Considering the remaining 66 patients, 39 (59.1%) received sandwich chemoradiotherapy and 27 (40.9%) received CT alone.

The most commonly identified histological subtype was endometrioid (43 cases, 65.2%), followed by mix-epithelial (13 cases, 19.7%), serous (8 cases, 12.1%), and clear cell (2 cases, 3.0%). Each enrolled patient underwent BPLND in addition to TH and BSO. Most of these patients (92.4%) also received PALND. The median number of pelvic lymph nodes retrieved was 21 in the sandwich group and 25 in the CT-alone group. Considering para-aortic lymph nodes, 12 and nine nodes were retrieved from the sandwich and CT-alone groups, respectively. Table 1 summarizes the patients' baseline characteristics. No difference was detected between the two treatment groups in terms of patient age, surgical stage, histology, and pathological risk factors.

All patients received a platinum-based CT with either carboplatin (area under the curve [AUC] 4–6) or cisplatin (50 mg/m^2) plus paclitaxel (135–175 mg/m^2) or epirubicin (60–80 mg/m^2), or doxorubicin liposome injection (Lipodox®) (30 mg/m^2). The different combinations of chemotherapy regimens were similar in both arms. The median number of chemotherapy cycles per patient was 6 (4–8). In the sandwich sequence, irradiation was initiated within 3 weeks of the third chemotherapy cycle. RT was administered using external beam radiation therapy (EBRT) and delivered with intensity-modulated radiation therapy (IMRT) to the pelvis. The radiation fields were extended to the para-aortic region if metastasis was pathologically confirmed. The majority of these patients received a dose ranging between 5,040 and 5,400 cGy. Patients with cervical stromal invasion received additional vaginal brachytherapy (dose: 400–1,000 cGy. Details of CT and RT are shown in Table 1.

Outcomes

The median follow-up period was 50 months in the sandwich group and 85 months in the CT-alone group (p = 0.035). Disease recurrence was documented in 17 patients. These recurrences included 16 cases of distant metastasis, one case with pelvic recurrence, and two cases with concurrent distant and pelvic recurrences. No vaginal recurrence was detected in the

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TABLE 1 Characteristics of the patients (N = 66).

	Sandwich (n = 39)		CT alone (n = 27)		*p*-value
Median follow-up interval (months)	50.1	(26.0-77.7)	85.3	(36.9-112.4)	0.035*
Age	55.0	(48.0-64.0)	55.0	(47.0-57.0)	0.330
BMI	23.9	(21.3-26.1)	22.9	(18.5-26.2)	0.235
FIGO stage no. (%)‡					0.688
IIIA	9	(23.1%)	5	(18.5%)	
IIIB	2	(5.1%)	1	(3.7%)	
IIIC1	15	(38.5%)	8	(29.6%)	
IIIC2	13	(33.3%)	13	(48.1%)	
Histology					0.329
Endometrioid grade 1 and 2	18	(46.2%)	10	(37.0%)	
Endometrioid grade 3	10	(25.6%)	5	(18.5%)	
Serous	4	(10.3%)	4	(14.8%)	
Mixed-epithelial	5	(12.8%)	8	(29.6%)	
Clear cell	2	(5.1%)	0	(0.0%)	
Histology grading					0.609
Grade 1	4	(10.3%)	1	(3.7%)	
Grade 2	14	(35.9%)	10	(37.0%)	
Grade 3	21	(53.8%)	16	(59.3%)	
Gross residual disease					–
Absent	39	(100.0%)	27	(100.0%)	
Present	0	(0.0%)	0	(0.0%)	
No. of dissected lymph nodes					
Pelvic lymph node	21.0	(15.0-32.0)	25.0	(17.0-34.0)	0.270
Para-aortic lymph node	12.0	(6.0-17.0)	9.0	(4.0-14.0)	0.176
No. of cases receiving PALND	36	(92.3%)	25	(92.6%)	1.000
Minimal invasive approach	13	(33.3%)	0	(0.0%)	0.002†
LVSI					1.000
Absent	11	(28.2%)	7	(28.0%)	
Present	28	(71.8%)	18	(72.0%)	
Deep myometrial invasion					0.817
Absent	15	(38.5%)	12	(44.4%)	
Present	24	(61.5%)	15	(55.6%)	
Comorbidities					
Hypertension	8	(20.5%)	1	(3.7%)	0.071
Type II DM	8	(20.5%)	4	(14.8%)	0.748
HBV carrier	4	(10.3%)	0	(0.0%)	0.138
Others	6	(15.4%)	5	(18.5%)	0.749
Radiotherapy					–
EBRT dose 46.8 Gy	1	(2.6%)	–		
EBRT dose 50.4–54.0 Gy	33	(86.8%)	–		
EBRT dose >54.0 Gy	4	(10.5%)	–		
Vaginal brachytherapy	13	(33.3%)	0	(0.0%)	0.002†
No. of CT cycles					0.460
4–5 cycles	1	(2.6%)	1	(3.7%)	
6 cycle	38	(97.4%)	25	(92.6%)	
>6 cycle	0	(0.0%)	1	(3.7%)	
CT regimen					0.979
Platinum + paclitaxel	29	(74.4%)	21	(77.8%)	

(Continued)

TABLE 1 Continued

	Sandwich (n = 39)		CT alone (n = 27)		*p*-value
Platinum + doxorubicin	10	(25.6%)	6	(22.2%)	
CT delay or dose reduction					0.795
Absent	23	(59.0%)	17	(65.4%)	
Present	16	(41.0%)	9	(34.6%)	

Chi-square test or Mann–Whitney U test. *p < 0.05, †p < 0.01.
Values are presented as median (interquartile range) or number (%).
‡Stages were allocated according to the International Federation of Gynecology and Obstetrics (FIGO) 2009.
CT, chemotherapy; BMI, body mass index; PALND, para-aortic lymph node dissection; LVSI, lymphovascular space invasion; DM, diabetes mellitus; HBV, hepatitis B; ERBT, external beam radiotherapy.

present cohort. The most common site of distant metastasis was the lung (five cases, 7.6%), followed by the retroperitoneum (four cases, 6.1%), bone (four cases, 6.1%), and liver (four cases, 6.1%). During the follow-up period, 16 patients died, with 13 attributed to EC.

The Kaplan–Meier analyses revealed a 5-year PFS of 77.2% and 64.8% in the sandwich and CT-alone groups, respectively ($p = 0.209$) (Figure 1A). The sandwich arm was associated with a lower rate of pelvic recurrence than the CT-alone group (0% *vs.* 11.1%, $p = 0.031$) (Figure 1B), whereas the PFS for distant metastasis was similar in both groups (77.2% *vs.* 67.6%, $p = 0.328$) (Figure 1C). Although the difference in 5-year OS between the two groups did not reach statistical significance (86.7% *vs.* 69.6%, $p = 0.097$) (Figure 2A), a significantly improved (DSS) was observed in the sandwich group (91.8% *vs.* 69.6%, $p = 0.041$) (Figure 2B).

Based on univariate and multivariable analyses, grade 3 histology and deep myometrial invasion were identified as independent risk factors for 5-year OS and 5-year DSS. The sandwich sequence was a positive predictor for 5-year DSS (HR = 0.23, 0.06–0.86, $p = 0.029$). For PFS, grade 3 histology was the only negative predictor that attained statistical significance (Table 2).

Treatment-related toxicity

The sandwich sequence was associated with higher incidence and greater severity of neutropenia (grades 3–4: 56.4% *vs.* 18.5%, $p = 0.005$) and hematologic toxicity (grades 3–4: 59.0% *vs.* 25.9%, $p = 0.016$) than the CT-alone group (Table 3). Dose reduction was performed in one patient from 50.4 to 46.8 Gy, owing to skin irritation during RT. The proportions of patients requiring a dose delay or reduction during CT (34.6% *vs.* 41.0%, $p = 0.795$) were comparable. Furthermore, treatment completion rates were similar between the two groups (97.4% *vs.* 96.2%, $p = 0.642$). Five patients experienced lymphoceles after receiving RT, and they all resolved spontaneously within 18 months of the follow-up period. No patient reported hematuria during the follow-up. Grade 1–2 hematochezia was documented in five patients, and after medical treatment, no patient experienced sustained hematochezia. No patient died from treatment-associated adverse events in this cohort.

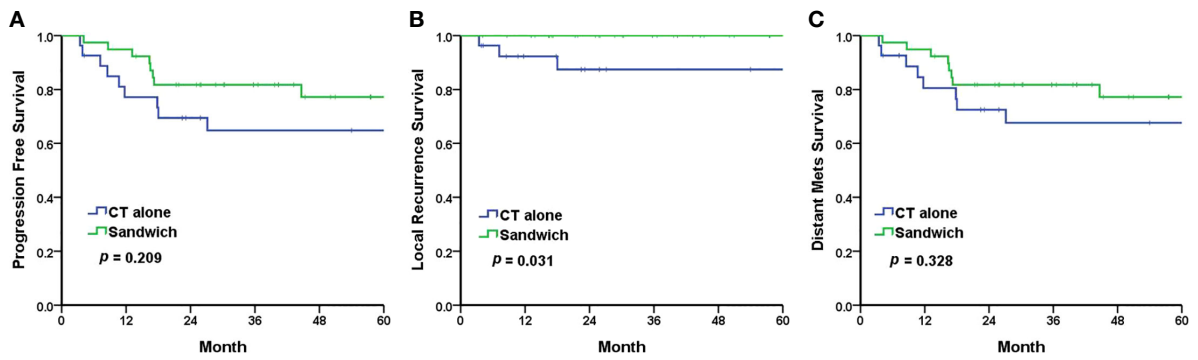

FIGURE 1
The Kaplan–Meier survival curves for 5-year progression-free survival **(A)**, local recurrence **(B)**, and distant metastasis **(C)**. CT, chemotherapy; mets, metastasis.

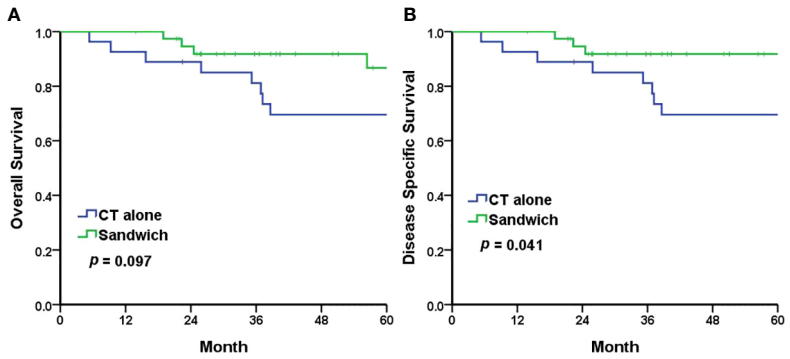

FIGURE 2
The Kaplan–Meier survival curves for 5-year overall survival **(A)** and 5-year disease-specific survival **(B)**. CT chemotherapy.

Discussion

In the present study, we detected a significant improvement in 5-year DSS and local control in patients treated with the sandwich sequence when compared with those treated with six consecutive cycles of CT alone. Multivariable analyses revealed that sandwich chemoradiotherapy was a positive prognostic factor for 5-year DSS, whereas both grade 3 histology and deep myometrial invasion were negative predictors for 5-year OS and DSS. Moreover, grade 3 histology was associated with a worse 5-year PFS. The proportions of treatment completion were similarly high in both groups, despite a significantly higher incidence and greater severity of neutropenia and hematologic toxicity in the sandwich sequence than in the CT-alone group.

Over the last 3 years, observational cohorts from the NCDB database have examined different sequences of adjuvant treatment. Goodman et al. have reported a longer 5-year OS in patients with stage III–IV, grade I–II endometrioid ECs who were treated with the CT-RT sequence when compared with those treated with RT-CT or either therapy alone (7). In patients with stage IIIC disease, a survival benefit was documented following treatment with the CT-RT sequence when compared with concurrent chemo-radiotherapy (CCRT) (8, 9). Xiang et al. have shown that the addition of pelvic irradiation to CT, irrespective of the sequence, affords a superior survival in patients with stage IIIC2 endometrioid ECs and stage IIIB, IIIC non-endometrioid ECs (10). These results supported the importance of RT as an adjuvant treatment of locally advanced ECs and the trend toward better survival in patients who had upfront CT in their adjuvant treatments.

Sandwich chemoradiotherapy was first reported in two pilot phase II studies. Both studies showed encouraging outcomes for locally advanced EC presenting high-risk histologies (12, 13). Subsequent single-armed studies also revealed a modest efficacy with acceptable toxicity in patient groups exhibiting different stages and histologic compositions (14–17). According to Secord et al., adjuvant sandwich therapy could improve the 3-year OS and PFS when compared with sequential CT-RT or RT-CT in patients with stage III–IV disease (11). In patients with stage III endometrioid EC, Lu et al. have reported comparable OS, PFS, and toxicity between sandwich and sequential chemoradiotherapy. However, the authors found that the group survival outcomes appeared similar, possibly due to small sample sizes (18). In comparison, although the 5-year OS in our study also failed to reach statistical significance between treatment groups, the 5-year DSS was significantly improved in the sandwich arm.

Recently, a multicenter retrospective analysis examining 179 patients with stage IIIC disease reported a significantly improved 5-year OS in the sandwich arm when compared with the sequential arm (74% *vs.* 56%). A trend toward a better PFS (65% *vs.* 54%, $p = 0.05$) was also reported (19). In a later cohort study assessing the same group of patients, the authors also identified a better 5-year OS (62% *vs.* 35%) and PFS (57% *vs.* 35%) using subgroup analyses among stage IIIC2 patients treated with a sandwich sequence when compared with sequential chemoradiotherapy (20). In addition, McEachron et al. have demonstrated OS and PFS benefits in patients with stage III–IV EC treated with sandwich therapy when compared with those treated with alternate sequences (21). In this multicenter analysis assessing 152 patients with relatively poor histology, 44% had endometrioid, 47.5% presented serous EC, and 8.5% had clear cell EC. With 20% of patients exhibiting stage IV disease, the authors found a 3-year OS advantage in the sandwich group when compared with CT-RT and RT-CT (71% *vs.* 52% *vs.* 50%), along with similar results for 3-year PFS (55% *vs.* 34% *vs.* 37%). In a more recent cohort study by Ko et al., using the SEER-Medicare database, the authors identified 44 cases treated with sandwich therapy in a subclassification analysis out of 2,870 patients with stage III disease (22). The best 5-year OS was observed in endometrioid EC treated with the sandwich regimen (82%), serous EC treated with the CCRT regimen

TABLE 2 Univariate and multivariable analyses of prognostic factors for 5-year OS, PFS, and DSS.

5-year overall survival

	Univariate			Multivariable		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Age group						
<60	Reference					
≥60	1.22	(0.33-4.53)	0.763			
FIGO stage						
IIIA and IIIB and IIIC1	Reference					
IIIC2	1.74	(0.56-5.41)	0.337			
Histology grading						
Grades 1 and 2	Reference			Reference		
Grade 3	9.69	(1.25-75.10)	0.030*	10.44	(1.34-81.10)	0.025*
Treatment						
CT alone	Reference			Reference		
Sandwich	0.38	(0.11-1.25)	0.110	0.31	(0.09-1.02)	0.054
LVSI						
Absent	Reference					
Present	4.19	(0.54-32.44)	0.170			
Deep myometrial invasion						
Absent	Reference			Reference		
Present	8.83	(1.14-68.42)	0.037*	10.52	(1.35-82.01)	0.025*
BMI						
<25	Reference					
≥25	0.64	(0.17-2.36)	0.502			
Cervical stromal involvement						
Absent	Reference					
Present	1.38	(0.44-4.36)	0.581			

5-year disease-specific survival

	Univariate			Multivariable		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age group						
<60	Reference					
≥60	1.34	(0.36-5.07)	0.665			
FIGO stage						
IIIA and IIIB and IIIC1	Reference					
IIIC2	1.45	(0.44-4.75)	0.541			
Histology grading						
Grades 1 and 2	Reference			Reference		
Grade 3	8.70	(1.11-68.01)	0.039*	9.16	(1.17-71.70)	0.035*
Treatment						
CT alone	Reference			Reference		
Sandwich	0.27	(0.07-1.04)	0.056	0.23	(0.06-0.87)	0.030*
LVSI						
Absent	Reference					
Present	3.82	(0.49-29.89)	0.201			
Deep myometrial invasion						
Absent	Reference			Reference		
Present	7.85	(1.00-61.34)	0.050	9.44	(1.20-74.15)	0.033*

(Continued)

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TABLE 2 Continued

	Univariate			Multivariable		
5-year overall survival	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
BMI						
<25	Reference					
≥25	0.72	(0.19-2.70)	0.622			
Cervical stromal involvement						
Absent	Reference					
Present	1.11	(0.33-3.81)	0.863			
5-year progression-free survival						
Age group						
<60	Reference					
≥60	0.79	(0.23-2.74)	0.705			
FIGO stage						
IIIA and IIIB and IIIC1	Reference					
IIIC2	2.03	(0.78-5.28)	0.145			
Histology grading						
Grades 1 and 2	Reference			Reference		
Grade 3	15.80	(2.09-119.33)	0.007†	11.57	(1.52-87.80)	0.018*
Treatment						
CT alone	Reference			Reference		
Sandwich	0.55	(0.21-1.42)	0.216	0.50	(0.19-1.30)	0.155
LVSI						
Absent	Reference			Reference		
Present	7.31	(0.97-55.16)	0.054	4.54	(0.56-36.87)	0.157
Deep myometrial invasion						
Absent	Reference			Reference		
Present	1.99	(0.70-5.64)	0.198	1.50	(0.51-4.45)	0.461
BMI						
<25	Reference					
≥25	1.22	(0.46-3.20)	0.693			
Cervical stromal involvement						
Absent	Reference					
Present	1.23	(0.46-3.33)	0.682			

Cox proportional hazard regression. *p < 0.05, †p < 0.01.
CT, chemotherapy; LVSI, lymphovascular space invasion; BMI, body mass index.

(48%), and clear cell EC treated with the CCRT regimen (66%). These comparative cohorts demonstrated promising results corroborating the efficacy of the sandwich sequence. However, prospective randomized control trials are warranted to further validate its efficacy.

On the other hand, GOG-258 failed to display a superior relapse-free survival with chemoradiotherapy when compared with CT alone (59% *vs*. 58%, $p = 0.20$) in patients with stage III–IVA EC. Although the chemoradiotherapy group was associated with improved local control exhibiting fewer pelvic/para-aortic (11% *vs*. 20%) and vaginal recurrences (2% *vs*. 7%), more distant recurrences were also detected (27% *vs*. 21%). The chemoradiotherapy protocol consisted of RT, with 2 cycles of concurrent cisplatin, followed by 4 cycles of CT with paclitaxel plus carboplatin. Data on this combination of CCRT plus CT remain limited. In the study by Ko et al. assessing 2,870 patients with stage III EC from the SEER-Medicare database, the authors identified <11 patients receiving CCRT plus CT, similar to that reported in GOG-258 and PORTEC-3 trials (22). Although CCRT alone was found to afford improved local control (24), recent large retrospective cohorts have reported less favorable survival when compared with the CT-RT sequence (8, 9). In the GOG-258 trial, the higher incidence of distant metastasis observed in the chemoradiotherapy group could be associated

TABLE 3 Adverse events.

	Grades 1–2					Grades 3–4				
Sandwich	**(N = 39)**	**CT alone**	**(N = 27)**	***p*-value**	**Sandwich**	**(N = 39)**	**CT alone**	**(N = 27)**	***p*-value**	
Anemia	31	(79.5%)	20	(74.1%)	0.828	3	(7.7%)	2	(7.4%)	1.000
Neutropenia	15	(38.5%)	8	(29.6%)	0.633	22	(56.4%)	5	(18.5%)	0.005†
Thrombocytopenia	19	(48.7%)	5	(18.5%)	0.025*	3	(7.7%)	0	(0.0%)	0.264
Hematologic toxicity	16	(41.0%)	17	(65.4%)	0.095	23	(59.0%)	7	(25.9%)	0.016*
Liver toxicity	15	(38.5%)	3	(11.5%)	0.036*	1	(2.6%)	0	(0.0%)	1.000
Renal toxicity	1	(2.6%)	2	(7.7%)	0.559	0	(0.0%)	0	(0.0%)	–

Chi-square test. *$p < 0.05$, †$p < 0.01$.
Values are presented as number (%).
CT, chemotherapy.

with the two fewer cycles of carboplatin and paclitaxel administered, as the two cycles of cisplatin cannot be regarded as equally potent to carboplatin plus paclitaxel. Moreover, the chemoradiotherapy arm was found to exhibit a lower CT completion rate (75% *vs.* 85%). Given that these results from recent large cohorts indicate the importance of upfront CT, the chemoradiotherapy sequence administered in GOG-258 appeared to be a relatively suboptimal choice. In contrast, several comparative cohorts have reported a superior survival benefit with the sandwich regimen over sequential chemoradiotherapy. Whether the sandwich sequence affords additional survival benefits in patients with stage III EC when compared with CT alone warrants further investigation.

In the current study, we excluded patients with gross residual tumors to ensure that both arms were comparable in postsurgical status before initiating adjuvant therapy. Extensive lymph node dissection reportedly affords a survival benefit in locally advanced endometrioid EC (25–27). Algkiozidis et al. have reported improved survival in patients undergoing dissections of ≧17 lymph nodes (26). In the present cohort, all patients had received BPLND, with PALND performed in >90% of patients. The median number of lymph nodes removed was 33 and 34 in the sandwich sequence and CT-alone groups, respectively. Given the extent of lymph node dissection, we aimed to achieve a complete excision of all metastatic lymph nodes. Hence, the risk of missing occult metastasis was minimized, facilitating the determination of precise areas for adjuvant RT, thus more accurately reflecting its efficacy.

After, 2012, minimally invasive approaches for preoperatively suspected early-staged EC were widely employed at our institution. This explains why the sandwich group comprised patients staged with the minimally invasive approach. No difference was detected in terms of OS, DSS, and PFS between the different surgical approaches. Furthermore, our preference for adjuvant therapy had shifted since, 2010 as growing numbers of publications have supported the efficacy of sandwich chemoradiotherapy. All patients in the sandwich arm were treated after, 2010; in the CT-alone arm, 17 patients (63%) were treated after, 2010. CT regimens in our patients were either carboplatin plus paclitaxel or carboplatin/cisplatin plus epirubicin/Lipodox®, with equal combinations in both groups. The choice of chemotherapeutic regimen has remained unaltered over the 15-year span. Given that these combinations provide a similar potency and treatment completion rate (28), the non-uniformity of CT regimens should minimally impact our results.

Hematologic adverse events were the most common cause of a CT dose delay or reduction in the current study. Previous cohorts have reported a dispersed level of toxicity with the sandwich therapy. However, most of these studies failed to specify their surveillance protocol during treatment. Onal et al. have documented a considerably low toxicity profile, with >grade 2 neutropenia observed only in 9% of cases treated with sandwich sequence (19). Frimer et al. have reported a 35% incidence of >grade 2 hematologic toxicity, as estimated by CT cycles rather than the proportion of patients (16). In our analysis, the rates of grade 3–4 hematologic toxicity were 59% in the sandwich arm and 26% in the CT-alone arm; in GOG-258, these rates were 40% for CCRT plus CT and 52% for CT alone. Despite the significant bone marrow toxicity noted in the sandwich arm of our study, both groups exhibited a comparable rate of CT dose delay or reduction (41.0% *vs.* 34.6%, $p = 0.795$), as well as treatment completion rate (97.4% *vs.* 96.2%, $p = 0.642$). Accordingly, although incorporating irradiation does increase toxicity, the adverse events were eventually tolerable in most of our patients.

The major limitation of the present study is its retrospective nature and limited sample size collected from a single institution. Selection bias is also a concern, as patients exhibiting high risks or superior performance status are likely to receive more aggressive adjuvant treatments. Nevertheless, we analyzed potential risk factors and did not identify any selection bias. Secondly, our cases were reviewed over a span of 15 years, during which the routine practice and clinician preferences were likely altered. Our institute initiated adjuvant sandwich chemoradiotherapy only after, 2012, resulting in imbalanced

monitoring times between the two treatment groups. The more recently enrolled patients likely benefited more from newly developed treatment modalities. Thus, these treatment modalities may have lengthened patient life spans after recurrence. In addition, while most retrospective studies addressing sandwich chemoradiotherapy have examined OS and PFS as their primary outcome, our study did not detect a significantly improved 5-year OS in the sandwich group, although the 5-year DSS showed improvement. Furthermore, outcomes of our cohort were likely improved owing to the exclusion of carcinosarcoma and undifferentiated and dedifferentiated carcinoma in both arms. The latter two histologies, which are less specifically described and excluded in other studies, also carry a distinctly poor prognosis. Finally, the toxicity profile can only be assessed by reviewing the laboratory tests and charts during adjuvant treatment. Chronic toxicity could not be reliably assessed as documentation of symptoms may be inconsistent among clinicians, and the reporting bias of patients may also affect outcomes. Therefore, the current study did not analyze neurotoxicity, constitutional symptoms, and other late events. The strength of our study is the uniformity of postsurgical status and the extent of lymph node assessment. Over 90% of our patients underwent PALND, which possibly reduced occult para-aortic metastasis and more appropriately reflected the efficacy of RT. Despite a significantly shorter follow-up period in the sandwich arm, both arms were monitored for a longer period when compared with other cohort studies. Furthermore, compared with the 75% and 85% chemotherapy cycle completion rates reported in GOG-258, almost all our patients completed their scheduled treatment. Hence, our results may better reflect the true potency of both treatment arms.

In conclusion, we documented a better 5-year DSS and local control in the sandwich chemoradiotherapy sequence than in the CT-alone group. The sandwich sequence was associated with increased hematologic toxicity, which appeared tolerable in most patients and did not impact the treatment completion rate. To the best of our knowledge, this is the first study that directly compared the sandwich sequence with CT alone. As survival outcomes are yet to be established in the GOG-258 trial, the survival benefits shown in our study provide additional information supporting the efficacy of sandwich chemoradiotherapy. Further prospective randomized studies are required to validate the efficacy of the sandwich regimen and identify the optimal adjuvant therapy.

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

This study was reviewed and approved by Institutional Review Board I &II of Taichung Veterans General Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

The authors confirm their contribution to the paper as follows: S-JW and C-HL were responsible for the study conception, design, and draft manuscript. LW, LS, Y-HS, C-KL and S-FH were responsible for data acquisition. S-TH and C-HL helped with data interpretation and performed statistical analyses. S-JW, LS and Y-HS wrote the original draft of the manuscript. 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.2022.946113/full#supplementary-material

References

1. American Cancer Society. *Cancer statistics center*. Atlanta, GA: American Cancer Society (2022).

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* (2017) 67(1):7–30. doi: 10.3322/caac.21387

3. Abu-Rustum NR, Yashar CM, Bradley K, Campos SM, Chino J, Chon HS, et al. NCCN guidelines® insights: Uterine neoplasms, version 3.2021. *J NatlComprCancNetw.* (2021) 19(8):888–95. doi: 10.6004/jnccn.2021.0038

4. Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, Fowler J, et al. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a gynecologic oncology group study. *J Clin Oncol* (2006) 24(1):36–44. doi: 10.1200/JCO.2004.00.7617

5. Matei D, Filiaci V, Randall ME, Mutch D, Steinhoff MM, DiSilvestro PA, et al. Adjuvant chemotherapy plus radiation for locally advanced endometrial cancer. *N Engl J Med* (2019) 380(24):2317–26. doi: 10.1056/NEJMoa1813181

6. de Boer SM, Powell ME, Mileshkin L, Katsaros D, Bessette P, Haie-Meder C, et al. Adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (PORTEC-3): final results of an international, open-label, multicentre, randomised, phase 3 trial. *Lancet Oncol* (2018) 19(3):295–309. doi: 10.1016/S1470-2045(18)30079-2

7. Goodman CR, Hatoum S, Seagle BL, Donnelly ED, Barber EL, Shahabi S, et al. Association of chemotherapy and radiotherapy sequence with overall survival in locoregionally advanced endometrial cancer. *Gynecol Oncol* (2019) 153(1):41–8. doi: 10.1016/j.ygyno.2019.01.007

8. Latham AH, Chen L, Hou JY, Tergas AI, Khoury-Collado F, St Clair CM, et al. Sequencing of therapy in women with stage III endometrial carcinoma receiving adjuvant combination chemotherapy and radiation. *Gynecol Oncol* (2019) 155(1):13–20. doi: 10.1016/j.ygyno.2019.07.021

9. Modh A, Ghanem AI, Burmeister C, Hanna RK, Elshaikh MA. What is the optimal adjuvant treatment sequence for node-positive endometrial cancer? results of a national cancer database analysis. *Int J Gynecol Cancer* (2018) 28(2):248–53. doi: 10.1097/IGC.0000000000001162

10. Xiang M, English DP, Kidd EA. Defining the survival benefit of adjuvant pelvic radiotherapy and chemotherapy versus chemotherapy alone in stages III-IVA endometrial carcinoma. *Gynecol Oncol* (2019) 154(3):487–94. doi: 10.1016/j.ygyno.2019.06.020

11. Secord AA, Havrilesky LJ, O'Malley DM, Bae-Jump V, Fleming ND, Broadwater G, et al. A multicenter evaluation of sequential multimodality therapy and clinical outcome for the treatment of advanced endometrial cancer. *Gynecol Oncol* (2009) 114(3):442–7. doi: 10.1016/j.ygyno.2009.06.005

12. Geller MA, Ivy JJ, Ghebre R, Downs LSJr, Judson PL, Carson LF, et al. A phase II trial of carboplatin and docetaxel followed by radiotherapy given in a "Sandwich" method for stage III, IV, and recurrent endometrial cancer. *Gynecol Oncol* (2011) 121(1):112–7. doi: 10.1016/j.ygyno.2010.12.338

13. Fields AL, Einstein MH, Novetsky AP, Gebb J, Goldberg GL. Pilot phase II trial of radiation "sandwiched" between combination paclitaxel/platinum chemotherapy in patients with uterine papillary serous carcinoma (UPSC). *Gynecol Oncol* (2008) 108(1):201–6. doi: 10.1016/j.ygyno.2007.09.025

14. Lupe K, D'Souza DP, Kwon JS, Radwan JS, Harle IA, Hammond JA, et al. Adjuvant carboplatin and paclitaxel chemotherapy interposed with involved field radiation for advanced endometrial cancer. *Gynecol Oncol* (2009) 114(1):94–8. doi: 10.1016/j.ygyno.2009.03.027

15. Abaid LN, Rettenmaier MA, Brown JV3rd, Micha JP, Mendivil AA, Wabe MA, et al. Sequential chemotherapy and radiotherapy as sandwich therapy for the treatment of high risk endometrial cancer. *J Gynecol Oncol* (2012) 23(1):22–7. doi: 10.3802/jgo.2012.23.1.22

16. Frimer M, Miller EM, Shankar V, Girda E, Mehta K, Smith HO, et al. Adjuvant pelvic radiation "Sandwiched" between Paclitaxel/Carboplatin chemotherapy in women with completely resected uterine serous carcinoma: Long-term follow-up of a prospective phase 2 trial. *Int J Gynecol Cancer* (2018) 28(9):1781–8. doi: 10.1097/IGC.0000000000001359

17. Raspagliesi F, Bogani G, Pinelli C, Casarin J, Cerrotta AM, DelleCurti CT, et al. Patterns of failure after adjuvant "sandwich" chemo-radio-chemotherapy in locally advanced (stage III-IVA) endometrial cancer. *J Cancer Res Clin Oncol* (2021) 147(3):813–20. doi: 10.1007/s00432-020-03339-y

18. Lu SM, Chang-Halpenny C, Hwang-Graziano J. Sequential versus "sandwich" sequencing of adjuvant chemoradiation for the treatment of stage III uterine endometrioid adenocarcinoma. *Gynecol Oncol* (2015) 137(1):28–33. doi: 10.1016/j.ygyno.2015.01.546

19. Onal C, Sari SY, Yildirim BA, Yavas G, Gultekin M, Guler OC, et al. A multi-institutional analysis of sequential versus 'sandwich' adjuvant chemotherapy and radiotherapy for stage IIIC endometrial carcinoma. *J Gynecol Oncol* (2019) 30(3): e28. doi: 10.3802/jgo.2019.30.e28

20. Onal C, Yildirim BA, Sari SY, Yavas G, Gultekin M, Guler OC, et al. Treatment outcomes of endometrial cancer patients with paraaortic lymph node metastasis: a multi-institutional analysis. *Int J Gynecol Cancer* (2019) 29(1):94–101. doi: 10.1136/ijgc-2018-000029

21. McEachron J, Zhou N, Spencer C, Shanahan L, Chatterton C, Singhal P, et al. Evaluation of the optimal sequence of adjuvant chemotherapy and radiation therapy in the treatment of advanced endometrial cancer. *J Gynecol Oncol* (2020) 31(6):e90. doi: 10.3802/jgo.2020.31.e90

22. Ko EM, Brensinger CM, Cory L, Giuntoli RL2nd, Haggerty AF, Latif NA, et al. Utilization and survival outcomes of sequential, concurrent and sandwich therapies for advanced stage endometrial cancers by histology. *Gynecol Oncol* (2020) 159(2):394–401. doi: 10.1016/j.ygyno.2020.07.105

23. *Common terminology criteria for adverse events (CTCAE) version 5*. US Department of Health and Human Services, National Institutes of Health, National Cancer Institute. Published: November 27.

24. Greven K, Winter K, Underhill K, Fontenesci J, Cooper J, Burke T. Final analysis of RTOG 9708: adjuvant postoperative irradiation combined with cisplatin/paclitaxel chemotherapy following surgery for patients with high-risk endometrial cancer. *Gynecol Oncol* (2006) 103(1):155–9. doi: 10.1016/j.ygyno.2006.02.007

25. Chan JK, Cheung MK, Huh WK, Osann K, Husain A, Teng NN, et al. Therapeutic role of lymph node resection in endometrioid corpus cancer: a study of 12,333 patients. *Cancer* (2006) 107(8):1823–30. doi: 10.1002/cncr.22185

26. Alagkiozidis I, Weedon J, Grossman A, Wang P, Mize B, Wilson K, et al. Extent of lymph node dissection and overall survival in patients with uterine carcinosarcoma, papillary serous and endometrioid adenocarcinoma: A retrospective cohort study. *Int J Surg (Lond Engl)* (2015) 24(Pt A):9–13. doi: 10.1016/j.ijsu.2015.10.006

27. Todo Y, Kato H, Kaneuchi M, Watari H, Takeda M, Sakuragi N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. *Lancet* (2010) 375(9721):1165–72. doi: 10.1016/S0140-6736(09)62002-X

28. Nomura H, Aoki D, Michimae H, Mizuno M, Nakai H, Arai M, et al. Japanese Gynecologic oncology group. effect of TaxanePlus platinum regimens *vs* doxorubicin plus cisplatin as adjuvant chemotherapy for endometrial cancer at a high risk of progression: A randomized clinical trial. *JAMA Oncol* (2019) 5(6):833–40. doi: 10.1001/jamaoncol.2019.0001

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