MASARYK UNIVERSITY

FACULTY OF MEDICINE

Individualized Approach to Patients with Endometrial Cancer

(a collection of annotated publications)

Habilitation thesis

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Contents

1 Objectives of the Habilitation Thesis

This habilitation thesis is structured as a collection of previously published papers, reflecting the author's long-term scientific work focused on individualizing care for patients with endometrial cancer. The presented results were achieved primarily during her tenure at the Department of Obstetrics and Gynecology, University Hospital Brno and the Faculty of Medicine, Masaryk University, through both local unicentric projects and international collaboration—particularly with the ENITEC group (European Network of Individual Treatment in Endometrial Cancer), of which the author is an active member.

The author's research activities cover a broad range of topics related to endometrial cancer. The publications selected for this thesis are grouped into four main thematic areas:

- **Diagnostic and Screening**

Endometrial cancer is typically detected at an early stage due to early symptomatology. This chapter aims to describe the role of ultrasound, endometrial biopsy, and other diagnostic methods, as well as their potential for screening, based on the author's own patient cohort.

- **Molecular Classification and Prognostic Markers**

The introduction of molecular classification into routine clinical practice has marked a significant advancement in personalizing therapy for patients with endometrial cancer. This chapter presents initial clinical experience with molecular profiling and explores the potential utility of additional prognostic markers, even those not included in the current classification systems.

- **Lymph Node Staging**

A major recent change in endometrial cancer management concerns lymph node staging. The traditional approach of systematic pelvic and para-aortic lymphadenectomy has been gradually replaced by sentinel lymph node biopsy. This

chapter provides an in-depth overview of this technique, including results from both the author's institution and a multicenter study.

- **Predictive models**

Modern technologies, including artificial intelligence, are increasingly being applied in medicine; nevertheless, traditional mathematical models are still being developed to personalize patient care. The final chapter introduces basic types of predictive models used to estimate final pathology results, prognosis, and survival in patients with endometrial cancer. It includes both the author's team model and validation of an existing one.

2 Introduction

Tumors of the uterine corpus, particularly endometrial carcinoma, remain among the most common gynecological malignancies in developed countries, including the Czech Republic, with an incidence of 37 per 100,000 women in 2022[1]. The disease predominantly affects women in the 6th and 7th decades of life. Due to the early onset of symptoms, the majority of cases (67%) are diagnosed at stage I according to the FIGO (International Federation of Gynecology and Obstetrics) classification, with excellent five-year survival rates approaching 90%[1,2]. However, prognosis significantly worsens in more advanced stages and in tumors exhibiting adverse prognostic features.

Currently, there is no population-wide screening program for endometrial cancer. The use of transvaginal ultrasound examination to assess endometrial thickness followed by biopsy in asymptomatic women is not generally recommended, except for patients with a genetic predisposition to endometrial carcinoma, specifically Lynch syndrome[3].

Over the past decade, there has been a significant shift in the understanding of endometrial carcinomas. It is now clear that the previously used simple didactic division into two types (Type I: 80–90%, estrogen-dependent, endometrioid/mucinous type arising from hyperplastic endometrium; Type II: 10–20%, estrogen-independent, non-endometrioid types arising from atrophic endometrium)[4] is no longer adequate. The introduction of molecular classification has led to the categorization of endometrial carcinomas into four prognostically distinct groups, offering a more accurate reflection of tumor biology and patient prognosis. Efforts are ongoing to refine further the classification of the largest and most heterogeneous group – tumors with a non-specific molecular profile.

Significant advancements have also been made in the surgical treatment of uterine tumors and lymph node staging. Systematic pelvic and para-aortic lymphadenectomy, once a routine procedure, has been gradually replaced by the less invasive sentinel lymph node biopsy, which has become the preferred approach. Emphasis is placed on

minimally invasive surgical techniques, now regarded as an important quality indicator of surgical care in patients with endometrial carcinoma[5].

3 Diagnostic and Screening

Due to its characteristic early symptomatology, endometrial carcinoma is most often diagnosed at an early stage. Typical presenting symptoms include postmenopausal bleeding and irregular or excessive menstrual bleeding in premenopausal women[6,7]. Less frequently, the disease may first present with signs of more advanced disease, such as lower abdominal pain, abdominal discomfort, abdominal distension, or constipation.

In a patient with abnormal uterine bleeding, in addition to a clinical gynecological examination, a transvaginal ultrasound should be performed to assess the endometrium, myometrium, cervix, and surrounding uterine structures in detail. For ultrasound findings, the use of IETA (International Endometrial Tumor Analysis) terminology is recommended[8]. A thin, well-defined, homogeneous, hyperechogenic endometrium measuring ≤3 mm on ultrasound has a negative predictive value of up to 99% for excluding endometrial carcinoma. In contrast, typical ultrasound features suggestive of premalignant or malignant pathology include endometrial thickening, disruption of the endomyometrial junction, myometrial or cervical invasion, and abnormal tumor vascularization of Doppler imaging[9]. If a suspicious endometrial lesion is observed, intrauterine pathology should be suspected, and an endometrial biopsy (using a Pipelle device, dilation and curettage, or hysteroscopy) is indicated, as it remains definitive diagnostic method.

Given the generally favorable prognosis of the disease, which is largely due to early clinical symptoms (90% of patients)[6] and the predominance of less aggressive histological types, endometrial carcinoma is not considered an ideal candidate for population screening. While transvaginal ultrasound may seem to be a suitable non-invasive method, it has very low specificity and results in an excessive number of biopsies in asymptomatic women, potentially leading to complications. If considering biopsy in asymptomatic women, a reasonable threshold has been defined: endometrial thickness >11 mm, at which the risk of cancer is comparable to that of symptomatic women with endometrial thickness of 4–5 mm[10]. In the case of thinner endometrium and no symptoms, biopsy should only be considered if high-risk ultrasound features

are present (increased vascularization, disrupted endomyometrial junction, non-uniform endometrium)[11].

However, before performing an invasive procedure involving endometrial sampling, it is important to keep in mind that no clear survival benefit has been demonstrated in cases where carcinoma is detected in asymptomatic patients or shortly after the first episode of bleeding[12–14].

Routine ultrasound-based screening for endometrial cancer in asymptomatic women is not recommended, even in those with risk factors such as significant obesity, metabolic syndrome, or polycystic ovary syndrome[3]. The exception is women with a hereditary predisposition to endometrial cancer (primarily Lynch syndrome), in whom annual transvaginal ultrasound (preferably by an expert sonographer) is advised, along with annual to biennial endometrial biopsy starting at age 35. If abnormal uterine bleeding occurs, biopsy should be performed regardless of the surveillance schedule. These patients should also be offered prophylactic hysterectomy with bilateral salpingo-oophorectomy after completing childbearing (ideally by age 40), as a preventive measure against endometrial and ovarian cancer[3].

3.1 Vinklerová P, Felsinger M, Frydová S, Ovesná P, Hausnerová J, Weinberger V. Je nález hyperplazie či polypu dutiny děložní automatickou indikací k biopsii? Čes. Gynek., 2020, 85(2):84-93.

The clinical approach to endometrial pathology in the Czech Republic is distinct from that of many other countries, particularly due to the widespread availability of high-resolution ultrasound. As a result, asymptomatic women are frequently referred for endometrial biopsy—often repeatedly—based on ultrasound findings such as endometrial polyps or hyperplasia, despite the absence of clinical symptoms.

This retrospective study analyzed the primary indications for endometrial biopsy referral at the Department of Gynecology and Obstetrics at University Hospital Brno, in women over 50. We assessed the histological outcomes in relation to ultrasound findings, with particular focus on the detection of premalignant or malignant pathology. The sensitivity and specificity of ultrasound-detected hyperplasia and polyps were evaluated, using various endometrial thickness and polyp size thresholds.

Our findings indicate that the detection of a polyp or endometrial hyperplasia on ultrasound examination does not, in isolation, constitute an automatic indication for histopathological verification. Ultrasound should be viewed as one component of a broader diagnostic strategy—one that also accounts for clinical symptoms, patient comorbidities, and procedural risks. These include the relatively frequent failure to obtain a representative tissue sample for histological evaluation.

In postmenopausal women, any occurrence of bleeding or spotting was confirmed to be a strong predictor of underlying premalignant or malignant pathology, independent of endometrial thickness or polyp size. In contrast, asymptomatic patients with incidental ultrasound findings may be more appropriately managed conservatively. Biopsy can reasonably be deferred unless endometrial thickness exceeds 12 mm or other high-risk features are present. In such cases, focal lesions should be addressed using hysteroscopy, while blind dilatation and curettage should be reserved for situations involving heavy uterine bleeding or clearly abnormal intrauterine findings—such as a large-volume mass.

The study *"Is the finding of endometrial hyperplasia or corporal polyp a mandatory indication for biopsy?"* was published in *Česká gynekologie* in 2020.

The author's contribution: first author, conceptualization, methodology, data curation, and manuscript writing – original draft.

RETROSPEKTIVNÍ STUDIE

Je nález hyperplazie či polypu děložní dutiny automatickou indikací k biopsii?

Is the finding of endometrial hyperplasia or corporal polyp an mandatory indication for biopsy?

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ABSTRACT

Objective: The aim of our study was to analyze a group of patients referred for endometrial biopsy. To evaluate the ultrasound finding of hyperplasia/polyp, the symptomatology of patients related to the result of definitive histology, to determine the severity of individual variables in connection with the detection of precancerosis/cancer. Due to the complexity of information identify women who are suitable for conservative approach.
Design: Unicentric retrospective observational study.
Setting: Department of Obstetrics and Gynecology, Masaryk University, University Hospital Brno.
Methods: All patients over 50 years who underwent surgical endometrial biopsy at our department in the period of 2017–2018 (n = 754) were included. We were interested in reasons of indication, the age of patients at the time of the procedure and at the menopause, the presence of risk factors for development precancerosis/cancer (hypertension, diabetes mellitus, using of tamoxifen), number of deliveries and pregnancies, symptomatology, the description of ultrasound scans, the result of histology examination, peroperative and postoperative complications.
Results: Perimenopause – the median of endometrial thickness in both benign and malignant histology was 8 mm (p = 0.448), the median of the largest polyp dimension was 18 mm. All patients with precancerosis/malignancy were symptomatic with irregular/excessive bleeding, no carcinoma was found in polyp. Postmenopause – the median of endometrial thickness in benign histology was 7 mm versus 16 mm in precancerosis/malignancy (p < 0.001), the median of the largest polyp dimension was the same in both histologies (13 mm, p = 0.274). The risk of malignancy was more than threefold in bleeding versus asymptomatic patients with both hyperplasia and polyp (OR 3.39, 3.79). In asymptomatic patients the risk of cancer was similar for selected cut-offs (5, 8 and 12 mm), statistically significant only for 12 mm (OR 3.54), while in symptomatic patients the risk was high for all cut-offs, however with wide confidence intervals, statistically significant for cut-offs of 8 mm (minimum 3.58) and 12 mm (minimum 4.94).
Conclusion: We have shown that symptomatology is a strong risk factor for the presence of precancerosis/malignancy in patients with endometrial hyperplasia or polyp. The thickness of the endometrium or polyp size in asymptomatic patients does not play a major role. Ultrasound alone does not have sufficient accuracy for detection or even screening of endometrial cancer. We recommend a conservative procedure, monitoring changes in the ultrasound scan and symptomatology of the patient over time.

KEYWORDS

curettage, endometrial cancer, endometrial hyperplasia, hysteroscopy, polyp, postmenopausal bleeding

SOUHRN

Cíl studie: Cílem naší práce byla analýza souboru pacientek doporučených k biopsii endometria. Vyhodnotit ultrazvukový nález hyperplazie/polypu, symptomatologii pacientek v souvislosti s výsledky definitivní histologie, určit míru závažnosti jednotlivých proměnných v souvislosti s detekcí prekancerózy/karcinomu. Díky komplexnosti dat identifikovat ženy, které jsou vhodné pro konzervativní postup.

Typ studie: Unicentrická retrospektivní observační studie.
Název a sídlo pracoviště: Gynekologicko-porodnická klinika LF MU a FN Brno.
Metodika: Zařazeny byly všechny pacientky ve věku vyšším než 50 let, které podstoupily na naší klinice v období let 2017–2018 operační biopsii endometria (n = 754). Sledovali jsme důvody indikace k biopsii, věk pacientek v době zákroku a při nástupu menopauzy,

přítomnost rizikových faktorů vzniku prekancerózy/karcinomu děložního těla (hypertenze, diabetes mellitus, užívání tamoxifenu), počet porodů a těhotenství, symptomy, ultrazvukový popis děložní dutiny, výsledek histologického vyšetření, peroperační a pooperační komplikace.
Výsledky: Perimenopauza – medián výšky endometria u benigní i maligní histologie byl 8 mm (p = 0,448), medián největšího rozměru polypu byl 18 mm. Všechny pacientky s prekancerózou/malignitou byly symptomatické nepravidelným/nadměrným krvácením, žádná malignita nebyla nalezena v polypu. Postmenopauza – medián výšky endometria u benigní histologie byl 7 mm oproti 16 mm při prekanceróze/malignitě (p < 0,001), medián největšího rozměru polypu byl u obou histologií shodný (13 mm, p = 0,274). Riziko přítomnosti malignity bylo více než trojnásobné při krvácení oproti asymptomatickým pacientkám v případě hyperplazie i polypu (OR 3,39; 3,79). U asymptomatických pacientek bylo riziko přítomnosti karcinomu obdobné pro vybrané cut-off (5, 8 a 12 mm), statisticky významné pouze pro 12 mm (OR 3,54), zatímco u symptomatických pacientek bylo riziko vysoké u všech cut-off, nicméně při širokých intervalech spolehlivosti, statisticky významné pro cut-off 8 mm (minimálně 3,58) a 12 mm (minimálně 4,94).
Závěr: Prokázali jsme, že symptomatologie je silným rizikovým faktorem přítomnosti prekancerózy/malignity u pacientek s nálezem hyperplazie či polypu endometria. Samotná výška endometria či velikost polypu u asymptomatických pacientek nehraje hlavní roli. Ultrazvuk sám o sobě nemá dostatečnou přesnost pro detekci, či dokonce screening karcinomu endometria. Doporučujeme konzervativní postup, sledování změn ultrazvukového obrazu a symptomatologie pacientky v čase.

KLÍČOVÁ SLOVA

hyperplazie endometria, hysteroskopie, karcinom endometria, korporální polyp, postmenopauzální krvácení, separovaná abraze

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ÚVOD

Hyperplazie endometria a korporální polyp jsou poměrně běžné nálezy, které můžeme pomocí ultrazvukového (UZ) vyšetření detekovat u 10–20 % pacientek po menopauze [2, 11]. Vzhledem k dostupnosti ultrazvukového vyšetření se dostáváme do situace, že jsou často objeveny náhodně u asymptomatické pacientky a pro obavy z malignity indikovány k bioptické verifikaci. Ženy tak podstupují i opakovaně zákroky (separovaná abraze a/nebo hysteroskopie), které mohou v některých případech vést k závažným peroperačním či pooperačním komplikacím (iatrogenní perforace dělohy, poranění okolních orgánů, krvácení, infekce, fluid-overload syndrom, anesteziologické komplikace atd. [6, 11]).

Při popisu endometria v UZ obraze využíváme terminologii navrženou mezinárodní skupinou pro endometriální tumory IETA (International Endometrial Tumor Analysis), která si dala za cíl sjednotit názvosloví pro lepší reprodukovatelnost nálezů především pro účely klinických studií [12]. Kromě samotné výšky endometria popisujeme echogenitu, heterogenitu, pravidelnost a neporušenost endo-myometriální junkce, vaskularizaci, přítomnost a charakter tekutiny v děložní dutině. Některé z těchto znaků nás mohou upozornit na přítomnost maligního procesu lépe než pouhá detekce fokální léze (polypu) či měření výšky endometria v UZ obraze [4].

Průměrná výška endometria u postmenopauzálních žen je 3–5 mm, o hyperplazii pak obvykle mluvíme, pokud je sliznice ≥ 5 mm [11, 15]. Při této výšce endometria u symptomatické pacientky je riziko nádoru 7 %, u pacientky asymptomatické je srovnatelné riziko až při endometriu > 11 mm [15]. Medián výšky endometria u karcinomu je 17–20 mm a v 75–90 % případů se projevuje krvácením nebo špiněním [7, 15, 17]. V České republice neexistuje jednotné doporučení zabývající se touto problematikou, a většina gynekologů se proto drží striktní hranice 5 mm nebo jedná empiricky. V doporučeném postupu pro ultrazvukový staging karcinomu endometria [7] autoři zmiňují jen nutnost bioptické verifikace pacientek s postmenopauzálním krvácením a endometriem nad 3 mm [6].

Polyp je definován jako lokalizovaná hypertrofie endometriální tkáně tvořená žlázkami, stromatem a cévami obklopená epitelem [2]. Riziko malignity v polypu se celkově udává kolem tří procent [2, 16]. Výrazně vyšší riziko je u žen postmenopauzálních (5 %) oproti premenopauzálním (1 %) a dále u pacientek s krvácením či špiněním (5 %) oproti asymptomatickým (2 %) [16]. Ani u polypu neexistuje v České republice jednotné doporučení, kdy jej biopticky ověřovat, a část gynekologů takovou pacientku bez ohledu na symptomatologii referuje k histologické verifikaci, druhá část zvolí empiricky konzervativní postup.

Cílem naší práce bylo identifikovat důvody, pro které byly pacientky referovány na naši kliniku k bioptickému ověření endometria. Vyhodnotili jsme výsledky histologie z pohledu výskytu prekancerózy a zhoubného nádoru dělohy u perime-

nopauzálních a postmenopauzálních pacientek ve věku vyšším než 50 let. Z pohledu ultrazvukového nálezu hyperplazie nebo polypu jsme zhodnotili senzitivitu a specificitu pro vybrané cut-off výšky endometria či velikosti polypu v detekci prekancerózy, karcinomu u asymptomatických a symptomatických pacientek. K nálezu prekancerózy a karcinomu děložní dutiny jsme vztáhli konkrétní ultrazvukový nález (hyperplazie/polyp) a zaznamenali symptomatologii dané pacientky. Díky těmto kritériím a komplexnosti klinického obrazu jsme se v závěru pokusili identifikovat ženy, které jsou vhodné pro konzervativní postup – místo biopsie pouhé sledování změny UZ obrazu v čase a/nebo vyčkávání na klinické projevy.

METODIKA A SOUBOR PACIENTEK

Jedná se o retrospektivní studii, do které byly zařazeny konsekutivně všechny pacientky starší než 50 let, které podstoupily separovanou abrazi (SA) nebo hysteroskopii (HSK) od ledna 2017 do prosince 2018 na Gynekologicko-porodnické klinice FN Brno a LF MU. Pacientky byly rozděleny do dvou skupin. V první se jednalo o perimenopauzální ženy, u kterých uplynulo méně než rok od poslední menstruace v době zákroku, a druhá skupina byly postmenopauzální ženy, u kterých uběhl více než rok od poslední menstruace v době zákroku. U obou skupin jsme sledovali důvody indikace k biopsii, věk pacientek v době zákroku a v době menopauzy (pouze u druhé skupiny), přítomnost rizikových faktorů vzniku prekancerózy a karcinomu děložního těla (hypertenze, diabetes mellitus, užívání tamoxifenu), počet porodů a těhotenství, symptomy (krvácení, špinění), UZ popis děložní dutiny (výška endometria, přítomnost polypu a jeho velikost), výsledek histologického vyšetření, peroperační a pooperační komplikace.

Metodika předoperačního UZ vyšetření

Každá pacientka podstoupila transvaginální ultrazvukové vyšetření maximálně 21 dní před přijetím na kliniku k provedení hysteroskopie a/nebo separované abraze. Ultrazvukové vyšetření prováděli lékaři s kmenem či ukončenou specializací v oboru gynekologie a porodnictví v rámci vyšetření pacientek na všeobecné příjmové ambulanci. Každé ultrazvukové vyšetření bylo popsáno v písemné zprávě, tyto záznamy byly použity pro analýzu studie. Popisy ultrazvukového obrazu a zpráva z vyšetření vycházely ze standardů užívaných na naší klinice podle IETA terminologie [12]. Během ultrazvukového vyšetření byla děloha hodnocena v sagitální rovině (hrdlo – istmus – tělo – fundus) od jednoho rohu ke druhému a v transverzálním řezu od hrdla k fundu, byla hodnocena děložní dutina, myometrium, cervix [7]. Za hyperplazii byla označena výška endometria v UZ obraze ≥ 5 mm [11, 15]. Jako polyp byla označena fokální hypertrofie endometriální tkáně rostoucí exofyticky do děložní dutiny a ohraničená od zbylého endometria [2, 7].

Sběr dat a statistické zhodnocení

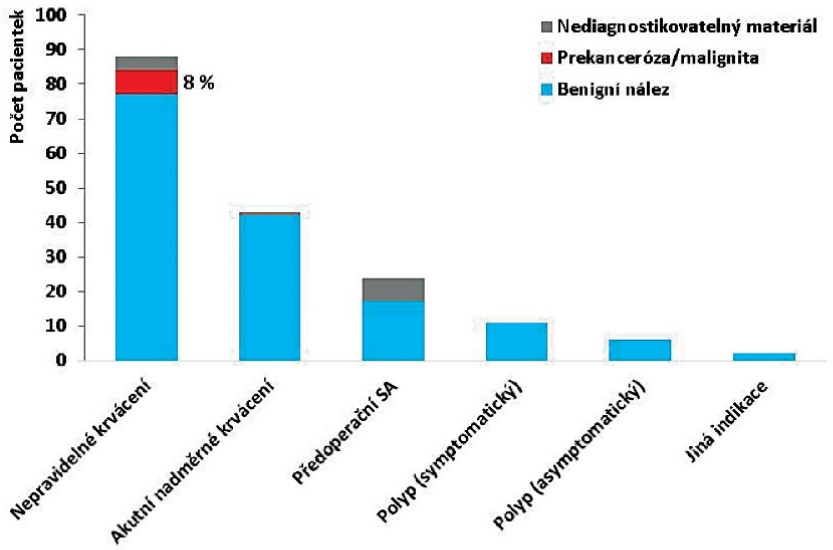
Veškerá klinická data byla vyhledána pomocí nemocničního informačního systému AMIS z ambulantních záznamů a operačních protokolů, shrnuta a vyjádřena pomocí absolutních a relativních četností výskytu, v případě spojitých dat pak pomocí mediánu a rozsahu nebo průměru se směrodatnou odchylkou. Srovnání četností kategoriálních proměnných bylo provedeno Fisherovým exaktním testem, rozdíl ve spojitých parametrech mezi pacientkami s benigním a maligním nálezem byl posouzen Mannovým-Whitneyovým U testem. Riziko přítomnosti hodnocených faktorů bylo vyjádřeno pomocí poměru šancí (OR, odds ratio) včetně 95% intervalu spolehlivosti (IS) a příslušné p-hodnoty. Všechny testy byly provedeny jako oboustranné na hladině významnosti 5‰.

VÝSLEDKY

V letech 2017–2018 bylo na Gynekologicko-porodnické klinice FN Brno a LF MU provedeno celkem 1352 hysteroskopií a/nebo separovaných abrazí. Z toho 754 (56 %) pacientek bylo starších než 50 let a bylo zařazeno do studie.

Perimenopauza

Celkem 174 pacientek bylo zařazeno do skupiny perimenopauzálních žen, průměrný věk 52 let, z nich bylo 145 (83 %) symptomatických. Nejčastějšími důvody k biopsii v této skupině žen (graf 1) bylo nepravidelné (n = 88; 51 %) nebo akutní nadměrné krvácení (n = 43; 25 %), dále pak SA u asymptomatické (ve smyslu krvácení/špinění) pacientky před plánovanou hysterektomií (n = 24; 14 %), podezření na polyp (symptomatický: n = 11; 6 %; asymptomatický: n = 6; 3 %), jiná indikace (n = 2; 1 %). Histologicky se jednalo o benigní endometrium (n = 114; 65 %), polyp (n = 36; 21 %), myom (n = 4; 2 %), karcinom endometria (n = 5; 3 %), atypickou hyperplazii (n = 2; 1 %), u jedné pacientky se vyskytla kompletní mola (1 %) a u 12 (7 %) nebyl získán hodnotitelný materiál. Všechny pacientky s prekancerózou a karcinomem byly symptomatické nepravidelným nebo nadměrným krvácením. Medián výšky endometria v UZ obraze u pacientek s prekancerózou/malignitou byl shodný s mediánem u benigních nálezů (8 mm vs. 8 mm, p = 0,448). Medián největšího rozměru polypu byl 18 mm, u žádné pacientky nebyla nalezena atypic-



Graf 1 Perimenopauzální pacientky – důvody k provedení HSK/SA a výsledek histologie

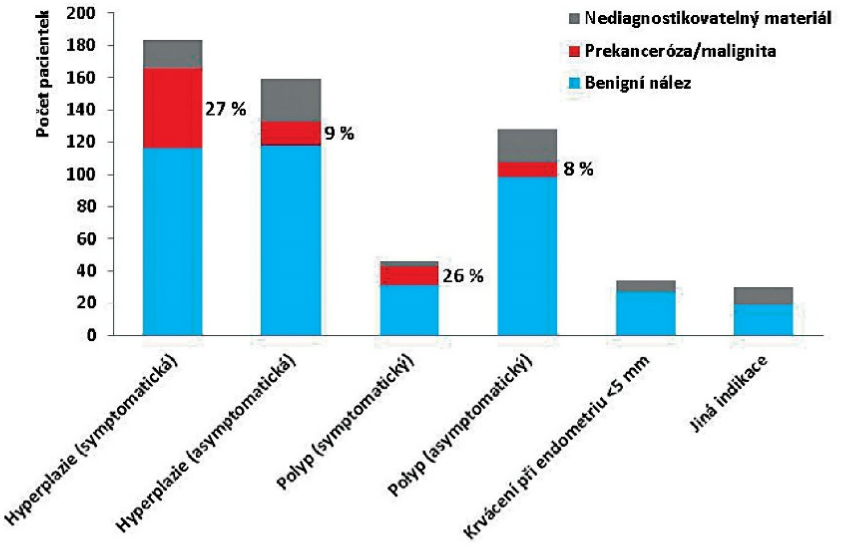
Tab. 1 Postmenopauzální pacientky indikované k biopsii – charakteristika souboru

	Celý soubor n = 580*	Benigní nález n = 408	EIN**, malignita n = 6	p-hodnota
Věk průměr ± SD	65 ± 8,7	64 ± 8,5	69 ± 8,4	**<0,001**
Gravidita medián (rozmezí)	2 (0–11)	2 (0–9)	2 (0–11)	0,417
Parita medián (rozmezí)	2 (0–5)	2 (0–5)	2 (0–5)	0,460
Věk menopauzy medián (rozmezí)	50 (40–60)	50 (40–60)	51,5 (40–58)	**0,043**
Hypertenze	346 (60 %)	226 (55 %)	68 (79 %)	**<0,001**
Diabetes mellitus	102 (18 %)	59 (14 %)	27 (31 %)	**<0,001**
Tamoxifen	19 (3 %)	13 (3 %)	2 (2 %)	1,000
Symptomy***	268 (46 %)	177 (43 %)	61 (71 %)	**<0,001**
Výška endometria (mm) medián (rozmezí)	8 (1–50)	8 (1–45)	15 (2–50)	**<0,001**
Polyp podle UZ	189 (33 %)	140 (34 %)	23 (27 %)	0,207
Největší rozměr polypu (mm)**** medián (rozmezí)	13 (3–42)	13 (3–42)	13 (3–40)	0,274

*celý soubor obsahuje i data pacientek, u kterých nebyl získán diagnostikovatelný materiál
**EIN (endometrial intraepithelial neoplasia) = atypická hyperplazie
***krvácení nebo špinění
****polyp detekovaný UZ a potvrzený histologicky

Tab. 2 Hyperplazie v UZ obraze – charakteristika UZ obrazu, výsledná histologie

	Počet	Výška endometria (mm) medián (rozmezí)	Histologie		
			Benigní nález	EIN, malignita	Nediagnost. materiál
Symptomatická hyperplazie	183	11 (5–46)	116 (64 %)	50 (27 %)	17 (9 %)
Asymptomatická hyperplazie	159	10 (5–45)	118 (74 %)	15 (9 %)	26 (17 %)
		p = 0,005	**OR 3,391** (95% CI: 1,804–6,375); **p < 0,001**		



Graf 2 Postmenopauzální pacientky – důvody k provedení HSK/SA a výsledek histologie

ká hyperplazie/karcinom v polypu. Při hodnocení známých rizikových faktorů (hypertenze, diabetes mellitus, užívání tamoxifenu) nebyl statisticky významný rozdíl mezi pacientkami s benigní histologií a prekancerózou/malignitou.

Postmenopauza

Do skupiny postmenopauzálních žen bylo zařazeno 580 pacientek, průměrný věk 65 let, medián nástupu menopauzy 50 let, 268 (46 %) bylo symptomatických. Podrobná charakteristika souboru je uvedena v tabulce 1. Nejčastějším důvodem k bioptické verifikaci (graf 2) byla hyperplazie v UZ obraze (n = 342; 59 %), dále polyp (n = 174; 30 %), krvácení při endometriu < 5 mm (n = 34; 6 %) nebo jiná indikace u asymptomatické pacientky (n = 30; 5 %).

Nález hyperplazie/polypu v UZ obraze – jejich rizikovost z pohledu výskytu prekancerózy/malignity s ohledem na klinické projevy

U pacientek s hyperplazií v UZ obraze (tab. 2) se u asymptomatických (n = 159) histologicky potvrdila prekancerózа/malignita v 15 případech (9 %), u symptomatických (n = 183) to bylo v 50 (27 %) případech. Symptomatické pacientky měly zvýšené riziko výskytu karcinomu více než třikrát (OR 3,39, 95% IS: 1,804–6.375; p < 0,001). Podle ultrazvukového obrazu nálezu hyperplazie jsme vypočítali senzitivitu a specificitu ultrazvuku v detekci karcinomu při výšce endometria 5, 8 a 12 mm (tab. 3). U asymptomatických pacientek bylo riziko statisticky významné až u cut-off 12 mm (3,54, 95% IS: 1,186–10,587; p = 0,024).

Tab. 3 Senzitivita a specificita diagnostiky karcinomu endometria podle ultrazvuku při vybraných cut-off výškách endometria u pacientky asymptomatické a symptomatické; stanovení rizika výskytu malignity u pacientky v závislosti na kombinaci ultrazvukové hyperplazie a symptomatologie (OR)

	Výška endometria v UZ obraze (mm)		
Cut-off	**≥ 5**	**≥ 8**	**≥ 12**
Asymptomatická pacientka			
Senzitivita (%)	100	87	60
Specificita (%)	38	52	86
OR	3,787	2,383	3,543
95% IS	0,214–66,954	0,510–11,131	1,186–10,587
p-hodnota	0,364	0,269	**0,024**
Symptomatická pacientka			
Senzitivita (%)	100	94	86
Specificita (%)	24	48	71
OR	16,593	15,374	11,886
95% IS	0,985–279,569	3,584–65,949	4,935–28,627
p-hodnota	0,051	**<0,001**	**<0,001**

U symptomatických pacientek bylo riziko vysoké u všech cut-off, ale při širokém intervalu spolehlivosti, statisticky významné u cut-off 8 mm (15,37, 95% IS: 3,584–65,949; p < 0,001) a 12 mm (11,88, 95% IS: 4,935–28,627; p < 0,001).

V případě polypu v UZ obraze (tab. 4) je zřejmé, že symptomatické polypy byly statisticky významně větší než asymptomatické (p = 0,017). U asymptomatických pacientek s polypem (n = 128) byla v 10 případech (8 %) potvrzena atypická hyperplazie/karcinom, u symptomatických (n = 46) to bylo ve 12 případech (26 %). Ve skupině symptomatických pacientek bylo více než trojnásobně zvýšené riziko (OR 3,79, 95% IS: 1,495–9,628; p = 0,005) nálezu karcinomu v polypu.

Prekanceróza/karcinom – jeho ultrazvukový obraz a klinické projevy

Histologicky benigní nálezy častěji korespondovaly s UZ nálezem nižšího endometria než případy, kdy se jednalo o prekancerózu/malignitu (medián 7 vs. 16 mm, p < 0,001), 74 % případů oproti 59 % (p = 0,058) bylo doprovázeno krvácením nebo špiněním (tab. 5). Histologicky se jednalo o atypickou hyperplazii (n = 13; 15 %), karcinom endometria (n = 70; 80 %), metastázy do dělohy (n = 2; 3 %), sarkom (n = 1; 1 %), karcinom děložního čípku (n = 1; 1 %).

Medián největšího rozměru benigního polypu byl 13 mm, stejný rozměr byl pak i v případě atypické hyperplazie/karcinomu v polypu (p = 0,274). Karcinomy v polypu častěji krvácely (p = 0,016).

Peroperační a pooperační komplikace způsobené biopsií endometria

Peroperační komplikace se v obou skupinách vyskytla u 13 pacientek (2 %) – ve čtyřech případech byl výkon označen jako technicky náročný a byl přivolán zkušenější lékař, třikrát došlo k perforaci dělohy, třikrát byl výkon předčasně ukončen pro riziko perforace, u dvou pacientek došlo k poranění čípku a krvácení, v jednom případě došlo k anesteziologické komplikaci (neúspěšná intubace). Závažné pooperační komplikace jsme nezaznamenali. Selhání metody ve smyslu nezískání diagnostikovatelného materiálu bylo v 97 případech (13 %). U pěti z těchto pacientek (5 %) byl z definitivní histologie potvrzen karcinom, z toho čtyři pacientky byly symptomatické a všechny byly postmenopauzální.

DISKUSE

Hyperplazie a polyp endometria patří k běžným nálezům při ultrazvukovém vyšetření, které se v dnešní době stalo součástí pravidelné gynekologic-

ké prohlídky. Nejčastějším důvodem k biopsii endometria je obava z maligního procesu, ale vzhledem k četnosti výskytu těchto patologií děložní dutiny by snaha o bioptickou verifikaci všech nálezů vedla k neúměrnému množství zákroků a operační zátěži pacientek. Selekce případů vhodných ke konzervativnímu postupu je proto velmi aktuální téma.

V perimenopauze byly v našem souboru všechny nálezy prekancerózy/karcinomu symptomatické, nezaznamenali jsme jediný takový nález v polypu. To podporuje závěry metaanalýzy souboru více než 35 000 žen [16], jejíž autoři doporučují premenopauzální asymptomatické pacientky dispenzarizovat pomocí UZ a biopsii indikovat pouze v případě symptomatologie nebo infertility. U malých polypů může dojít ve 27 % případů dokonce ke spontánní regresi nálezu [9].

Postmenopauzální pacientky s nálezem karcinomu endometria v našem souboru vykazovaly známé epidemiologické vlastnosti: vyšší věk, hypertenze, cukrovka, byly většinou symptomatické a s nálezem vyššího endometria podle UZ (u všech $p \leq 0{,}001$).

V roce 2004 byla vydána dosud největší metaanalýza na kohortě 100 000 žen ve věku vyšším než 50 let, ve které autoři stanovili riziko výskytu karcinomu endometria vztažené k jeho výšce měřené ultrazvukem jak u asymptomatických, tak symptomatických žen [15]. Jako optimální cut-off k selekci asymptomatických postmenopauzálních pacientek ve vysokém riziku výskytu malignity (a tedy indikovaných k biopsii) stanovili výšku endometria více než 11 mm (odpovídalo riziku výskytu karcinomu 6,7 %). Toto riziko je srovnatelné s pacientkami, které jsou symptomatické s endometriem nad 5 mm.

Následně vyšla celá řada studií, ve kterých se autoři snaží nalézt optimální hranici výšky endometria rozdělující pacientky na nízce a vysoce rizikové. Závěry těchto studií a z nich vyplývající doporučení se často velmi liší. Od velmi striktního čínského doporučení referovat všechny postmenopauzální pacientky s endometriem > 5 mm nebo polypem k hysteroskopii [10], přes kanadské guidelines a další studie doporučující zmíněnou hranici 11 mm [1, 18], najdeme i autory, kteří preferují konzervativní postup u všech asymptomatických pacientek [8, 11].

V našem souboru asymptomatických postmenopauzálních pacientek klesala senzitivita záchytu karcinomu podle ultrazvukové hranice hyperplazie 5 mm, 8 mm a 12 mm ze 100 % na 87 %, respektive 60 %, při nárůstu specificity z 38 % na 52 % respektive 86 %. Riziko nálezu karcinomu při asymptomatické hyperplazii se nehledě na výšku endometria v zásadě neměnilo (OR 3,79; 2,38 a 3,54). Statisticky

Tab. 4 Polyp v UZ obraze – charakteristika UZ obrazu, výsledná histologie

	Počet	Největší rozměr (mm) medián (rozmezí)	Histologie		
			Benigní nález	EIN, malignita	Nediagnost. materiál
Symptomatický polyp	46	19 (3–42)	31 (67 %)	12 (26 %)	3 (7 %)
Asymptomatický polyp	128	12 (3–40)	98 (76 %)	10 (8 %)	20 (16 %)
		0,017	**OR 3,794** (95% IS: 1,495–9,628); **p = 0,005**		

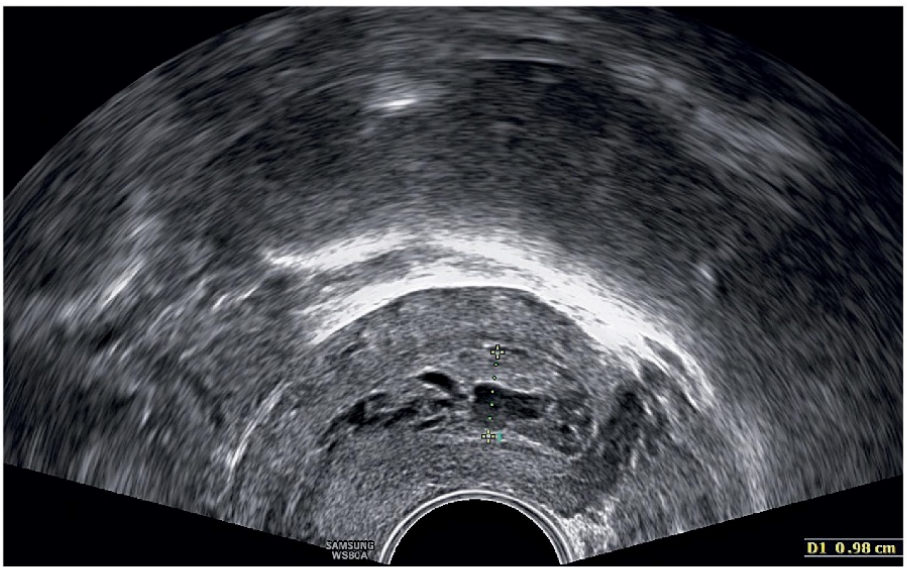
Tab. 5 Výsledek histologie – charakteristika UZ obrazu, symptomatologie

	Počet	Výška endometria (mm) medián (rozmezí)	p	Symptomy	p
Benigní nálezy endometria	145	7 (1–45)	**<0,001**	86 (59 %)	0,058
EIN, malignita	62	16 (6–50)		46 (74 %)	
Nediagnostikovatelný materiál	84	7 (1–46)		29 (35 %)	
	Počet	**Největší rozměr polypu (mm) medián (rozmezí)**	**p**	**Symptomy**	**p**
Benigní polyp	264	13 (3–42)	0,274	91 (34 %)	**0,016**
EIN/malignita v polypu	25	13 (3–40)		15 (60 %)	

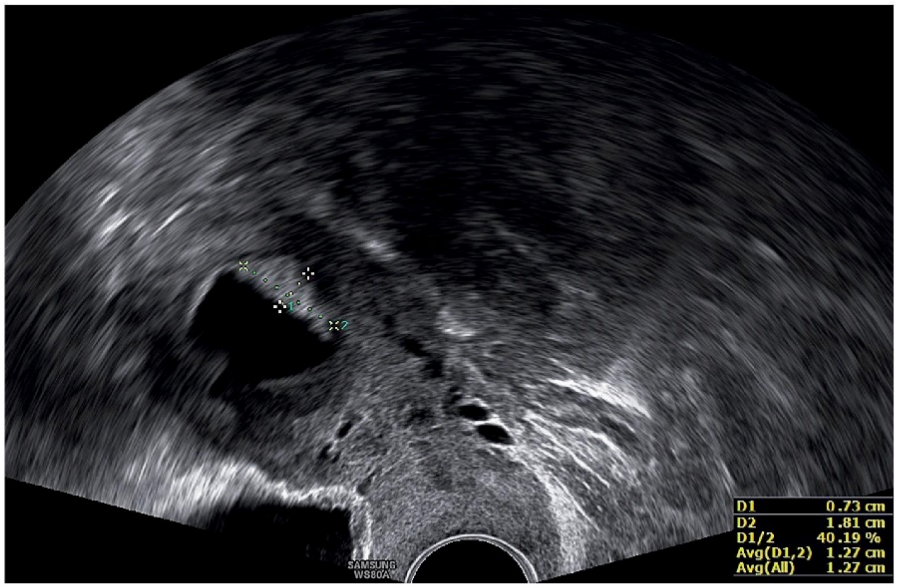
významné riziko bylo pouze u cut-off 12 mm (p = 0,024). Oproti tomu u symptomatických pacientek bylo riziko výskytu malignity dramaticky vyšší (OR 16,59; 15,37 a 11,88), nicméně při velmi širokých intervalech spolehlivosti. Statisticky významné riziko bylo u cut-off 8 mm (minimálně 3,58) a 12 mm (minimálně 4,93). Senzitivita detekce karcinomu klesala ze 100 % přes 94 % na 86 % a specificita stoupala z 24 % na 48 % a 71 %. Senzitivita a specificita pro jednotlivé výšky endometria byla v našem souboru srovnatelná s dalšími studiemi [15], ale hodnocení rizika pro jednotlivé výšky endometria stanovením OR není v literatuře k dispozici. I přesto, že až 90 % karcinomů se projevuje krvácením a riziko se dramaticky zvyšuje až právě se symptomatologií pacientky [7, 17], stále jsou histologicky ověřovány i asymptomatické pacientky ve snaze o brzký záchyt maligního onemocnění ještě před vznikem příznaků. Tento postup sice vede k diagnostice více pacientek v počátečním stadiu onemocnění (T1a stadium v 82 % u asymptomatických vs. 66 % u symptomatických) a méně časté indikaci adjuvantní radioterapie (31 % vs. 41 %), nicméně v souboru 1607 pacientek s karcinomem endometria nebyl shledán žádný statisticky významný rozdíl u pětiletého přežití bez recidivy (79 % vs. 79 %), specifického přežití (83 % vs. 82 %) a celkového přežití (80 % vs. 77 %) [8]. Autoři studie tak doporučují indikovat bioptické vyšetření pouze u pacientek symptomatických anebo při výrazné změně UZ obrazu v čase.

V našem souboru byla statisticky významně větší výška endometria u symptomatické pacientky (p = 0,005) za současně častějšího nálezu karcinomu (p < 0,001). Symptomatický polyp byl větší než asymptomatický (p = 0,017), kdy ve skupině symptomatických byl nález karcinomu častější (p = 0,005). Z definitivní histologie ale nebyl shledán rozdíl mezi velikostí benigního a maligního polypu (p = 0,274). Karcinomy se významně častěji projevovaly krvácením (p = 0,016). To je v souladu se studií, kde se autorům také nepodařil stanovit cut-off velikosti polypu v UZ obraze pro diagnostiku karcinomu [2]. Například Cavkaytar et al. stanovili hranici velikosti polypu 11,5 mm, u které byla při specificitě 86 % senzitivita pouze 54 % [3]. Fernández-Para et al. dokonce prokázali, že největší riziko malignity v polypu je při velikosti od dvou do tří centimetrů a s dalším růstem velikosti pak riziko klesá [5]. Samotná velikost polypu velmi pravděpodobně tedy nehraje zásadní roli a je třeba prospektivně definovat relevantní UZ parametry pro karcinom v polypu (obr. 1, 2).

Nedoporučujeme rutinně provádět biopsii endometria u asymptomatických pacientek s nesuspektním ultrazvukovým obrazem před plánovanou hysterektomií. V našem souboru bylo u perimenopauzálních pacientek při této indikaci nejčastější selhání metody (až ve 29 % případů). U pacientek referovaných k biopsii z jiného důvodu, než byl nález polypu či hyperplazie (krvácení při endometriu < 5 mm, SA před plánovanou



Obr. 1 Degenerativně změněný polyp bez patologické vaskularizace – nesuspektní



Obr. 2 Hyperechogenní nepravidelné ložisko, fluidometra a hematometra – suspektní

hysterektomií u asymptomatické pacientky, SA při extrakci nitroděložního tělíska, UZ obraz mukometry), se žádná prekanceróza či malignita neobjevila.

Výskyt peroperačních komplikací v našem souboru byl poměrně nízký a odpovídá dolní hranici rozmezí udávané v literatuře [6, 11]. Nicméně pokud bychom za komplikaci považovali i selhání metody ve smyslu nezískání diagnostikovatelného materiálu (v 79 % případů z pouhé SA bez HSK), dostali bychom se až na 15 % komplikací. V případě ložiskové léze podezřelé z malignity bychom tedy i za cenu větší zátěže pacientky měli indikovat HSK s odběrem biopsie pod vizuální kontrolou, kde je menší riziko selhání metody, a samostatnou SA rezervovat pro případy akutního krvácení [16].

Slabinou naší studie je retrospektivní sběr dat, která tímto mohou být neúplná. Jedná se o data z univerzitního pracoviště, kde se na diagnostice i operačních biopsiích podílejí mladší lékaři v přípravě na atestaci, což může mít negativní vliv na kvalitu dat. Na druhou stranu tento přístup zcela odpovídá reálné klinické praxi a situace, kdy by tyto ženy byly vyšetřeny expertním sonografistou či biopsii prováděl pouze atestovaný lékař, jsou iluzí. Výhodou studie je poměrně velký počet případů za pouhé dva roky, s kompletními klinickými, patologickými i ultrazvukovými charakteristikami, což dává možnost do budoucna soubor rozšířit a zaměřit se na onkologické výsledky časné diagnostiky karcinomu endometria pomocí ultrazvuku a symptomatologie. Díky těmto kritériím a komplexnosti klinického obrazu zpochybňujeme paušální indikaci biopsie děložní dutiny na základě rutinního UZ vyšetření bez ohledu na symptomatologii pacientky. Ultrazvuk sám o sobě nemá dostatečnou přesnost pro detekci karcinomu endometria a pro screening tohoto onemocnění není dostatečně spolehlivý. Je nutné hledat nové metody pro časnou a přesnou diagnostiku tohoto zhoubného onemocnění, které budou velmi pravděpodobně založeny na detekci specifických molekulárně biologických markerů ze stěru pochvy, hrdla dělohy či odběru periferní krve pacientky [13, 14].

ZÁVĚR

Nález polypu a hyperplazie děložní dutiny na ultrazvukovém vyšetření nejsou automatickou indikací k bioptické verifikaci. Ultrazvuk není přesnou metodou v diagnostice prekancerózy a karcinomu endometria. Je jen jedním dílem mozaiky, která je složena z výsledků zobrazovací metody, ze symptomatologie, celkového zdravotního stavu s přihlédnutím k rizikům zákroku, včetně relativně vysokého procenta rizika selhání metody z důvodů nezískání dostatečně reprezentativního materiálu k histologické verifikaci. U postmenopauzálních pacientek jsme prokázali, že krvácení nebo špinění je velmi silným rizikovým faktorem

pro přítomnost prekancerózy či malignity endometria, a to v případě podezření na hyperplazii i polyp bez ohledu na jejich velikost. Ve skupině perimenopauzálních pacientek byly dokonce všechny ženy s tímto histologickým nálezem symptomatické.

U všech asymptomatických pacientek doporučujeme zvážit konzervativní postup s přihlédnutím ke zdravotnímu stavu a případným rizikovým faktorům, případně provádět biopsii až u výšky endometria 12 a více mm. U ložiskových lézí by metodou volby měla být hysteroskopie a samostatná separovaná abraze by měla být rezervována pro případy silného krvácení nebo jednoznačně patologického nálezu v děložní dutině ve smyslu přítomnosti velkoobjemového tumoru.

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LITERATURA

1. **Alcázar, JL., Bonilla, L., Marucco, MD., et al.** Risk of endometrial cancer and endometrial hyperplasia with atypia in asymptomatic postmenopausal women with endometrial thickness ≥ 11 mm: A systematic review and meta-analysis. J Clin Ultrasound, 2018, 46, p. 565–570.
2. **Bel, S., Billard, C., Godet, J., et al.** Risk of malignancy on suspicion of polyps in menopausal women. Eur J Obstet Gynecol Reprod Biol, 2017, 216, p. 138–142.
3. **Cavkaytar, S., Kokanali, MK., Ceran, U., et al.** Roles of sonography and hysteroscopy in the detection of premalignant and malignant polyps in women presenting with postmenopausal bleeding and thickened endometrium. Asian Pac J Cancer Prev, 2014, 15, 13, p. 5355–5358.
4. **Epstein, E., Fischerova, D., Valentin, L., et al.** Ultrasound characteristics of endometrial cancer as defined by International Endometrial Tumor Analysis (IETA) consensus nomenclature: prospective multicenter study. Ultrasound Obstet Gynecol, 2018, 51, 6, p. 818–828.
5. **Fernández-Parra, J., Rodríguez Oliver, A., López Criado, S., et al.** Hysteroscopic evalutaion of endometrial polyps. Int J Gynaecol Obstet, 2006, 95, 2, p. 144–148.
6. **Ferrazzi, E., Zupi, E., Leone, FP., et al.** How often are endometrial polyps malignant in asymptomatic postmenopausal women? A multicenter study. Am J Obstet Gynecol, 2009, 200, p. 235.e1–235.e6.
7. **Frühauf, F., Dvořák, M., Haaková, L., et al.** Ultrazvukový staging karcinomu endometria – doporučená metodika vyšetření. Čes Gynek, 2014, 79, 6, s. 466–476.
8. **Gemer, O., Segev, Y., Helpman, L., et al.** Is there a survival advantage in diagnosing endometrial cancer in asymptomatic postmenopausal patients? An Israeli Gynecology Oncology Group study. Am J Obstet Gynecol, 2018, 219, p. 181.e1–181e6.
9. **Gynecological endokrinology collaboration of Chinese Society of Obstetrics and Gynecology.** Diagnosis and treatment of abnormal uterine bleeding. Chin J Obstet Gynecol, 2014, 39, 11, p. 80–86.
10. **Jiang, T., Yuan, Q., Zhou, Q., et al.** Do endometrial lesions require removal? A retrospective study. BMC Womens Health, 2019, 6, 19, p. 61.
11. **Jokubkiene, L., Sladkevicius, P., Valentin, L.** Transvaginal ultrasound examination of the endometrium in postmenopausal women without vaginal bleeding. Ultrasound Obstet Gynecol, 2016, 48, p. 390–396.
12. **Leone, FPG., Timmerman, D., Bourne, T., et al.** Terms, definitions and measurements to describe the sonographic features of the endometrium and intrauterine lesions: a consensus opinion from the International Endometrial Tumor Analysis (IETA) group. Ultrasound Obstet Gynecol, 2010, 35, p. 103–112.
13. **Martiqnetti, JA., Pandya, D., Naqarsheth, N., el al.** Detection of endometrial precancer by a targeted gynecologic cancer liquid biopsy. Cold Spring Harb Mol Case Stud, 2018, 4(6).
14. **Rižner, TL.** Discovery of biomarkers for endometrial cancer: current status and prospects. Expert Rev Mol Diagn, 2016, 16(12), p. 1315–1336.
15. **Smith-Bindman, R., Weiss, E., Feldstein, V.** How thick is too thick? When endometrial thickness should prompt biopsy in postmenopausal women without vaginal bleeding. Ultrasound Obstet Gynecol, 2004, 24, 5, p. 558–565.
16. **Uglietti, A., Buggio, L., Farella, M., et al.** The risk of malignancy in uterine polyps: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol, 2019, 237, p. 48–56.
17. **Van den Bosch, T., Ameye, L., Van Schoubtoeck, D., et al.** Intra-cavitary uterine pathology in women with abdormall uterine bleeding: a prospective study of 1220 women. Facts views Vis Obgyn, 2015, 7, 1, p. 17–24.
18. **Wolfman, W.** Asymptomatic endometrial Thickening. J Obstet Gynaecol Can, 2018, 40, 5, p. e367–e377.

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3.2 Koblížková M, Bretová P, Felsinger M, Minář L, Bednaříková M, Weinberger V. Faktory zvyšující riziko malignity při nálezu endometriálního polypu v ultrazvukovém obraze. Čes. Gynek. 2024; 89(1): 44–51.

This article presents a comprehensive review of preoperative risk factors associated with an increased likelihood of malignancy in endometrial polyps diagnosed by ultrasound, intending to refine patient selection for endometrial biopsy.

Ultrasonographic features indicative of possible malignancy include a thickened endometrium ranging from 11 to 26 mm, heterogeneous echogenicity, absence or indistinct visualization of the endometrial midline, and an irregular endomyometrial junction. Doppler imaging often reveals a high color score (3 to 4), corresponding to increased vascularity, branching of vessels from one or multiple entry points, and an overall inhomogeneous perfusion pattern. In contrast, a benign polyp typically appears as a sharply demarcated, hyperechoic or mixed-echogenic lesion with a single dominant feeding vessel without branching and a low color score.

Besides ultrasound features, the most significant clinical predictors of malignancy in an endometrial polyp are abnormal uterine bleeding (particularly in postmenopausal women), advanced age, particularly over 59 years, and elevated BMI. Another notable risk factor is Lynch syndrome, which is associated with a lifelong elevated risk of developing endometrial carcinoma. Other potential, yet inconsistently confirmed, risk factors include polyp size, type 2 diabetes mellitus, positive family history, and tamoxifen use. Histopathological examination remains the diagnostic gold standard for distinguishing between benign and malignant lesions. Ultrasound serves as a critical tool for guiding further diagnostic and therapeutic interventions, with its diagnostic value significantly enhanced when interpreted in the context of the patient's clinical presentation and overall risk profile.

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Faktory zvyšující riziko malignity při nálezu endometriálního polypu v ultrazvukovém obraze

Ultrasound finding of endometrial polyp and factors increasing risk of malignancy

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Souhrn: Cílem článku je přinést ucelený přehled rizikových faktorů a jejich vztahu ke vzniku malignity endometria při ultrazvukovém nálezu endometriálního polypu. Přehledová práce byla vytvořená systematickým sběrem a tříděním původních prací, review a metaanalýz se vztahem k problematice endometriálních polypů a riziku přítomnosti malignity. Každý jednotlivý předpokládaný rizikový faktor byl samostatně podroben rozboru literatury. V případě abnormálního děložního krvácení, vyššího věku pacientky a hodnoty indexu tělesné hmotnosti se výsledky studií poměrně jednotně shodují na vysokém riziku přítomnosti malignity v děložním polypu. Vysoké riziko mají také pacientky s Lynchovým syndromem. Co se týče potenciálních rizikových faktorů, jako je počet a velikost polypu, diabetes, hypertenze či pozitivní rodinná anamnéza, není dostatek dat k jasnému závěru a/nebo se publikované výsledky liší.

Klíčová slova: endometriální polyp – rizikové faktory – karcinom endometria – velikost polypu – tamoxifen – abnormální děložní krvácení

Summary: This article presents a comprehensive review of factors that increase the risk of malignancy in ultrasound findings of an endometrial polyp. We collected original studies, reviews, and meta-analyses that dealt with the topic of endometrial polyps and the risk of developing endometrial cancer. Each presumed risk factor was analysed individually. According to searched studies, abnormal uterine bleeding, old age, and body mass index are valid risk factors for developing endometrial cancer in endometrial polyps. Lynch syndrome patients are also in a high-risk group for endometrial cancer. On the other hand, the number of polyps, their size, diabetes mellitus, hypertension, and positive family history are factors with inconclusive results. There are either not enough data or different results among several studies.

Key words: endometrial polyp – risk factors – endometrial carcinoma – polyp size – tamoxifen – abnormal uterine bleeding

Úvod

Endometriální polyp vzniká lokálním růstem endometriální tkáně skládající se ze žlázek, stromatu a cév endometria, které jsou pokryty epiteliální tkání. Jejich velikost se pohybuje od pár milimetrů do několika centimetrů. V děložní dutině se mohou nacházet jednotlivě i ve větším počtu [1]. Endometriální polyp je nejčastějším benigním pre- i postmenopauzálním patologickým nálezem v dutině děložní. Polypy mohou být klinicky symptomatické i asymptomatické. Dle studie IETA3 (International Endometrial Tumor Analysis) publikované v roce 2022 byla u 1 745 asymptomatických pacientek (bez přítomnosti postmenopauzálního krvácení nebo krvácení mimo cyklus) incidence nálezu endometriálního polypu 58,9 % (66 % premenopauzální, 52 % postmenopauzální) [2]. Základní prebioptickou diagnostickou metodou je transvaginální ultrazvuk (TVS). V případě rozhodnutí o chirurgické léčbě endometriálních polypů je zlatým standardem jejich resekce za pomoci operačního hysteroskopu [3].

Dosud prokázanými rizikovými faktory pro vznik endometriálních polypů jsou vysoký věk, hypertenze, obezita, syndrom polycystických ovarií, pozdní menopauza, užívání tamoxifenu a další stavy spojené s hyperestrinizmem (časná menarche, pozdní menopauza, nulliparita), zatížená rodinná anamnéza a genetická predispozice [3–5]. Tyto faktory se shodují s rizikovými faktory vzniku karcinomu endometria. Endometriální karcinom (EC) je nejčastější gynekologicko-onkologické onemocnění v rozvinutých zemích [5]. V ČR byla v roce 2021 incidence onemocnění 35/100 000 osob [6]. V závislosti na jednotlivých rizikových faktorech a jejich kombinaci se prevalence vzniku EC u premenopauzálních i postmenopauzálních

Tab. 1. Srovnání výsledků studií zabývajících se abnormálním ultrazvukovým nálezem v dutině děložní u post- i premenopauzálních pacientek.
Tab. 1. A comparison of studies dealing with the uterine cavity abnormal ultrasound findings in post- and premenopausal patients.

Histopatologické nálezy	Garuti et al. [17], n = 1 519	Patrizi et al. [10], n = 1 020
	% (n)	
endometriální polyp	92,4 (1 404)	83,5 (852)
polyp s atypickou hyperplazií	1,1 (17)	2,2 (22)
karcinom endometria	1,0 (15)	1,2 (12)
ostatní benigní nálezy	5,5 (83)	13,1 (134)

n – počet

žen v souvislosti s výskytem polypu pohybuje v rozmezí 0,5–3 % [7–9]. Ve studii Garudi et al. zaměřené na pacientky s ultrazvukovým nálezem endometriálního polypu byla provedena hysteroskopická polypektomie u 1 519 pacientek. Nález polypu bez atypií byl u 92,4 % (1 404) pacientek. V 1,1 % (17) případů byl nález atypického polypu s atypickou hyperplazií a v 1,0 % (15) případů nález EC [3].

V roce 2022 byla uskutečněna rozsáhlá studie Patrizi et al., kdy byla provedena hysteroskopie 1 020 pacientkám s abnormálním ultrazvukovým nálezem v dutině děložní. Jednalo se o pacientky premenopauzální (403) i postmenopauzální (617). U premenopauzálních pacientek byl potvrzen v 90,0 % (399) benigní nález, kterým byl nejčastěji (v 77,4 %) endometriální polyp. EC se u dané skupiny nevyskytl, v 1 % byl nález atypické hyperplazie. V případech postmenopauzálních pacientek se benigní nález vyskytl u 95,1 % (587), kdy endometriální polyp byl přítomen v 87,5 % případů (540). EC se našel v 1,9 % (12) případů, atypická hyperplazie v 2,9 % (18) případů (tab. 1) [10].

Diagnóza benigního polypu je stanovena definitivním histologickým vy-

Tab. 2. Přehled použitých studií zabývajících se rizikovými faktory vzniku endometriálního karcinomu v polypu.
Tab. 2. An overview of studies dealing with risk factors for the endometrial cancer development in a polyp.

Autor	Rok	Země	Design studie	Počet pacientek	Rizikový faktor
Mbatsogo et al. [38]	2005	Francie	retrospektivní	108	tamoxifen
Fernándes-Para et al. [16]	2006	Španělsko	retrospektivní	653	velikost polypu
Giordano et al. [33]	2007	Itálie	retrospektivní	6	DM 2. typu BMI
Ferazzi et al. [18]	2009	Itálie	retrospektivní multicentrická	1 922	velikost polypu
Lee et al. [25]	2010	Jižní Korea	retrospektivní	287	abnormální děložní krvácení tamoxifen
Wethington et al. [1]	2011	USA	retrospektivní	1 011	věk abnormální děložní krvácení
Manchanda et al. [30]	2012	VB	prospektivní	69	Lynchův syndrom
Win et al. [26]	2015	USA	metaanalýza	86 856	rodinná anamnéza
Elfayomy et al. [35]	2015	Egypt	prospektivní	150	BMI
Bel et al. [14]	2017	Francie	retrospektivní	631	věk rodinná anamnéza
Ghoubara et al. [34]	2018	VB	prospektivní	2 625	DM 2. typu BMI tamoxifen
Garuti et al. [17]	2019	Itálie	prospektivní	1 436	velikost polypu BMI
Uglietti et al. [21]	2019	Itálie	metaanalýza	35 345	abnormální děložní krvácení
Yela et al. [39]	2019	Brazílie	retrospektivní	675	tamoxifen
Vinklerová et al. [15]	2020	ČR	retrospektivní	754	velikost polypu
Wong et al. [11]	2021	VB	prospektivní	240	věk BMI
Xu et al. [19]	2022	Čína	retrospektivní multicentrická	16 020	velikost polypu věk abnormální děložní krvácení
Patrizi et al. [10]	2022	Itálie	prospektivní	1 020	DM 2. typu BMI

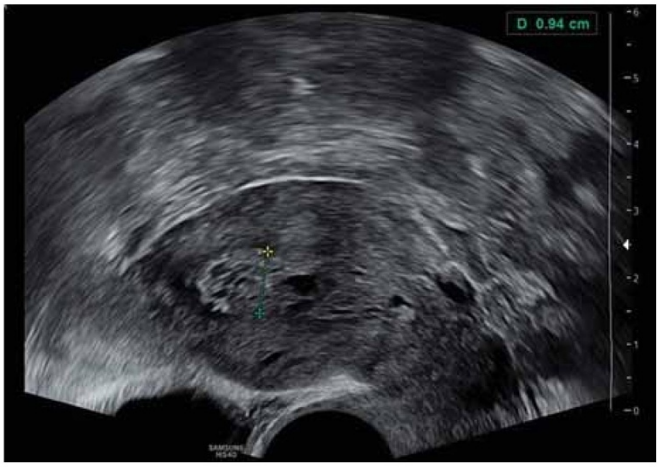
BMI – index tělesné hmotnosti, DM – diabetes mellitus, USA – Spojené státy americké, VB – Velká Británie

šetřením. Jedná se ovšem o invazivní metodu, která má svoje limity a rizika. U některých pacientek není technicky možné provedení biopsie z dutiny děložní, u některých je samotný výkon z důvodů vážných komorbidit kontraindikovaný. V určitém procentu bude operační zákrok nevýtěžný, neboť nebude získáno adekvátní množství materiálu pro histologické vyšetření, a přitom obraz patologické léze v dutině děložní bude perzistovat. V těchto specifických případech přistupujeme k nálezu konzervativně a snažíme se provádět pravidelná ultrazvuková vyšetření, jejichž cílem bude odhalit změnu v ultrazvukovém obraze a dát ji do korelace s případnými klinickými projevy pacientky [11].

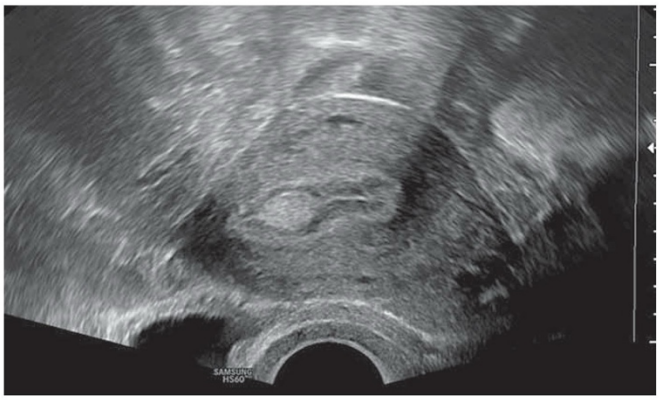
Cílem článku je přinést ucelený přehled o možných rizikových faktorech vzniku malignity v endometriálním polypu a jejich významnosti. Kromě epidemiologických rizikových faktorů zmíníme i rizikové ultrazvukové charakteristiky, které byly definovány v rámci terminologie IETA (International Endometrial Tumor Analysis) [2,12,13]. Jedná se o kritické informace, které slouží k adekvátní informovanosti pacientky, pomohou lékaři v dalším diagnosticko-terapeutickém přístupu a ve stanovení adekvátního, na míru šitého postupu.

Metodika

Jedná se o přehledový článek. K vyhledání publikací byly použity databáze MEDLINE (PubMed) a EMBASE (ELSEVIER) za užití klíčových hesel pro vyhledávání: endometrial polyp* AND risk* AND (malignan* OR cancer OR carcinom*), polyp* AND endometrial* (malignan* OR cancer OR carcinom*), risk factor* AND endometrial (malignan* OR cancer OR carcinom*), tamoxifen AND endometrial (malignan* OR cancer OR carcinom*). Použity byly jednak původní práce, retrospektivní a prospektivní studie a také review a metaanalýzy. Ze sebíraných článků byly vyřazeny kazuistiky (tab. 2).



Obr. 1. Endometriální polyp cystického vzhledu u postmenopauzální pacientky.
Fig. 1. Cystic endometrial polyp in postmenopausal patient.

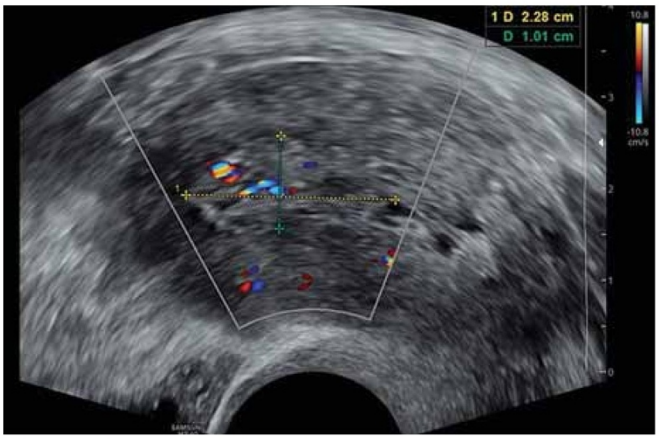


Obr. 2. Hyperechogenní homogenní endometriální polyp u premenopauzální pacientky.
Fig. 2. Hyperechogenic homogenous endometrial polyp in premenopausal patient.

Rizikové faktory

Ultrazvukové charakteristiky

Vyšetření TVS zlepšilo naši schopnost správně diagnostikovat intrauterinní abnormality a nastavit odpovídající terapii. V roce 2010 publikovala IETA konsenzus sjednocující názvosloví a definice užívané při popisu endometria a děložní dutiny za použití zobrazovacích metod – ultrazvukové sonografie, color flow dopplerovského měření a sonohysterografie [12]. Endometriální polyp je popisován při užití TVS jako lokalizovaný, ostře ohraničený, hyperechogenní nebo smíšeně echogenní útvar s cystickou strukturou (častěji v případě postmenopauzálních žen: 48 %, obr. 1), anebo bez přítomnosti cyst (více u žen premenopauzálních: 64 %, obr. 2). V případě postmenopauzálních žen se nezobrazuje střední linie (87 %). Kromě jednoho případu měly všechny polypy cévní



Obr. 3. Zásobení endometriálního polypu z jedné přívodné cévy bez větvení.
Fig. 3. Single dominant vessel without branching of endometrial polyp.

Tab. 3. Ultrazvukové charakteristiky endometriálních polypů u pacientek s abnormálním děložním krvácením dle Van den Bosch et al. [13]. Výsledky uvedeny jako: % (%CI).
Tab. 3. Ultrasound characteristics of endometrial polyps in patients with abnormal uterine bleeding according to Van den Bosch et al. [13]. Results reported as: % (%CI).

	Abnormální děložní krvácení	
Endometriální polyp	**premenopauzální**	**postmenopauzální**
lokalizovaný	97 % (95–100)	95 % (92–98)
pendulující	71 % (64–78)	72 % (66–78)
hyperechogenní	64 % (57–71)	27 % (22–33)
smíšené echogenní s cystami	8 % (4–12)	46 % (9–53)
ostře ohraničený	97 % (95–100)	92 % (89–96)
CS > 1, dominantní zásobní céva bez větvení	85 % (79–91)	71 % (64–79)

CS – color score

Tab. 4. Ultrazvukové nálezy endometriálních polypů u pacientek bez přítomnosti abnormálního děložního krvácením dle Heremans et al. [2].
Tab. 4. Ultrasound findings of endometrial polyps in patients without abnormal uterine bleeding according to Heremans et al. [2].

	Bez abnormálního děložního krvácení	
Endometriální polyp	**premenopauzální**	**postmenopauzální**
hyperechogenní	20 %	24 %
smíšeně echogenní	68 %	69 %
světlý okraj	80 %	51 %
pravidelná endo – myometriální junkce	95 %	79 %
CS 1	7 %	43 %
CS > 1, dominantní zásobní céva bez větvení	72 %	38 %

CS – color score

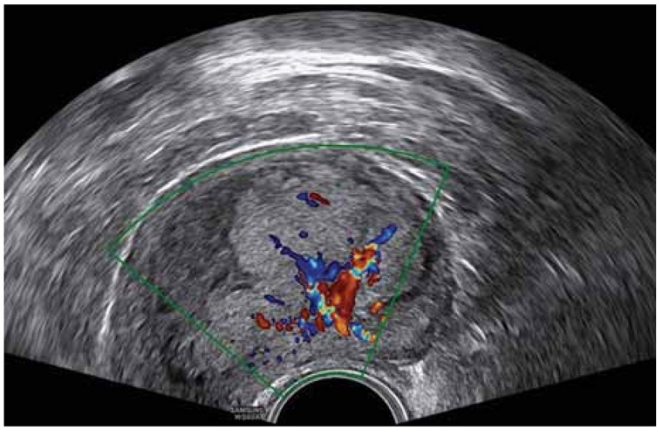
zásobení tvořeno jednou dominantní zásobující cévou (obr. 3). Celkem 71 % polypů při zobrazení pomocí sonohysterografie mělo pendulující charakter [13]. Novější studie Heremans et al. potvrzuje charakteristiky typické pro nález polypu uvedené v předchozí studii. Většina endometriálních polypů má přítomnu jednu dominantní zásobující cévu s dalším větvením, nebo bez něj. Při porovnání post- a premenopauzálních žen byl výskyt polypů u postmenopauzálních žen spojen s vyšším výskytem tekutiny v děložní dutině, cystickým endometriem a nižším color score. Pokud srovnáme případy s abnormálním děložním krvácením a bez něj, mají u asymptomatických žen polypy typicky smíšeně echogenní charakter, světlý okraj, pravidelnou hranici endometria s myometriem, dominantní zásobující cévu bez větvení a nižší color score (tab. 3, 4) [2].

V dostupné literatuře a dosud publikovaných pracích IETA group zabývajících se terminologií a popisem ultrazvukových nálezů není jasně definován popis EC v polypu. V hodnocení se proto vychází z charakteristik obrazu karcinomu endometria (obr. 4), kde je endometrium zesíleno (11–26 mm) s heterogenní echogenitou, nelze odlišit středovou linii a je nepřehledná endomyometriální junkce. Při vyšetření pomocí dopplerovského zobrazení je vysoké color score (3–4) s velkým množstvím cév větvených z jednoho nebo více vstupních míst [9].

Velikost polypu

Rozměry endometriálních polypů se obecně pohybují od několika milimetrů až po velmi objemné nálezy vyplňující dutinu děložní a případně i utlačující okolní myometrium. V menší práci z našeho pracoviště jsme neshledali signifikantní rozdíl mezi velikostí benigního a maligního polypu (medián největšího rozměru polypu v obou skupinách 13 mm; p = 0,274), což je v souladu i s některými dalšími studiemi, kde autoři nenachází vhodný cut-off velikosti polypu v UZ obraze pro diagnostiku kar-

cinomu [14,15]. Fernández-Para et al. dokonce prokázali, že největší riziko malignity v polypu je při velikosti od 2 do 3 cm a s dalším růstem velikosti pak riziko klesá [16]. Další autoři se snaží definovat statisticky významné rozdíly v rozměrech benigních a maligních nálezů, nicméně nenachází společný konsenzus. Garuti et al. uvádějí průměrnou velikost polypů s nálezem atypické histologie 24,5 mm v porovnání s polypy s normálním histologickým nálezem, kde se velikost pohybuje okolo 17,3 mm [17]. Retrospektivní multicentrická studie Ferrazzi et al. uvádí rozměr polypu 18 mm a více jako statisticky významný faktor pro výskyt abnormální histologie polypu [18]. Xu et al. popisují průměrný rozměr benigních polypů 13,4 ± 7 mm. Průměrné rozměry maligních polypů udávají 19,1 ± 10 mm (tab. 5) [19].



Obr. 4. Karcinom endometria – vysoké endometrium, nepravidelná endo-myometrální junkce, bohaté cévní zásobení (CS 4), více cév z jednoho vstupu.
Fig. 4. Endometrial cancer – thick endometrium, irregular endo-myometrial junction, abundant color flow (CS 4), multiple vessels with multifocal origin.

Věk

Endometriální polypy se u žen vyskytují ve všech věkových skupinách [20]. Zatímco pro premenopauzální ženy je výskyt polypu spíše spojen s reprodukčními problémy a malignita se objevuje vzácně (1,1 %), postmenopauzální status ženy se stává významným rizikem možného maligního zvratu (4,9 %) [21]. Analýza databáze 1 011 pacientek publikovaná Wethington et al. pracovala se skupinou, kde průměrný věk výskytu polypu byl 52 let. Endometriální karcinom nebo atypická hyperplazie, vzniklé v souvislosti s polypem, se diagnostikovaly u 18 žen, přičemž 15 (83 %) z nich bylo starších 50 let. Pokud tato data vztáhneme k celé kohortě zkoumaných pacientek, výskyt atypické hyperplazie a karcinomu endometria je u žen < 50 let 0,7 %, zatímco u žen > 50 let bylo detekováno 5,1 % případů [1]. Podle Bel et al. je významná věková hranice rizika vzniku malignity 59 let. Při dosažení tohoto věku se riziko rapidně zvyšuje, ale dále již s narůstajícím věkem nestoupá. A zatímco u pacientek mladších 59 let nebyl prokázán vztah abnormálního děložního krvácení s vyšším rizikem vzniku malignity, u žen po 59. roku je s preexistujícím postmenopauzálním děložním krvácením spojeno až 12,3% riziko maligního zvratu [14]. Autor Wong et al. ve své 5leté retrospektivní studii uvádějí významný faktor pro maligní zvrat věkovou skupinu > 60 let [9]. Retrospektivní studie Xu et al. sesbírala data 16 020 pacientek (2001–2018). Závěrem studie byl poznatek, že věk jako rizikový faktor u pacientek s benigním a maligním nálezem polypu není statisticky signifikantní, nicméně pacientky s benigním nálezem byly průměrně mladší (41,9 ± 11,5 let) oproti pacientkám s maligním nálezem (53,3 ± 11,6 let) [19].

Abnormální děložní krvácení

Abnormální děložní krvácení, zejména silné a nepravidelné menstruační

Tab. 5. Rozměry polypů vztaženy k histologickému nálezu. Výsledky uvedeny jako: průměr (min.–max.).
Tab. 5. Size of polyps related to histological findings. Results reported as: mean (Min.–Max.).

Studie	Rozměry polypu		p
	benigní nález (mm)	maligní nález (mm)	
Fernándes-Para et al. [16]	20 (1–50)	20 (10–40)	NS
Ferazzi et al. [18]	11 (8–18)	19 (16–23)	0,0002
Garuti et al. [17]	17 (9–25)	24 (1–48)	0
Vinklerová et al. [15]	13 (3–42)	13 (3–40)	0,274
Xu et al. [19]	13 (6–20)	19 (9–29)	< 0,001

p – hodnota

a postmenopauzální krvácení, patří mezi „red flags" symptomy možného děložního nebo cervikálního karcinomu [22]. U postmenopauzálních žen s krvácením je v závislosti na věku a dalších rizikových faktorech riziko přítomnosti karcinomu endometria až 10 % [23]. Endometriální polypy bez přítomnosti malignity nacházíme u 10–30 % žen s abnormálním děložním krvácením [24]. Dle systematické review Lee et al. je přítomnost endometriální malignity v polypu u 4,5 % žen s děložním krvácením a pouze u 1,5 % asymptomatických pacientek [25]. Již dříve zmíněná publikace Wethington et al. uvádí výskyt atypické hyperplazie a EC v 18 případech. U 11 pacientek se projevilo atypické vaginální krvácení (u čtyř nebyla symptomatologie uvedena). Vztaženo na celou kohortu – atypická hyperplazie a EC asociovaný s přítomností polypu se vyskytly ve 2,2 % pacientek s krvácením v porovnání s 1,2 % pacientek bez děložního krvácení [1]. Velká metaanalýza 51 studií s více než 35 tisíci pacientkami autorů Uglietti et al. popisuje zvýšené riziko karcinomu u symptomatických pacientek (5,1 %) oproti asymptomatickým (1,9 %; $p < 0{,}001$), ale s velkými rozdíly mezi jednotlivými publikacemi [21]. Nově publikovaná retrospektivní studie Xu et al. popisuje incidenci abnormálního děložního krvácení ve skupině s benigním nálezem polypu významně nižší (35,1 %) v porovnání s maligním nálezem v terénu polypu (82,2 %) [19].

Rodinná anamnéza

Rodinná anamnéza je nutnou součástí každého vyšetření pacientky. Týká se kompletních údajů o zdravotním stavu pacienta, o morbiditě a mortalitě v rodině. Některé studie uvedené v metaanalýze Win et al. prezentují zvýšené riziko vzniku endometriální malignity u pacientek s postiženou alespoň jednou prvostupňovou příbuznou (matka, sestra, dcera), ale konkrétní hodnota rizika se v rámci studií liší. Ke vztahu endometriální polyp – endometriální malignita – rodinná anamnéza se studie vůbec nevyjadřují [26]. Bel et al. ve své retrospektivní studii uvádějí, že rodinná anamnéza se zaměřením na prvostupňové příbuzné se jeví statisticky nesignifikantní. Pouze v kombinaci s dalšími faktory (studie neuvádí konkrétní případy) se stává statisticky významnou [14].

Důležitou kapitolou jsou pacientky s Lynchovým syndromem. Jedná se o autozomálně dominantně dědičné onemocnění způsobené mutacemi mistmatch repair genů DNA zárodečných buněk. Se stanovením této diagnózy souvisí zvýšené riziko pro rozvoj nádorových onemocnění nejčastěji v oblasti kolorektální a endometriální oblasti [27]. Pacientky s Lynchovým syndromem mají celoživotní riziko vzniku endometriálního karcinomu 40–60 % [28]. V častých případech bývá diagnóza Lynchova syndromu stanovena právě až na základě výskytu malignity. EC je nejčastější extraintestinální karcinom vznikající v souvislosti s tímto syndromem [29]. V prospektivní observační studii Manchansa et al. prováděli ultrazvukové a hysteroskopické sledování 69 pacientek s Lynchovým syndromem s cílem porovnat obě metody a jejich výtěžnost za pomoci histopatologické verifikace. U šesti žen (10 %) byl nález endometriálního polypu, z nichž pouze u jedné pacientky byl v polypu nalezen adenokarcinom [30]. Pacientky s Lynchovým syndromem jsou jediná skupina žen, kde je dle evropských guidelines doporučený UZ screening se zaměřením na patologii endometria i v případě absence abnormálního krvácení. Transvaginální ultrazvuk (preferenčně expertním sonografistou) by měl být proveden jednou ročně a od 35 let by měly pacientky podstoupit jednou za 1 rok nebo jednou za 2 roky biopsii endometria pipelou nebo hysteroskopii. Při abnormálním děložním krvácení je namístě provedení biopsie i mimo tento interval [31].

Diabetes mellitus 2. typu a index tělesné hmotnosti

Diabetes mellitus 2. typu je systémové metabolické onemocnění, jehož incidence v posledních desetiletích narůstá. Společně s obezitou se řadí mezi civilizační choroby a rizikové faktory pro vznik nádorových onemocnění [32]. Ve studii Giardano et al. uvádějí, že většina pacientek s maligním endometriálním polypem měla některý z rizikových faktorů pro rozvoj endometriálního karcinomu – hypertenze, obezita, estrogenní hormonální terapie. Není však uvedeno, kolik pacientek s nálezem benigního polypu také patřilo do rizikové skupiny pro jeden nebo více přítomných rizikových faktorů [33]. Studie Ghoubara et al. sledující rizikové faktory malignizace polypu u krvácejících postmenopauzálních žen došla k závěru, že diabetes u těchto pacientek nepatří k významnému rizikovému faktoru [34]. Observační studie Patrizi et al. sledující celkem 1 020 pacientek popisuje celkem 34 pacientek s atypickou hyperplazií nebo karcinomem endometria. U 26 z nich (89 %) byl při hysteroskopickém vyšetření nalezen polyp. Následné histologické došetření potvrdilo atypický nález u deseti polypů (30,3 %). Z celkového počtu pacientek s atypickou hyperplazií nebo karcinomem endometria bylo osm (24 %) sledováno pro diabetes mellitus. Pacientky s benigním nálezem (986) měly diagnostikovaný polyp v 758 případech (92 %). Léčeno pro diabetes mellitus bylo 77 pacientek (8 %) [10].

Garuti et al. ve své studii definují cut-off BMI (index tělesné hmotnosti) 25,3 kg/m^2 jako hodnotu, od které se další nárůst BMI stává signifikantním rizikovým faktorem přítomnosti EC [17]. Elfayomy et al. v prospektivní observační studii uvádějí obezitu až s BMI > 30 kg/m^2 jako statisticky významnou [35]. Tuto hodnotu (BMI ≥ 30 kg/m^2) jako významný rizikový faktor potvrzují Wong et al. ve své 5leté retrospektivní studii [9]. Podle již zmíněné observační studie Patrizi et al. sledující 1 020 pacientek narůstá riziko pro vznik maligní léze s rostoucím BMI, nicméně statisticky významným se stává až při překročení hodnoty BMI ≥ 40 kg/m^2 [10]. V pro-

spektivní studii Ghoubara et al. sledující ženy s postmenopauzálním krvácením docházejí k závěru, že BMI se stává signifikantním rizikovým faktorem při překročení hodnoty 32,5 kg/m^2 [34].

Užívání tamoxifenu

Tamoxifen, antagonista nesteroidních receptorů, je nejčastěji užívaný lék pro hormonální léčbu estrogen receptor pozitivního karcinomu prsu [36]. V některých studiích je uváděno užívání tamoxifenu jako rizikový faktor vzniku objemných endometriálních benigních polypů [37], nicméně na užívání tamoxifenu a jeho vliv na vznik malignity v terénu endometriálního polypu není dosud jednotný konsenzus. Některé starší práce řadí užívání tamoxifenu mezi rizikové faktory, jako např. francouzské review autora Mbatsogo, které uvádí maligní transformaci u 4,6 % endometriálních polypů pacientek podstupujících léčbu tamoxifenem pro karcinom prsu [38]. Novější studie se kloní spíše ke statistické nevýznamnosti užívání tamoxifenu. Při sledování postmenopauzálního krvácení u žen s endometriálními polypy uvádějí Ghoubara et al. ve své prospektivní práci užívání tamoxifenu jako nevýznamné [34]. Ve studii Garuti et al. užívalo 2,5 % z celkového počtu pacientek tamoxifen (38/1 481). Garudi et al. docházejí k závěru, že vliv užívání tamoxifenu se jeví pro riziko vzniku endometriálního karcinomu na podkladě polypu jako statisticky nevýznamné [17]. Yela et al. ve své retrospektivní studii probíhající 5 let (2010–2015) pozoruje 675 žen užívajících tamoxifen při nálezu endometriálního polypu. Dle výsledků studie není užívání tamoxifenu faktor spojený s větší prevalencí endometriální malignity u těchto žen [39].

Závěr

Tento článek přináší ucelený přehled a rozbor předpokládaných rizikových faktorů, které mohou přispívat k malignímu zvratu při ultrazvukovém nálezu endometriálního polypu. Názory na některé faktory se v jednotlivých studiích liší. V případě rozměru polypu jsou poznatky studií velmi rozdílné a v posledních letech se této problematice nevěnuje mnoho pozornosti. Informace o rodinné anamnéze se sledovaly jen v malém množství studií, a je tedy pouze omezené množství dat. Jako významná se předpokládá pozitivní anamnéza v případě prvostupňových příbuzných. I zde není jednoznačně prokázána míra významnosti a ovlivnění dalšími faktory, proto nelze vyvodit jasné závěry. Výjimkou jsou pacientky s Lynchovým syndromem, což je významný rizikový faktor, a pacientky je potřeba pečlivě dispenzarizovat. Dalším faktorem, kde panuje nejednotný názor, je přítomnost diabetes mellitus. Jen málo studií přistupuje k diabetu jako k jednotlivému faktoru. Většinou je posuzován multifaktoriálně spolu s dalšími interními komorbiditami přítomnými u pacientek, jako např. hypertenze a obezita. Pokud bychom chtěli riziko blíže specifikovat, bylo by potřeba se těmto faktorům věnovat jednotlivě a získat větší množství dat. V případě abnormálního děložního krvácení se výsledky studií poměrně jednotně shodují na přítomnosti významného rizika endometriálního karcinomu v terénu polypu. Jako rizikové potvrzují studie i vyšší věk pacientky a vyšší hodnoty BMI. v žádném z těchto dvou případů však není jasně definována jednotná cut-off hodnota.

Literatura

1. Wethington SL, Herzog TJ, Burke WM et al. Risk and predictors of malignancy in women with endometrial polyps. Ann Surg Oncol 2011; 18(13): 3819–3823. doi: 10.1245/s10434-011-1815-z.
2. Heremans R, Van den Bosch T, Valentin L et al. Ultrasound features of endometrial pathology in women without abnormal uterine bleeding: results from the International Endometrial Tumor Analysis study (IETA3). Ultrasound Obstet Gynecol 2022; 60(2): 243–255. doi: 10.1002/uog.24910.
3. American Association of Gynecologic Laparoscopists. AAGL practice report: practice guidelines for the diagnosis and management of endometrial polyps. J Minim Invasive Gynecol 2011; 19(1): 3–10. doi: 10.1016/j.jmig.2011.09.003.
4. Vitale SG, Haimovich S, Lagana AS et al. Endometrial polyps. An evidence-based diagnosis and management guide. Eur J Obstet Gynecol Reprod Biol 2021; 260: 70–77. doi: 10.1016/j.ejogrb.2021.03.017.
5. Colombo N, Creutzberg C, Amant F 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.
6. Dušek L, Mužík J, Kubásek M et al. Epidemiologie zhoubných nádorů v České republice. 2023 [online]. Dostupné z: https://svod.cz/report.php?diag=C54,C55.
7. Clark TJ, Stevenson H. Endometrial polyps and Abnormal Uterine Bleeding (AUB-P): what is the relationship, how are they diagnosed and how are they treated. Best Pract Res Clin Obstet Gynaecol 2017; 40: 89–104. doi: 10.1016/j.bpobgyn.2016.09.005.
8. Uglietti A, Mazzei C, Deminico N et al. Endometrial polyps detected at ultrasound and rate of malignancy. Arch Gynecol Obstet 2014; 289(4): 839–843. doi: 10.1007/s00404-013-3037-z.
9. Wong CL, So PL. Prevalence and risk factors for malignancy in hysteroscopy-resected endometrial polyps. Int J Gynecol Obstet 2021; 155(3): 433–441. doi: 10.1002/ijgo.13656.
10. Patrizi L, Ticconi C, Borelli B et al. Clinical significance of endometrial abnormalities: an observational study on 1020 women undergoing hysteroscopic surgery. BMC Womens Health 2022; 22(1): 106. doi: 10.1186/s12905-022-01682-5.
11. Wong M, Thanatsis N, Nardelli F et al. Risk of pre-malignancy or malignancy in postmenopausal endometrial polyps: a CHAID decision tree analysis. Diagnostics (Basel) 2021; 11(6): 1094. doi: 10.3390/diagnostics11061094.
12. Leone FP, Timmerman D, Bourne T et al. Terms, definitions and measurements to describe the sonographic features of the endometrium and intrauterine lesions: a consensus opinion from the International Endometrial Tumor Analysis (IETA) group. Ultrasound Obstet Gynecol 2010; 35(1): 103–112. doi: 10.1002/uog.7487.
13. Van Den Bosch T, Verbakel JY, Velantin L et al. Typical ultrasound features of various endometrial pathologies described using International Endometrial Tumor Analysis (IETA) terminology in women with abnormal uterine bleeding. Ultrasound Obstet Gynecol 2021; 57(1): 164–172. doi: 10.1002/uog.22109.
14. Bel S, Bilard C, Godet J et al. Risk of malignancy on suspicion of polyps in menopausal women. Eur J Obstet Gynecol Reprod Biol 2017; 216: 138–142. doi: 10.1016/j.ejogrb.2017.07.013.
15. Vinklerová P, Felsinger M, Frydová S et al. Je nález hyperplazie či polypu děložní dutiny automatickou indikací k biopsii? Ceska Gynekol 2020; 85(2): 84–93.
16. Fernández-Parra J, Rodríguez OA, López CS et al. Hysteroscopic evaluation of endometrial polyps. Int J Gynaecol Obstet 2016; 95(2): 144–148. doi: 10.1016/j.ijgo.2006.07.007.

17. Garuti G, Luerti M, Leone FP et al. Prevalence and predictors of atypical histology in endometrial polyps removed by hysteroscopy: A secondary analysis from the SICMIG hysteroscopy trial. Facts Views Vis Obgyn 2019; 11(2): 127–134.
18. Ferrazzi E, Zupi E, Leone FP et al. How often are endometrial polyps malignant in asymptomatic postmenopausal women? A multicenter study. Am J Obstet Gynecol 2009; 200(3): 235.e1–235.e6. doi: 10.1016/j.ajog.2008.09.876.
19. Xu J, Rao X, Lu W et al. Noninvasive predictor for premalignant and cancerous lesions in endometrial polyps diagnosed by ultrasound. Front Oncol 2022; 11: 812033. doi: 10.3389/fonc.2021.812033.
20. Mansour T, Chowdhury YS. Endometrial Polyp. StatPearls Publishing. 2022 [online]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK557824/.
21. Uglietti A, Buggio L, Farella M et al. The risk of malignancy in uterine polyps: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 2019; 237: 48–56. doi: 10.1016/j.ejogrb.2019.04.009.
22. Kolhe S. Management of abnormal uterine bleeding – focus on ambulatory hysteroscopy. Int J Womens Health 2018; 10: 127–136. doi: 10.2147/IJWH.S98579.
23. Smith-Bindman R, Weiss E, Feldstein V. How thick is too thick? When endometrial thickness should prompt biopsy in postmenopausal women without vaginal bleeding. Ultrasound Obstet Gynecol 2004; 24(5): 558–565. doi: 10.1002/uog.1704.
24. Ricciardi E, Vecchione A, Matci R et al. Clinical factors and malignancy in endometrial polyps. Analysis of 1,027 cases. Eur J Obstet Gynecol Reprod Biol 2014; 183: 121–124. doi: 10.1016/j.ejogrb.2014.10.021.
25. Lee SC, Kaunitz AM, Sanchez-Ramos L et al. The oncogenic potential of endometrial polyps: a systematic review and meta-analysis. Obstet Gynecol 2010; 116(5): 1197–1205. doi: 10.1097/AOG.0b013e3181f74864.
26. Win AK, Reece JC, Ryan S. Family history and risk of endometrial cancer: a systematic review and meta-analysis. Obstet Gynecol 2015; 125(1): 89–98. doi: 10.1097/AOG.0000000000000563.
27. Yokoyama T, Takehara K, Sugimoto N et al. Lynch syndrome-associated endometrial carcinoma with MLH1 germline mutation and MLH1 promoter hypermethylation: a case report and literature review. BMC Cancer 2018; 18(1): 576. doi: 10.1186/s12885-018-4489-0.
28. Zhao S, Chen L, Zang Y et al. Endometrial cancer in Lynch syndrome. Int J Cancer 2022; 150(1): 7–17. doi: 10.1002/ijc.33763.
29. Tafe LJ, Riggs ER, Tsongalis GJ. Lynch syndrome presenting as endometrial cancer. Clin Chem 2014; 60(1): 111–121. doi: 10.1373/clinchem.2013.206888.
30. Manchanda R, Saridogan E, Abdelraheim A et al. Annual outpatient hysteroscopy and endometrial sampling (OHES) in HNPCC/Lynch syndrome (LS). Arch Gynecol Obstet 2012; 286(6): 1555–1562. doi: 10.1007/s00404-012-2492-2.
31. Concin N, Matias-Guiu X, Vergote I et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer 2021; 31(1): 12–39. doi: 10.1136/ijgc-2020-002230.
32. Češka R et al. Diabetes mellitus. In: Češka R et al (eds). Interna. 3. vydání. Praha: Triton 2020: 238–267.
33. Giordano G, Gnetti L, Merisio C et al. Postmenopausal status, hypertension and obesity as risk factors for malignant transformation in endometrial polyps. Maturitas 2007; 56(2): 190–197. doi: 10.1016/j.maturitas.2006.08.002.
34. Ghoubara A, Sundar S, Ewies A. Predictors of malignancy in endometrial polyps: study of 421 women with postmenopausal bleeding. Climacteric 2018; 21(1): 82–87. doi: 10.1080/13697137.2017.1410783.
35. Elfayomy AK, Soliman BS. Risk factors associated with the malignant changes of symptomatic and asymptomatic endometrial polyps in premenopausal women. J Obstet Gynaecol India 2015; 65(3): 186–192. doi: 10.1007/s13224-014-0576-6.
36. Lee M, Piao J, Jeon MJ. Risk factors associated with endometrial pathology in premenopausal breast cancer patients treated with tamoxifen. Yonsei Med J 2020; 61(4): 317–322. doi: 10.3349/ymj.2020.61.4.317.
37. Jeon J, Kim SE, Lee DY et al. Factors associated with endometrial pathology during tamoxifen therapy in women with breast cancer: a retrospective analysis of 821 biopsies. Breast Cancer Res Treat 2020; 179(1): 125–130. doi: 10.1007/s10549-019-05448-w.
38. Mbatsogo BA, Bouëdec BL, Michy T et al. Endometrial cancers arising in polyps associated with tamoxifen use. Gynecol Obstet Fertil 2005; 33(12): 975–979. doi: 10.1016/j.gyobfe.2005.10.021.
39. Yela DA, Ikejiri TA, Machado CR et al. Tamoxifen use as a malignancy risk factor in postmenopausal women with endometrial polyps. Menopause 2019; 26(8): 863–866. doi: 10.1097/GME.0000000000001340.

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3.3 Vinklerová P, Ovesná P, Bednaříková M, Minář L, Felsinger M, Hausnerová J, Weinberger V. Does an Endometrial Cancer Diagnosis among Asymptomatic Patients Improve Prognosis? Cancers. 2021;14(1):115.

In another study, we aimed to determine whether a diagnosis of EC (endometrial cancer) in asymptomatic patients—those without abnormal uterine bleeding—conferred a prognostic advantage in terms of DFS (disease-free survival), OS (overall survival), and DSS (disease-specific survival), compared to symptomatic patients.

The study analyzed data from 625 women with histologically confirmed EC who underwent primary surgical treatment. Of these, 144 patients (23%) were asymptomatic at diagnosis, while the remaining 481 (77%) reported bleeding symptoms. Univariable analysis showed that symptomatic patients had a significantly increased risk of disease recurrence (HR 3.1; 95% CI 1.24–7.77; $p = 0.016$). However, no statistically significant differences in OS or DSS were observed between the two groups. Importantly, in multivariable analysis adjusting for relevant clinical and pathological variables—including age, tumor histology and grade, FIGO stage, and LVSI (lymphovascular space invasion)—symptomatology lost its prognostic significance. The increased risk of adverse outcomes in symptomatic patients was thus attributed to more advanced disease and higher-risk pathological features, rather than the presence of bleeding itself.

The findings of this study underscore that abnormal bleeding, while useful for prompting earlier diagnosis, is not an independent predictor of poor prognosis. The results challenge the rationale for performing invasive diagnostic procedures such as endometrial biopsy or hysteroscopy solely based on ultrasound findings in asymptomatic postmenopausal women, a practice still prevalent in some clinical settings despite lacking support from current European guidelines. The clinical decisions regarding biopsy should be based on the presence of symptoms and/or other high-risk imaging or histopathological findings, rather than symptomatology alone. Early-stage detection among asymptomatic women does not appear to translate into improved survival outcomes when tumors are matched for stage and histologic characteristics.

This study represents one of the largest single-center evaluations of symptomatology as a prognostic factor in endometrial cancer and reinforces the importance of individualized patient assessment based on objective risk parameters.

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The author's contribution: first author, conceptualization, methodology, data curation, and manuscript writing – original draft.

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Does an Endometrial Cancer Diagnosis among Asymptomatic Patients Improve Prognosis?

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Simple Summary: Endometrial cancer is common malignancy with an excellent prognosis due to its early symptoms—abnormal bleeding. It is still common in some countries to provide a biopsy in asymptomatic patients based on ultrasound findings; even though, it is not supported by the European guidelines. The aim of our study was to find out if there is a prognostic difference among symptomatic and bleeding-free patients with similar clinical histological characteristics.

Abstract: Background: Endometrial cancer is the most common gynecological malignancy in developed countries with no screening available. There is still a tendency to provide invasive bioptic verification in asymptomatic women with abnormal ultrasound findings to diagnose carcinoma in a preclinical phase; even though, it is not supported by European guidelines. Our goal was to determine DFS (disease-free survival), OS (overall survival), and DSS (disease-specific survival) differences between symptom-free and symptomatic (bleeding, or spotting) endometrial cancer patients with similar stage and tumor/clinical characteristics. Methods: All of our patients with endometrial cancer following surgical treatment between 2006 and 2019 were assessed, evaluating risk factors for recurrence and death while focusing on bleeding using univariable and multivariable analysis. Results: 625 patients meeting the inclusion criteria were divided into asymptomatic (n = 144, 23%) and symptomatic (n = 481, 77%) groups. The median follow-up was 3.6 years. Using univariable analysis, symptomatic patients had a three times higher risk of recurrence (HR 3.1 (95% CI 1.24–7.77), p = 0.016). OS (HR 1.35 (0.84–2.19), p = 0.219) and DSS (HR 1.66 (0.64–4.28), p = 0.3) were slightly worse without reaching statistical significance. In our multivariable analysis, symptomatology was deemed completely insignificant in all monitored parameters (DFS: HR 2.03 (0.79–5.24), p = 0.144; OS: HR 0.72 (0.43–1.21), p = 0.216). Conclusions: The symptomatic endometrial cancer patients risk factor of earlier recurrence and death is insignificantly higher when compared with the asymptomatic cohort. However, multivariable analysis verifies that prognosis worsens with other clinically relevant parameters, not by symptomatology itself. In terms of survival outcome in EC patients, we recognized symptomatology as a non-significant marker for the patient's prognosis.

Keywords: endometrial cancer; postmenopausal bleeding; prognosis

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1. Introduction

In developed countries, endometrial cancer (EC) is the most common gynecological malignancy with more than 380,000 new cases in 2018 [1]. It is usually diagnosed in the

first stage with an excellent prognosis identifying thickened or abnormal endometrium via ultrasound use in daily practice and/or early symptoms including postmenopausal or abnormal bleeding, which occurs in 90% of cancers [2]. Among European women, the relative 5-year survival is 76% [3]. The 5-year overall survival rate is 89% with stage I, 78% stage II, 61% stage III, and 21% with stage IV [4]. Along with FIGO (The International Federation of Gynecology and Obstetrics) stage, other conventional prognostic factors are histology, grade, and lymphovascular space invasion (LVSI) [5]. Currently, these factors still determine patient risk and the indication of adjuvant treatment.

Furthermore, additional immunohistochemical markers seem to be beneficial in predicting prognosis, even though they have never been part of risk classification. Abnormal expression of L1CAM (L1 Cell Adhesion Molecule), mutated tumor protein p53, a loss of estrogen (ER), and progesterone receptors (PR), for example, are associated with a worse prognosis [6].

The latest EC classification trend is grouping according to molecular features, first introduced in 2013 by the Cancer Genome Atlas (TCGA) Research Network [7]. They divided EC into four prognostic subclasses. The polymerase-epsilon (POLE) ultramutated was characterized by a very favorable outcome. The hypermutated group (also termed microsatellite instable, MSI) and the copy-number low (microsatellite stable, MSS) were associated with intermediate results. The copy-number high (mainly serous histotype) was defined by tumor protein p53 mutations and poor prognosis. Recently published guidelines by the European Society of Gynecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP), now recommend this EC classification; however, it is not yet comprehensive. Worldwide use in daily practice is hindered by its cost and availability.

General population screening is not recommended, apart from patients with Lynch syndrome [5,8]. Women should be advised to report any postmenopausal or abnormal bleeding, which is at higher risk for malignity presence. Daily routine ultrasound use may lead to incidental findings of thickened endometrium or polyps and invasive procedures (resulting from fear of malignancy). Based on a thickened endometrium $\geq$5 mm, it is still common in many countries to perform routine endometrial sampling in asymptomatic postmenopausal women, even though this is not supported by European guidelines [8]. Endometrial cancer prevalence among asymptomatic women is low, and biopsy is not recommended in the case of a postmenopausal patient with endometrial thickness or polyp without bleeding [9,10]. On the other hand, the rationale for an active approach could relate to a doctor's concern about neglecting a woman's health following late detection of uterine cancer—a real and clinically serious issue. So far, there are no robust data published regarding whether there is a difference and if it matters when endometrial cancer is diagnosed in the asymptomatic or symptomatic phase.

Our study, accordingly, aimed to answer the question of whether bleeding is a strong prognostic factor in endometrial cancer patients. We evaluated symptomatology concerning DFS (disease-free survival), OS (overall survival), and DSS (disease-specific survival). Is there an advantage when diagnosing endometrial cancer of the same stage and characteristics in the preclinical (asymptomatic) phase from the perspective of DSF, OS, and DSS?

2. Materials and Methods

Our retrospective observational study took place between January 2006 and December 2019 at the Department of Gynecology and Obstetrics, University Hospital Brno, Czech Republic. All surgically treated patients with EC diagnosis were consecutively included in the study. Patients were divided into two groups depending on symptoms while focusing on postmenopausal bleeding, spotting, pinkish discharge, or irregular and excessive bleeding in premenopausal women. An ultrasound finding of an endometrial tumor in premenopausal age and endometrial thickness ($\geq$5 mm) or a polyp was a signal for biopsy among asymptomatic postmenopausal patients. We excluded cases with no surgical treatment, uterine sarcoma histology, and unknown symptomatology status.

All patients underwent a total abdominal or laparoscopic hysterectomy with bilateral salpingo-oophorectomy (ovaries were spared when patients were younger than 45 years with endometrioid histology, grade 1). Pelvic and paraaortic lymphadenectomy was performed according to actual national recommendations (only pelvic until 2013 and since that pelvic and paraaortic lymphadenectomy in high-risk carcinomas including non-endometrioid and endometrioid cancer stage ≥1B of any grading) and patient performance status [11]. We have introduced sentinel node biopsy (using indocyanine green) instead of systematic lymphadenectomy since 2019. Only women with complete remission after primary treatment were included. During the follow-up period, regular check-ups were effected every 3–4 months following primary treatment for the first two years, biannually over the next three years, and once a year thereafter. Gynecologic examination and transvaginal/transrectal plus abdominal ultrasound were obligatory. A CT (computed tomography) scan was undertaken when recurrence was suspected. We monitored all types of recurrence—local (vaginal vault), regional (pelvic structures including lymphatic nodes), and distant (extrapelvic metastasis).

Patient's age at the time of diagnosis and relevant data regarding histological type (endometrioid, mucinous, serous, clear-cell, and carcinosarcoma), grade, LVSI, pathological stage (according to FIGO 2009), lymphadenectomy, and adjuvant treatment provided such as radiotherapy (RT), chemotherapy (CHT), and their combination (CHRT) were obtained from medical records. We divided women into five categories according to their age for univariable analysis. A continuous variable was used for multivariable analysis.

We selected DFS, OS, and DSS as prognostic parameters to compare patients in both groups. DFS is the length of time after primary cancer treatment that a patient survives without any signs or symptoms of the disease. OS is the time from either the date of diagnosis or the start of treatment for the disease patients are still alive. DSS is the time from the diagnosis date or treatment onset to the date of death from the disease. Patients who die from causes unrelated to the cancer are not counted in this measurement [12].

Patients without an event were censored upon the date of the last follow-up visit. The impact of symptomatology on DFS and OS was assessed using Cox proportional hazards model, which gives hazard ratios (HR) accompanied by a 95% confidence interval (CI). Results from univariate analysis (crude HR) were adjusted for other clinical parameters in the multivariable Cox model giving adjusted HRs. Histology, grade, and FIGO stage categories were combined in multivariate models due to low number of events in some strata. The DSS was estimated by a cumulative incidence in the presence of death from other causes than EC as a competing risk. Comparison between groups was undertaken using the Fine and Gray method. Analyses were detailed with R software (4.0.3) including survival and cmprsk (Subdistribution Analysis of Competing Risks) packages.

3. Results

Between 2006 and 2019, our database included 722 endometrial cancer patients of which 625 met the inclusion criteria—144 (23%) were asymptomatic and 481 (77%) reported symptoms. Table 1 summarizes clinical/histological characteristics and adjuvant treatment. Data collection and statistical analysis were detailed in March 2021, when median (interquartile range, IQR) follow-up was 3.6 years (4 days–13.8 years).

Table 1. Clinical patients' characteristics.

Clinical Characteristics		Asymptomatic (n = 144)	Symptomatic (n = 481)	p-Value
Age (years)	<50	13 (9.0%)	46 (9.6%)	0.32
	51–60	27 (18.8%)	95 (19.8%)	
	61–70	68 (47.2%)	191 (39.7%)	
	71–80	32 (22.2%)	117 (24.3%)	
	>80	4 (2.8%)	32 (6.7%)	
Age (years)	Mean (SD)	64.5 (9.3%)	65.2 (10.6%)	0.476
Lymphadenectomy	No	124 (86.1%)	335 (69.6%)	<0.001
	Yes	20 (13.9%)	146 (30.4%)	
Adjuvant therapy	None	111 (77.6%)	264 (56.4%)	<0.001
	RT	25 (17.5%)	166 (35.5%)	
	CHT	4 (2.8%)	19 (4.1%)	
	CHRT	3 (2.1%)	19 (4.1%)	
LVSI	No	138 (95.8%)	398 (83.3%)	<0.001
	Yes	6 (4.2%)	80 (16.7%)	
Histology + grade	Endometrioid G1	60 (41.7%)	86 (17.9%)	<0.001
	Endometrioid G2	70 (48.6%)	270 (56.1%)	
	Endometrioid G3	5 (3.5%)	70 (14.6%)	
	Non-endometrioid	9 (6.2%)	55 (11.4%)	
FIGO stage	Ia	117 (81.2%)	277 (57.6%)	<0.001
	Ib	13 (9.0%)	89 (18.5%)	
	II	10 (6.9%)	60 (12.5%)	
	IIIa	1 (0.7%)	12 (2.5%)	
	IIIb	2 (1.4%)	8 (1.7%)	
	IIIc	1 (0.7%)	26 (5.4%)	
	IVa	0 (0.0%)	0 (0.0%)	
	IVb	0 (0.0%)	9 (1.9%)	

RT = radiotherapy, CHT = chemotherapy, CHRT = chemoradiotherapy, LVSI = lymphovascular space invasion, G = grade, FIGO = The International Federation of Gynecology and Obstetrics.

3.1. Disease-Free Survival

Recurrence occurred in 56 patients during the follow-up period. Five years following primary treatment, there were no signs of uterine cancer relapse among 96.1% (92.8–99.5%) asymptomatic and 86.2% (82.5–90%) symptomatic women. Using a univariable model, symptomatic cases had a three times higher risk of recurrence (HR 3.1 (95% CI 1.24–7.77), $p = 0.016$) than asymptomatic (Figure 1A). Furthermore, we observed increased recurrence risk among elderly patients, with NEC (non-endometrioid carcinoma) histology, LVSI presence, endometrioid EC grade and stage increases, following lymphadenectomy, and CHT treatment (Table 2).

Table 2. Disease-free survival—univariable and multivariable Cox proportional hazards model.

Clinical Characteristics		Crude HR (95% CI, p-Value)	Adjusted HR (95% CI, p-Value)
Symptomatology	No	1	1
	Yes	3.1 (1.24–7.77, $p = 0.016$)	2.03 (0.79–5.24, $p = 0.144$)
Age (years)	<50	1	
	51–60	2.18 (0.47–10.08, $p = 0.320$)	
	61–70	2.49 (0.58–10.58, $p = 0.217$)	
	71–80	3.11 (0.71–13.68, $p = 0.134$)	
	>80	9.91 (2.14–45.92, $p = 0.003$)	
Age (years)	Mean (SD)	1.05 (1.02–1.08, $p = 0.002$)	1.04 (1.01–1.07, $p = 0.013$)
Lymphadenectomy	No	1	
	Yes	1.75 (1.02–3, $p = 0.042$)	
Adjuvant therapy	None	1	1
	RT	1.47 (0.81–2.69, $p = 0.209$)	0.82 (0.42–1.61, $p = 0.569$)
	CHT	9.61 (4.43–20.86, $p < 0.001$)	1.68 (0.50–5.63, $p = 0.404$)
	CHRT	2.18 (0.65–7.25, $p = 0.205$)	0.36 (0.08–1.63, $p = 0.186$)

Table 2. *Cont.*

Clinical Characteristics		Crude HR (95% CI, *p*-Value)	Adjusted HR (95% CI, *p*-Value)
LVSI	No	1	1
	Yes	3.75 (2.09–6.73, $p < 0.001$)	1.34 (0.58–3.06, $p = 0.494$)
Histology + grade	Endometrioid G1	1	
	Endometrioid G2	3.52 (1.06–11.63, $p = 0.039$)	1 (ref. G1 + G2)
	Endometrioid G3	7.15 (1.99–25.64, $p = 0.003$)	1.70 (0.76–3.79, $p = 0.194$)
	Non-endometrioid	15.61 (4.55–53.61, $p < 0.001$)	3.20 (1.59–6.43, $p = 0.001$)
FIGO stage	Ia	1	
	Ib	2.69 (1.33–5.47, $p = 0.006$)	
	II	3 (1.36–6.63, $p = 0.007$)	
	IIIa	6.51 (2.21–19.22, $p = 0.001$)	
	IIIb	4.11 (0.55–30.82, $p = 0.169$)	
	IIIc	7.60 (3.03–19.07, $p < 0.001$)	
	IVa	NA	
	IVb	21.67 (7.32–64.17, $p < 0.001$)	
FIGO stage	I–II	1	1
	III–IV	5.37 (2.96–9.73, $p < 0.001$)	3.55 (1.40–8.96, $p = 0.007$)

RT = radiotherapy, CHT = chemotherapy, CHRT = chemoradiotherapy, LVSI = lymphovascular space invasion, G = grade, FIGO = The International Federation of Gynecology and Obstetrics.

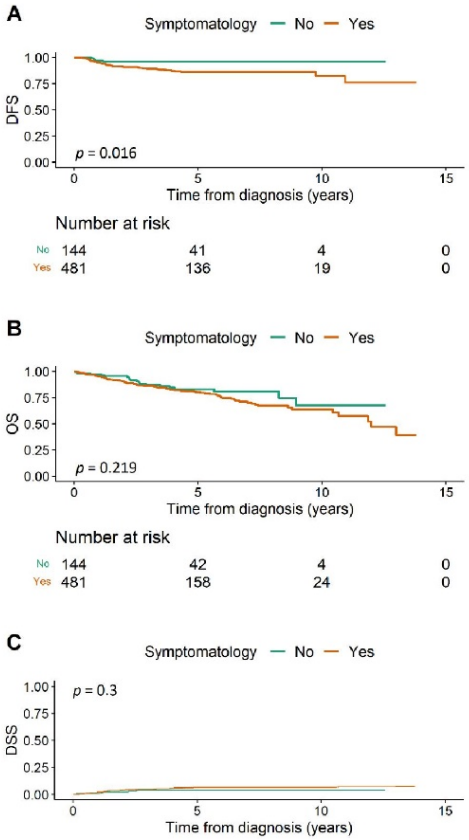

Figure 1. (**A**) Disease-free survival, (**B**) overall survival, (**C**) disease-specific survival. DFS = disease-free survival, OS = overall survival, DSS = disease-specific survival.

However, symptomatology became non-significant in the multivariable analysis when it was adjusted with other clinical parameters (HR 2.03 (0.79–5.24), $p = 0.144$). Concurrently, histology, grade, and stage remained risky (Table 2).

3.2. Overall Survival

Overall, 117 patients died during the follow-up period (20 symptom-free and 97 bleeding). We recorded a worsening OS trend in bleeding patients without reaching statistical significance with the univariable model (HR 1.35 (0.84–2.19), $p = 0.219$; Figure 1B). Five-year OS was 82.9% (75.5–90.9%) in asymptomatic and 80.1% (76–84.5%) in symptomatic cases. Reduced survival was notable among women over age 70, after lymphadenectomy, following CHT treatment, with LVSI, with a grade 3 endometrioid or NEC histology, and advancing disease stage (Table 3).

Table 3. Overall survival—univariable and multivariable Cox proportional hazards model.

Clinical Characteristics		Crude HR (95% CI, *p*-Value)	Adjusted HR (95% CI, *p*-Value)
Symptomatology	No	1	1
	Yes	1.35 (0.84–2.19, $p = 0.219$)	0.72 (0.43–1.21, $p = 0.216$)
Age (years)	<50	1	
	51–60	1.4 (0.46–4.27, $p = 0.551$)	
	61–70	1.9 (0.68–5.33, $p = 0.222$)	
	71–80	4.63 (1.66–12.89, $p = 0.003$)	
	>80	7.44 (2.48–22.31, $p < 0.001$)	
Age (years)	Mean (SD)	1.07 (1.05–1.09, $p < 0.001$)	1.07 (1.05–1.10, $p < 0.001$)
Lymphadenectomy	No	1	
	Yes	1.42 (0.98–2.07, $p = 0.066$)	
Adjuvant therapy	None	1	1
	RT	0.98 (0.64–1.51, $p = 0.938$)	0.64 (0.40–1.03, $p = 0.067$)
	CHT	5.91 (3.19–10.96, $p < 0.001$)	1.16 (0.50–2.73, $p = 0.727$)
	CHRT	1.65 (0.66–4.14, $p = 0.284$)	0.28 (0.09–0.85, $p = 0.024$)
LVSI	No	1	1
	Yes	4.55 (3.06–6.75, $p < 0.001$)	2.05 (1.13–3.72, $p = 0.018$)
Histology + grade	Endometrioid G1	1	
	Endometrioid G2	1.17 (0.66–2.07, $p = 0.584$)	1 (ref. G1 + G2)
	Endometrioid G3	2.63 (1.4–4.95, $p = 0.003$)	2.05 (1.17–3.61, $p = 0.013$
	Non-endometrioid	5.43 (2.97–9.95, $p < 0.001$)	2.89 (1.77–4.72, $p < 0.001$)
FIGO stage	Ia	1	
	Ib	2.38 (1.45–3.91, $p = 0.001$)	
	II	2.54 (1.44–4.49, $p = 0.001$)	
	IIIa	3.78 (1.60–8.94, $p = 0.003$	
	IIIb	16.01 (7.06–36.3, $p < 0.001$)	
	IIIc	8.7 (4.71–16.09, $p < 0.001$)	
	IVa	NA	
	IVb	14.89 (6.58–33.72, $p < 0.001$)	
FIGO stage	I–II	1	1
	III–IV	5.69 (3.80–8.52, $p < 0.001$)	3.63 (1.93–6.85, $p < 0.001$)

RT = radiotherapy, CHT = chemotherapy, CHRT = chemoradiotherapy, LVSI = lymphovascular space invasion, G = grade, FIGO = The International Federation of Gynecology and Obstetrics.

Nonetheless, symptomatology was recognized in the multivariable model with slightly longer survival in the symptomatic group (HR 0.72 (0.43–1.21), $p = 0.216$) when it was adjusted for other parameters. We recorded shorter survival in patients with grade 3 endometrioid or NEC histology and with disease stages increasing following adjustment. Adjuvant therapy had a protective effect (radiotherapy $p = 0.067$, chemoradiotherapy, $p = 0.024$) as well (Table 3).

3.3. Disease-Specific Survival

Thirty-six deaths were caused by endometrial cancer. Disease-specific survival was insignificantly worse with bleeding patients in the univariable model (HR 1.66 (0.64–4.28), $p = 0.3$, Figure 1C). Survival rates were substantially reduced following lymphadenectomy, CHT, with LVSI and disease stage escalation (Table 4). NEC histology and endometrioid EC grade >1 was also considered a risk, since all fatalities came from these groups of patients. The multivariate model of DSS was not performed due to the low number of events.

Table 4. Disease-specific survival—univariable model of cumulative incidence with competing risk.

Clinical Characteristics		Crude HR (95% CI, *p*-Value)
Symptomatology	No	1
	Yes	1.66 (0.64–4.28, $p = 0.300$)
Age (years)	<50	1
	51–60	1.14 (0.22–5.89, $p = 0.870$)
	61–70	1.48 (0.34–6.37, $p = 0.600$)
	71–80	1.7 (0.37–7.74, $p = 0.500$)
	>80	3.94 (0.77–20.05, $p = 0.099$)
Age (years)	Mean (SD)	1.03 (0.99–1.07, $p = 0.170$)
Lymphadenectomy	No	1
	Yes	2.20 (1.14–4.26, $p = 0.019$)
Adjuvant therapy	None	1
	RT	1.12 (0.49–2.53, $p = 0.790$)
	CHT	11.93 (5.17–27.53, $p < 0.001$)
	CHRT	3.75 (1.1–12.74, $p = 0.034$)
LVSI	No	1
	Yes	8.08 (4.21–15.48, $p < 0.001$)
Histology + grade	Endometrioid G1 + 2	1
	Endometrioid G3	5.19 (2.22–12.13, $p < 0.001$)
	Non-endometrioid	8.74 (4.06–18.78, $p < 0.001$)
FIGO stage	I–II	1
	III–IV	10.33 (5.36–19.90, $p < 0.001$)

RT = radiotherapy, CHT = chemotherapy, CHRT = chemoradiotherapy, LVSI = lymphovascular space invasion, G = grade, FIGO = The International Federation of Gynecology and Obstetrics.

4. Discussion

Endometrial cancer is a common malignancy with a generally favorable prognosis. There is no recommended screening for the general population; however, ultrasound use in daily practice may lead to fortuitous findings of uterine polyps or hyperplasia. Since it is not possible to provide biopsies for all patients, there is a clinically driven need to establish a cut-off for identifying high-risk EC patients. A 12% prevalence of thickened endometrium ≥5 mm in gynecologically healthy asymptomatic postmenopausal women was identified with a Swedish population study [13]. A reasonable endometrial thickness threshold seems to be ≥11 mm for biopsy in asymptomatic patients when the EC incidence probability is about 6.7% compared to 1.7% in women with endometrium thickness <11 mm [10,14]. We omitted the division of asymptomatic patients according to endometrial thickness in our current study. However, we postulated before, that significant risk of malignancy is only when threshold of 12 mm was used (OR 3.54, $p = 0.024$) comparing to 8 and 5 mm [15].

Malignancy risk is less than 2% with asymptomatic polyps, and small ones can even vanish spontaneously in premenopausal women [9]. An invasive approach should be reserved for bleeding patients and in cases of infertility.

A hysteroscopy (instead of dilatation and curettage) is recommended to obtain a representative sample or remove a focal lesion, although complication risk (uterine perforation, bowel damage, bleeding, infection, fluid-overload syndrome, etc.) is not negligible [9,13,16].

Scrimin and al. published their study of 1070 patients undergoing hysteroscopy, where nearly half of the indication was inappropriate [17]. We should consider the consequences of each invasive procedure to avoid unnecessary overtreatment and potential adverse events. Even when strictly respecting the 11 mm threshold for symptom-free postmenopausal women, 19 redundant endometrial biopsies have to be undertaken to diagnose one endometrial cancer or precancerosis [10].

In our previous study, we took a closer look at the exact description of symptom-free and symptomatic endometrial cancer tumors. Asymptomatic tumors were more often endometrioid grade 1 (41.7%) compared to bleeding (17.9%), which were more frequently endometrioid grade 3 (14.6% vs. 3.5%) or NEC (11.4% vs. 6.2%). Although immunohistochemical markers L1CAM, p53, ER, PR are strongly associated with patient prognosis and survival, we did not find any significant difference in their expression between symptomatic and symptom-free EC patients. A deep myometrial and/or cervical invasion was more commonly observed in symptomatic cases. The bleeding may correspond more with the local status of the spread and very probably has no connection to the EC patient's survival outcome [18].

Only a few studies have focused on symptomatology in EC with the view of survival. Gemer et al. presented the largest retrospective multicentric study of 1607 postmenopausal women and detailed no difference between asymptomatic and bleeding EC patients in terms of 5-year recurrence-free survival (79.1% vs. 79.4%, $p = 0.85$), disease-specific survival (83.2% vs. 82.2%, $p = 0.57$), and overall survival (79.7% vs. 76.8%, $p = 0.37$) using univariable analysis [19]. Interestingly, they did not recognize a difference in low and high-grade histology between groups; however, there was a lower deep myometrial invasion rate in symptom-free patients. Comparing our results, we affirmed that bleeding patients had a three times higher risk of recurrence (HR 3.1 (1.24–7.77), $p = 0.016$), while overall and disease-specific survival was insignificantly worse (HR 1.35 (0.84–2.19), $p = 0.219$; HR 1.66 (0.64–4.28), $p = 0.300$). Nonetheless, when using multivariable analysis symptomatology became insignificant replaced by other factors which worsened patient prognosis (LVSI, histology, grade, FIGO stage). We confirmed factors that improve patient prognosis including radiotherapy and chemoradiotherapy in endometrial cancer patients.

Similar prognostic factors were identified in another multicentric study of 543 postmenopausal women [20]. Seebacher et al. demonstrated that tumor stage, grade, patients' age—but not symptomatology—were associated with disease-free (HR 0.9, $p = 0.7$) and overall survival (HR 0.8, $p = 0.4$) in multivariable analysis. Their conclusions are compatible with our results: Symptomatology was not a significant risk factor when a multivariable analysis was used (DFS—HR 2.03 (0.79–5.24), $p = 0.144$; OS—HR 0.72 (0.431–1.21), $p = 0.216$).

The Israeli authors divided EC patients into three groups: Asymptomatic, bleeding up to 3 months, and bleeding more than 3 months. They presented consistent results regarding deep myometrial invasion in stage I (21%, 24%, 26%, $p = 0.84$), grade 3 tumors (10%, 13%, 14%, $p = 0.42$), and advanced-stage disease (12%, 14%, 15%, $p = 0.92$) in 220 endometrioid EC patients. The only non-significant trend toward better survival in the asymptomatic and short-term bleeding group was reported ($p = 0.172$) using univariable analysis [21]. In our study, we were unable to subdivide patients according to symptom duration owing to a lack of that specific information in medical records.

The most recent study about symptomatology as a prognostic factor is concerning only patients with preoperative suspicion of the endometrial polyp [22]. This means in majority only patients in the early stage with no signs of advanced disease. Authors find no difference in survival rates and recommended follow-up instead of biopsy in asymptomatic women. In our study we included all patients after surgical treatment, so the cohort differs and we have more advanced diseases especially in symptomatic group.

In our study, we observed that the non-bleeding group differed significantly (p = <0.001) in stage IA (81%) compared to the symptomatic (57%). Although the bleeding EC patients were diagnosed at the higher stage according to FIGO, there was no difference between the patients in terms of specific survival and overall survival even when using univariable analysis. Bleeding and spotting alone are not significant markers that worsen the patient's prognosis. In terms of the diagnostic, bleeding is just one of the markers, which may, in particular cases, lead to the shift towards earlier stage detection.

To the best of our knowledge, our study represents the largest unicentric cohort dealing with symptomatology as a prognostic factor in endometrial cancer. There was an earlier recurrence and death (resulting from EC or other reasons) in bleeding patients. However, a poorer prognosis is related to other clinical and histological features, not the symptomatology itself. DFS, OS, and DSS were not worse among symptomatic patients at a similar disease stage.

Our study's strength is reflected in the significant number of patients with guaranteed consistent treatment decisions and high-quality follow-up data. The retrospective design, and the absence of selective detail such as symptom duration, might be considered a shortcoming of sorts.

Consequently, we should educate our patients to immediately report postmenopausal or irregular bleeding and to make arrangements for dilatation and curettage or a hysteroscopy when necessary. Since there is no prognostic advantage in detecting EC in the preclinical asymptomatic phase, we recommend an expectation approach and consider the necessity of invasive biopsy in terms of possible complications and comorbidities among elderly patients.

5. Conclusions

Symptomatic endometrial cancer patients are at higher risk of earlier recurrence and death (both from EC and other terminal conditions, with an insignificant difference compared to the asymptomatic cohort). However, a worse prognosis resulted from other specific clinically relevant parameters, not from the bleeding itself. DSF, OS, and DSS are similar in patients at the same disease stage irrespective of symptomatology. The bleeding is not the marker worsening the prognosis. Nonetheless, EC diagnosis in the asymptomatic phase would lead to earlier stage detection. In the clinical practice, the decision regarding biopsy should be based on symptomatology and/or a significant change in the finding on the imaging method.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of University Hospital Brno, Approval Number 01-070218/EK (confirmed on 7 February 2018).

Informed Consent Statement: All patients signed informed consent with histology sample storing and using for scientific and publication purposes.

Data Availability Statement: Data are available upon reasonable request.

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Abbreviations

DFS	disease-free survival
DSS	disease-specific survival
EC	endometrial cancer
FIGO	The International Federation of Gynecology and Obstetrics
CHRT	chemoradiotherapy
CHT	chemotherapy
LVSI	lymphovascular space invasion
NEC	non-endometrioid carcinoma
OS	overall survival
RT	radiotherapy
HR	hazard ratio
CI	confidence interval

References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; 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. [CrossRef] [PubMed]
2. Clarke, M.A.; Long, B.J.; Del Mar Morillo, A.; Arbyn, M.; Bakkum-Gamez, J.N.; Wentzensen, N. Association of Endometrial Cancer Risk with Postmenopausal Bleeding in Women. *JAMA Intern. Med.* **2018**, *178*, 1210–1222. [CrossRef] [PubMed]
3. Sant, M.; Lopez, M.D.C.; Agresti, R.; Pérez, M.J.S.; Holleczek, B.; Bielska-Lasota, M.; Dimitrova, N.; Innos, K.; Katalinic, A.; Langseth, H.; et al. Survival of women with cancers of breast and genital organs in Europe 1999–2007: Results of the EUROCARE-5 study. *Eur. J. Cancer* **2015**, *51*, 2191–2205. [CrossRef] [PubMed]
4. Quinn, M.A.; Benedet, J.L.; Odicino, F.; Maisonneuve, P.; Beller, U.; Creasman, W.T.; Heintz, A.P.; Nyan, H.Y.; Pecorelli, S. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int. J. Gynaecol. Obstet.* **2006**, *95* (Suppl. 1), S105–S143.
5. Colombo, N.; Creutzberg, C.; Amant, F.; Bosse, T.; Martín, A.G.; Ledermann, J.; Marth, C.; Nout, R.A.; Querleu, D.; Mirza, M.R.; et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-up. *Int. J. Gynecol. Cancer* **2016**, *26*, 2–30. [CrossRef] [PubMed]
6. Vrede, S.; van Weelden, W.; Visser, N.; Bulten, J.; van der Putten, L.; van de Vijver, K.; Santacana, M.; Colas, E.; Gil-Moreno, A.; Moiola, C.; et al. Immunohistochemical biomarkers are prognostic relevant in addition to the ESMO-ESGO-ESTRO risk classification in endometrial cancer. *Gynecol. Oncol.* **2021**, *161*, 787–794. [CrossRef] [PubMed]
7. Getz, G.; Gabriel, S.B.; Cibulskis, K.; Lander, E.; Sivachenko, A.; Sougnez, C.; Lawrence, M.; Kandoth, C.; Dooling, D.; Fulton, R.; et al. Integrated genomic characterization of endometrial carcinoma. *Nature* **2013**, *497*, 67–73.
8. Concin, N.; Matias-Guiu, X.; Vergote, I.; Cibula, D.; Mirza, M.R.; Marnitz, S.; Ledermann, J.; Bosse, T.; Chargari, C.; Fagotti, A.; et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int. J. Gynecol. Cancer* **2021**, *31*, 12–39. [CrossRef] [PubMed]
9. Uglietti, A.; Buggio, L.; Farella, M.; Chiaffarino, F.; Dridi, D.; Vercellini, P.; Parazzini, F. The risk of malignancy in uterine polyps: A systematic review and meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2019**, *237*, 48–56. [CrossRef] [PubMed]
10. Alcázar, J.L.; Bonilla, L.; Marucco, J.; Padilla, A.I.; Chacón, E.; Manzour, N.; Salas, A. Risk of endometrial cancer and endometrial hyperplasia with atypia in asymptomatic postmenopausal women with endometrial thickness ≥11 mm: A systematic review and meta-analysis. *J. Clin. Ultrasound* **2018**, *46*, 565–570. [CrossRef] [PubMed]
11. Onkogynekologie.com. Available online: http://www.onkogynekologie.com/ (accessed on 19 October 2021).
12. NCI Dictionary of Cancer Terms—National Cancer Institute. Published 2 February 2011. Available online: https://www.cancer.gov/publications/dictionaries/cancer-terms/ (accessed on 26 June 2021).
13. Jokubkiene, L.; Sladkevicius, P.; Valentin, L. Transvaginal ultrasound examination of the endometrium in postmenopausal women without vaginal bleeding. *Ultrasound Obstet. Gynecol.* **2016**, *48*, 390–396. [CrossRef] [PubMed]
14. Smith-Bindman, R.; Weiss, E.; Feldstein, V. How thick is too thick? When endometrial thickness should prompt biopsy in postmenopausal women without vaginal bleeding. *Ultrasound Obstet. Gynecol.* **2004**, *24*, 558–565. [CrossRef] [PubMed]
15. Vinklerová, P.; Felsinger, M.; Frydová, S.; Ovesná, P.; Hausnerová, J.; Weinberger, V. Is the finding of endometrial hyperplasia or corporal polyp an mandatory indication for biopsy? *Ceska Gynekol.* **2020**, *85*, 84–93. [PubMed]
16. Ferrazzi, E.; Zupi, E.; Leone, F.P.; Savelli, L.; Omodei, U.; Moscarini, M.; Barbieri, M.; Cammareri, G.; Capobianco, G.; Cicinelli, E.; et al. How often are endometrial polyps malignant in asymptomatic postmenopausal women? A multicenter study. *Am. J. Obstet. Gynecol.* **2009**, *200*, 235.e1–235.e6. [CrossRef] [PubMed]
17. Scrimin, F.; Wiesenfeld, U.; Galati, E.F.; Monasta, L.; Ricci, G. Hysteroscopic chasing for endometrial cancer in a low-risk population: Risks of overinvestigation. *Arch. Gynecol. Obstet.* **2016**, *293*, 851–856. [CrossRef] [PubMed]
18. Vinklerová, P.; Bednaříková, M.; Minář, L.; Felsinger, M.; Hausnerová, J.; Ovesná, P.; Weinberger, V. Tumor Characteristic Variations between Symptomatic and Asymptomatic Endometrial Cancer. *Healthcare* **2021**, *9*, 902. [CrossRef] [PubMed]

19. Gemer, O.; Segev, Y.; Helpman, L.; Hag-Yahia, N.; Eitan, R.; Raban, O.; Vaknin, Z.; Leytes, S.; Ben Arie, A.; Amit, A.; et al. Is there a survival advantage in diagnosing endometrial cancer in asymptomatic postmenopausal patients? An Israeli Gynecology Oncology Group study. *Am. J. Obstet. Gynecol.* **2018**, *219*, 181.e1–181.e6. [CrossRef] [PubMed]
20. Seebacher, V.; Schmid, M.; Polterauer, S.; Hefler-Frischmuth, K.; Leipold, H.; Concin, N.; Reinthaller, A.; Hefler, L. The presence of postmenopausal bleeding as prognostic parameter in patients with endometrial cancer: A retrospective multi-center study. *BMC Cancer* **2009**, *9*, 460. [CrossRef] [PubMed]
21. Barak, F.; Kalichman, L.; Gdalevich, M.; Milgrom, R.; Laitman, Y.; Piura, B.; Lavie, O.; Gemer, O. The influence of early diagnosis of endometrioid endometrial cancer on disease stage and survival. *Arch. Gynecol. Obstet.* **2013**, *288*, 1361–1364. [CrossRef] [PubMed]
22. Namazov, A.; Helpman, L.; Eitan, R.; Vaknin, Z.; Lavie, O.; Ben-Arie, A.; Amit, A.; Levy, T.; Volodarsky, M.; Atlas, I.; et al. The diagnosis of endometrial cancer in women with asymptomatic endometrial polyp does not increase survival rates: An Israel gynecologic oncology group study. *Maturitas* **2021**, *148*, 18–23. [CrossRef] [PubMed]

3.4 Hrstka R, Zavadil-Kokas F, Moukova L, Kolarova T, Shahidianakbar M, Anton M, Ovesna P, Munzova D, Bednarikova M, Bretova P, et al. Genetic analysis of uterine lavage fluids to identify women at high risk of endometrial cancer. BMC Res Notes. 2025;18:117.

The final article included in this chapter presents a prospective case-control analysis investigating the potential of uterine lavage fluid as a non-invasive medium for early detection of endometrial cancer and its precursors. Despite the rising incidence of EC and the absence of population-level screening, current diagnostic methods largely rely on symptom-driven detection, most notably postmenopausal bleeding. This study aimed to determine whether targeted NGS (next-generation sequencing) of uterine lavage fluid could effectively distinguish patients with EC or EIN (endometrial intraepithelial neoplasia) from healthy controls.

A total of 257 women undergoing hysterectomy were enrolled and categorized into three groups: 89 with histologically confirmed EC, 80 with EIN, and 88 as controls. DNA extracted from the lavage samples was sequenced for mutations in a panel of 22 genes commonly associated with endometrial carcinogenesis. Mutations were found across all groups, with no significant association between specific mutations and the presence of EC or EIN. Paradoxically, some cancer-associated mutations, were more prevalent in the control group than in the cancer group. Additionally, the overall mutation burden was higher in the control group, suggesting that background somatic alterations may occur independently of neoplastic transformation.

The study highlights critical limitations in using uterine lavage fluid for early EC detection, chiefly the lack of specificity of identified mutations and the risk of false positives. While uterine lavage sampling is minimally invasive and well-tolerated, its clinical utility remains uncertain without further refinement of biomarker panels. The diagnostic potential of this method might improve when integrated with additional genomic markers such as copy number variations, DNA methylation patterns, or even microbiome profiling.

In conclusion, while molecular profiling of uterine lavage fluid holds promise for the early detection of EC, this study did not support its diagnostic validity in the current form. The findings underline the need for broader biomarker discovery, improved methodological specificity, and further prospective validation before such an approach can be implemented in routine clinical practice.

The study, titled *"Genetic analysis of uterine lavage fluids to identify women at high risk of endometrial cancer",* was published in *BMC Research Notes* (IF 1.6, Q2) in 2025. The author's contribution: clinical methodology, data curation, manuscript writing – review and editing.

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RESEARCH NOTE Open Access

Genetic analysis of uterine lavage fluids to identify women at high risk of endometrial cancer

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Abstract

Objectives Endometrial cancer (EC) is the most common malignancy of the female genital tract in developed countries, yet preventive screening remains unavailable, and diagnostic approaches are largely limited to symptomatic women. Despite advancements in precision oncology, the biology of precancerous lesions is less understood compared to advanced disease. To address this gap, we conducted a prospective case-control study analysing uterine lavage fluid from women undergoing diagnostic evaluation. The study included 257 participants: 80 diagnosed with endometrial intraepithelial neoplasia (EIN), 89 with early-stage EC, and 88 healthy controls. Using targeted next-generation sequencing, we examined genetic alterations in 22 selected genes associated with EC development.

Results Our findings did not confirm a direct association between specific genetic mutations in uterine lavage fluid and the presence of EIN or early-stage EC ($p=0.501$). Mutations were detected in both cases and controls, with a higher overall mutation burden observed in controls, suggesting potential background genomic alterations unrelated to EC development. In conclusion, while molecular profiling of uterine lavage fluid remains a promising concept for non-invasive diagnosis, our results highlight significant challenges in specificity. Further studies with larger cohorts and additional biomarkers are necessary to clarify its diagnostic relevance and clinical applicability.

Keywords Endometrial cancer, Endometrial intraepithelial neoplasia, Precancer screening, Uterine lavage fluids, DNA sequencing

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Introduction

Endometrial cancer (EC) is the most prevalent cancer of the female genital tract in developed countries and the sixth most common cancer in women worldwide, with its incidence and mortality steadily rising [1, 2]. Current diagnostic practices rely on the presence of clinical symptoms, meaning that most cases are diagnosed only after symptoms appear, underscoring the lack of effective screening strategies for early detection [3]. Epidemiologically, EC risk is most strongly associated with estrogen exposure, however, other factors such as obesity, diabetes, early menarche, nulliparity, late menopause, advanced age, and tamoxifen use also play significant roles [4, 5]. Clinically, abnormal uterine bleeding or spotting, sometimes accompanied by vaginal discharge, is the most common presenting symptom. The standard diagnostic approach involves ultrasonography followed by dilatation and curettage or curettage followed by hysteroscopy to confirm the diagnosis through histopathological examination.

While targeted prevention efforts are emerging, they are limited by gaps in understanding the biology of precancerous endometrial lesions, as our knowledge largely pertains to advanced disease [6]. Endometrial cancers are broadly classified into two types based on precursor lesions: Type I EC, which commonly originates from atypical hyperplasia, and Type II EC, often arising from atrophic endometrium or within an endometrial polyp. Molecular distinctions between these precancerous lesions and normal endometrial tissue are clear, with Type I EC frequently exhibiting mutations in PTEN, KRAS, and beta-catenin, alongside microsatellite instability [7]. In contrast, Type II EC typically shows TP53 mutations, HER2/neu amplification, and loss of E-cadherin as well as p16 function due to either mutation or hypermethylation [8]. These molecular changes, evident even in early lesions, suggest the potential for early detection through genetic analysis.

In this study, we conducted a prospective analysis of uterine lavage fluid collected from women undergoing diagnostic evaluation, employing targeted next-generation sequencing (NGS) to detect genetic alterations associated with endometrial intraepithelial neoplasia (EIN) and early-stage endometrial carcinoma in comparison with healthy controls. This approach aims to establish a minimally invasive method for identifying high-risk individuals, potentially enabling earlier intervention for EC.

Materials and methods

Patient enrolment and sample collection

The local ethical committee of the University Hospital in Brno (FN Brno) approved the case-control study starting from 1 May 2021 and lasting until 31 December 2024, i.e. 44 months in total, with informed consent obtained from each patient included in the study. Only patients planning to undergo a hysterectomy (due to cancer, precancer, or other reasons– for example, uterine fibroids, uterine prolapse.) with a final histopathological examination were included in the study. The uterine lavage was done immediately before hysteroscopy or hysterectomy in the operating theatre. Cases were divided into three groups: (a) EC– histologically confirmed endometrial cancer, (b) EIN– histologically confirmed endometrial intraepithelial neoplasia, and (c) control– cases with benign histology. Cases with age under 18 years, with any previous malignity or cancer duplicity as well as with different histology from biopsy and final histological specimen were excluded. Clinical data were evaluated by oncologist from the hospital's patient records. Besides histological results, potential clinical risk factors for endometrial cancer (age, BMI, parity, hormonal contraceptives, arterial hypertension, and diabetes mellitus) were also recorded.

Fluids from uterine lavages were stored at 4 °C and processed within 24 h. Briefly, samples were centrifuged at 3,200 ×g/ 4 °C for 20 min. Pellets were resuspended in erythrocyte buffer (RBC) and incubated for 15 min. The samples were then centrifuged again for 20 min at 3,200 ×g/ 4 °C and the resulting pellets were frozen at -80 °C until DNA isolation.

Library preparation and sequencing

DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen, Germany) according to manufacturer´s instructions. Sequencing libraries were prepared using KAPA HyperPlus Library Preparation Kit (Roche, Switzerland). Briefly, gDNA was subjected to enzymatic fragmentation, then the sample library was amplified and purified, and the multiplexed DNA sample library pool was hybridized to enrichment probes. We designed a custom endometrial tumor amplicon panel to cover 22 genes with the highest mutation frequencies specific for EC (list of genes: POLE, POLD1, PTEN, PIK3CA, TP53, CTNNB1, KRAS, NRAS, HRAS, AKT1, EGFR, FGFR2, FBXW7, RB1, ATM, APC, ARID1A, ARID5B, PIK3R1, CDKN2A, PPP2R1A, RPL22) [9]. The genomic target regions were designed to cover all coding exons and all known hotspot loci localized outside the exons. For target enrichment, we designed hybridization probes using HyperDesign tool (Roche), which is an intuitive, user-friendly interface that combines KAPA Target Enrichment technology with KAPA HyperCap Probe and KAPA HyperPETE Primer designs to achieve the best possible coverage of regions of interest, with an estimated coverage of 98.1 %. Sequencing reads from sequencing of custom endometrial tumor amplicon panel were evaluated for quality control by FastQC [10] and aligned to reference genome hg38.p14 [11] by TopHat2 [12]. BAM files which were obtained were sorted (by samtools [13]) and used for detecting

of variants in DNA by VarScan2 [14]. Detected variants were used for statistical analysis between defined groups of samples.

Statistical analyses

Standard descriptive statistics - absolute and relative frequencies for categorical variables and median and interquartile range (IQR) for continuous variables - were used to summarize the data. Comparisons of frequencies or distributions between the EC, EIN and control groups were made using Pearson's Chi-squared test and Kruskal-Wallis rank sum test, respectively. To assess the association between the presence of specific mutations and disease status, univariate logistic regression analysis was performed. Any *p*-values presented are considered nominal in nature and no adjustment for multiplicity has been done. The analysis was performed using the R software (version 4.3.2). All tests were set as two-sided and tested at 5% significance level.

Data obtained from NGS were processed using the maftools (v2.18.0) package. Only mutations occurring in exons that resulted in protein-level changes were included in the analysis; thus, alterations in ncRNA, splicing regions, 3´UTR, 5´UTR, and synonymous mutations were excluded. Benign and likely benign mutations defined according to NCBI ClinVar database [15] were also excluded. Thus, clinically significant mutations and variants of uncertain significance (VUS) were included in the analysis. Finally, duplicate mutations were excluded during data processing. The occurrence of mutations is presented at the gene level.

Results

Patients' characteristics

We sequenced and analysed 257 lavage samples from patients enrolled in the study. Of these, 89 samples were histologically confirmed to have endometrial cancer (EC), 80 were from patients with endometrial intraepithelial neoplasia (EIN), and 88 were from the control group. Among these samples, 100 were free of any significant pathogenic mutations across the 22 sequenced genes, while exon mutations in one or more of these genes were detected in 157 samples (Table 1). Overall, we observed a similar frequency of all mutations in the 22 selected genes between the control and EIN groups, while a lower frequency was observed in the EC group. Table 1 also presents the associations between clinicopathological parameters and EC malignancy status. Women in the control group were significantly younger and had a lower BMI compared to those in the EIN and EC groups ($p < 0.001$ and $p = 0.006$, respectively). This finding suggests that age alone is unlikely to be the primary factor driving mutation accumulation, as one would expect an older population to exhibit a higher mutation frequency. Significant differences were also observed in the use of hormonal contraceptives ($p = 0.001$), prevalence of hypertension ($p = 0.002$), and presence of peroral antidiabetic drugs (PAD) ($p = 0.007$).

Analysis of mutations in DNA isolated from lavage fluid collections

Of the 257 uterine lavage samples that were analysed, at least one mutation was detected in 157 cases, and a total of 641 mutations were identified at 466 unique sites in 22 genes that were analysed. The mutation frequencies in each group are presented in Fig. 1.

Comparison of mutation prevalence between different groups of patients

We performed a pairwise analysis of controls vs. EIN, controls vs. EC, and EIN vs. EC. However, the frequency of any of the identified mutations was not significant enough within the control and EIN groups to distinguish the presence of EIN from controls (Fig. 2a). In fact, when comparing the mutation incidence between controls and ECs (Fig. 2b), the opposite trend was observed, with mutations in the KRAS and TP53 genes occurring significantly more frequently in the control group than in patients diagnosed with ECs. Similarly, when comparing EIN vs. EC, mutations in the KRAS gene were observed significantly more frequently in EIN (Fig. 2c). Notably, the mutation frequency of the KRAS gene was significantly higher in the control and EIN groups compared to the EC group ($p = 0.007$ and $p = 0.002$, respectively), further supporting this trend.

Discussion

A key role in cancer elimination is given to cancer prevention. However, most cases of EC are diagnosed in symptomatic women, and there is currently no reliable screening tool to identify high-risk individuals suspected of having EC [16]. However, the group of women who are overweight, have hereditary non-polyposis colon cancer, Lynch syndrome, or have had tamoxifen treatment would benefit from an effective screening strategy. Annually performed clinical examination and transvaginal ultrasound are insufficient. On the other hand, an additional endometrial biopsy and outpatient hysteroscopy could improve screening results, but are not well tolerated and acceptable by all women. Pipelle sampling can be used only in cases with a non-representative biopsy specimen or cervical stenosis [5]. Therefore, we focused on targeted sampling using uterine fluid lavage considered a minimally invasive sampling procedure that, in conjunction with molecular testing, could be useful in both diagnosis and screening. This straightforward and cost-effective method is well-tolerated by women and could be more

Table 1 Patient characteristics and presence of mutations by group

Characteristic	Overall, $N=257$[1]	Controls, $N=88$[1]	EIN, $N=80$[1]	EC, $N=89$[1]	*p*-value[2]
Age (years)	56 (48, 67)	47 (44, 51)	53 (48, 62)	66 (59, 73)	**<0.001**
BMI (kg/m^2)	30 (25, 36)	29 (23, 31)	31 (25, 41)	31 (27, 35)	**0.006**
Parity					0.589
0	24 (15.8%)	10 (19.2%)	6 (14.6%)	8 (13.6%)	
1	33 (21.7%)	10 (19.2%)	7 (17.1%)	16 (27.1%)	
2	80 (52.6%)	24 (46.2%)	24 (58.5%)	32 (54.2%)	
3	14 (9.2%)	7 (13.5%)	4 (9.8%)	3 (5.1%)	
4	1 (0.7%)	1 (1.9%)	0 (0.0%)	0 (0.0%)	
Hormonal contraceptives					**0.001**
No	112 (74.2%)	34 (65.4%)	27 (65.9%)	51 (87.9%)	
Yes	37 (24.5%)	18 (34.6%)	14 (34.1%)	5 (8.6%)	
Not known	2 (1.3%)	0 (0.0%)	0 (0.0%)	2 (3.4%)	
Hypertension					**0.002**
No	78 (51.3%)	36 (69.2%)	21 (51.2%)	21 (35.6%)	
Yes	74 (48.7%)	16 (30.8%)	20 (48.8%)	38 (64.4%)	
DM					0.070
No	125 (82.2%)	47 (90.4%)	36 (87.8%)	42 (71.2%)	
DM I	1 (0.7%)	0 (0.0%)	0 (0.0%)	1 (1.7%)	
DM II	25 (16.4%)	5 (9.6%)	5 (12.2%)	15 (25.4%)	
Not known	1 (0.7%)	0 (0.0%)	0 (0.0%)	1 (1.7%)	
PAD[3]					**0.007**
No	130 (85.5%)	48 (92.3%)	38 (92.7%)	44 (74.6%)	
Yes	21 (13.8%)	3 (5.8%)	3 (7.3%)	15 (25.4%)	
Not known	1 (0.7%)	1 (1.9%)	0 (0.0%)	0 (0.0%)	
Any mutation NGS					0.501
No	100 (38.9%)	32 (36.4%)	29 (36.2%)	39 (43.8%)	
Yes	157 (61.1%)	56 (63.6%)	51 (63.7%)	50 (56.2%)	
POLE EDM[4]	4 (1.6%)	1 (1.1%)	1 (1.3%)	2 (2.2%)	>0.999
AKT1	6 (2.3%)	2 (2.3%)	2 (2.5%)	2 (2.2%)	>0.999
APC	23 (8.9%)	6 (6.8%)	10 (12.5%)	7 (7.9%)	0.395
ARID1A	58 (22.6%)	24 (27.3%)	16 (20.0%)	18 (20.2%)	0.428
ARID5B	17 (6.6%)	9 (10.2%)	2 (2.5%)	6 (6.7%)	0.132
ATM	50 (19.5%)	22 (25.0%)	16 (20.0%)	12 (13.5%)	0.152
CDKN2A	6 (2.3%)	2 (2.3%)	2 (2.5%)	2 (2.2%)	>0.999
CTNNB1	21 (8.2%)	7 (8.0%)	5 (6.2%)	9 (10.1%)	0.655
EGFR	5 (1.9%)	2 (2.3%)	2 (2.5%)	1 (1.1%)	0.745
FBXW7	16 (6.2%)	8 (9.1%)	5 (6.2%)	3 (3.4%)	0.285
FGFR2	15 (5.8%)	3 (3.4%)	4 (5.0%)	8 (9.0%)	0.298
HRAS	1 (0.4%)	1 (1.1%)	0 (0.0%)	0 (0.0%)	0.654
KRAS	35 (13.6%)	15 (17.0%)	16 (20.0%)	4 (4.5%)	**0.007**
NRAS	3 (1.2%)	1 (1.1%)	0 (0.0%)	2 (2.2%)	0.776
PIK3CA	40 (15.6%)	18 (20.5%)	12 (15.0%)	10 (11.2%)	0.236
PIK3R1	26 (10.1%)	9 (10.2%)	8 (10.0%)	9 (10.1%)	0.999
POLD1	14 (5.4%)	6 (6.8%)	3 (3.8%)	5 (5.6%)	0.697
POLE	30 (11.7%)	11 (12.5%)	9 (11.2%)	10 (11.2%)	0.957
PPP2R1A	11 (4.3%)	6 (6.8%)	3 (3.8%)	2 (2.2%)	0.313
PTEN	71 (27.6%)	25 (28.4%)	23 (28.7%)	23 (25.8%)	0.896
RB1	12 (4.7%)	5 (5.7%)	2 (2.5%)	5 (5.6%)	0.572
RPL22	19 (7.4%)	6 (6.8%)	8 (10.0%)	5 (5.6%)	0.536
TP53	12 (4.7%)	7 (8.0%)	4 (5.0%)	1 (1.1%)	0.081

[1]Median (IQR); n (%)

[2]Kruskal-Wallis rank sum test; Pearson's Chi-squared test

[3]Peroral Antidiabetic Drugs

[4]POLE exonuclease domain mutations

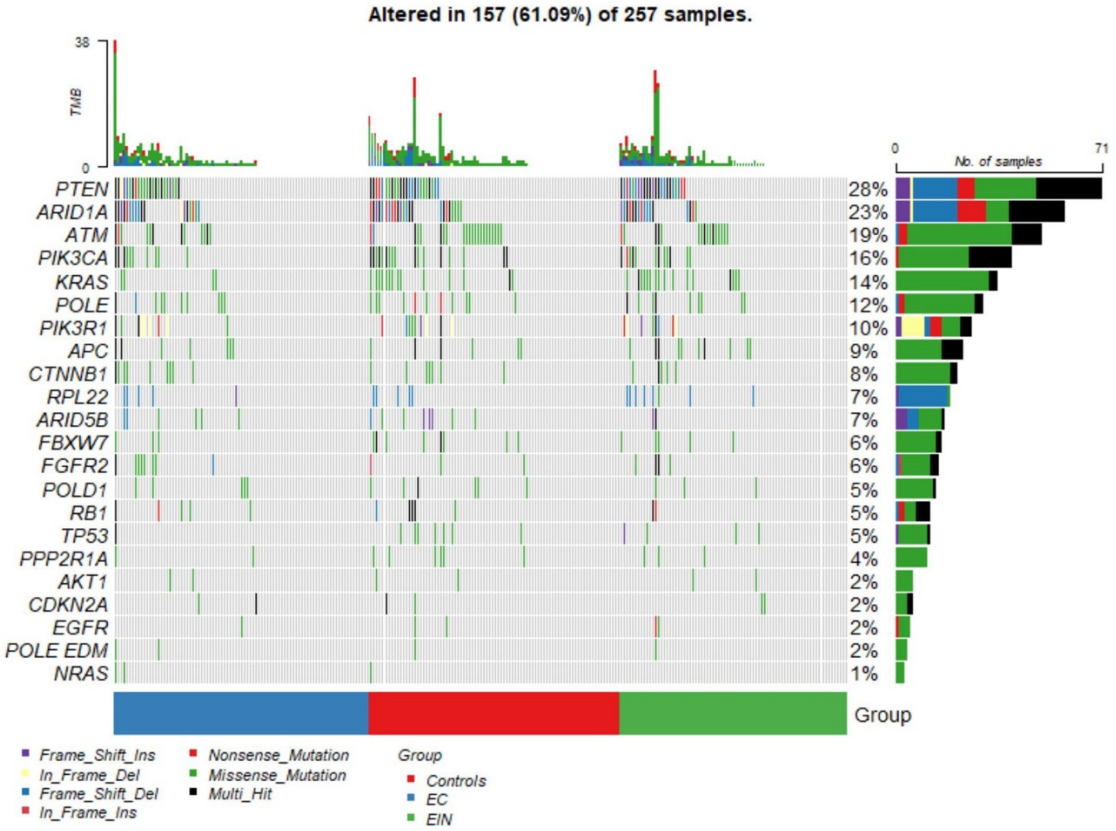

Fig. 1 Specific mutations in the genes of interest (including exon resolution) in individual samples. The rows show individual mutations, the columns show the patients assigned to each group

widely used not only in symptomatic patients, but also in asymptomatic women with an adverse medical history.

An ideal screening method for identifying high-risk patients with EC should be accurate, cost-effective, patient-friendly and at the same time to reliably identify cases requiring invasive testing while providing reassurance to low-risk women. Minimally invasive biofluid sampling has improved early gynecologic cancer detection by enabling the identification of cancer-specific genomic biomarkers, especially in blood, uterine lavage, and cervicovaginal fluid [17]. Examinations of uterine fluid lavages concerning the presence of EC are well-established, as demonstrated by various studies. For instance, a comprehensive genomic analysis of uterine lavage fluid has been shown to detect early endometrial cancers and reveal prevalent driver mutations even in women without histopathologic evidence of cancer [18, 19]. Moreover, recent advancements in NGS have enabled a more precise identification of oncogenic mutations in uterine lavage fluid, highlighting its potential as a liquid biopsy tool for early detection. Targeted molecular analysis of uterine lavage fluid has identified oncogenic mutations that precede clinical symptoms, underscoring the potential for early cancer screening [20]. For example, Chao et al. demonstrated that massively parallel sequencing of uterine lavage specimens successfully detects tumor-associated mutations, allowing risk stratification of patients before conventional histopathological confirmation [21]. In addition, a study by Weng et al. explored the role of circulating free DNA (cfDNA) in uterine lavage fluid, revealing that cfDNA mutations correlate with early-stage EC progression, further strengthening the case for non-invasive molecular diagnostics [22]. Accordingly, Mayo-de-Las-Casas et al. showed that the detection of somatic mutations in peritoneal lavages and plasma of EC patients can be used as a diagnostic tool, offering a broader perspective on the molecular landscape of endometrial cancer [23].

As a proof-of-concept study, we prospectively collected a cohort of 257 women to perform a genetic analysis of DNA extracted from uterine fluid lavages, aiming to identify specific mutations or mutation patterns indicative

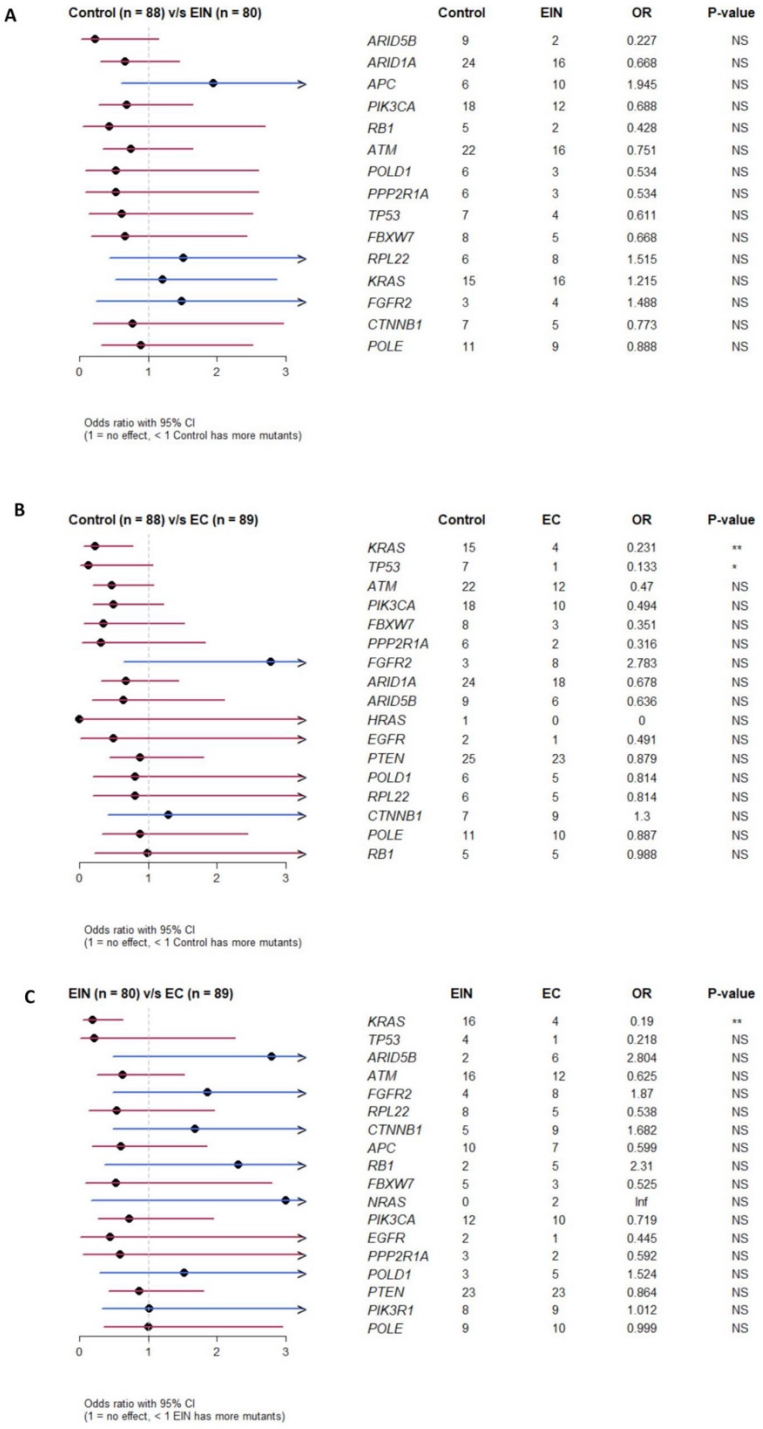

Fig. 2 **A**) Risk of EIN vs. control according to the occurrence of mutations in the monitored genes. **B**) Risk of EC vs. control according to the occurrence of mutations in the monitored genes. **C**) Risk of EC vs. EIN according to the occurrence of mutations in the monitored genes

of endometrial cancer or precancer. However, statistical analysis revealed no significant association between the presence of these mutations and the occurrence of cancer or precancer. These findings highlight inherent limitations and contextual challenges associated with this diagnostic approach. For instance, Nair et al. reported that while uterine lavage fluid could detect cancer-associated mutations in patients with endometrial cancer, similar mutations were present in nearly half of individuals without histopathologic evidence of cancer, raising concerns about specificity and clinical applicability [19]. Similarly, Maritschnegg et al. demonstrated the detection of mutations in uterine lavage fluid but noted inconsistencies in identifying some early-stage cancers, particularly certain subtypes [18]. Moreover, mutations associated with cancer have also been found in benign conditions, further complicating diagnostic accuracy. Genetic heterogeneity within tumors also poses a challenge; Mota et al. highlighted that intra-tumor heterogeneity may lead to under-detection of mutations when relying solely on uterine fluid samples [24]. These issues underscore the need for more refined approaches to enhance the specificity and reliability of uterine lavage as a diagnostic tool.

In conclusion, although genetic analysis of uterine fluid lavage could be a promising tool for early detection of EC, we did not confirm this trend in our study. This may be due to the limited specificity of this approach, or its sensitivity or potential overlap with benign conditions, which ultimately highlights the need for complementary diagnostic tools and more thorough validation. Other emerging factors warrant further investigation for their potential in the early detection of EC. These include copy number variation (CNV) analysis, which provides valuable insights into genomic alterations associated with EC [25]; and the identification of specific gene methylation patterns, which have demonstrated diagnostic accuracy comparable to endometrial biopsy and have been validated in prospective studies [26]. In addition, the role of the gut microbiome is an intriguing and emerging field of research. Some studies have suggested that specific bacterial species, such as *Porphyromonas somerae*, may have predictive value, particularly in postmenopausal and obese patients [27, 28]. However, no study has yet conclusively demonstrated that the microbiome alone has the same diagnostic potential as other molecular markers, and further validation is needed before it can be considered a reliable clinical tool. Integrating these biomarkers into a multifaceted diagnostic framework could enhance early detection strategies, improve risk assessment, and ultimately lead to better patient outcomes.

Limitations

While targeted sampling using uterine fluid lavage combined with genetic analysis of selected genes has been investigated as a potential approach for detecting endometrial cancer, our findings did not identify a specific mutational pattern, and even mutational load alone did not differentiate cases from controls. This highlights important limitations, including the possibility that genetic alterations detected in the lavage may not always indicate malignancy, leading to false positives, while tumors lacking mutations in the selected genes can result in false negatives. Additionally, the genetic diversity of endometrial cancer and population-specific variations in baseline genetic markers further complicate interpretation, as a limited gene panel may fail to capture the full spectrum of relevant mutations, potentially missing key diagnostic markers.

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Author contributions
R.H. designed experiments and wrote the main manuscript text; F.Z.K. was responsible for bioinformatics analysis of sequencing data; L.M., M.A., D.M., M.B., P.B. and L.M. conducted the clinical study and made several contributions to the paper; T.K. and M.S. performed the laboratory analysis; P.O. executed the statistical analysis; J.H. was responsible for histological verifications and participated on the manuscript writing; V.W. designed experiments, secured funding and participated on the manuscript writing. Each author has reviewed and consented to their contribution as detailed in the submission.

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Data availability
The experimental data that support the findings of this study are available in NCBI database as BioProject PRJNA1196106 and can be also accessible via link: http://www.ncbi.nlm.nih.gov/bioproject/1196106.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethics Committee of the University Hospital in Brno, Czech Republic (protocol number 03-100620/EK) for research involving human participants. All participants provided written informed consent prior to their participation in the study.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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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.
2. Mazidimoradi A, Momenimovahed Z, Khalajinia Z, Allahqoli L, Salehiniya H, Alkatout I. The global incidence, mortality, and burden of uterine cancer in

2019 and correlation with SDI, tobacco, dietary risks, and metabolic risk factors: an ecological study. Health Sci Rep. 2024;7(1):e1835.
3. Sangtani A, Wang C, Weaver A, Hoppman NL, Kerr SE, Abyzov A, et al. Combining copy number, methylation markers, and mutations as a panel for endometrial cancer detection via intravaginal Tampon collection. Gynecol Oncol. 2020;156(2):387–92.
4. Hrstka R, Podhorec J, Nenutil R, Sommerova L, Obacz J, Durech M, et al. Tamoxifen-Dependent induction of AGR2 is associated with increased aggressiveness of endometrial Cancer cells. Cancer Invest. 2017;35(5):313–24.
5. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. Lancet. 2016;387(10023):1094–108.
6. Spira A, Yurgelun MB, Alexandrov L, Rao A, Bejar R, Polyak K, et al. Precancer atlas to drive precision prevention trials. Cancer Res. 2017;77(7):1510–41.
7. Schlosshauer PW, Ellenson LH, Soslow RA. Beta-catenin and E-cadherin expression patterns in high-grade endometrial carcinoma are associated with histological subtype. Mod Pathol. 2002;15(10):1032–7.
8. Holcakova J, Bartosik M, Anton M, Minar L, Hausnerova J, Bednarikova M et al. New trends in the detection of gynecological precancerous lesions and Early-Stage cancers. Cancers (Basel). 2021;13(24).
9. 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.
10. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data. http://www.bioinformaticsbabrahamacuk/projects/fastqc/. 2010.
11. Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, et al. Ensembl 2022. Nucleic Acids Res. 2022;50(D1):D988–95.
12. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14(4):R36.
13. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO et al. Twelve years of samtools and BCFtools. Gigascience. 2021;10(2).
14. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012;22(3):568–76.
15. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42(Database issue):D980–5.
16. Asaturova A, Zaretsky A, Rogozhina A, Tregubova A, Badlaeva A. Advancements in minimally invasive techniques and biomarkers for the early detection of endometrial cancer: A comprehensive review of novel diagnostic approaches and clinical implications. J Clin Med. 2024;13:24.
17. Shen Y, Yang W, Liu J, Zhang Y. Minimally invasive approaches for the early detection of endometrial cancer. Mol Cancer. 2023;22(1):53.
18. Maritschnegg E, Wang Y, Pecha N, Horvat R, Van Nieuwenhuysen E, Vergote I, et al. Lavage of the uterine cavity for molecular detection of mullerian duct carcinomas: A Proof-of-Concept study. J Clin Oncol. 2015;33(36):4293–300.
19. Nair N, Camacho-Vanegas O, Rykunov D, Dashkoff M, Camacho SC, Schumacher CA, et al. Genomic analysis of uterine lavage fluid detects early endometrial cancers and reveals a prevalent landscape of driver mutations in women without histopathologic evidence of cancer: A prospective Cross-Sectional study. PLoS Med. 2016;13(12):e1002206.
20. Martignetti JA, Pandya D, Nagarsheth N, Chen Y, Camacho O, Tomita S et al. Detection of endometrial precancer by a targeted gynecologic cancer liquid biopsy. Cold Spring Harb Mol Case Stud. 2018;4(6).
21. Chao A, Wu KY, Lin CY, Lee YS, Huang HJ, Tang YH, et al. Towards less invasive molecular diagnostics for endometrial cancer: massively parallel sequencing of endometrial lavage specimens in women attending for an office hysteroscopy. J Mol Med (Berl). 2022;100(9):1331–9.
22. Weng CH, Wu KY, Wang CJ, Huang HJ, Tsai CL, Lin CY, et al. Massively parallel sequencing of endometrial lavage specimens for the detection of cancer-associated mutations in atypical and non-atypical endometrial hyperplasia. Front Med (Lausanne). 2022;9:1090788.
23. Mayo-de-Las-Casas C, Velasco A, Sanchez D, Martinez-Bueno A, Garzon-Ibanez M, Gatius S, et al. Detection of somatic mutations in peritoneal lavages and plasma of endometrial cancer patients: A proof-of-concept study. Int J Cancer. 2020;147(1):277–84.
24. Mota A, Colas E, Garcia-Sanz P, Campoy I, Rojo-Sebastian A, Gatius S, et al. Genetic analysis of uterine aspirates improves the diagnostic value and captures the intra-tumor heterogeneity of endometrial cancers. Mod Pathol. 2017;30(1):134–45.
25. Dugo E, Piva F, Giulietti M, Giannella L, Ciavattini A. Copy number variations in endometrial cancer: from biological significance to clinical utility. Int J Gynecol Cancer. 2024;34(7):1089–97.
26. Zheng H, Yu C, Yang L, Zhou F, Liu A. Research progress of DNA methylation markers for endometrial carcinoma diagnosis. J Cancer. 2025;16(3):812–20.
27. Di Tucci C, De Vito I, Muzii L. Immune-Onco-Microbiome: A new revolution for gynecological cancers. Biomedicines. 2023;11(3).
28. Zheng W, Lin X, Chen H, Yang Z, Zhao H, Li S, et al. Gut microbiota and endometrial cancer: research progress on the pathogenesis and application. Ann Med. 2025;57(1):2451766.

Publisher's note

4 Molecular Classification and Prognostic Markers

Recent advances in the pathological and molecular characterization of endometrial carcinoma have demonstrated the limitations of the traditional dualistic classification system. This binary model fails to capture the biological heterogeneity and prognostic variability of EC adequately. A pivotal development in the refinement of tumor classification came with the 2013 publication of TCGA (The Cancer Genome Atlas) study, which introduced a molecular taxonomy delineating four distinct EC subtypes based on genomic alterations[15]. This molecular framework provides a more precise prognostic stratification than histopathological assessment alone. Nevertheless, widespread implementation of TCGA methodology in routine clinical diagnostics remains limited due to significant technical and financial barriers. Consequently, subsequent research efforts have prioritized the identification of surrogate markers—particularly immunohistochemically assessable features—that correlate with TCGA subtypes[16,17].

The currently endorsed molecular classification integrates mutational analysis of the *POLE* (DNA Polymerase Epsilon) gene with IHC (Immunohistochemistry) profiling of p53 and MMR (mismatch repair) proteins—MLH1 (MutL Homolog 1), PMS2 (Postmeiotic Segregation Increased 2), MSH2 (MutS Homolog 2), and MSH6 (MutS Homolog 6)[18]. Based on these assessments, endometrial carcinomas are stratified into the following risk groups:

- ***POLE*-ultramutated carcinomas (5–15%)**

This group includes carcinomas with pathogenic mutations in exons 9–14 of the exonuclease domain of the *POLE* gene[19,20], detected via molecular diagnostic methods such as Sanger sequencing or NGS. Despite otherwise adverse pathological features (high grade, substantial lymphovascular space invasion, deep myometrial invasion), these tumors exhibit excellent prognosis. Surgical intervention is considered sufficient for stage I–II disease, and neither chemotherapy nor radiotherapy has been shown to add therapeutic benefit[16,20]. In the rare instances of disease progression, sensitivity to immunotherapy has been documented[21].

- **Mismatch Repair Deficient Carcinomas (25–30%)**

This group encompasses carcinomas with defects in one of the MMR system genes, leading to the accumulation of mutations due to faulty DNA base-pair mismatch repair[15]. MMR deficiency is determined by IHC detection of the MLH1, PMS2, MSH2, and MSH6 proteins[3]. Additional molecular testing for MSI (microsatellite instability) is recommended in cases of ambiguous expression. Prognosis is intermediate and depends on the disease stage.

If MLH1/PMS2 expression is lost, methylation analysis of the MLH1 promoter is necessary to distinguish between sporadic MMR deficiency (methylation present) and possible Lynch syndrome-associated tumors (methylation absent), warranting referral for genetic counseling. Hereditary predisposition to endometrial carcinoma, i.e., Lynch syndrome, occurs in 10% of MMR-deficient cases (compared to 3–5% of all endometrial carcinomas)[22]. Carcinomas in this category generally respond well to immunotherapy[21].

- **p53-Mutant Carcinomas (15–25%)**

This is the most aggressive group of carcinomas, associated with the poorest prognosis and responsible for the majority of endometrial cancer-related deaths. These are typically serous carcinomas, carcinosarcomas, clear cell carcinomas, or high-grade endometrioid carcinomas. Detection of a p53-mutant carcinoma (with myometrial invasion) is sufficient to classify the patient as high-risk, where adjuvant chemotherapy in addition to surgery is recommended, and in selected cases, radiotherapy as well[3]. p53 status is primarily assessed using IHC, with *TP53* mutational status confirmed via NGS if necessary.

- **Carcinomas with No Specific Molecular Profile (40–60%)**

This is the largest and most heterogeneous group of tumors, lacking a distinct molecular signature. Prognosis is generally intermediate, depending on stage and other tumor characteristics. A significant potential for future refinement of this group remains, based on additional prognostic molecular markers[3].

- **Multiple Classifiers (~5%)**

In approximately 5% of cases, both IHC and molecular genetic assessments reveal multiple concurrent features, referred to as "multiple classifiers"[23]. This often occurs in the context of an ultramutated phenotype (POLEmut), where secondary mutations—including in *TP53*—accumulate rapidly and chaotically, or in tumors with high genomic instability (e.g., MSI), where subsequent mutations such as *TP53* are common. Classification follows this scheme:

- Pathogenic *POLE* mutation, MMR deficient, and p53 mutation → classified as POLEmut
- Pathogenic *POLE* mutation, MMR proficient, and p53 mutation → classified as POLEmut
- No *POLE* mutation, MMR deficient, and p53 mutation → classified as MMRd

In addition to molecular classification, numerous other IHC-detectable biomarkers with demonstrated prognostic relevance have been identified, although they are not formally included in the classification of endometrial carcinoma. These markers, visualized using immunohistological (antibody-based) staining, indicate the presence of specific molecules within or on the surface of tumor cells. They can help identify patients at higher risk of recurrence, lymph node involvement, or death, while others may predict therapeutic response. For example:

- **Estrogen and Progesterone Receptors (ER/PR)**

ER and PR are frequently expressed in endometrioid EC and are associated with excellent prognosis[24,25]. Conversely, their absence is typical for non-endometrioid subtypes and correlates with increased lymph node metastasis[26], worse DFS[27], and poor response to hormonal therapy[28,29]. A 10% positivity cut-off, historically adopted from breast cancer protocols[27,30], is commonly used, although this threshold is now recognized as suboptimal for endometrial carcinoma.

The ENITEC group recently proposed a new ER/PR expression-based risk stratification scheme following a multicenter study: high risk (0–10%), intermediate risk (20–80%), and low risk (90–100%), which correlates more accurately with patient prognosis[31]. In clinical practice, it is advisable to confirm ER and PR expression by IHC before initiating hormonal therapy in advanced or recurrent disease, as the presence of receptors predicts response to hormonal therapy[32].

- **Human Epidermal Growth Factor Receptor 2 (HER2)**

Erb-B2 Receptor Tyrosine Kinase 2, commonly referred to as HER2 is a transmembrane receptor encoded by the *HER2* (*ERBB2*) oncogene and plays a pivotal role in oncogenesis and the aggressive behavior of certain cancers. In HER2-positive tumors, treatment options include monoclonal antibodies (trastuzumab, pertuzumab) and tyrosine kinase inhibitors (lapatinib)[33].

In endometrial carcinoma, HER2 overexpression has been linked to high-grade, non-endometrioid (serous) histology, advanced stage, and poor survival[34,35]. HER2 is therefore considered a potential target for biologic therapy in uterine tumors. The addition of trastuzumab to systemic chemotherapy has been shown to improve progression-free survival in patients with advanced serous EC[36].

Assessment of HER2 expression is increasingly being incorporated into clinical practice, alongside the therapeutic use of targeted monoclonal antibodies.

- **L1 Cell Adhesion Molecule (L1CAM)**

L1CAM is an immunoglobulin involved in embryonic brain development and neurohistogenesis (including axon growth, neuronal migration, and regeneration). In cancer cell lines, L1CAM promotes tumor growth and aggressive behavior[37]. It is one of the most potent prognostic IHC markers studied prior to the advent of molecular classification. L1CAM positivity is associated with advanced stage, high grade, non-endometrioid histology, LVSI, lymph node metastasis, and reduced DFS[27,38]. A 10% cut-off is most commonly used[38–40].

In the context of new molecular classification, L1CAM positivity has been observed in up to 80% of p53-mutant cases, underscoring the poor prognosis associated with both

markers[41,42]. In tumors with nonspecific molecular profiles, L1CAM IHC assessment enables further prognostic stratification based on DSS[42]. L1CAM expression is not unique to endometrial cancer and is also found in other malignancies. Preclinical mouse models are currently investigating monoclonal antibodies targeting L1CAM as potential adjuncts to chemotherapy and inhibitors of tumor progression[43,44].

It is important to note that estrogen receptor is now considered as a standard part of IHC examination according to recently updated European guidelines[45].

4.1 Bednaříková M, Hausnerová J, Minář L, Taslerová R, Vinklerová P, Ehrlichová L, Trizuljak J, Blaháková I, Princ D, Matulová K, Ovesná P, Slabý O, Weinberger V. Molekulární testování karcinomu endometria – analýza prvních zkušeností z klinické praxe. Klin Onkol 2023; 36(3): 215-223.

In the first study of this chapter, we focused on analyzing the initial experience following the implementation of molecular classification into clinical practice at the Department of Gynecology and Obstetrics, University Hospital Brno. This was a prospective data collection conducted from May 2021 to May 2022, during which molecular testing was performed as part of routine histopathological examination whenever a sufficiently representative tumor sample was available for immunohistochemical and molecular-genetic analyses.

Immunohistochemical evaluation of p53 and MMR proteins was conducted in all patients newly diagnosed with endometrial carcinoma. *POLE* mutational status was assessed by Sanger sequencing in all patients classified outside the low-risk category. In cases where MMR deficiency (MMRd) and/or p53 mutation (p53mut) were detected, *POLE* sequencing was performed regardless of clinical parameters, to resolve potential multiple classifiers.

A total of 85 patients were included in the analysis. The results of molecular testing were as follows: 22 patients (26%) were MMRd, 8 patients (9%) showed p53mut, and among the 40 patients in the non-low-risk category in whom *POLE* mutational analysis was performed, no ultramutated tumor was identified. When comparing patient stratification based on risk of recurrence according to the ESGO/ESTRO/ESP (European Society of Gynaecological Oncology/ European Society for Radiotherapy and Oncology/ European Society of Pathology) 2021 guidelines with the corresponding 2016 recommendations, differences were observed in the distribution across nearly all risk groups, except for patients diagnosed with advanced or metastatic disease.

Genetic counseling was offered to 24 patients in total—22 based on the identification of MMRd and 2 based on family history. At the time of analysis, genetic testing results

were available for 18 patients. Of these, 4 (22%) were confirmed carriers of a pathogenic variant in one of the genes associated with Lynch syndrome, 8 (45%) had a negative result, and 6 (33%) had a variant of uncertain clinical significance.

In summary, the updated European guidelines incorporating molecular classification introduce significant changes to the management of patients with newly diagnosed endometrial carcinoma. Our experience demonstrated that implementing a combination of IHC analysis of MMR and p53 proteins in all patients, along with *POLE* sequencing in those at non-low risk, is feasible in routine clinical practice and did not delay decision-making regarding adjuvant therapy. Evaluation of MMR protein expression helps identify patients who are carriers of inherited cancer predisposition syndromes and who are also potential candidates for targeted immunotherapy (*note: not available at the time of publication*).

A remaining question is why no patient with a *POLE* mutation was identified in the cohort. This may be explained by the small sample size of this pilot study, the fact that *POLE* status was assessed only in patients in the non-low-risk group, where it may influence treatment decisions, and the use of Sanger sequencing, which has lower sensitivity compared to next-generation sequencing (*note: Sanger sequencing has been recently replaced by NGS at University Hospital Brno*).

This pilot study, entitled *"Molecular testing of endometrial carcinoma in real-world clinical practice",* was published in 2023 in the journal *Klinická onkologie*.

The author's contribution: clinical methodology, data curation, manuscript writing – review and editing.

Molekulární testování karcinomu endometria – analýza prvních zkušeností z klinické praxe

Molecular testing of endometrial carcinoma in real-world clinical practice

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Souhrn

Východiska: Molekulární klasifikace přináší zásadní změny do diagnosticko-léčebného algoritmu u pacientek s karcinomem endometria (endometrial cancer – EC). Cílem sdělení je analýza prvních zkušeností se zavedením molekulárního testování do reálné klinické praxe. ***Materiál a metody:*** Od května 2021 je ve FN Brno u všech pacientek s nově diagnostikovaným EC v rámci standardního histopatologického vyšetření stanovován také imunohistochemicky status proteinů p53 a mismatch repair (MMR). U tumorů nesplňujících klinická kritéria pro nízké riziko a vždy při průkazu deficience *MMR* nebo mutace *p53* je prováděno také molekulárně genetické testování genu *POLE*. U každé pacientky je vyhodnoceno riziko recidivy podle nejnovějších guidelines z roku 2020 a na jeho základě doporučen další postup. Všem pacientkám s MMR-deficientními tumory a/nebo pozitivní rodinnou anamnézou je doporučena také genetická konzultace. ***Výsledky:*** Do analýzy bylo od května 2021 do května 2022 zařazeno celkem 85 pacientek s mediánem věku 66 let. MMR-deficientní tumory mělo 22 pacientek (26 %) a p53-mutované tumory 8 pacientek (9 %). U žádné ze 40 pacientek v nenízkém riziku s provedenou analýzou mutačního stavu genu *POLE* nebyl prokázán ultramutovaný typ tumoru. Celkem 46 (51 %) pacientek mělo v době diagnózy nízké riziko, 2 pacientky (2 %) střední, 14 pacientek (16 %) vyšší střední a 20 pacientek (24 %) vysoké riziko recidivy. Celkem 6 pacientek (7 %) bylo diagnostikováno s pokročilým nebo metastatickým onemocněním. Medián doby od operace po projednání na multidisciplinární komisi byl 21 dní (8–36). Celkem 76 pacientek (90 %) absolvovalo léčbu v plném rozsahu v souladu s rizikem recidivy. Z 18 pacientek s dostupnými výsledky genetického vyšetření byla u 4 (22 %) prokázána hereditární forma onemocnění. ***Závěr:*** Racionálně indikované molekulární testování kombinující imunohistochemické analýzy proteinů MMR i p53 u všech pacientek s EC a sekvenační analýzu genu *POLE* u pacientek v nenízkém riziku recidivy je v běžné praxi proveditelné a neprodlužuje dobu nutnou pro rozhodnutí o adjuvantní léčbě.

Klíčová slova

karcinom endometria – molekulární testování – p53 – mismatch repair systém – POLE

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.

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Summary

Background: Molecular classification has brought significant changes in the management of endometrial cancer (EC). In this article, we aim to analyze our first experience with an implementation of molecular testing into daily clinical practice. ***Materials and methods:*** In all newly diagnosed EC, the status of mismatch repair (MMR) and *p53* proteins has been evaluated immunohistochemically as a part of the routine histopathological examination since May 2021. In tumors that do not meet clinical criteria for a low risk and those with *MMR* deficiency or *p53* mutation, the molecular genetic testing of the *POLE* gene is performed as well. Recommendations for adjuvant treatment or follow-up are subsequently made based on the risk of recurrence. Genetic counselling is proposed to all patients with MMR-deficient tumors or family history of cancer. ***Results:*** A total of 85 patients with newly diagnosed EC between May 2021 and May 2022 were enrolled in the analysis. The median age was 66 years. The results of molecular testing were as follows: 22 (26%) MMR-deficient, 8 (9%) p53-mutated and none POLE-ultramutated of those 40 tumors with performed *POLE* sequencing. A total of 46 (51%) patient had a low risk, 2 (2%) intermediate, 14 (16%) high-intermediate and 20 (24%) patients had a high risk of recurrence. Advanced or metastatic diseases were diagnosed in 6 (7%) patients. The median time between surgery and multidisciplinary tumor board decision was 21 days (8–36). A total of 76 (90%) patients underwent the whole treatment plan according to the recurrence risk. At the time of analysis, the results of genetic testing were available in 18 patients and revealed 4 (22%) carriers of a pathogenic variant in any of the genes associated with Lynch syndrome. ***Conclusion:*** Molecular testing combining immunohistochemical analyses of MMR and p53 proteins in all newly diagnosed EC patients with sequencing analysis of *POLE* in those with non-low-risk disease is feasible and does not prolong the time needed for treatment decision.

Key words

endometrial cancer – molecular testing – p53 – mismatch-repair system – POLE

Úvod

Adenokarcinomy endometria (EC) představují výrazně heterogenní skupinu nádorů s často velmi odlišným biologickým chováním (tab. 1). Zásadní léčebnou modalitou je chirurgický výkon mající význam nejen kurativní, ale také stagingový. Zatímco u pacientek s nízkým rizikem recidivy je samotná operace považována za dostačující výkon, u pacientek nesplňujících kritéria pro nízké riziko je následně indikována adjuvantní léčba ať již ve formě radioterapie, chemoterapie, nebo kombinace obou metod, a to v návaznosti na míru rizika recidivy onemocnění [1]. Mezi základní prognostické parametry definující riziko rekurence patřily až do zavedení nové molekulární klasifikace stadium onemocnění v době diagnózy (tab. 2), histologický typ a přítomnost, nebo absence nádorové lymfangioinvaze (lymphovascular space invasion – LVSI) (tab. 3) [2].

Molekulární klasifikace EC byla navržena v roce 2013 na základě výsledků komplexních genomických, transkriptomických a proteomických analýz a rozděluje karcinom endometria do čtyř skupin: 1) ultramutované tumory s patogenní variantou genu *POLE*; 2) hypermutované mikrosatelitově instabilní tumory; 3) copy-number high tumory většinově s přítomnou patogenní variantou genu *TP53*; 4) ostatní tumory, tzv. copy-number low. Kromě molekulárně biologických charakteristik se jed-

Tab. 1. Histologické typy adenokarcinomu endometria, zjednodušené dělení podle [15].

Histologický typ	Četnost (%)
Endometroidní adenokarcinom	80
Serózní karcinom	< 10
Clear cell karcinom	< 10
Nediferencovaný/ dediferencovaný karcinom	2
Ostatní (vzácné): smíšený karcinom, mezonefrický adenokarcinom, skvamózní karcinom, mucinózní karcinom (intestinální typ), mesonephric-like adenokarcinom, karcinosarkom	

Tab. 2. TNM a FIGO klasifikace karcinomů endometria [16].

TNM	FIGO	Charakteristika
T1	I	nádor omezen na tělo dělohy
T1a	IA	nádor omezen na endometrium nebo postihuje méně než polovinu myometria
T1b	IB	nádor postihuje polovinu či více myometria
T2	II	nádor postihuje cervikální stroma, nešíří se však mimo dělohu
T3a, N1	III	lokální a/nebo regionální šíření specifikované níže:
T3a	IIIA	nádor postihuje serózu těla děložního nebo adnexa
T3b	IIIB	postižení pochvy nebo parametrií
N1	IIIC	metastázy do pánevních nebo paraaortálních mízních uzlin
	IIIC1	metastázy do pánevních mízních uzlin
	IIIC2	metastázy do paraaortálních mízních uzlin
T4	IVA	nádor postihuje sliznici močového měchýře / sliznici střeva
M1	IVB	vzdálené metastázy

FIGO – International Federation of Gynecology and Obstetrics, TNM – primární tumor, regionální lymfatické uzliny

Tab. 3. Charakteristiky prognostických skupin pacientek s karcinomem endometria podle doporučení z roku 2016 a 2020 [2,8].

Riziko	DOP_2016	DOP_2020	
		Molekulární klasifikace neznámá	Molekulární klasifikace známá
nízké	stadium IA endometroidní grade 1–2 LVSI negativní	stadium IA endometroidní low grade LVSI negat./fokálně	stadium I–II **POLEmut** bez reziduálního onemocnění
			stadium IA **MMRd/NSMP** endometroidní low grade LVSI negat./fokálně
střední	stadium IB endometroidní grade 1–2 LVSI negativní	stadium IB endometroidní low grade LVSI negat./fokálně	stadium IB **MMRd/NSMP** endometroidní low grade LVSI negat/fokálně
		stadium IA endometroidní high grade LVSI negat./fokálně	stadium IA **MMRd/NSMP** endometrioidní high grade LVSI negat./fokálně
		stadium IA non-endometroidní bez invaze do myometria	stadium IA **p53abn** a/nebo non-endometroidní bez invaze do myometria
vyšší střední	stadium IA endometroidní grade 3 LVSI +/–	stadium I endometroidní LVSI-pozitivní	Stadium I **MMRd/NSMP** endometroidní LVSI pozitivní
	stadium IA/B endometroidní grade 1–2 LVSI pozitivní	stadium IB endometroidní high grade	stadium IB **MMRd/NSMP** endometroidní high grade
		stadium II	stadium II **MMRd/NSMP** endometroidní
vysoké	stadium IB endometroidní grade 3 LVSI +/–	stadium III–IVA operace s R0	stadium III–IVA **MMRd/NSMP** operace s R0
	non-endometroidní	stadium I–IVA non-endometroidní s invazí do myometria operace s R0	stadium I – IVA **p53abn** s invazí do myometria operace s R0
	stadium II		stadium I – IVA **MMRd/NSMP** serózní, nediferencovaný karcinom nebo karcinosarkom s invazí do myometria; operace s R0
	stadium III endometroidní operace s R0		
pokročilý	stadium III s reziduem	stadium III–IVA reziduální onemocnění	stadium III–IVA reziduální onemocnění jakýkoliv typ
	stadium IVA		
metastatický	stadium IVB	stadium IVB	stadium IVB jakýkoliv typ

DOP_2016 – doporučení pro diagnostiku a léčbu karcinomu endometria z roku 2016, DOP_2020 – doporučení pro diagnostiku a léčbu karcinomu endometria z roku 2020, LVSI – nádorová lymfangioinvaze, MMRd – mismatch repair-deficientní, NSMP – nespecifický molekulární profil, POLEmut – ultramutované tumory s mutací genu *POLE*, p53abn – p53-mutovaný, R0 – operace s nulovým reziduem

notlivé skupiny významně odlišují také svou biologickou povahou. Zatímco ultramutované nádory s patogenní variantou genu *POLE* mají vynikající prognózu, nádory patřící do skupiny copy-number high mají naopak prognózu nejhorší a skupiny hypermutovaných mikrosatelitně instabilních i copy-number low tumorů vykazují intermediární prognózu [3]. Vzhledem k finanční nákladnosti komplexního genomového profilování, relativně dlouhé době odezvy a stále ještě limitované dostupnosti v běžné klinické praxi byla následně navržena a na nezávislých souborech pacientek validována alternativní metodika molekulárního testování založená na molekulárně genetické sekvenční analýze genu *POLE* a imunohistochemickém stanovení exprese proteinů MMR (mismatch repair systému, tj. MLH1, MSH2, MSH6 a PMS2) a p53 [4–7].

Na základě robustních dat dokladujících prognostický význam molekulární klasifikace bylo v roce 2020 její stanovování inkorporováno do aktualizace evropských doporučení pro management pacientek s EC (DOP_2020). Tato doporučení definují kritéria pro zařazení pacientek do jednotlivých skupin podle míry rizika recidivy onemocnění jak v situaci, kdy molekulární klasifikace známa není, tak v situaci, kdy jsou výsledky molekulárního testování k dispozici (tab. 3) [8]. Oproti předchozím doporučením z roku 2016 (DOP_2016) přinesla DOP_2020 některé zásadní změny. Vedle modifikace klinických prognostických parametrů (např. přesunutí stadia II z vysokého do vyššího středního rizika) je to především fakt, že průkaz patogenní sekvenční varianty v genu *POLE* svědčící pro ultramutovaný typ tumoru nebo mutovaný stav genu *TP53* zásadním způsobem ovlivňují terapeutický přístup u pacientek s nově diagnostikovaným EC. Nejenom pacientky v klinickém stadiu IA, ale i pacientky ve stadiu IB a II s POLE-ultramutovanými tumory jsou bez ohledu na další parametry zařazeny do skupiny s nízkým rizikem bez nutnosti adjuvantní terapie. Naopak tumory s aberantní expresí p53 (resp. mutací *TP53*) a prokázanou invazí do myometria jsou i při diagnóze ve stadiu IA bez ohledu na histologický typ zařazeny do skupiny s vysokým rizikem, kdy je standardně doporučena adjuvantní léčba (tab. 3) [8]. V roce 2021 bylo publikováno také společné národní doporučení čtyř odborných společností České lékařské společnosti J. E. Purkyně (ČLS JEP) detailně popisující metodiku molekulárního testování EC v podmínkách ČR [9].

Cílem předkládané práce je analýza prvních zkušeností s prospektivním stanovováním molekulárního testování u pacientek s nově diagnostikovaným EC a vyhodnocení přínosu zavedení molekulární klasifikace do reálné klinické praxe.

Materiál a metody

Od jara roku 2021 je molekulární testování EC ve FN Brno prováděno prospektivně v rámci standardního histopatologického vyšetření vždy, pokud je k dispozici dostatečně reprezentativní vzorek tumoru pro imunohistochemické a molekulárně genetické analýzy. Vyšetření jsou prováděna na vzorcích tumoru odebraných při operaci, fixovaných formaldehydem a zalitých v parafínu (formalin-fixed parafin-embeded – FFPE). Pokud nebyl operační výkon proveden, jsou vyšetření v indikovaných případech doplněna ze vzorků získaných při diagnostickém výkonu.

Proteiny p53 a MMR jsou stanovovány imunohistochemicky (IHC) u všech pacientek s nově diagnostikovaným EC.

Metodika IHC analýzy p53

K imunohistochemické analýze byla použita komerčně dostupná protilátka p53 (klon D0-7, 1 : 200; Agilent, USA) a automatický systém Ventana BenchMark Ultra. Dle míry exprese proteinu je nález hodnocen jako wild type (fokálně slabá disperzní jaderná pozitivita) či mutovaný typ exprese (silná jaderná exprese ve více než 80 % nádorových buněk, nulová jaderná exprese či cytoplazmatická exprese).

Metodika IHC analýzy MMR

K imunohistochemické analýze byly použity komerčně dostupné protilátky MSH2 (klon BSB-147, 1 : 50; BioSB, USA), MLH1 (klon ES05, RTU; Agilent, USA), PMS2 (klon EP51, RTU; Agilent, USA), MSH6 (klon PU29, 1 : 70; Novocastra, Leica Biosystems Newcastle Ltd, Anglie) a automatický systém Ventana BenchMark Ultra. Hodnocení a interpretace výsledků se řídí dle doporučení College of American Pathologists.

Metodika analýzy mutačního stavu genu *POLE*

Mutační stav genu *POLE* je vyšetřován u všech tumorů s výjimkou těch, které splňují kritéria pro zařazení do skupiny s nízkým rizikem recidivy, tj. low-grade endometroidní karcinomy s hloubkou invaze do méně než poloviny šířky myometria, bez podstatné LVSI (tab. 3). V případě průkazu MMRd a/nebo p53MUT je mutační stav genu *POLE* došetřen bez ohledu na klinické parametry, a to pro dořešení případných double či multiple classifiers.

Po identifikaci vhodného FFPE bloku k analýze a zhodnocení procentuálního zastoupení nádorových buněk erudovaným patologem je izolována genomová DNA s podílem nádorové DNA ≥ 50 %. Amplifikace a sekvenční analýza exonů 9–14 genu *POLE* (dle referenční sekvence NM_006231.4) se řídí aktuálními standardy vydávanými Společností českých patologů ČLS JEP a aktuální verzí National Comprehensive Cancer Network (NCCN) guidelines. Jednotlivé sekvence použitých primerů (Generi Biotech, Česká republika) jsou uvedeny v tab. 4. Nález je hodnocen jako negativní v případě, že nebyla detekována žádná z dosud popsaných patogenních sekvenčních variant genu *POLE* v exonech 9–14, nebo jako pozitivní v případě, že byla detekována některá z dosud popsaných patogenních sekvenčních variant genu *POLE* v exonech 9–14.

Stanovení molekulárního subtypu EC

Specifikace molekulárního subtypu EC je reportována u tumorů, které mají stanoveny všechny potřebné parametry, tj. status MMR, p53 a mutační stav genu *POLE*. V souladu s národním doporučením je používána terminologie [9]:

1) POLE-ultramutovaný typ tumoru – průkaz známých patogenních variant v exonu 9–14 genu *POLE*
2) MMR-deficientní typ – průkaz deficience MMR a současně *POLEwt* při jakémkoli statusu p53

3) p53-mutovaný typ – p53-aberantní fenotyp a současně MMR proficience i *POLEwt*
4) s nespecifickým molekulárním profilem

Stratifikace pacientek podle míry rizika recidivy

Na základě stagingu, histologického typu, gradingu, stanovení LVSI a výsledků molekulárního testování je pacientka v rámci projednání dalšího postupu na Indikační onkogynekologické komisi (IOGK) zařazena do příslušné rizikové skupiny podle DOP_2020 (tab. 3) [8]. Pokud bylo provedeno kompletní molekulární testování včetně analýzy genu *POLE*, je definitivní riziko stanoveno na základě DOP_2020 – Molekulární klasifikace známa. V situacích, kdy by případný průkaz patogenní varianty genu *POLE* neměnil léčebný postup, jako jsou tumory splňující kritéria pro nízké riziko, nebo naopak diseminované tumory v době diagnózy, je riziko stanoveno na základě klinických parametrů (tab. 3).

Kritéria pro indikaci genetické konzultace

Všem pacientkám s MMR-deficientními tumory a/nebo s pozitivní rodinnou anamnézou je v rámci projednání na IOGK doporučena také genetická konzultace k vyloučení hereditární formy onemocnění.

Soubor pacientek

Do analýzy byly zařazeny všechny pacientky Onkogynekologického centra FN Brno s nově diagnostikovaným EC v období květen 2021 – květen 2022. Pro posouzení významu začlenění molekulárního testování do klinické praxe byly pacientky retrospektivně zařazeny do některé z prognostických skupin také podle předchozích doporučení DOP_2016 (tab. 3) a výsledky stratifikací podle jednotlivých doporučení byly porovnány [2].

Statistická analýza

K popisu charakteristik pacientek a tumorů byly použity absolutní a relativní četnost pro kategoriální proměnné a pro spojité medián a mezikvartilové rozpětí. Vzhledem k tomu, že se jednalo o deskriptivní studii, nebylo provedeno žádné statistické testování.

Tab. 4. Sekvence použitých primerů.

PRIMER	Sekvence
POLE-9F	GAGCTTGGCTTTATGCTTATTTTG
POLE-9R	GGCAGATGCTGCTGTAGTATG
POLE-10F	TCTCTAGGCAGAGTGTGTGG
POLE-10R	ACATGTCCGTTCTTCCCAC
POLE-11F	CTTTGGGAGAGGAATTTGGAATAG
POLE-11R	CAGGAGCCACCTCCTAAGTC
POLE-12F	GGGCATTAGAGCCTGACC
POLE-12R	GTGACAGCACAGTCTGCAAG
POLE-13F1	TGCCTGTTAGGAACTTGCATC
POLE-13R1	ATGTCCTCCGGGTCTAGC
POLE-13F2	ACAGTTACCTTCCTGTGGGC
POLE-13R2	TGTCCCGGAGACACAGC
POLE-14F	GTGCTTCACACTTGACCCTG
POLE-14R	TGCCGACAGGACAGATAATG

Výsledky

Do analýzy bylo zařazeno celkem 85 pacientek s mediánem věku 66 let, z toho 58 (68 %) v klinickém stadiu I, 7 (8 %) ve stadiu II, 16 (19 %) ve stadiu III a 4 (5 %) ve stadiu IV. Celkem 67 (79 %) pacientek mělo low-grade endometroidní karcinom, 13 (15 %) high-grade endometroidní karcinom a 5 (6 %) non-endometroidní karcinomy (tab. 5). I přes implementaci molekulárního testování byl medián doby od operace po projednání na IOGK 21 dní (8–36).

Výsledky molekulárního testování byly následující: celkem 22 pacientek (26 %) mělo MMR-deficientní tumory a 8 pacientek (9 %) p53-mutované tumory. U žádné ze 40 pacientek v nenízkém riziku s provedenou analýzou mutačního stavu genu *POLE* nebyl prokázán ultramutovaný typ tumoru. U jedné pacientky nebyl vyšetřen status p53 a u dvou pacientek v nenízkém riziku nebyla provedena analýza genu *POLE* (tab. 6). V obou případech se jednalo o tumory diagnostikované v klinickém stadiu IVB, kdy doplnění molekulárních analýz by nevedlo ke změně terapeutického postupu. Z pohledu zařazení pacientek do skupin dle míry rizika recidivy podle DOP_2020 bylo 46 pacientek (51 %) v nízkém riziku, 2 pacientky (2 %) ve středním, 14 pacientek (16 %) ve vyšším středním a 20 pacientek (24 %) ve vysokém riziku. Celkem 6 pacientek (7 %) bylo diagnostikováno s pokročilým nebo metastatickým onemocněním.

U 76 pacientek (90 %) byl léčebný postup stanoven i dokončen plně v souladu s DOP_2020 podle míry rizika recidivy onemocnění [8]. U jedné pacientky (1 %) musela být doporučená adjuvantní léčba ukončena předčasně z důvodu nepřiměřené toxicity chemoterapie. Pouze u 8 pacientek (9 %) byla výsledná léčebná doporučení modifikována z důvodu celkového stavu nebo vůle pacientky, kdy navzdory riziku recidivy nebyla doporučená adjuvantní chemoterapie nebo radioterapie aplikována.

Při porovnání rozdílů ve stratifikaci pacientek podle míry rizika recidivy na základě DOP_2020 a předchozích DOP_2016 jsou patrné rozdíly v počtech zařazených pacientek prakticky ve všech rizikových skupinách s výjimkou pacientek diagnostikovaných s pokročilým nebo metastatickým onemocněním, kde se kritéria pro zařazení v mezidobí nijak nezměnila (graf 1) [2,8]. K modifi-

Tab. 5. Klinické charakteristiky souboru.

Klinické charakteristiky	n = 85
věk, medián (IQR)	66 (58–72)
BMI, medián (IQR)	33 (29 – 35)
menopauzální stav, n (%)	
pre/perimenopauza	14 (16 %)
postmenopauza	71 (84 %)
parita, medián (IQR)	2,00 (1,00–2,00)
Klinické stadium, n (%)	
I	
IA	51 (60 %)
IB	7 (8,2 %)
II	7 (8,2 %)
III	
IIIA	4 (4,7 %)
IIIB	1 (1,2 %)
IIIC	11 (13 %)
IV	
IVA	0
IVB	4 (4,7 %)
Histologie, n (%)	
endometroidní LG	67 (79 %)
endometroidní HG	13 (15 %)
non-endometroidní	
serózní	2 (2,4 %)
clear-cell	1 (1,2 %)
karcinosarkom	2 (2,4 %)
Doba do IOGK, medián (IQR), dny	21,0 (19,0 –23,2)

BMI – body mass index, HG – high-grade, IOGK – indikační onkogynekologická komise, IQR – medzikvartilové rozpětí, LG – low-grade, n – počet případů

Tab. 6. Výsledky molekulárního testování.

Výsledky testování	n = 85
POLE, n (%)	
MUT	0
WT	40 (100 %)
% nádorové tkáně, medián (IQR)	80 (50–90)
neprovedeno	45
MMR, n (%)	
MMRd	22 (26 %)
MMRp	63 (74 %)
p53, n (%)	
MUT	8 (9 %)
WT	76 (90 %)
neprovedeno	1

kaci rizika oproti dříve užívanému doporučení došlo v našem pilotním souboru celkem u 11 (13 %) pacientek, z toho u 4 (36 %) na základě molekulárních charakteristik (konkrétně průkaz p53-mutovaného tumoru při absenci průkazu MMRd a nemutovaném stavu genu *POLE*) a u 7 (64 %) pacientek na základě změny v posuzování významnosti některého z klinických parametrů (tab. 7).

Genetická konzultace byla nabídnuta celkem 24 pacientkám z celého souboru – 22 pacientkám na základě průkazu MMR-deficientního tumoru a 2 pacientkám s MMR-proficientními tumory pro rodinnou zátěž. V době analýzy byly známy výsledky genetického vyšetření u 18 pacientek, z nichž u 4 (22 %) bylo prokázáno nosičství patogenní varianty v některém z genů asociovaných s Lynchovým syndromem, u 8 (45 %) byl výsledek negativní a u 6 (33 %) pacientek byla detekována varianta nejasného klinického významu.

Diskuze

Aktualizace DOP_2020 obsahující implementaci molekulární klasifikace přináší zásadní změny do managementu pacientek s nově diagnostikovaným karcinomem endometria [8]. Předkládaná analýza shrnuje pilotní zkušenosti s aplikací těchto doporučení do běžné klinické praxe ve FN Brno. V návaznosti na publikovaná národní doporučení pro molekulární testování u karcinomu endometria jsme se v rámci Mezioborové pracovní skupiny pro onkogynekologii dohodli na algoritmu vyšetření a v průběhu jara 2021 bylo zahájeno molekulární testování jako nedílná součást rutinního histopatologického vyšetření. Námi zvolená metodika molekulárního testování reflektuje klinickou potřebu a vychází z úvahy, že vyšetření mutačního stavu genu *POLE* není nutné provádět paušálně, protože u pacientek s EC splňujících klinická kritéria pro nízké riziko recidivy nemění případná znalost o ultramutovaném typu tumoru klinický postup, kterým je doporučení dispenzarizace po operačním výkonu bez nutnosti adjuvantní léčby. Vyšetření stavu p53 a MMR indikujeme u všech pacientek s nově diagnostikovaným EC. Průkaz p53-mutovaného typu tumoru totiž automaticky zařazuje pacientku do skupiny s vysokým rizikem, kdy je standardně doporučována adjuvantní léčba ve snaze o redukci rizika recidivy onemocnění. Průkaz MMR-deficientního tu-

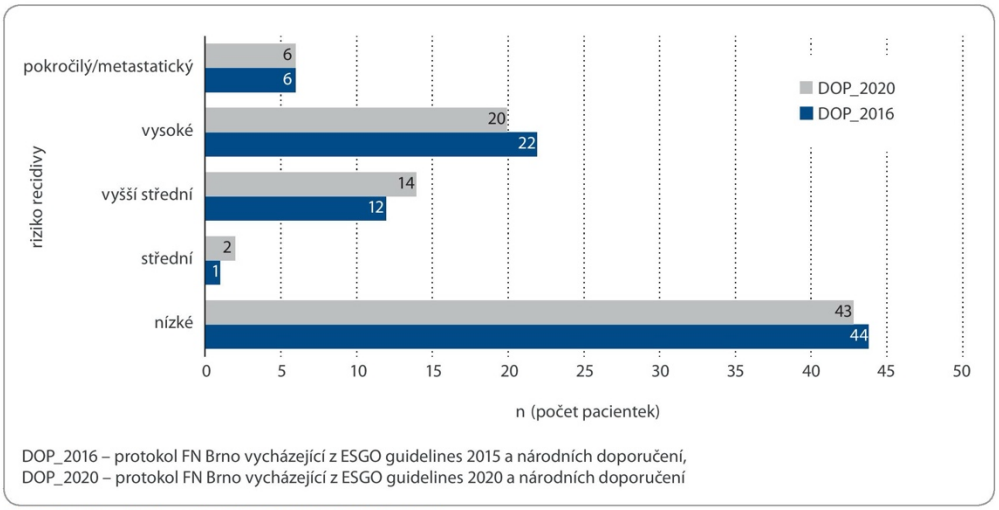
Tab. 7. Molekulární klasifikace karcinomu endometria – přehled publikovaných studií.

Charakteristiky	TCGA 2013 [3] n = 232	Talhouk 2015 [4] n = 143	Stelloo 2016 [6] n = 834	Talhouk 2017 [10] n = 319	Bosse 2018 [11] n = 376	Kommoss 2018 [7] n = 452	Devereaux 2022 [12] n = 310
typ studie	retrospektivní	retrospektivní	retrospektivní	retrospektivní	retrospektivní	retrospektivní	prospektivní
věk, medián	NA	63	68	67	66	65	64
stadium, n (%)							
I	254 (68 %)	102 (71 %)	834 (100 %)	221 (70 %)	291 (77 %)	365 (81 %)	177 (57 %)
II–IV	116 (32 %)	41 (29 %)		94 (30 %)	85 (23 %)	57 (19 %)	102 (33 %)
NA	3			4			31 (10 %)
histologie, n (%)							
EEC	304 (82 %)	119 (83 %)	834 (100 %)	215 (67 %)	376 (100 %)	320 (71 %)	220 (71 %)
non-EEC	66 (18 %)	25 (17 %)		104 (33 %)		132 (29 %)	90 (39 %)
grade, n (%)							
LG	193 (52 %)	90 (63 %)	724 (87 %)	123 (39 %)		357 (79 %)	185 (60 %)
HG	111 (30 %)	53 (37 %)	110 (13 %)	196 (61 %)	376 (100 %)	95 (21 %)	32 (10 %)
NA	66 (18 %)						93 (30 %)
POLE, n (%)							
MUT	17 (7 %)	12 (8 %)	49 (6 %)	30 (9 %)	49 (13 %)	42 (9 %)	15 (5 %)
WT	215 (93 %)	131 (92 %)	785 (94 %)	289 (91 %)	327 (87 %)	410 (91 %)	295 (95 %)
metodika	NGS	NGS/Sanger	Sanger	NGS/Sanger	NGS/Sanger	NGS/Sanger	SNaPshot
MMR, n (%)							
MMRd	65 (28 %)	41 (29 %)	219 (26 %)	64 (20 %)	138 (36 %)	127 (28 %)	59 (25 %)
MMRp	167 (72 %)	102 (71 %)	615 (74 %)	255 (80 %)	238 (64 %)	325 (72 %)	251 (75 %)
metodika	NGS/MSI	IHC	MSI	IHC	IHC	IHC	IHC
p53							
P53MUT	60 (26 %)	25 (18 %)	74 (9 %)	86 (27 %)	79 (21 %)	55 (12 %)	81 (26 %)
P53WT	172 (74 %)	118 (82 %)	760 (91 %)	233 (73 %)	297 (79 %)	397 (88 %)	229 (73 %)
metodika	NGS	IHC	IHC	IHC	IHC	IHC	IHC
NSMP/P53WT	90 (39 %)	63 (44 %)	492 (59 %)	139 (44 %)	228 (50 %)	228 (50 %)	135 (44 %)

EEC – endometroidní karcinom, HG – high-grade, IHC – imunohistochemie, LG – low-grade, MMR – mismatch-repair system, MMRd – MMR-deficientní, MSI –mikrosatelitní instabilita, MUT – mutovaný, n – počet, NA – nestanoveno, NGS – sekvenování nové generace, non-EEC – non-endometroidní karcinom, NSMP – s nespecifickým molekulárním profilem, Sanger – analýza metodou Sangerova sekvenování, SNaPshot – single nucleotide polymorphism analyses, WT – wild type

moru sice podle aktuálních doporučení neovlivňuje zařazení pacientky do některé z rizikových skupin, ale umožňuje identifikaci pacientek, u nichž je indikováno další došetření k vyloučení hereditární formy onemocnění. Při zohlednění počtu nových pacientek v našem centru a potřebě rychlého zavedení molekulárního testování jsme se dohodli, že v úvodní fázi budeme status p53 a MMR stanovovat v souladu s národním doporučením imunohistochemicky a mutační stav genu *POLE* metodou Sangerova sekvenování. Sekvenování nové generace využíváme v současnosti u pacientek s EC při vyčerpaných možnostech standardní systémové léčby podávané s paliativním záměrem, a to v rámci pátrání po eventuální targetovatelné lézi v situacích, kdy je pacientka stále kandidátkou aktivního terapeutického přístupu.

Jak dokladují naše první zkušenosti, námi zvolená metodika je v praxi proveditelná a nijak neprodlužuje dobu nutnou k projednání dalšího postupu v rámci primární léčby u pacientek s nově diagnostikovaným EC (medián doby od data diagnózy do projednání dalšího postupu s výsledky histologie byl v našem souboru 21 dní (tab. 5). Při hodnocení pilotních výsledků v kontextu s dosud publikovanými daty mů-



DOP_2016 – protokol FN Brno vycházející z ESGO guidelines 2015 a národních doporučení,
DOP_2020 – protokol FN Brno vycházející z ESGO guidelines 2020 a národních doporučení

Graf 1. Predikce rizika podle DOP_2016 a DOP_2020 [2,8].

žeme konstatovat, že věkové složení naší kohorty (medián věku 66 let) i proporcionální rozložení jednotlivých stadií v době diagnózy (68 % diagnostikováno ve stadiu I a 32 % ve stadiu II–IV) odpovídá klinickým parametrům souborů v dosud publikovaných retrospektivních analýzách [3,4,6,7,10,11] i ve vůbec první práci popisující zkušenosti s prospektivním stanovováním molekulární klasifikace u EC [12] (tab. 5 a 8).

Skutečnost, že v našem souboru nebyla zachycena žádná pacientka s ultramutovaným typem tumoru, může být vysvětlena především relativně malou velikostí naší pilotní kohorty v porovnání s velikostí souborů pacientek v rámci dosud publikovaných studií, obzvláště vezmeme-li do úvahy udávanou frekvenci těchto typů tumorů (5–13 %; tab. 8). Podle dosud publikovaných dat se POLE-ultramutované tumory vyskytují především u pacientek s endometroidními tumory, a to u tumorů s vysokým i nízkým gradem. Např. ve studii Stelloo et al. bylo 49 % pacientek s POLE-ultramutovaným typem tumoru ve skupině s nízkým rizikem recidivy [6]. Je tedy evidentní, že řada pacientek s potenciálně POLE-ultramutovaným tumorem nebyla v našem souboru vůbec testována, neboť znalost výsledku testování by nezměnila náš klinický postup. Velký důraz je kladen na dostatečné procentuální zastoupení nádorové tkáně ve vzorku vybraném patologem pro účely Sangerova sekvenování, a to vzhledem k možné limitaci této metody v senzitivitě záchytu patogenních variant. Udávaný detekční limit námi užívané metodiky je 15–20 % mutované alely, tj. nejméně 30–40 % buněk s mutací. Při mediánu 80 % nádorové tkáně ve vzorcích určených k testování *POLE* v naší pilotní kohortě nepředpokládáme významný podíl falešně negativních výsledků (tab. 6). Navíc výsledky studie Temko et al. dokladují, že somatické patogenní varianty exonukleázové domény genu *POLE* vznikají velmi časně v průběhu kancerogeneze a jsou detekovatelné již v prekurzorových lézích. Nepředpokládá se tedy významný podíl falešně negativních výsledků při analýze mutačního stavu *POLE* v důsledku heterogenity tumoru [13].

Relativně nízký podíl pacientek s p53-mutovaným typem tumoru v našem souboru (n = 8, tj. 9 %) je vysvětlitelný zejména nízkým podílem pacientek s non-endometroidními karcinomy, resp. serózními karcinomy (tab. 6). Tato hodnota je v naprosté korelaci s výsledky studie Stelloo et al., ve které byly retrospektivně analyzovány pacientky ze studií PORTEC-1 a PORTEC-2, tj. pouze s endometroidními karcinomy při podílu HG tumorů 13 % (tab. 8).

Podíl pacientek s MMR-deficientními tumory 26 % (tab. 6) je plně v souladu s výsledky dosud publikovaných studií (tab. 8). Vysoký záchyt pacientek s hereditární formou EC při genetickém testování indikovaném na základě průkazu MMR-deficientního tumoru a/nebo rodinné anamnézy (22 %) dokladuje oprávněnost námi zvolených kritérií ke genetické konzultaci i skutečnost, že plošné zavedení testování MMR u všech pacientek s nově diagnostikovaným EC napomáhá identifikovat pacientky s dědičnou dispozicí ke vzniku nádorových onemocnění.

Konkordance mezi skutečně absolvovanou léčbou a léčbou doporučenou v návaznosti na zařazení pacientky do rizikové skupiny podle recentních evropských doporučení s využitím molekulárního testování (89 %) je v našem pilotním souboru vysoká, vyšší než např. v holandské studii posuzující compliance lékařů s aplikací doporučených postupů v běžné klinické praxi [14]. Naše pilotní zkušenosti tak dokladují praktickou realizovatelnost nových doporučení pro management pacientek s EC. Vý-

znamnost změn, které tato nová doporučení přináší, ilustruje porovnání s predikcí rizika recidivy podle předchozího DOP_2016 (graf 1) [2] a také detailní charakteristika parametrů, které byly důvodem pro změnu ve stratifikaci rizika u jednotlivých případů (tab. 7).

Hlavním kladem naší pilotní studie je skutečnost, že se jedná o první práci svého druhu vyhodnocující pragmatickou implementaci molekulárního testování do běžné klinické praxe u pacientek s nově diagnostikovaným karcinomem endometria, kdy jsou stanovovány parametry p53 a MMR u všech pacientek a mutační stav genu *POLE* je vyhodnocován pouze u pacientek s nenízkým rizikem recidivy onemocnění. Relativním nedostatkem je velikost souboru podmíněná faktem, že se jedná o pilotní studii vyhodnocující dosavadní zkušenosti před případnou modifikací zavedeného postupu.

Závěr

Pilotní analýza našich zkušeností s implementací molekulárního testování u pacientek s nově diagnostikovaným karcinomem endometria dokladuje, že kombinace imunohistochemických analýz proteinů MMR a p53 u všech pacientek a sekvenační analýzy genu *POLE* u pacientek v nenízkém riziku je v běžné klinické praxi proveditelné a neprodlužuje dobu nutnou k rozhodnutí o adjuvantní léčbě. Stanovování nových markerů v rámci molekulárního testování pomáhá identifikovat pacientky se špatnou, nebo naopak excelentní prognózou (p53-mutované tumory, resp. ultramutované tumory s patogenní variantou genu *POLE*), a lépe tak cílit doporučení stran případné adjuvantní léčby. Samotný výsledek analýzy proteinů MMR sice doposud neměl vliv na volbu optimální strategie v rámci primární léčby, nicméně její plošné provádění u všech pacientek s nově diagnostikovaným karcinomem endometria napomáhá v širším kontextu identifikovat ty pacientky, které jsou nosičkami dědičné dispozice ke vzniku nádorových onemocnění.

Dedikace

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Literatura

1. Modrá kniha České onkologické společnosti. [online]. Dostupné z: https://www.linkos.cz/lekar-a-multidisciplinarni-tym/personalizovana-onkologie/modra-kniha-cos/aktualni-vydani-modre-knihy/.
2. Colombo N, Preti E, Landoni F et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013; 24 (Suppl 6): vi33–vi38. doi: 10.1093/annonc/mdt353.
3. The Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. Nature 2013; 497(7447): 67–73. doi: 10.1038/nature12113.
4. Talhouk A, McConechy MK, Leung S 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.
5. León-Castillo A, de Boer SM, Powell ME 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): 3388–3397. doi: 10.1200/JCO.20.00549.
6. Stelloo E, Nout RA, Osse EM 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): 4215–4224. doi: 10.1158/1078-0432.CCR-15-2878.
7. Kommoss S, McConechy MK, Kommoss F et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol 2018; 29(5): 1180–1188. doi: 10.1093/annonc/mdy058.
8. Concin N, Matias-Guiu X, Vergote I et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer 2020; 31(1): 12–39. doi: 10.1136/ijgc-2020-002230.
9. Dundr P, Cibula D, Doležel M et al. Molecular testing in endometrial carcinoma – joint recommendation of Czech Oncological Society, Oncogynecological Section of the Czech Gynecological and Obstetrical Society, Society of Radiation Oncology, Biology and Physics, and the Society of Czech Pathologists. Ceska Gynekol 2021; 86(4): 264–272. doi: 10.48095/cccg2021264.
10. Talhouk A, McConechy MK, Leung S et al. Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer: molecular classification of EC. Cancer 2017; 123(5): 802–813. doi: 10.1002/cncr.30496.
11. Bosse T, Nout RA, McAlpine JN et al. Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups. Am J Surg Pathol 2018; 42(5): 561–568. doi: 10.1097/PAS.0000000000001020.
12. Devereaux KA, Weiel JJ, Pors J et al. Prospective molecular classification of endometrial carcinomas: institutional implementation, practice, and clinical experience. Mod Pathol 2022; 35(5): 688–696. doi: 10.1038/s41379-021-00963-y.
13. Temko D, Van Gool IC, Rayner E et al. Somatic POLE exonuclease domain mutations are early events in sporadic endometrial and colorectal carcinogenesis, determining driver mutational landscape, clonal neoantigen burden and immune response. J Pathol 2018; 245(3): 283–296. doi: 10.1002/path.5081.
14. Eggink FA, Mom CH, Boll D et al. Compliance with adjuvant treatment guidelines in endometrial cancer: room for improvement in high risk patients. Gynecol Oncol 2017; 146(2): 380–385. doi: 10.1016/j.ygyno.2017.05.025.
15. Kurman RJ, Carcangiu ML, Herrington CS et al. WHO classification of tumours of female reproductive organs. International Agency for Research on Cancer 2014.
16. Brierley J, Gospodarowicz MK, Wittekind C et al. TNM klasifikace zhoubných novotvarů. [online]. Available from: https://dwn.alza.cz/ebook/nahled/pdf2/EK34089.
17. Colombo N, Creutzberg C, Amant F et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up. Radiother Oncol 2015; 117(3): 559–581. doi: 10.1016/j.radonc.2015.11.013.

4.2 Chacon E, Boria F, Lyer RR, Fanfani F, Malzoni M, Bretová P, et al. SENECA study: staging endometrial cancer based on molecular classification. Int J Gynecol Cancer. 2024;34(9):1313-1321.

The Department of Gynecology and Obstetrics at University Hospital Brno has extensive experience with the sentinel lymph node biopsy method (*see Chapter 5 for details*) in endometrial carcinoma, which enabled us to make a significant contribution to the international multicenter study SENECA (Staging ENdomEtrial CAncer based on molecular classification).

This study sought to assess the relationship between SLN (sentinel lymph node) involvement and molecular subtypes of endometrial carcinoma in patients with early-stage disease (FIGO 2009 stage I–II).

This large, retrospective, multicenter observational study included 2139 patients treated surgically for stage I–II endometrial cancer between January 2021 and December 2022 across 66 institutions in 16 countries. All patients underwent sentinel lymph node mapping according to ESGO recommendations, with SLNs evaluated by ultrastaging or one-step nucleic acid amplification.

Sentinel lymph node metastases were detected in 9.6% of cases, with the majority (67.8%) representing low-volume disease such as micrometastases or isolated tumor cells. Among the molecular subtypes, SLN involvement was most frequent in p53abn (12.5%) and MMRd (12.4%) tumors, compared to 7.8% in NSMP and 6.3% in POLEmut subtypes. These differences were statistically significant, with both p53abn and MMRd tumors showing increased odds of nodal metastasis compared to the NSMP (no specific molecular profile) group.

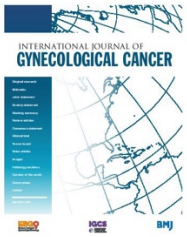
Risk stratification according to the updated 2021 ESGO molecular classification revealed SLN involvement in 2.84% of patients classified as low-risk, 6.62% in the intermediate-risk group, 21.63% in the high–intermediate-risk group, and 22.51% in the high-risk category. However, the predictive performance for lymph node metastasis of the 2021 classification was comparable to that of the 2016 system (AUC:

0.74 vs. 0.75; p = 0.73), suggesting that molecular stratification does not significantly improve the identification of patients at risk for nodal disease.

In conclusion, the SENECA study demonstrated that sentinel lymph node involvement varies significantly across molecular subtypes of endometrial carcinoma, with p53abn and MMRd tumors exhibiting the highest rates of nodal metastasis. While molecular classification adds substantial biological insight and refines prognostic stratification, it does not currently outperform traditional histopathological criteria in predicting lymph node status. Therefore, molecular profiling should not yet replace established surgical staging algorithms, although it remains a crucial component of individualized risk assessment and should be incorporated into future clinical research and trial design.

The *"SENECA study: staging endometrial cancer based on molecular classification"* was published in the *International Journal of Gynecological Cancer* (IF 4.5, Q1) in 2024.

The author's contribution: data curation, manuscript writing – review and editing.



SENECA study: staging endometrial cancer based on molecular classification

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ABSTRACT

Objective Management of endometrial cancer is advancing, with accurate staging crucial for guiding treatment decisions. Understanding sentinel lymph node (SLN) involvement rates across molecular subgroups is essential. To evaluate SLN involvement in early-stage (International Federation of Gynecology and Obstetrics 2009 I–II) endometrial cancer, considering molecular subtypes and new European Society of Gynaecological Oncology (ESGO) risk classification.

Methods The SENECA study retrospectively reviewed data from 2139 women with stage I–II endometrial cancer across 66 centers in 16 countries. Patients underwent surgery with SLN assessment following ESGO guidelines between January 2021 and December 2022. Molecular analysis was performed on pre-operative biopsies or hysterectomy specimens.

Results Among the 2139 patients, the molecular subgroups were as follows: 272 (12.7%) p53 abnormal (p53abn, 1191 (55.7%) non-specific molecular profile (NSMP), 581 (27.2%) mismatch repair deficient (MMRd), 95 (4.4%) POLE mutated (POLE-mut). Tracer diffusion was detected in, at least one side, in 97.2% of the cases; with a bilateral diffusion observed in 82.7% of the cases. By ultrastaging (90.7% of the cases) or one-step nucleic acid amplification (198 (9.3%) of the cases), 205 patients were identified with affected sentinel lymph nodes, representing 9.6% of the sample. Of these, 139 (67.8%) had low-volume metastases (including micrometastases, 42.9%; and isolated tumor cells, 24.9%) while 66 (32.2%) had macrometastases. Significant differences in SLN involvement were observed between molecular subtypes, with p53abn and MMRd groups having the highest rates (12.50% and 12.40%, respectively) compared with NSMP (7.80%) and POLE-mut (6.30%), (p=0.004); (p53abn, OR=1.69 (95% CI 1.11 to 2.56), p=0.014; MMRd, OR=1.67 (95% CI 1.21 to 2.31), p=0.002). Differences were also noted among ESGO risk groups (2.84% for low-risk patients, 6.62% for intermediate-risk patients, 21.63% for high–intermediate risk patients, and 22.51% for high-risk patients; p<0.001).

Conclusions Our study reveals significant differences in SLN involvement among patients with early-stage endometrial cancer based on molecular subtypes. This underscores the importance of considering molecular characteristics for accurate staging and optimal management decisions.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The molecular profile of endometrial cancer is a strong independent prognostic factor and predicts the response to adjuvant treatment. However, its influence on lymph node involvement in early endometrial cancer is unknown.

WHAT THIS STUDY ADDS

⇒ Molecular subgroups of endometrial cancer have distinctive sentinel node involvement patterns. The p53 abnormal and mismatch repair deficient molecular profile are the two groups with the highest tendency to present nodal involvement. The European Society of Gynaecological Oncology high–intermediate risk and high prognostic risk groups are groups with high lymph node involvement.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ In this study, molecular profiling emerges as a predictor of nodal involvement. This suggests the potential of molecular classification in the personalization of surgical lymph node staging protocols for patients with endometrial cancer.

INTRODUCTION

Endometrial cancer is the most common gynecological cancer in Europe, encompassing a 5-year prevalence of 34.7%, amounting to 445 805 cases.[1] In recent years, sentinel lymph node biopsy (SLN) has emerged as a viable alternative to complete lymph node dissection in early-stage disease.[2 3] Prospective

clinical trials have confirmed the high sensitivity to detect lymph node metastasis and the high negative predictive value using a standardized SLN algorithm in high-risk/high-grade endometrial cancer.[4–7] In particular, when performed according to state-of-the-art principles, a negative SLN is acceptable to confirm pN0.[8 9]

The Cancer Genome Atlas Research Network identified in 2013 four molecular subgroups of endometrial cancer with different clinical and prognostic outcomes.[10–14] The significance of this development led the European Society of Gynecologic Oncology (ESGO) to the integration of the new molecular classification into the prognostic risk classification of endometrial cancer.[8] This transition involved shifting from a risk classification based purely on histopathologic factors to a new prognostic risk classification that incorporates the molecular subtype in addition to the different histologic features. However, there is a lack of evidence on the role of molecular classification in the sentinel node biopsy algorithm.[9]

Therefore, the primary objective of this study was to assess the rate of SLN involvement according to the different molecular subtypes in patients with stage I–II endometrial cancer (International Federation of Gynecology and Obstetrics (FIGO) 2009).[15] Second, we aimed to evaluate the accuracy of the new ESGO prognostic risk classification (including molecular profiling) for the prediction of SLN involvement with respect to the classic risk classification (based on histological factors).

METHODS

Study Design

The study was a retrospective multicentric international observational study reviewing data of patients diagnosed with early-stage (FIGO stage 2009 I–II)[15] endometrial cancer who underwent standard surgical protocol according to ESGO guidelines[8] including total hysterectomy and bilateral salpingo-opherectomy together with the SLN algorithm[9] between January 2021 and December 2022. Patients were considered eligible if all the following criteria were met: age 18 years or older; histological confirmation of endometrial cancer with endometrioid histology or high-risk histology (serous, clear cell, carcinosarcoma, and mixed histologies); pre-operative FIGO stage I or II by MRI or ultrasound; pre-operative CT scan or PET-CT without evidence of local or distant disease (could be omitted in low-risk and intermediate-risk endometrial carcinoma with low-grade histology according to the ESGO guidelines.[8] In addition, a detailed SLN study protocol had to be accredited, either by ultrastaging or one-step nucleic acid amplification.[16 17] Molecular analysis had to be performed on the pre-operative biopsy or hysterectomy specimen.

The definition of POLE was predicated on the identification of exonuclease domain mutations within the gene. The participating centers employed diverse DNA sequencing methodologies, encompassing next-generation sequencing and Sanger sequencing. Definition of mismatch repair deficient (MMRd): An MMRd tumor was discerned via the immunostaining of at least two (PMS2 and MSH6), or preferably four (PMS2, MLH1, MSH6, and MSH2) MMR proteins. The complete absence of expression in one or more of these MMR proteins constituted a diagnostic criterion for MMRd endometrial cancer. Analysis of p53: p53 immunostaining was regarded as a near-flawless surrogate marker for an underlying TP53 mutation in nearly all cases studied. In only a handful of instances, the determination of TP53 was additionally corroborated by extensive DNA sequencing techniques; both results were admitted for classification purposes.

According to ESGO guidelines, POLE mutation analysis could be omitted in low-risk and intermediate-risk endometrial carcinoma with low-grade histology (stage IA endometrioid+low-grade + lymphovascular space invasion (LVSI) negative or focal or stage IB endometrioid+low-grade + LVSI negative or focal) while information on MMRd and p53 abnormal (p53abn) status was available in all cases.[8] Patients were excluded if they were pregnant; if they had undergone previous hysterectomy and/or previous pelvic/para-aortic lymphadenectomy; if extra-uterine disease (peritoneal, visceral, or suspicious lymph node metastasis) was present; or they had a past medical history of any invasive tumor, previous abdominal or pelvic radiotherapy of any type (including brachytherapy), and history of pre-operative neoadjuvant chemotherapy.

Accrual and Data Source

Gynecological cancer centers/units/hospitals regularly performing elective surgeries for endometrial cancer internationally were invited to participate. Invitations were sent through international/national and informal networks. Participating sites registered with the central audit team at Clinica Universidad de Navarra and were provided with unique user access credentials for the database.

Each participating site identified a principal investigator who was responsible for coordinating data entry at their local site. After obtaining ethical consent from our central institutional review board, we required a certificate of approval from the local ethics committees from all the investigators. An anonymized complete case record form, including 140 items by Google Forms database was sent to all the principal investigators. Before completing the case collection, all researchers signed a final declaration affirming that all the submitted data matched the data in the patients' charts. The trial was registered in clinicaltrials.gov under the identification number NCT05707312.

Statistical Analysis

A sample size of 1032 patients may provide sufficient statistical power to evaluate the association between molecular subgroups and sentinel lymph node status. We assumed a 90% power for a two-sided p value of 0.05 and a minimum difference of 4.4 percentage points in prevalence rates of positive lymph nodes. We expected a potential dropout rate of 10%. Quantitative data will be presented as mean and SD and qualitative variables with absolute values and percentages. Additionally, qualitative variables among groups will be compared by X^2 test or Fisher exact test; and quantitative variables with t-test and analysis of variance test.

The primary objective was to evaluate the lymph node involvement rate (sentinel) for each molecular subtype in patients with stage I–II endometrial cancer. The sentinel lymph node involvement rate included isolated tumor cells (isolated tumor cells <0.2 mm or less than 200 tumorous cells in a single histologic section), micrometastases (0.2–2 mm or more than 200 tumorous cells in a single histologic section) and macrometastases (metastases >2 mm). The SLN involvement was compared among molecular subtype groups (POLE-mutated (POLE-mut); MMRd; non-specific molecular profile (NSMP); and p53abn. Patients harboring more

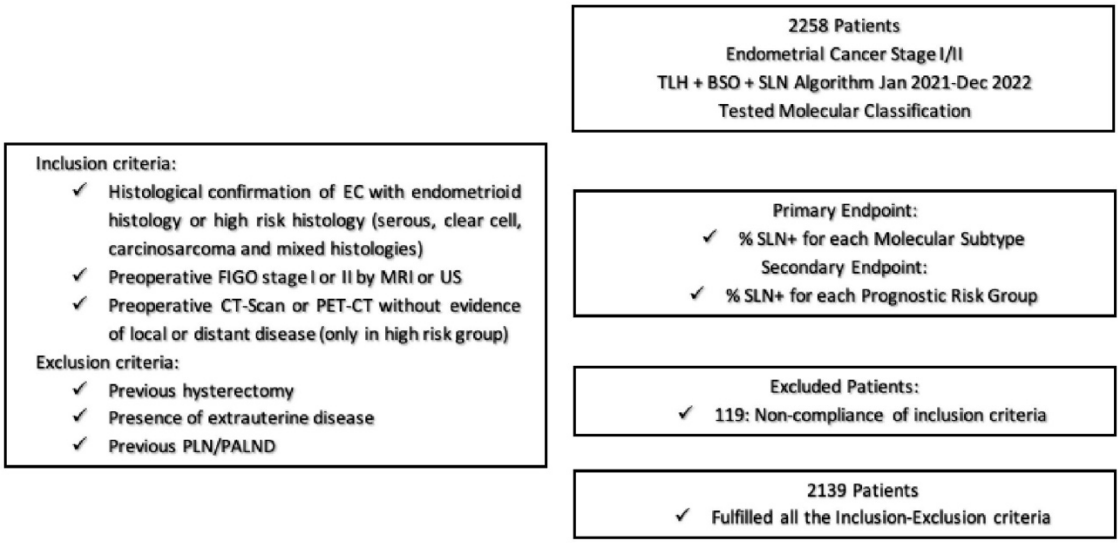

Figure 1 Flowchart of study population. BSO, bilateral salpingo-opherectomy; EC, endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics; PALND, para-aortic lymph node dissection; PLN, pelvic lymph node; SLN, sentinel lymph node; TLH, total hysterectomy.

than one molecular feature were classified according to the guideline's recommendations.[8] The rate of sentinel lymph node involvement was studied including isolated tumor cells; however, for staging purposes, isolated tumor cells were considered as pN0i+.

The hypothesis is that there will be differences in the lymph node involvement rate among molecular subtype groups. The z-test for independent proportions and the logistic regression will be used to test this hypothesis.

The secondary objectives include evaluation of the lymph node involvement rate (sentinel) for each ESGO prognostic risk group (new risk classification including molecular profile versus classic risk classification without including molecular profile). The patients were categorized according to the ESGO classification criteria into: low-risk, intermediate-risk, high–intermediate and high-risk groups.[8] Nodal status was not taken into account to establish the groups since it was the target variable. For the multivariate analysis, a logistic regression model will be used. All analyses were performed with the IBM SPSS 26.0 and the Stata 14 packages.

RESULTS

From January 1, 2023, to September 1, 2023, we collected data from 2258 patients across 66 institutions spanning 16 different countries. A total of 119 patients did not meet the inclusion–exclusion criteria or had missing information and were excluded (Figure 1). Tables 1 and 2 show the patient characteristics. Mean age was 64.5 years (SD 10.80). Mean body mass index was 30.2 kg/m^2 (SD 6.65). The diagnostic method used in most patients was hysteroscopy (998 (46.7%)) followed by blind biopsy (630 (29.5%)) and curettage (476 (22.3%)). Regarding the surgical approach, a total of 2026 patients (94.7%) underwent minimally invasive procedures and 113 were operated by laparotomy (5.3%). Among patients who underwent minimally invasive surgery, 594 patients (27.8%) were operated on robotically.

Focusing on the SLN approach, the majority of the cases, 2059 (96.2%), were performed with indocyanine green as a tracer (alone or in combination), injected at a volume of 4 cc (1544 (72.2%) patients). In 1686 (78.8%) patients lymph node staging was performed exclusively by sentinel lymph node biopsy. The median number of sentinel nodes was two per patient (range 0–6). Tracer diffusion was detected in, at least one side, in 97.2% of the cases; with a bilateral diffusion observed in 82.7% of the cases. By ultrastaging (1941 (90.7%) of the cases)) or one-step nucleic acid amplification (198 (9.3%) of the cases), 205 patients were identified with affected sentinel lymph nodes, representing 9.6% of the sample. Of these, 139 (67.8%) patients had low-volume metastases (including micrometastases, 42.9%; and isolated tumor cells, 24.9%) while 66 (32.2%) patients had macrometastases. The most common final pathology was low-grade (1655 (77.4%) cases, including G1 and G2 tumors) endometrioid tumors (1866 (87.2%) cases) without lymphovascular space invasion (1649 (76.7%) cases). FIGO 2009 stages I and II were recorded in 1946 (90.9%) of the cases.

Molecular profiling was predominantly tested in the final postoperative specimen (64.5% of the cases vs 35.5% tested preoperatively). A complete molecular profile was obtained in 1217 (56.8%) cases, while in 922 (43.2%) patients, POLE-mut analysis was omitted due to low-risk or intermediate-risk endometrial cancer with low-grade histologies. Concerning the distribution of the groups, the most prevalent groups were NSMP in 1191 (55.7%) cases and MMRd in 581 (27.2%), followed by p53abn in 272

Table 1 Baseline characteristics

Baseline characteristics	n=2139
Age (years), mean (SD)	64.55 (10.80)
Body mass index (kg/m^2),mean (SD)	30.24 (6.65)
Diagnostic method, N (%)	
Hysteroscopy	998 (46.7)
Blind biopsy	630 (29.5)
Gynecologic curettage	476 (22.3)
Not reported	35 (1.6)
Surgical approach, N (%)	
Laparoscopic	1432 (66.9)
Robotic	594 (27.8)
Open	113 (5.3)
Nodes approach, N (%)	
SLNB	1686 (78.8)
SLNB+PLND (only one pelvic side)	131 (6.1)
SLNB+PLND (both pelvic sides)	188 (8.8)
SLNB+PLND (one side) + PALND	7 (0.3)
SLNB+PLND (both sides) + PALND	115 (5.4)
SLNB+PALND	12 (0.6)
Tracer, N (%)	
Indocyanine green	1865 (87.2)
Radiocolloid and indocyanine green	189 (8.8)
Blue dye	74 (3.5)
Blue dye and indocyanine green	5 (0.2)
Radiocolloid and blue dye	2 (0.1)
Not reported	4 (0.2)
Tracer volume, N (%)	
4 cc	1544 (72.2)
2 cc	371 (17.3)
1 cc	90 (4.2)
Not reported	134 (6.3)
SLN median, number (range)	2 (0–6)
SLN distribution, N (%)	
Both pelvic sides	1729 (80.8)
Right pelvic side	152 (7.1)
Left pelvic side	150 (7.0)
Both pelvic sides+aortic area	41 (1.9)
Left pelvic side+aortic area	8 (0.4)
Right pelvic side+aortic area	1 (0.05)
Aortic area	1 (0.05)
No SLN identified	57 (2.7)
SLN diagnostic method, N (%)	
Ultrastaging	1941 (90.7)
OSNA	198 (9.3)
SLN involvement, N (%)	205 (9.6)
Isolated tumor cells	51 (24.9)
Micrometastases	88 (42.9)
Macrometastases	66 (32.2)

OSNA, one-step nucleic acid amplification; PALND, para-aortic lymph node dissection; PLND, pelvic lymph node dissection; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy.

(12.7%) patients. The lowest prevalence group was POLE ultramutated in 4.4% of cases (95 patients).

Among the 205 patients with sentinel node involvement, we observed significant differences between molecular subtypes, with the p53abn and MMRd subgroups being the two groups with the highest rate of sentinel node involvement, 12.5% and 12.4%,

Table 2 Histopathological and molecular characteristics

Histology, N (%)	
Endometrioid	1866 (87.2)
Serous	129 (6.0)
Mixed histology	63 (2.9)
Carcinosarcoma	42 (2.0)
Clear cell	30 (1.4)
Not reported	9 (0.4)
Grade, N (%)	
Low grade	1655 (77.4)
High grade	432 (20.2)
Not reported	52 (2.4)
LVSI, N (%)	
No	1649 (76.7)
Yes	479 (22.4)
Not reported	20 (0.9)
FIGO stage 2009, N (%)	
IA	1278 (59.7)
IB	518 (24.2)
II	150 (7.0)
IIIA	28 (1.3)
IIIB	5 (0.3)
IIIC1	154 (7.2)
IIIC2	4 (0.2)
IV	2 (0.1)
Molecular profile, N (%)	
POLE-mut	95 (4.4)
MMRd	581 (27.2)
NSMP	1191 (55.7)
p53abn	272 (12.7)

FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; MMRd, mismatch repair deficient; NSMP, non-specific molecular profile; p53abn, p53 abnormal ; POLE-mut, POLE-mutated.

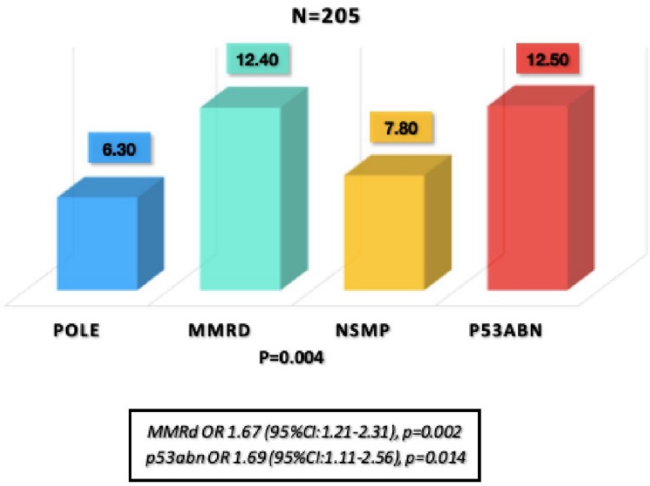

Figure 2 Rate of sentinel lymph node involvement according to the molecular profile. MMRD, mismatch repair deficient; NSMP, non-specific molecular profile; p53ABN, p53 abnormal; POLE, polymerase epsilon.

respectively, compared with 7.8% in NSMP and 6.3% in POLE ultramutated (p=0.004) (Figure 2). Patients with MMRd and p53abn had a 1.6 times higher chance of having sentinel lymph node involvement (OR=1.67 (95% CI 1.21 to 2.31), p=0.002 and OR=1.69 (95% CI 1.11 to 2.56), p=0.014, respectively). In this context, a higher rate of deep myometrial invasion (40.1%) was observed in the MMRd group, as well as a higher prevalence of high-grade (62.1%) non-endometrioid tumors (61.4%) with positive LVSI (31.6%) in the p53abn group (p<0.001).

Finally, significant differences in the SLN involvement rate were observed between the different groups of the new (molecular profile known) ESGO prognostic risk classification (2.84% for low-risk patients, 6.62% for intermediate-risk patients, 21.63% for high–intermediate risk patients and 22.51% for high-risk patients; p<0.001) (Figure 3A). This rate of nodal involvement remained very similar compared to the old (molecular profile unknown) ESGO prognostic risk classification (2.6% in low risk, 7.0% in intermediate risk, 20.6% in high–intermediate risk and 23.4% for high-risk patients) with no significant differences in the area under the curve between the two models (AUC 0.74 vs 0.75; p=0.73) (Figure 3B).

DISCUSSION

Summary of Main Results

In this retrospective study we showed that there are significant differences in sentinel node involvement for patients with early-stage endometrial cancer according to their molecular subtypes (p53abn: 12.50%; MMRd 12.40%; NSMP: 7.80%; POLE ultramutated: 6.30%). Second, we have defined the rate of SLN involvement for each of the new ESGO prognostic risk groups including molecular profiling (2.8% for low-risk patients, 6.6% for intermediate-risk patients, 21.6% for high–intermediate risk patients, and 22.5% for high-risk patients; p<0.001). This rate of nodal involvement remained very similar to that of the old ESGO prognostic risk classification (without including molecular profiling), with no significant differences in the area under the curve between the two models.

Results in the Context of Published Literature

This is a real-life study, in which a tracer distribution rate of 97.2% was revealed, with a bilateral mapping rate of 82.7%. These figures are in line with previous prospective studies.[4–7] However, this bilateral tracer distribution rate highlights a lack of standardization of the sentinel node dissection technique. A competency assessment tool for performing SLN biopsy in surgical quality assurance is now available from Moloney et al.[18] This might help to reduce the morbidity associated with lymphadenectomy in cases of tracer non-diffusion and to increase the rate of bilateral mapping.

Our study shows that 205 patients with stage I–II (FIGO 2009) endometrial cancer had sentinel lymph node involvement (9.6%), of which 24.9% had isolated tumor cells. With the introduction of ultrastaging and the one-step nucleic acid amplification protocol,[16 17 19] isolated tumor cells are increasingly identified in routine practice. Isolated tumor cells are not considered as pTN+, although they seem to have prognostic implications. Recently, Cucinella et al[20] conducted a multicenter retrospective study comparing the prognosis of patients with negative nodes versus those with isolated tumor cells in sentinel lymph nodes who are considered low risk—namely, FIGO 2009 IA cases with endometrioid grade 1 or 2. From 15 centers worldwide, 494 patients (42 isolated tumor cells and 452 node negative) were included. Twenty-one recurrences (4.3%) were identified, including in five patients with isolated tumor cells and 16 patients with negative lymph nodes. The study

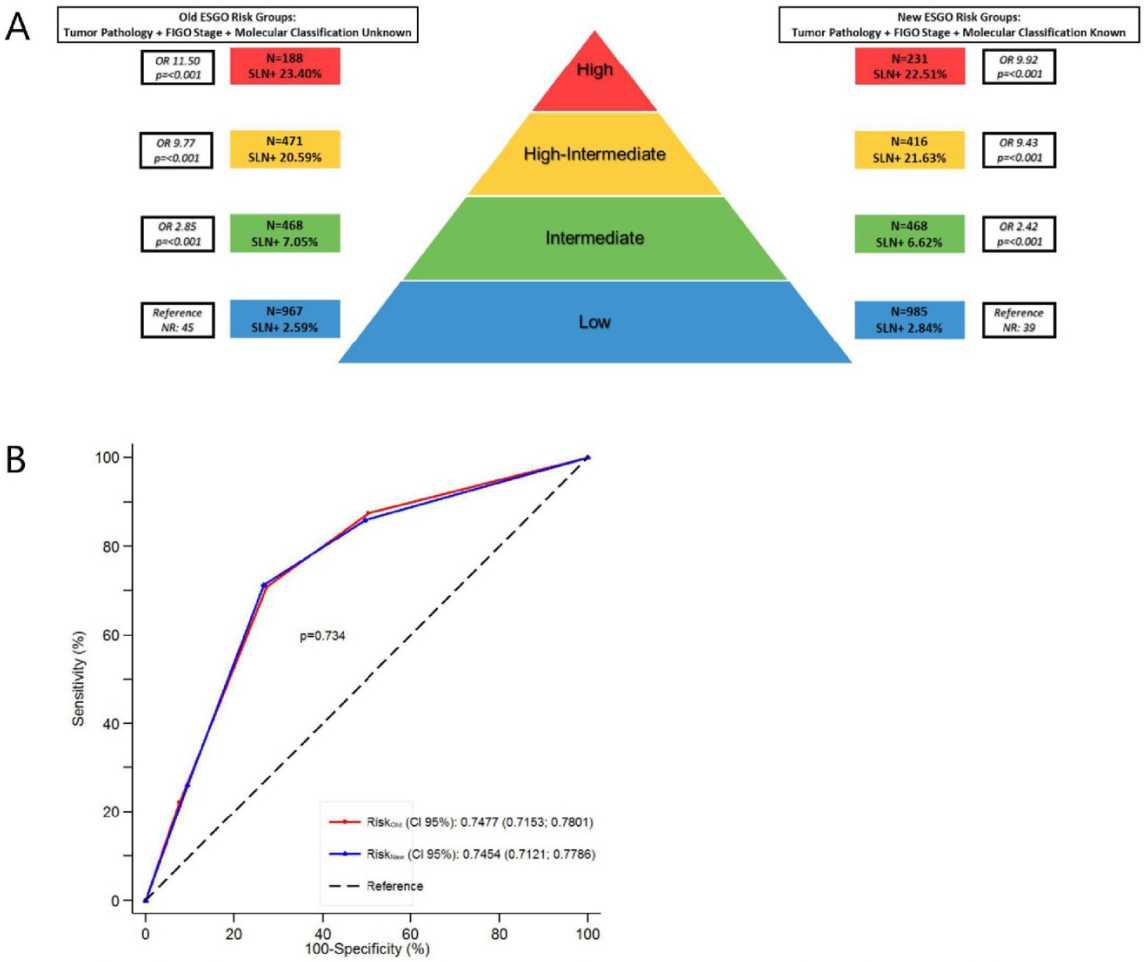

Figure 3 Rate of sentinel lymph node (SLN) involvement according to the European Society of Gynaecological Oncology (ESGO) prognostic risk classification (A). Area under the curve for the risk of sentinel lymph node involvement for the two models (B).

found that isolated tumor cells, grade 2, and lymphovascular space invasion were all associated with worse recurrence-free survival in the univariate analysis. Even when considering patients with negative lymphovascular space invasion, the presence of isolated tumor cells was still associated with higher non-vaginal recurrence (HR=4.47, 95% CI 1.21 to 16.6, p=0.03). Currently the author's group are conducting prospective studies to decide what is best when making recommendations in low-grade endometrioid endometrial cancer with isolated tumor cells. Until then, we should focus on uterine factors and molecular profiling of endometrial tumors to make the most educated decision for our patients.[21]

Regarding the molecular profile, a similar distribution was observed to that described by Kommoss et al[13] in the final validation of the ProMisE study where 452 patients with endometrial carcinoma and molecular profile were identified. Of these, 55.7% belonged to the NSMP group, 28.1% to MMRd followed by 12.2% belonging to the p53abn group, with ultramutated POLE being the least prevalent group with 9.3% of patients. In our study we obtained information from 2139 patients with a molecular profile; of these, the percentage of patients identified with ultramutated POLE was slightly lower (4.4% vs 9.3%) probably because their analysis was omitted in 43.2% of the sample due to low risk or intermediate risk endometrial cancer with low-grade histologies. As in the study by Kommoss et al, p53abn and MMRd remained the two molecular groups with the greatest lymph node involvement (34.5% p53abn and 9.4% MMRd vs 12.50% and 12.40%, respectively, in our study). This association was also observed by Jamieson et al[22] who reported retrospective data of 172 patients undergoing sentinel node mapping plus lymphadenectomy. The authors showed that molecular classification was correlated with the probability of nodal involvement (p53abn 44.8%; MMRd 14.9%; POLE mutated 14.2%; NSMP 10.8%). According to our findings, this greater likelihood of these two groups (p53abn and MMRd) of having positive SLNs could be influenced by the higher rate of deep

myometrial invasion in the MMRd group or the greater prevalence of LVSI-positive high-grade non-endometrioid tumors in the p53abn group.

Finally, we have defined the rate of sentinel lymph node involvement for each of the risk groups of the new ESGO prognostic risk classification. This rate of nodal involvement is similar to that described by Persson et al for the high-risk group in the SHREC trial (22.5% vs 21.0%),[4] but there were differences for the intermediate group with respect to that described by Bjørnholt et al in the SENTIREC trial (6.6% vs 22.5%).[23] These differences might be due to the definition of intermediate risk in the SENTIREC study, which did not consider molecular classification or lymphovascular status. These variables, as demonstrated in the latest 2023 FIGO classification,[24] are fundamental to define the stage of the patients adequately. In fact, in a recent analysis performed by Schwameis et al, 27.6% of the stages changed with respect to the 2009 FIGO classification when these variables were taken into account. Particularly in early-stage disease, the new substages (including molecular subtypes) added further prognostic granularity and identified treatment relevant subgroups.[25]

Strengths and Weaknesses

Overall, the strengths of this study include a collaborative effort of 66 institutions from 16 countries where comprehensive data were collected on 2139 patients. Another strength of our study was that all patients were staged following the SLN algorithm. It is also important to emphasize that our study is, to the best of our knowledge, the first study with a large cohort of patients showing that patients with stage I–II (FIGO 2009) endometrial cancer differ in sentinel node involvement with respect to molecular profile as well as prognostic risk group. However, we recognize that such groups, by definition, might already be at a lower risk of lymph node involvement.

Our study has several weaknesses due to the retrospective nature, including the fact that there was no formal auditing of the data. To account for these limitations, we provided the participating sites with a strict list of inclusion and exclusion criteria, and all investigators declared that the reported information adhered to the data in the reviewed charts. In addition, there is a 43.2% of incomplete molecular profile (without ultramutated POLE analysis) due to the presence of low-risk or intermediate-risk endometrial cancer with low-grade histologies; therefore, within the NSMP group there could potentially be some patients belonging to the ultramutated POLE group. While our study benefited from being able to collect data from multiple centers worldwide, it is essential to acknowledge the variability inherent in the equipment used for DNA sequencing methodologies and antibodies used for immunohistochemical determinations across these centers. Nonetheless, it is worth noting that each center has undergone rigorous quality assurance measures, contributing to the reliability of their respective results. However, it should be remarked that we did not perform a centralized data review. There are also 24.9% of patients with lymph nodes affected by isolated tumor cells with uncertain impact on oncologic outcomes. This, together with the small number of events, represents a further limitation.

Implications for Practice and Future Research

Molecular classification represents a paradigm shift in the knowledge of endometrial cancer. Currently, evidence is lacking on how molecular profiling impacts surgical staging. Correct staging of the disease is crucial to properly manage these patients and avoid undertreatment or overtreatment. The present study shows that there are two molecular groups (p53abn and MMRd) with a greater tendency to have lymph node involvement. However, molecular profiling did not improve the prediction of nodal status when compared with classic risk factors (FIGO stage and final histology) since the rate of nodal involvement remained very similar between groups with no significant differences in the area under the curve between the two models. For that reason, lymph node staging should not yet be adopted based on molecular profiling as prospective studies are needed to validate whether these differences affect survival.[26]

This trend was also observed in the prospective PROME trial,[27] in which molecular features were not associated with the risk of having nodal metastases (OR=1.03, 95% CI 0.21 to 5.05, p=0.969 for POLE-mut; OR=0.788, 95% CI 0.32 to 1.98, p=0.602 for p53abn; OR=1.14, 95% CI 0.53 to 2.42, p=0.733 for MMRd/microsatellite instability-high). Bogani et al observed at multivariable analysis that only deep myometrial invasion (OR=3.318, 95% CI 1.357 to 8.150, p=0.009) and lymphovascular space invasion (OR=6.584, 95% CI 2.663 to 16.279, p<0.001) were correlated with the increased risk of positive nodes.

Furthermore, we have defined the rate of sentinel lymph node involvement for each ESGO prognostic risk group. We believe that these data will be helpful for tailoring the surgery of these complex patients due to frequent obesity and adhesions. In this sense we would like to emphasize the importance of implementing the preoperative definition of the molecular profile as it has been shown to have a good correlation with the definitive biopsy.[28] This could be useful to define pre-operatively the prognostic risk groups and therefore facilitate decision-making during surgery.[29]

For all these reasons, the present study should be considered as a hypothesis-generating study to stimulate an international collaboration to prospectively investigate the potential role of molecular classification in the surgical staging of patients with endometrial cancer,[30] validating the results obtained by our group. In the meantime, we believe that from now on, in all prospective and retrospective studies on sentinel lymph node biopsy and endometrial cancer, the definition of the molecular profile should be considered as a variable to be weighted for the risk of lymph node involvement.

CONCLUSIONS

In this retrospective study, significant differences were found for nodal involvement in patients with stage I–II endometrial cancer (FIGO 2009) according to molecular profile. Patients belonging to the p53abn and MMRd groups were associated with a higher rate of sentinel lymph node involvement.

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REFERENCES

1 Corpus uteri source: globocan 2020. 2020. Available: https://gco.iarc.fr/today
2 Kitchener H, Swart AMC, Qian Q, *et al*. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet* 2009;373:125–36.
3 Burke WM, Orr J, *et al*, SGO Clinical Practice Endometrial Cancer Working Group. Endometrial cancer: a review and current management strategies: part I. *Gynecol Oncol* 2014;134:385–92.
4 Persson J, Salehi S, Bollino M, *et al*. Pelvic sentinel lymph node detection in high-risk endometrial cancer (SHREC-Trial)-the final step towards a paradigm shift in surgical staging. *Eur J Cancer* 2019;116:77–85.
5 Cusimano MC, Vicus D, Pulman K, *et al*. Assessment of sentinel lymph node biopsy vs lymphadenectomy for Intermediate- and high-grade endometrial cancer staging. *JAMA Surg* 2021;156:157–64.
6 Frumovitz M, Plante M, Lee PS, *et al*. Near-infrared fluorescence for detection of sentinel lymph nodes in women with cervical and uterine cancers (FILM): a randomised, phase 3, multicentre, non-inferiority trial. *Lancet Oncol* 2018;19:1394–403.

7 Rossi EC, Kowalski LD, Scalici J, *et al*. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. *Lancet Oncol* 2017;18:384–92.
8 Concin N, Matias-Guiu X, Vergote I, *et al*. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer* 2021;31:12–39.
9 Abu-Rustum NR. Sentinel lymph node mapping for endometrial cancer: a modern approach to surgical staging. *J Natl Compr Canc Netw* 2014;12:288–97.
10 GetzG, GabrielSB, Cibulskis K, *et al*. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013;497:67–73.
11 Talhouk A, McConechy MK, Leung S, *et al*. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer* 2015;113:299–310.
12 Talhouk A, McConechy MK, Leung S, *et al*. Confirmation of promise: a simple, genomics-based clinical classifier for endometrial cancer. *Cancer* 2017;123:802–13.
13 Kommoss S, McConechy MK, Kommoss F, *et al*. Final validation of the promise molecular classifier for endometrial carcinoma in a large population-based case series. *Ann Oncol* 2018;29:1180–8.
14 León-Castillo A, de Boer SM, Powell ME, *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;38:3388–97.
15 Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet* 2009;105:103–4.
16 Tsujimoto M, Nakabayashi K, Yoshidome K, *et al*. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. *Clin Cancer Res* 2007;13:4807–16.
17 Dundr P, Cibula D, Němejcová K, *et al*. Pathologic protocols for sentinel lymph nodes ultrastaging in cervical cancer. *Arch Pathol Lab Med* 2019;144:1011–20.
18 Moloney K, Janda M, Frumovitz M, *et al*. Development of a surgical competency assessment tool for sentinel lymph node dissection by minimally invasive surgery for endometrial cancer. *Int J Gynecol Cancer* 2021;31:647–55.
19 La Fera E, Bizzarri N, Petrecca A, *et al*. Evaluation of the one-step nucleic acid amplification method for rapid detection of lymph node metastases in endometrial cancer: prospective, multicenter, comparative study. *Int J Gynecol Cancer* 2023;33:1063–9.
20 Cucinella G, Schivardi G, Zhou XC, *et al*. Prognostic value of isolated tumor cells in sentinel lymph nodes in low risk endometrial cancer: results from an international multi-institutional study. *Int J Gynecol Cancer* 2024;34:179–87.
21 Hsu HC, Ramirez PT. Isolated tumor cells in low-risk endometrial cancer: are we ready for treatment decisions in 'isolation'? *Int J Gynecol Cancer* 2024;34:188–9.
22 Jamieson A, Thompson EF, Huvila J, *et al*. Endometrial carcinoma molecular subtype correlates with the presence of lymph node metastases. *Gynecol Oncol* 2022;165:376–84.
23 Bjørnholt SM, Sponholtz SE, Mogensen O, *et al*. The SENTIREC-endo study - risks and benefits of a national adoption of sentinel node mapping in low and intermediate risk endometrial cancer. *Gynecol Oncol* 2023;171:121–8.
24 Berek JS, Matias-Guiu X, Creutzberg C, *et al*. FIGO staging of endometrial cancer: 2023. *Int J Gynaecol Obstet* 2023;162:383–94.
25 Schwameis R, Fanfani F, Ebner C, *et al*. Verification of the prognostic precision of the new 2023 FIGO staging system in endometrial cancer patients - an international pooled analysis of three ESGO accredited centres. *Eur J Cancer* 2023;193.
26 Amant F, McCluggage WG, Werner HMJ, *et al*. Incorporating molecular profiling into endometrial cancer management requires prospective studies. *Int J Gynecol Cancer* 2021;31:944–5.
27 Bogani G, Lalli L, Casarin J, *et al*. Predicting the risk of nOdal disease with histological and molecular features in endometrial cancer: the prospective PROME trial. *Int J Gynecol Cancer* 2024;34:1366–72.
28 Abdulfatah E, Wakeling E, Sakr S, *et al*. Molecular classification of endometrial carcinoma applied to endometrial biopsy specimens: towards early personalized patient management. *Gynecol Oncol* 2019;154:467–74.
29 Cabrera S, Bebia V, López-Gil C, *et al*. Molecular classification improves preoperative risk assessment of endometrial cancer. *Gynecol Oncol* 2024;189:56–63.
30 Kasius JC, Trozzi R, Pijnenborg J, *et al*. Improving endometrial cancer assessment by combining the new techniqUe of GENomic profiling with surgical extra uterIne disEase assessment (EUGENIE). *Int J Gynecol Cancer* 2023;33:823–6.

4.3 Bretová P, Ndukwe MI, Laco J, Vošmiková H, Rešlová T, Pohanková D, Balcarová K, Haviger J, Havigerová JM, Sirák I. Preoperative Risk Stratification in Endometrial Cancer Using ESGO/ESTRO/ESP 2021 Guidelines: Accuracy with and without Molecular Classification. BMC Cancer. 2025; 25,1302.

The molecular classification has undoubtedly become a routine component of definitive histopathological assessment in many countries. It is primarily used to stratify patients into risk groups that guide decisions on adjuvant therapy. However, knowledge of the definitive risk group may also influence the surgical management approach. For example, it may determine whether to proceed with or omit a side-specific systematic pelvic lymphadenectomy in cases of intraoperative sentinel node mapping failure.

In this retrospective single-center study, conducted at the Department of Obstetrics and Gynecology, University Hospital Hradec Králové, we evaluated whether the integration of molecular classification improves the accuracy of preoperative risk stratification in patients with endometrial cancer following the 2021 ESGO/ESTRO/ESP guidelines. A total of 143 patients with apparent early-stage EC (FIGO 2009 stage I–II) who underwent surgical treatment between January 2022 and December 2024 were included.

Preoperative staging was based on expert transvaginal ultrasound, complemented by CT (computed tomography) imaging and histological assessment. Molecular classification was derived from IHC for mismatch repair proteins and p53, and NGS for *TP53* and *POLE* mutations.

The study demonstrated that adding molecular profiling significantly increased the accuracy of preoperative risk classification from 59.4% to 73.4%. The agreement between preoperative and postoperative risk groups improved accordingly, with Cohen's kappa increasing from 0.551 to 0.767, indicating a shift from moderate to good concordance.

The most significant benefit of molecular profiling was observed in correctly identifying high-risk patients. However, misclassification still occurred in 26.6% of patients, largely due to factors not reliably detectable preoperatively, such as LVSI, depth of invasion, or lymph node metastases.

The findings support the incorporation of molecular classification into preoperative assessment to refine surgical planning and guide the extent of lymph node staging.

The study entitled "Preoperative Risk Stratification in Endometrial Cancer Using ESGO/ESTRO/ESP 2021 Guidelines: Accuracy with and without Molecular Classification" was published in the *BMC Cancer* (IF 3.4, Q2) in 2025.

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Preoperative risk stratification in endometrial cancer using ESGO/ESTRO/ESP 2021 guidelines: accuracy with and without molecular classification

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Abstract

Background The study aimed to evaluate the impact of integrating molecular classification with imaging-based preoperative staging on risk stratification prediction in endometrial cancer patients in accordance with ESGO/ESTRO/ESP (European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology) 2021 guidelines.

Methods A retrospective cohort of 143 endometrial cancer patients was analyzed to assess changes in preoperative risk stratification after incorporating molecular classification into clinical evaluation. Preoperative clinical staging was primarily based on transvaginal ultrasound imaging. The overall agreement between preoperative risk group estimates (with/without molecular classification) and final postoperative outcomes was assessed using weighted Cohen's Kappa, with bootstrap 95% confidence intervals and quadratic weights.

Results The addition of molecular classification significantly improved preoperative risk stratification accuracy (from 59.4 to 73.4%), particularly for patients post-operatively classified as high-risk. Kappa values indicated an improvement in overall agreement between preoperative and postoperative risk stratification following the addition of molecular classification, from 0.551 (95% CI: 0.430–0.671) to 0.767 (95% CI: 0.675–0.849). The non-overlapping confidence intervals indicated statistical significance. Preoperative assessment without molecular input tended to underestimate risk stratification. However, 26.6% of patients remained misclassified due to other factors, mostly within the intermediate and high-intermediate risk groups.

Conclusions Incorporating molecular classification enhances preoperative risk stratification and has the potential to tailor surgical treatment. Further validation through prospective multicentric studies is needed to support our findings.

Keywords Endometrial cancer, Molecular classification, Sentinel node biopsy, Risk stratification, Ultrasonography, Next-generation sequencing, ESGO/ESTRO/ESP 2021 guidelines

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Background

Endometrial cancer remains the most common gynecological malignancy in developed countries, with incidence rates showing no signs of decline in recent years [1]. In the past decade, significant progress has occurred in clinical practice, particularly with the adoption of molecular classification (MC) and sentinel lymph node biopsy (SLNB). The 2021 ESGO/ESTRO/ESP (European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology) guidelines introduced updated risk stratification categories—low, intermediate, high-intermediate, and high-risk of recurrence—enabling more tailored treatment approaches [2].

Preoperative risk stratification guides surgical decision-making and is traditionally based on histological type, tumor grade, and disease stage determined through expert ultrasonography or MRI (magnetic resonance imaging), without molecular classification. Accurate imaging is essential for evaluating myometrial and cervical invasion. In cases of sentinel node biopsy failure, particularly among high-intermediate and high-risk patients, side-specific lymphadenectomy is recommended for accurate nodal staging [2]. Conversely, in low and intermediate-risk patients, particularly those without myometrial invasion, lymph node staging may be safely omitted in case of SLNB failure.

In clinical practice, postoperative risk classification incorporates definitive histological findings, including LVSI (lymphovascular space invasion), alongside molecular features, which may alter risk categories and guide adjuvant therapy. For example, a low-grade, FIGO (International Federation of Gynecology and Obstetrics) 2009 stage IA tumor (initially low-risk) may be upgraded to high-risk if a *TP53* (Tumor protein 53) mutation is found postoperatively, while high-intermediate risk tumors with cervical invasion may be downgraded if a *POLE* (Polymerase ε) mutation is identified [2]. Molecular classification refines the use of chemotherapy, radiotherapy, or hormonal therapy, ensuring tailored treatment. High-risk patients benefit from intensified therapy, while low-risk patients avoid unnecessary interventions. Moreover, molecular classification improves the prediction of treatment response [3].

This study aimed to evaluate whether incorporating MC into the preoperative staging of apparent early-stage (FIGO 2009 stages I-II) endometrial cancer improves risk stratification according to the ESGO/ESTRO/ESP guidelines. The study also assessed whether preoperative risk stratification without MC is more susceptible to underestimation or overestimation.

Methods

Patients and clinical data

This retrospective observational single-center study was conducted at the Gynecological Oncology Center of University Hospital Hradec Kralove, Czech Republic. Patients who underwent surgical treatment for histologically confirmed endometrial cancer (EC) between January 2022 and December 2024 were consecutively included.

Inclusion criteria included: apparent early-stage endometrial cancer (FIGO 2009 stages I-II), available histology from biopsy (dilatation and curettage or hysteroscopy) and definitive hysterectomy, preoperative staging by expert ultrasound, pelvic-abdominal-chest CT (computed tomography; waived in low-risk cases), and immunohistochemistry (IHC) for p53, mismatch-repair proteins (MMR), as well as next-generation sequencing (NGS) analysis for *TP53* and *POLE* mutations (waived in low-risk cases).

Exclusion criteria included patients with synchronous malignancies, apparent advanced-stage disease (FIGO 2009 stages III–IV), or missing essential data (molecular classification, imaging results). We also excluded patients without confirmed malignant histology from the preoperative biopsy—these were cases where only atypical hyperplasia/benign histology was reported preoperatively or where no biopsy was performed at all (e.g., surgery was indicated for other reasons such as fibroids or uterine prolapse, and endometrial cancer was diagnosed incidentally in the hysterectomy specimen).

Data collected included: age, BMI (body mass index), ultrasound and CT findings (depth of myometrial invasion, cervical involvement, lymphadenopathy, distant metastases), and histopathological details from biopsy and final histology (FIGO 2009 stage, histological type, grade, LVSI, IHC results for MMR proteins, p53, and NGS results for *POLE* and *TP53*). All data were extracted from the hospital's information systems.

Preoperative imaging

All patients underwent transvaginal and transabdominal ultrasound staging conducted by a certified ultrasonography expert (T.R.) evaluating tumor extent, myometrial and cervical involvement, and lymph nodes, using IETA (International Endometrial Tumor Analysis) terminology and Czech national guidelines [4, 5]. Pelvic-abdomen-chest CT was performed for non-low-risk cases.

Histopathology, immunohistochemistry and next-generation sequencing

The tissue specimens were fixed in formalin and processed routinely for histopathology. All endometrial carcinomas were initially diagnosed and/or finally reviewed and confirmed by an expert gynecologic pathologist

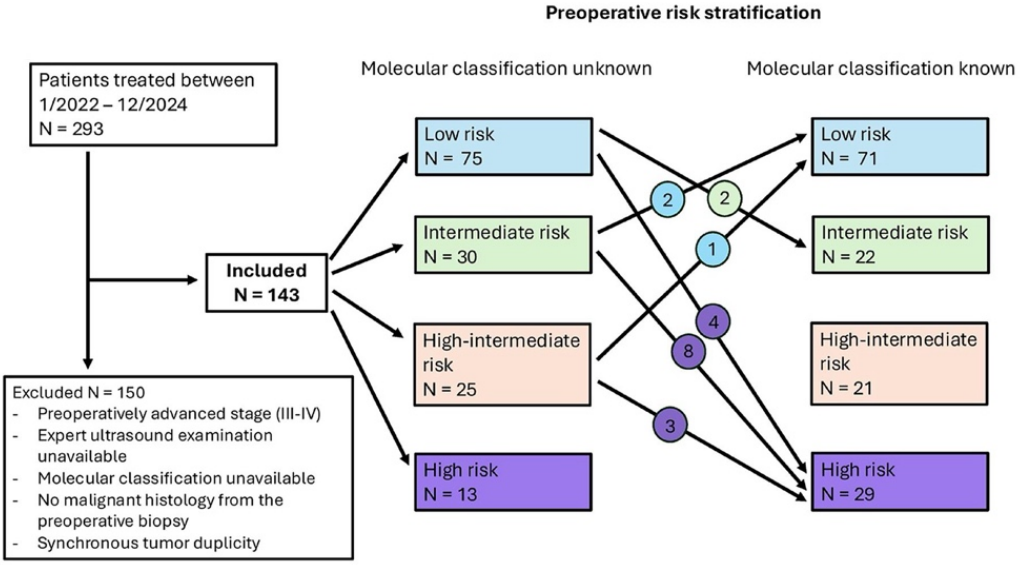

Fig. 1 Flowchart of patients included in the study

molecular classifiers and were assigned according to the recommended hierarchy. Detailed methodology is provided in Additional file 1.

The distribution of ESGO/ESTRO/ESP 2021 risk groups across the three assessment stages is shown in Table 2.

Without molecular classification, only 59.4% of EC patients were preoperatively classified into the correct risk group. The incorporation of molecular classification enhanced the accuracy of preoperative risk assessment in 14.0% of cases, increasing correct stratification from 59.4 to 73.4% of EC patients. However, a total of 26.6% of EC patients remained misclassified due to inaccurate preoperative assessment of cervical invasion (9.0%), myometrial invasion (6.3%), advanced-stage disease (4.2%), LVSI (3.5%), nodal metastases (2.8%), and tumor grade (0.7%).

Cohen's Kappa coefficient for agreement between preoperative and postoperative risk classification improved from 0.551 (95% CI, 0.430–0.671) without molecular classification to 0.767 (95% CI, 0.675–0.849) with molecular classification, indicating an improvement from moderate to good agreement. As the 95% confidence intervals did not overlap, this difference was statistically significant. Inclusion of molecular classification improved agreement from moderate to good. Confidence intervals did not overlap, indicating statistical significance.

The results of risk group prediction success aggregated into categories of correct, underestimate, and overestimate are shown in Table 3.

Notable shifts of more than 0.1 were observed in the correct and underestimation categories, though changes were not statistically significant (confidence intervals overlapped).

A detailed comparison of prediction accuracy for risk stratification (low risk, intermediate risk, high-intermediate risk, and high risk), using the metrics precision, recall, and the overall F1-score, is presented in Table 4. Bootstrap confidence intervals for each value were computed and they are presented in Additional file 2 for better clarity.

Positive shifts occurred in most measures. A statistically significant shift in F1-score was observed in high risk group, as shown in Fig. 2, including confidence intervals.

The obtained values indicate an improvement in prediction accuracy for the low-risk group, shifting the result from the acceptable range to the strong range. For the intermediate and high-intermediate risk group, only a slight increase was observed; however, both remained below the acceptable threshold. A statistically significant improvement was seen in the high-risk group, where the use of MC improved the results from below the acceptance level to the strong range.

Discussion

This analysis demonstrated that incorporating MC into preoperative staging significantly enhances the prediction of preoperative risk stratification in endometrial cancer patients, according to ESGO/ESTRO/ESP 2021 guidelines. The most significant improvement was observed in predicting the high-risk group, while MC also enhanced the accuracy in identifying the low-risk group preoperatively. However, distinguishing the intermediate and

Table 1 Baseline clinicopathologic characteristics of the study cohort ($n = 143$)

Variable	Category	*n* (%)
Histologic tumor type	Endometrioid	128 (89.5%)
	Serous	8 (5.6%)
	Carcinosarcoma	4 (2.8%)
	Undifferentiated/dedifferentiated	2 (1.4%)
	Clear cell	1 (0.7%)
Tumor grade	Low grade	116 (81.1%)
	High grade	27 (18.9%)
Cervical stromal invasion	Absent	116 (81.1%)
	Present	27 (18.9%)
Myometrial invasion	None	1 (0.7%)
	< 50%	88 (61.5%)
	≥ 50%	54 (37.8%)
Lymphovascular space invasion	Absent	111 (77.6%)
	Focal	11 (7.7%)
	Substantial	21 (14.7%)
Surgical FIGO 2009 stage	IA	73 (51.0%)
	IB	35 (24.5%)
	II	17 (11.9%)
	IIIA	4 (2.8%)
	IIIB	2 (1.4%)
	IIIC	10 (7.0%)
	IVA	0 (0.0%)
	IVB	2 (1.4%)
Molecular classification	NSMP	80 (55.9%)
	MMRd	30 (21.0%)
	p53abn	27 (18.9%)
	POLEmut	6 (4.2%)

Table 2 Distribution of ESGO/ESTRO/ESP 2021 risk groups across assessment stages

Risk Group	Preoperative without MC	Preoperative with MC	Postoperative
Low risk	75 (52.4%)	71 (49.7%)	61 (42.7%)
Intermediate risk	30 (21.0%)	22 (15.4%)	25 (17.5%)
High-intermediate risk	25 (17.5%)	21 (14.7%)	16 (11.2%)
High risk	13 (9.1%)	29 (20.3%)	41 (28.7%)

MC Molecular classification

Table 3 Prediction success rates for preoperative estimates by the categories of correct predictions, overestimation, and underestimation

Category	Without MC (CI)	With MC (CI)
Correct	0.587 (0.505–0.665)	0.706 (0.627–0.775)
Overestimate	0.091 (0.054–0.149)	0.077 (0.043–0.132)
Underestimate	0.322 (0.251–0.402)	0.217 (0.157–0.291)

MC Molecular classification, *CI* confidence interval

high-intermediate risk groups preoperatively remains challenging, even with MC. Without MC prior to surgery, there was a tendency to underestimate risk stratification (Table 3).

Molecular features are known to provide better risk stratification than standard histopathology, however, prior studies have focused on postoperative risk groups or novel classifications, making direct comparisons to our results difficult. For example, a small prospective study found that MC changed final risk group in 10.3% of patients [12]. The study was designed to evaluate standard definitive histopathological risk assessment and the additional value of molecular classification.

Another study evaluated the ability of demographic and sonographic variables, along with the ProMisE (Proactive Molecular Risk Classifier for Endometrial cancer) classification, to predict tumor recurrence or progression in 339 women with endometrial cancer. It found that a tumor size of less than 2 cm combined with p53 wild-type status identified approximately 50% of women at very low risk for recurrence or progression [13].

In a study of 658 patients, p53, combined with imaging tests, was found to be a reliable preoperative indicator of advanced disease [14]. However, unlike our study, the investigators included preoperatively advanced stages of EC according to imaging methods, which are already considered high risk regardless of molecular classification. We focused only on the preoperative early stages of EC, where the specific histopathological and molecular features can play a key role in risk stratification.

Our findings can also be viewed in light of the recent commentary by Betella et al., who reported that molecular classification changed FIGO 2023 stage in only 6% of 381 EC patients, meaning about 17 patients needed testing to change treatment for one [15]. In our study, molecular classification led to risk group reclassification in 14% of cases. This higher reclassification rate is likely due to the use of preoperative data, where not all parameters are fully known or accurately assessed. Consequently, adding molecular classification only at final histology typically results in fewer changes. While our findings support the added value of molecular profiling for preoperative risk stratification, the clinical and economic implications of routine use remain important considerations.

Although molecular classification improved preoperative risk stratification prediction in our study, 26.6% of patients were misclassified due to factors like LVSI, cervical and myometrial invasion, and lymph node metastasis, particularly in the intermediate and high-intermediate risk groups. Although this was not the primary focus of our study, it represents a relevant secondary observation that further highlights the limitations of preoperative assessment. Substantial LVSI is a strong prognostic factor that can upstage a case, but it is typically identified

(J.L.), following the WHO (World Health Organization) recommendations and international standards [2, 6–9]. All tumors were tested for p53 protein and MMR proteins (MLH1, MSH2, MSH6, and PMS2) expression using IHC.

For NGS, DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tumoral tissue using the Cobas DNA Sample Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). Subsequently, NGS-based technology was performed (Hybrid capture DNA Roche KAPA Evo Plus Kit, Roche Sequencing Solutions, Inc., USA) using a custom panel that targets the exons and flanking sequences (+/- 20 bp) of 96 cancer-related genes, including *TP53*, *POLE* genes, and 49 microsatellite regions for MSI status assessment.

Finally, all tumors were classified as POLEmut (*POLE* mutated), MMRd (mismatch-repair deficiency), p53abn (p53 abnormal), or NSMP (non-specific molecular profile).

Details are described in Additional file 1.

Preoperative and postoperative risk stratification

Patients were preoperatively classified according to the ESGO/ESTRO/ESP 2021 guidelines [2] based on histological type and grade, as well as clinical stage derived from imaging. The tumor board then recommended surgical management, typically consisting of hysterectomy with bilateral salpingo-oophorectomy and either SLNB or systematic lymphadenectomy. SLNB was preferred for patients with low- and intermediate-risk profiles, while systematic lymphadenectomy was performed in cases preoperatively assessed as high–intermediate- or high-risk. In selected low-risk cases without myometrial invasion, lymph node staging was omitted.

Postoperative risk stratification was reassessed using definitive histology, including the presence of LVSI, IHC, and NGS when indicated. *POLE* testing was not routinely performed in low-risk cases, as it would not influence risk categorization or treatment decisions [9].

To explore the potential improvement in preoperative risk stratification, we developed a modified model by integrating molecular classification with standard preoperative clinical and histopathological features. This allowed us to assess the impact of molecular classification on the accuracy of initial risk assessment.

Statistical analysis

Three risk stratification conditions were evaluated: (1) preoperative classification based on clinical and histopathological features without molecular classification; (2) preoperative classification including molecular classification; and (3) final postoperative classification based on final histopathological type, grade, pathological staging, LVSI, and molecular classification, all non-normally distributed (Shapiro-Wilk, $p < 0.05$).

Agreement between preoperative risk stratification (with and without MC) and postoperative risk stratification was assessed using Cohen's Kappa with 95% bootstrap confidence intervals. Kappa values were interpreted according to Fleisss et al. [10]: 0.41–0.60 moderate, 0.61–0.80 good, > 0.80 excellent.

To assess if preoperative risk stratification without MC were more prone to underestimation or overestimation compared to those with MC, misclassification patterns were analyzed by categorizing errors in the confusion matrix as underestimation (risk of recurrence rated below the postoperative risk group) and overestimation (risk of recurrence rated above the postoperative risk group).

Prediction performance across risk levels was assessed using multiclass confusion matrix metrics, including precision (proportion of correct severity estimates among those assigned), recall (proportion of actual severity cases correctly identified), and F1-score (harmonic mean of precision and recall, balancing both). Values > 0.70 are considered acceptable, and > 0.80 strong in medical diagnostics [11].

Software

Data processing and statistical analyses were performed using Python (version 3.10.15) with the following packages: pandas (2.1.4) for data manipulation, numpy (1.25.2) for numerical operations, statsmodels (0.14.4) for statistical modeling, sklearn (1.1.3) for machine learning metrics, scipy (1.9.3) for scientific computations, plotly (6.0.0) and matplotlib (3.6.3) for interactive and static visualizations, and seaborn (0.13.2) for enhanced statistical graphics.

Results

During the study period, 293 patients were diagnosed with endometrial cancer at our institution. Of these, 143 patients met the inclusion criteria and were included in the final analysis (Fig. 1). The median age was 66 years (mean 64, range 29–85), and the median BMI was 32 kg/m² (mean 33, range 20–56). The majority of patients 97 (67.8%) underwent SLNB, followed by systematic lymphadenectomy 27 (18.9%). In 19 (13.3%) patients, lymph node staging was omitted due to a preoperatively assessed low-risk tumor without myometrial invasion. Most tumors were endometrioid (89.5%) and low grade (81.1%). According to the final staging, 75.5% of patients had FIGO 2009 stage I disease. Detailed histopathological characteristics are displayed in Table 1. Based on molecular profiling, 6 cases (4.2%) were *POLE*mut, 30 (21.0%) MMRd, 27 (18.9%) p53abn, and 80 (55.9%) NSMP. Five tumors exhibited features of multiple

Table 4 Prediction accuracy for risk stratification (low risk, intermediate risk, high-intermediate risk, and high risk)

Grade	Precision		Recall		F1	
	Without MC	With MC	Without MC	With MC	Without MC	With MC
Low risk	**0.720**	**0.789**	**0.885**	**0.918**	**0.794**	**0.848**
Intermediate risk	0.367	0.500	0.440	0.440	0.400	0.468
High-intermediate risk	0.240	0.286	0.375	0.375	0.293	0.324
High risk	**1.000**	**0.966**	0.317	0.683	0.481	**0.800**

Precision, recall, and F1-score. Values > = 0.7 are highlighted

MC Molecular classification

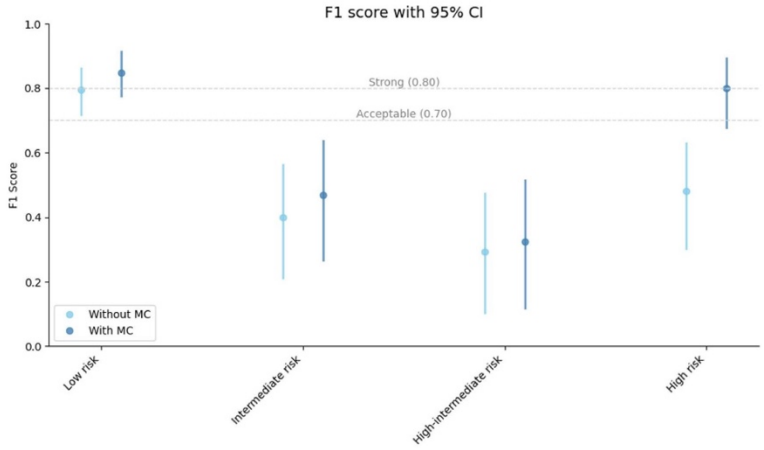

Fig. 2 Prediction F1-score with confidence intervals across severity levels

only in the definitive surgery specimen. It is important to note that the definition of substantial LVSI is not standardized internationally, with varying criteria used across guidelines [16]. In our study, we use the ESGO/ESTRO/ESP definition of substantial LVSI: diffuse or multifocal involvement of lymphovascular spaces or the presence of tumor cells in five or more lymphovascular spaces [2]. LVSI remains one of the last factors playing a key role in definitive risk stratification, which can not be assessed preoperatively.

Another source of pre- and postoperative bias arises from discrepancies in the assessment of myometrial (sensitivity 81%, specificity 82%) [17] and cervical invasion (sensitivity 63%, specificity 91%) [18] between preoperative imaging (ultrasound) and definitive histopathology. Although ultrasound shows high specificity (98%) for detecting lymph node metastasis, which aids in selecting candidates for debulking surgery rather than SLNB or systemic lymphadenectomy, its low sensitivity (41%) results in frequent postoperative upstaging [19].

Molecular analysis on preoperative biopsy samples is not only feasible but also shows excellent concordance with final pathology [20]. Incorporating this analysis into preoperative assessment can inform individualized surgical strategies. For instance, in cases where sentinel lymph node biopsy fails perioperatively, molecular classification may guide the decision to proceed with side-specific lymphadenectomy. However, the integration of MC into surgical planning introduces new challenges that warrant careful consideration and consensus among international endometrial cancer experts.

For example, preoperative knowledge of *POLE* mutation status could reclassify high-risk tumors (e.g., deep myometrial or cervical invasion, high grade) into the low-risk group, possibly avoiding lymphadenectomy in SLNB failure. However, lymph node status remains crucial for the *POLE*-mutated group, as there's no evidence on the prognosis of stage III-IV tumors without adjuvant treatment [21]. On the other hand, a large multicenter study showed that preoperatively staged I-II *POLE*-mutated tumors had the lowest frequency of lymph node metastasis compared to other risk groups [22].

Secondly, a *TP53* mutation in tumors with myometrial invasion automatically classifies patients as high-risk, requiring the same adjuvant treatment regardless of lymph node status. Since lymphadenectomy is primarily

for staging purposes (rather than a therapeutic one [23]), its necessity in this group is questionable. Furthermore, lymph node metastasis in p53abn tumors has been shown to carry the same prognosis (risk of recurrence and disease-specific death) as in lymph node-negative cases [24]. This suggests that lymph node status has no prognostic value in endometrial carcinomas with TP53 mutation.

Current guidelines suggest that lymph node staging can be waived for tumors without myometrial invasion [2]. However, for *TP53*-mutated tumors limited to the endometrial polyp, without myometrial invasion, adjuvant treatment is not recommended due to the excellent prognosis. Nevertheless, complete staging (including lymph nodes) must be performed as a significant risk of occult lymph node involvement may upstage these tumors to stage III [21]. Therefore, knowing p53 status is crucial for preoperatively low-risk tumors confined to the endometrium. If the tumor is *TP53*-mutated, SLNB should be performed, and in the case of failure, side-specific lymphadenectomy should be considered.

To our knowledge, this is the first study to address the additional value of molecular classification in preoperative stratification into ESGO/ESTRO/ESP risk groups. A statistically significant effect of adding molecular classification to preoperative stratification was observed based on the available data. However, the retrospective design may introduce biases, including information bias from incomplete or inaccurate data and selection bias due to patient selection based on data availability. Despite including only patients with complete data, the retrospective nature limits the ability to draw definitive conclusions. Additionally, as a single-center study, results may be influenced by institutional and personal factors affecting clinical practice.

Incorporating molecular classification into preoperative patient management enhances risk stratification and offers the potential to individualize surgical strategies. As discussed, preoperative identification of *POLE*mut tumors may support omitting lymphadenectomy even in cases of SLNB failure, while *TP53*mut tumors may justify lymph node staging despite limited invasion due to their high-risk profile. These molecular insights can influence whether lymphadenectomy is pursued, the interpretation of nodal involvement, and the overall extent of surgical staging. At the same time, expert ultrasonography remains a key tool for evaluating tumor invasion and guiding surgical planning, despite its known limitations. Together, imaging and molecular classification form a complementary basis for preoperative decision-making. However, their integration introduces new challenges that require further discussion and consensus within the gynecologic oncology community.

Conclusions

This study found that incorporating molecular classification into preoperative staging improved risk stratification prediction according to ESGO/ESTRO/ESP 2021 risk categories in a retrospective cohort. This approach introduces new challenges, particularly in tailoring surgical treatment, which require further discussion. A multicentric prospective study is needed to confirm these findings.

Abbreviations

ESGO	European Society of Gynaecological Oncology
ESTRO	European Society for Radiotherapy and Oncology
ESP	European Society of Pathology
MC	Molecular classification
SLNB	Sentinel lymph node biopsy
MRI	Magnetic resonance imaging
LVSI	Lymphovascular space invasion
FIGO	International Federation of Gynecology and Obstetrics
TP53	Tumor protein 53
POLE	Polymeraseε
EC	Endometrial cancer
CT	Computed tomography
IHC	Immunohistochemistry
MMR	Mismatch-repair proteins
NGS	Next-generation sequencing
BMI	Body mass index
IETA	International Endometrial Tumor Analysis
WHO	World Health Organization
NSMP	Non-specific molecular profile
ProMisE	Proactive Molecular Risk Classifier for Endometrial cancer

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

PB: Conceptualization, methodology, data curation, project administration, writing– original draft. MIN: Conceptualization, methodology, writing– original draft. JL: Investigation, methodology, writing– review, and editing. HV: Investigation, methodology, writing– review, and editing. TR: Methodology, writing– review, and editing. DP: Methodology, writing– review, and editing. KB: Data curation. JH: formal analysis, software, writing– review, and editing. JMH: formal analysis, software, writing– review, and editing. IS: Methodology, investigation, supervision, writing– review, and editing. All authors read and approved the final manuscript.

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Data availability

All relevant data are included in the manuscript and supplements. The whole DNA sequencing data are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled-access data storage at the database of the Fingerland Department of Pathology, University Hospital Hradec Kralove.

Declarations

Ethics approval and consent to participate
The study was conducted in compliance with the Helsinki Declaration and approved by the Ethics Committee of University Hospital Hradec Kralove (approval number 202411P15). All patients consented to tissue and data storage for scientific purposes. Given the retrospective nature of the study and the use of anonymized data, the requirement for specific informed consent was waived by the ethics committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63. https://doi.org/10.3322/caac.21834.
2. Concin N, Matias-Guiu X, Vergote I, 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. https://doi.org/10.1136/ijgc-2020-002230.
3. Ribeiro-Santos P, Martins Vieira C, Viana Veloso GG, et al. Tailoring endometrial cancer treatment based on molecular pathology: current status and possible impacts on systemic and local treatment. Int J Mol Sci. 2024;25(14): 7742. https://doi.org/10.3390/ijms25147742.
4. Leone FPG, Timmerman D, Bourne T, et al. Terms, definitions and measurements to describe the sonographic features of the endometrium and intrauterine lesions: a consensus opinion from the international endometrial tumor analysis (IETA) group. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol. 2010;35(1):103–12. https://doi.org/10.1002/uog.7487.
5. Frühauf F, Dvořák M, Haaková L, et al. Ultrasound staging of endometrial cancer - recommended methodology of examination. Ceska Gynekol. 2014;79(6):466–76.
6. WHO Classification of Tumours Editorial Board. Female genital tumours [Internet]. Lyon (France): International Agency for Research on Cancer; 2020 [cited 2025 Apr 6]. (WHO classification of tumours series, 5th ed.; vol. 4). https://tumourclassification.iarc.who.int/chapters/34
7. Matias-Guiu X, Anderson L, Buza N, Ellenson LH, Fadare O, Ganesan R, Ip PPC, Palacios J, Raspollini MR, Werner HMJ, Lax SF, McCluggage WG. Endometrial cancer histopathology reporting guide. 5th ed. Sydney: International Collaboration on Cancer Reporting; 2024.
8. Cho KR, Cooper K, Croce S, et al. International society of gynecological pathologists (ISGyP) endometrial cancer project: guidelines from the special techniques and ancillary studies group. Int J Gynecol Pathol. 2019;38(Supplement 1):S114–22. https://doi.org/10.1097/PGP.0000000000000496.
9. Dundr P, Cibula D, Doležel M, et al. Molecular testing in endometrial carcinoma (Joint recommendation of Czech oncological society, oncogynecological section of the Czech gynecological and obstetrical society, society of radiation oncology, biology and physics, and the society of Czech Pathologists). Cesk Patol. 2021;57(3):181–7.
10. Fleiss JL, Levin B, Paik MC. Statistical methods for rates and proportions. 1st ed. Hoboken: Wiley; 2003. https://doi.org/10.1002/0471445428.
11. Sokolova M, Lapalme G. A systematic analysis of performance measures for classification tasks. Inf Process Manag. 2009;45(4):427–37. https://doi.org/10.1016/j.ipm.2009.03.002.
12. Knez J, Sobocan M, Belak U, et al. Pre-treatment risk assessment of women with endometrial cancer: differences in outcomes of molecular and clinical classifications in the Slovenian patient cohort. Radiol Oncol. 2021;56(1):76–82. https://doi.org/10.2478/raon-2021-0036.
13. Eriksson LSE, Nastic D, Lindqvist PG, et al. Combination of proactive molecular risk classifier for endometrial cancer (ProMisE) with sonographic and demographic characteristics in preoperative prediction of recurrence or progression of endometrial cancer. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol. 2021;58(3):457–68. https://doi.org/10.1002/uog.23573.
14. Cabrera S, Bebia V, López-Gil C, et al. Molecular classification improves preoperative risk assessment of endometrial cancer. Gynecol Oncol. 2024;189:56–63. https://doi.org/10.1016/j.ygyno.2024.07.003.
15. Betella I, De Vitis LA, Calidona C, Multinu F, Colombo N. Letter to the editor-the new FIGO staging system for endometrial cancer: is the paradigm shift clinically feasible? Int J Gynaecol Obstet. 2024;164(1):364–5. https://doi.org/10.1002/ijgo.15265.
16. Sehnal B, Hruda M, Matej R, Robova H, Drozenova J, Pichlik T, Halaska MJ, Rob L, Dundr P. New FIGO 2023 staging system of endometrial cancer: an updated review on a current hot topic. Geburtshilfe Frauenheilkd. 2025;85:405–16. https://doi.org/10.1055/a-2494-8395.
17. Alcázar JL, Orozco R, Martinez-Astorquiza Corral T, et al. Transvaginal ultrasound for preoperative assessment of myometrial invasion in patients with endometrial cancer: a systematic review and meta-analysis: TVS for preoperative assessment of MI in endometrial cancer. Ultrasound Obstet Gynecol. 2015;46(4):405–13. https://doi.org/10.1002/uog.14905.
18. Alcázar JL, Pérez L, Güell O, et al. Diagnostic performance of transvaginal ultrasound for detecting cervical invasion in women with endometrial carcinoma: a systematic review and meta-analysis. J Ultrasound Med. 2019;38(1):179–89. https://doi.org/10.1002/jum.14682.
19. Borges AC, Veloso H, Galindo P, et al. Role of ultrasound in detection of lymph-node metastasis in gynecological cancer: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2024;64(2):155–63. https://doi.org/10.1002/uog.27633.
20. Kato MK, Fujii E, Yamaguchi M, et al. Excellent concordance of the molecular classification between preoperative biopsy and final hysterectomy in endometrial carcinoma. Gynecol Oncol. 2024;190:139–45. https://doi.org/10.1016/j.ygyno.2024.08.016.
21. Berek JS, Matias-Guiu X, Creutzberg C, et al. FIGO staging of endometrial cancer: 2023. Int J Gynecol Obstet. 2023;162(2):383–94. https://doi.org/10.1002/ijgo.14923.
22. Chacon E, Boria F, Lyer RR, et al. SENECA study: staging endometrial cancer based on molecular classification. Int J Gynecol Cancer Off J Int Gynecol Cancer Soc. 2024;34(9):1313–21. https://doi.org/10.1136/ijgc-2024-005711.
23. 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. https://doi.org/10.1016/S0140-6736(08)61766-3.
24. Siegenthaler F, Imboden S, Büchi C, et al. Added prognostic value of sentinel lymph node mapping in endometrial cancer to molecular subgroups. Gynecol Oncol. 2025;193:12–9. https://doi.org/10.1016/j.ygyno.2024.12.014.

Publisher's note

4.4 Vrede SW, Van Weelden WJ, Bulten J, Gilks CB, Teerenstra S, Huvila J, Matias-Guiu X, Gil-Moreno A, Asberger J, Sweegers S, van der Putten LJM, Küsters-Vandevelde HVN, Reijnen C, Colas E, Hausnerová J, Weinberger V, Snijders MPLM, Vinklerova P, et. al. Hormonal biomarkers remain prognostically relevant within the molecular subgroups in endometrial cancer. Gynecol Oncol. 2024; 192:15-23.

As an ENITEC member, the author had the opportunity to participate in the multicenter, retrospective study aimed to investigate the prognostic relevance of hormonal receptor expression—specifically ER and PR—within the established molecular subgroups of endometrial carcinoma.

With the integration of molecular classification into the standard management of EC, the traditional clinicopathological and immunohistochemical markers, such as ER and PR, have received less emphasis. However, the clinical utility of these hormonal biomarkers remains of interest, particularly regarding their potential to provide additional prognostic information beyond molecular subtyping alone.

The study cohort included 739 patients with endometrial carcinoma for whom both ER/PR immunohistochemistry and molecular classification data were available. Tumors were stratified into four molecular subtypes: *POLE*-ultramutated (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53mut), and NSMP. ER and PR expression was evaluated semi-quantitatively and categorized into three groups based on staining percentage: low (0–10%), intermediate (20–80%), and high (90–100%). The primary endpoint was DSS, assessed concerning receptor expression within each molecular subgroup.

The findings demonstrated a consistent and significant association between high hormonal receptor expression and improved disease-specific outcomes across all molecular classes. Notably, in the p53mut group—typically associated with poor prognosis—patients with high PR expression (90–100%) exhibited a 100% five-year DSS, in stark contrast to those with low PR expression (0–10%), who had a five-year DSS of only 48%. Similar trends were observed in the NSMP and MMRd groups,

although these differences were not statistically significant in every subgroup due to limited sample sizes. In multivariable Cox regression analysis, low PR expression (0–10%), p53mut status, presence of LVSI, and advanced stage (FIGO III–IV) were identified as independent negative prognostic factors. Conversely, *POLE*mut tumors and high PR expression were associated with significantly improved prognosis.

The study provides robust evidence that hormonal biomarkers—especially PR—retain significant prognostic value even when molecular classification is accounted for. These results support the continued use of ER and PR immunohistochemistry in routine pathological assessment of EC, as the combination of molecular and hormonal profiling may offer a more nuanced risk stratification. Importantly, the proposed three-tiered model of ER/PR evaluation (rather than the conventional binary positive/negative cut-off) appears to reflect the prognostic continuum better and may be more clinically informative.

In conclusion, this study reinforces the relevance of hormonal receptor status in the contemporary molecular framework of endometrial carcinoma. The findings advocate for integrating ER and PR expression—particularly PR—into risk stratification models and treatment planning. Future clinical trials and guidelines should consider incorporating detailed hormonal biomarker assessment alongside molecular classification to enhance individualized patient care.

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Hormonal biomarkers remain prognostically relevant within the molecular subgroups in endometrial cancer

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HIGHLIGHTS

- ER/PR expression is prognostic within the molecular subgroups.
- Within MMRd and NSMP EC the three-tiered risk classification of ER expression is prognostically significantly.
- Within p53mut and NSMP EC the three-tiered risk classification of PR expression is prognostically significantly.
- In the entire cohort, PR 0–10 % expression and p53mut are independent prognostic factors for decreased DSS.
- In the entire cohort, PR 90–100 % and *POLE*-mutant are independent prognostic factors for an improved DSS.

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ABSTRACT

Objective. The prognostic relevance of hormonal biomarkers in endometrial cancer (EC) has been well-established. A refined three-tiered risk model for estrogen receptor (ER)/progesterone receptor (PR) expression was shown to improve prognostication. This has not been evaluated in relation to the molecular subgroups. This study aimed to evaluate the ER/PR expression within the molecular subgroups in EC.

Methods. A retrospective multicenter cohort study was performed and data from the European Network for Individualized Treatment centers and Vancouver, Canada were used. ER/PR immunohistochemical expression was grouped as: ER/PR 0–10 %, 20–80 % or 90–100 %. Molecular subgroups were determined with full next-generation sequencing or combined with immunohistochemistry: *POLE*mut, mismatch repair deficient (MMRd), p53mut and no-specific molecular profile (NSMP).

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Pathology
Molecular classification
Immunohistochemical

Results. A total of 739 patients were included (median follow-up 5.0 years). Tumors were classified as *POLE*mut in 9.1 %($N = 67$), MMRd in 27.6 %($N = 204$), p53mut in 20.8 %($N = 154$) and NSMP in 42.5 %($N = 314$). Among all molecular subgroups, patients with ER/PR 90–100 % expression revealed the best disease-specific survival (DSS). Within p53mut, PR 90–100 % expression showed a 5-year DSS of 100.0 %. ER expression is prognostic more relevant in MMRd and NSMP tumors while PR expression in p53mut and NSMP tumors. Across all molecular subgroups, PR 0–10 %, p53mut, lympho-vascular space invasion and FIGO stage III-IV remained independently prognostic for reduced DSS Whereas PR 90–100 % and *POLE*mut remained independently prognostic for improved DSS.

Conclusion. We demonstrated that ER/PR expression remain prognostically relevant within the molecular subgroups, and that a three-tiered cutoff refines prognostication. These data support incorporating routine evaluation of ER/PR expression in clinical practice.

1. Introduction

Historically, endometrial cancer (EC) was divided into two histopathological subtypes [1]. Type 1 EC includes low-grade (grade 1 and 2) endometrioid EC (EEC), represents the majority (80 %) of patients, and is associated with obesity and good prognosis. Type 1 EC is considered to be hormone driven with high expression of estrogen (ER) and progesterone receptors (PR) [2]. Type 2 EC represents high-grade tumors (grade 3 EEC and non-endometrioid EC (NEEC)), generally have low ER expression and an unfavorable prognosis [1,3]. Despite the overall good prognosis of type 1 EC, mortality in absolute numbers is higher in type 1 compared to type 2 EC [4].

Hormone receptor expression (ER/PR) are prognostic biomarkers that predict lymph node metastasis (LNM) and outcome [5–7]. The current used cutoff for ER/PR expression within EC is not uniform, is adopted from breast cancer studies, and most frequently considered positive if >1 % or > 10 % expression [8,9]. Studies within EC showing the cutoff with the best prognostic impact are lacking, therefore in a previous explorative analysis different cutoff values for ER/PR expression were evaluated and showed the subgroups 0–10 % with unfavorable outcome, 20–80 % with intermediate outcome and 90–100 % with favorable outcome. This revised three-tiered risk classification model was shown to improve prognostication over the mostly used cutoff of 10 % [10]. Prospective validation is very much supported to verify these findings.

The Cancer Genome Atlas (TCGA) classified patients with EC into four important prognostic subgroups based on their genomic molecular signature: I) ultramutated tumors with polymerase epsilon (*POLE*) mutations, II) hypermutated tumors with microsatellite instability (MSI), III) copy-number-high (CNH) tumors with frequent tumor protein (*TP53*) mutations, IV) copy-number-low (CNL) tumors (also known as no-specific molecular profile (NSMP)) [11]. The Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) is a surrogate diagnostic algorithm using low cost clinically applicable immunohistochemistry (IHC); mismatch repair deficient (MMRd) instead of MSI and p53 instead of *TP53* [12,13].

The histopathological subtypes (type 1 and 2) are present within all molecular subgroups. Type 1 (EEC histology) is mainly represented by the *POLE*mut, MMRd and NSMP subgroup, with positive ER/PR expression. Type 2 (NEEC histology) is mainly represented by the p53mut subgroup, with generally negative ER/PR expression [11].

In this era of molecular profiling, the relevance of hormonal biomarkers needs to be redefined. Earlier study demonstrated that ER status was still important for the outcome of EC patients regardless of risk class and p53 or MMR status [14]. Within the NSMP subgroup loss of ER and/or PR expression (<1 % and < 10 %) was shown to be an important prognosticators for EC, but this was not found in the other molecular subgroups [15–17]. So far, it has not been investigated whether the previously mentioned three-tiered ER/PR risk model [10], has prognostic impact in the different molecular subgroups. Therefore, we studied the prognostic relevance of the three-tiered ER/PR classification within the molecular subgroups in EC. It is hypothesized that this three-tiered model refines prognostication within all molecular subgroups.

2. Materials and methods

2.1. Patients

A retrospective multicenter cohort study has been performed. Data was used from the European Network for Individualized Treatment (ENITEC) centers and Vancouver Hospital, Canada. Data from four previously published and one unpublished cohort were collected, resulting in 978 patients (flowchart Supplementary Fig. S1) [10,12,13,18,19]. Patients were treated between 1994 and 2019 (median 2007) and data on clinicopathological characteristics and outcome were collected.

Inclusion criteria were: (I) availability of ER/PR immunohistochemistry, (II) patients successfully classified with either full next-generation sequencing (NGS) or NGS combined with IHC according to ProMisE [12]. An exclusion criteria was: missing follow-up. Patients were aligned according to the diagnostic algorithm in Fig. 1 and final classified according to the World Health Organization (WHO) Classification of Female Genital Tumors [20]; *POLE*mut, MMRd, p53mut and NSMP.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2. DNA analysis

The molecular subgroups included in this study were determined by either full NGS or according to ProMisE. Both methods have been described previously [13,21] and details are provided for the different cohorts in the *Supplementary Method S1*. Briefly, for molecular profiling by full NGS, DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor blocks. Next, DNA was sequenced by NGS with single-molecule Molecular Inversion Probes (smMIPs) [22]. For the detection of MSI, 55 MSI markers were tested according to the previously published design [23]. Multiple-classifiers were classified as the molecular subgroup with the best prognosis [24]. For the molecular subgroups determined according ProMisE criteria, *POLE*mut analysis was performed by MiSeq, Sanger or NGS [13,19].

2.3. Immunohistochemical analysis

IHC was performed on 4 um FFPE tumor sections for the ENITEC centers and tissue microarrays (TMA) for Vancouver cohort, as described previously and detailed in the *Supplementary method S1* [10,13,17,19]. In brief, antibodies specific to MSH6, PMS2, p53, ER and PR were used. Staining for p53 was considered abnormal when more than 80 % of tumor cell nuclei showed strong expression (*overexpression*) or when there was complete loss of nuclear staining (*null-expression*) with a

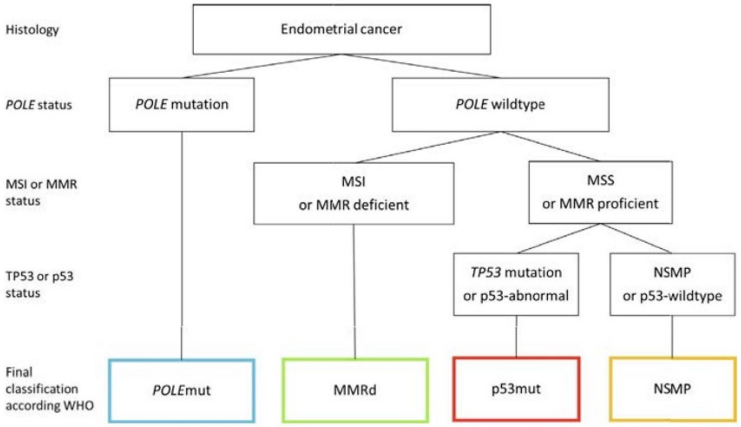

Fig. 1. Diagnostic algorithm of patients diagnosed with full next-generation sequencing or combined with immunohistochemistry, and the final classification according to the World Health organization (WHO) classification of female genital tumors.
Abbreviations: *POLE*, Polymerase epsilon; MSI, Microsatellite instability; MMRd, Mismatch repair deficient; *TP53*, Tumor protein 53; p53mut, p53-mutant; NSMP, No-specific molecular profile.

positive internal control. Mismatch repair deficiency (MMRd) was defined as complete absence of nuclear staining of PMS2 and/or MSH6, in the presence of a positive internal control. For the TMAs, staining for individual MMR proteins and ER/PR was repeated on whole sections whenever there was equivocal, uninterpretable, or aberrant staining. ER and PR expression was determined by estimating the percentage of positive nuclei in the whole invasive tumor area by 'eyeballing'. Scoring for ER and PR expression within the included cohorts was performed by two assessors (pathologists and researchers, who were trained by an expert gynecologic-pathologist), reviewing discrepancies in a consensus meeting. Within the different cohorts, the discrepancies were discussed between the two assessors to find a final agreement for the percentage [10,19]. In our previous study, explorative analysis was performed of the IHC samples with 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 % of expression. Cutoffs with the strongest prognostic impact were determined. According to this retrospective study the ER/PR risk groups were defined as: ER/PR 0–10 % as high-risk, ERPR 20–80 % as intermediate risk and 90–100 % as low risk, in this study the same cutoffs for the risk groups was used. The Cohen's k value for scoring ER/PR expression as per the three risk groups was 0.703 [10]. Percentages were scored by the pathologist as 0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 %. In a small amount of cases the percentages were scored in between, e.g. 85 %, these were rounded off into the nearest category (so 85 % was categorized as 90 %). When ER and PR were taken together, the subgroups were defined as ER + PR 0–10 %, ER + PR 20–80 %, ER + PR 90–100 % and discordant. Patients were grouped 'discordant' if the ER and PR percentages were not aligned in the same risk group (e.g. ER 10 % and PR 90 %). These patients were also included in the survival analysis to determine the relevance of this subgroup in clinical practice.

2.4. Primary objective

To study the prognostic relevance of the three-tiered ER/PR risk classification within the molecular subgroups in EC.

2.5. Statistical analysis

The molecular subgroups were compared with the dichotomous clinicopathological characteristics using the χ^2 or Fisher's exact test for categorical data, and the non-parametric Mann-Whitney *U* test for continuous variables. Survival analyses were performed using Kaplan-Meier curves and univariable and multivariable Cox-regression analysis. Associations are shown as hazard ratio (HR), 95 % CI and *P*-value. The including covariates in multivariable analysis are the main known prognostic biomarkers in EC. Myometrial invasion (MI) was excluded because this is already included in FIGO stage. Disease-specific survival (DSS) was defined as time from date of diagnosis to date of death by EC all censored by date of last contact. Patients who died within 1 year of survival were also included, due to this definition. The results were considered significant with *P*-value less than 0.05. Statistical Package for the Social Sciences, version 27.0 (IBM, New York, NY, USA) was used for statistical analyses.

3. Results

In total, 978 patients with known and classifiable ER/PR IHC status were available for molecular analysis. Only patients with a successful molecular analysis were included, resulting in 747 EC patients. In which 8 patients were excluded due to complete missing follow-up, leading to a total of 739 patients included in this study (Flowchart Supplementary Fig. S1). A baseline overview of each included cohort is shown in Supplementary Table 1. The baseline characteristics of the entire cohort are shown in Table 1. Median age was 65.0 (31.0–93.0) years, median BMI 29.0 (15.8–66.2) kg/m^2 and median follow-up 60.0 (1.0–283.0) months. The number of patients with <1 year follow-up ($n = 36$) consisted of patients who died due to EC ($n = 24$), died due to other cause than EC ($n = 5$) or did the rest of their follow-up in another hospital then they were operated ($n = 7$). Exclusion of these 7 cases did not affect results in the multivariable analysis (*data not shown*). The majority of the patients revealed EEC histology 80.4 % ($N = 594$), grade 1–2 EEC 53.5 % ($N = 394$) and FIGO stage I-II 75.5 % ($N = 558$). A minority of patients were diagnosed with ER + PR expression 0–10 % or 90–100 % (respectively, 16.8 % ($N = 124$) and 17.1 % ($N = 126$)). A total of 251 patients (34.0 %) were not aligned to one of the three risk groups and classified as 'discordant'. Most discordant cases are located in patients with ER 20–80 % + PR 0–10 % expression (13.4 %), and PR 20–80 % + ER 90–100 % expression (12.3 %) (*data not shown*).

Tumors were classified as *POLE*mut in 9.1 % ($N = 67$), MMRd in 27.6 % ($N = 204$), p53mut in 20.8 % ($N = 154$), and NSMP in 42.5 % ($N = 314$), in line with the original TCGA paper [11]. The majority

Table 1
Baseline.

		Total N = 739	*POLE*mut N = 67 (9.1)	MMRd N = 204 (27.6)	p53mut N = 154 (20.8)	NSMP N = 314 (42.5)	*P*
Patient characteristics							
Age (years)		65.0 (31.0–93.0)	57.3 (34.0–93.3)	65.1 (42.0–87.0)	72.6 (35.0–93.0)	62.9 (35.0–88.0)	<0.001*
BMI (kg/m^2)		29.0 (15.8–66.2)	27.6 (18.4–58.3)	29.1 (15.8–62.0)	28.4 (17.5–46.2)	29.6 (15.8–66.2)	0.142
Post-operative histology							
Histology	EEC	594 (80.4)	59 (88.1)	187 (91.7)	57 (37.0)	291 (92.7)	<0.001*
	NEEC	145 (19.6)	8 (11.9)	17 (8.3)	97 (63.0)	23 (7.3)	
Grade	1–2	394 (53.3)	30 (44.8)	102 (50.0)	22 (14.3)	240 (76.4)	<0.001*
	3	345 (46.7)	37 (55.2)	102 (50.0)	132 (85.7)	74 (23.6)	
MI[a]	<50 %	400 (54.1)	33 (50.0)	107 (52.5)	75 (50.0)	185 (59.3)	0.172
	>50 %	332 (44.9)	33 (50.0)	97 (47.5)	75 (50.0)	127 (40.7)	
LVSI	No	474 (64.1)	37 (55.2)	115 (56.4)	78 (50.6)	244 (77.7)	<0.001*
	Yes	265 (35.9)	30 (44.8)	89 (43.6)	76 (49.4)	70 (22.3)	
Lymph nodes	N0	231 (31.3)	31 (46.3)	61 (29.9)	38 (24.7)	101 (32.2)	0.170
	N1	54 (7.3)	3 (3.0)	13 (6.4)	15 (9.7)	20 (6.3)	
	Nx	454 (61.4)	34 (50.7)	129 (63.2)	101 (65.6)	190 (39.5)	
FIGO stage[a]	Early	558 (75.5)	60 (90.9)	154 (75.9)	87 (56.5)	257 (82.6)	<0.001*
	Advanced	176 (23.8)	6 (9.1)	49 (24.1)	67 (43.5)	54 (17.4)	
Hormonal receptor expression							
ER	0–10	142 (19.2)	16 (23.9)	39 (19.5)	55 (36.2)	32 (10.2)	<0.001*
	20-80	354 (47.9)	36 (53.7)	98 (49.0)	74 (48.7)	146 (46.6)	
	90–100	236 (31.9)	15 (22.4)	63 (31.5)	23 (15.1)	135 (43.1)	
PR	0–10	241 (32.6)	21 (31.3)	60 (29.7)	101 (66.9)	59 (18.8)	<0.001*
	20-80	344 (46.5)	39 (58.2)	108 (53.5)	38 (25.2)	159 (50.8)	
	90–100	148 (20.0)	7 (10.4)	34 (16.8)	12 (7.9)	95 (30.4)	
ER + PR	0–10	124 (16.8)	13 (19.4)	30 (14.7)	54 (35.1)	27 (8.6)	<0.001*
	20-80	238 (32.2)	29 (43.3)	69 (33.8)	29 (18.8)	111 (35.4)	
	90–100	126 (17.1)	6 (9.0)	29 (14.2)	10 (6.5)	81 (25.8)	
	Discordant	251 (34.0)	19 (28.4)	76 (37.3)	61 (39.6)	95 (30.3)	
Adjuvant treatment[a]							
None		287 (38.8)	24 (36.4)	75 (36.9)	46 (30.1)	142 (45.7)	<0.001*
Radiotherapy		247 (33.4)	25 (37.9)	73 (36.0)	35 (22.9)	114 (36.7)	
Chemotherapy		77 (10.4)	8 (12.1)	19 (9.4)	34 (22.2)	16 (5.1)	
Chemoradiation		122 (16.5)	9 (13.6)	36 (17.7)	38 (24.8)	39 (12.5)	
Outcome							
Recurrence		198 (26.8)	4 (6.1)	58 (29.1)	72 (51.4)	64 (20.5)	<0.001*
Mortality		216 (29.2)	8 (11.9)	57 (27.9)	83 (53.9)	68 (21.7)	<0.001*
EC-related mortality		152 (20.6)	1 (1.5)	38 (19.4)	67 (45.3)	46 (14.9)	<0.001*

Data is presented in number (%), median (IQR).
Abbreviations: *N*, number; *POLE*mut, polymerase epsilon mutant; MMRd, mismatch repair deficient; p53, protein 53; NSMP, No specific molecular profile; EEC, endometrioid endometrial cancer; NEEC, non-endometrioid endometrial cancer; LVSI, lympho-vascular space invasion; N0, negative lymph nodes, N1, positive lymph nodes; Nx, no information about the lymph nodes; FIGO, International Federation of Gynecology and Obstetrics, ER, estrogen receptor; PR, progesterone receptor; EC, endometrial cancer.
[a] Missing data for 7 cases MI, 5 cases for FIGO stage, 6 for adjuvant treatment.
* $P < 0.05$.

of patients within the *POLE*mut, MMRd and NSMP subgroups had EEC histology (respectively, 88.1 %, 91.7 %, 92.7 %), whereas the majority of patients within the p53mut subgroup had NEEC histology (63.0 %).

3.1. Outcome ER or PR expression

Figure 2A-E shows the 5-year DSS curve of the three-tiered ER risk classification within the entire cohort and the molecular subgroups. In the entire cohort, patients with ER 90–100 % expression showed a significantly better DSS when compared to ER 20–80 % ($P < 0.001$) and ER 0–10 % ($P < 0.001$). Patients with ER 20–80 % had a significant higher 5-year DSS compared to ER 0–10 % ($P < 0.001$) (Fig. 2A). Across all molecular subgroups, patients with ER 90–100 % expression showed the most favorable 5-year DSS (Fig. 2B-E). Within *POLE*mut EC, patients with ER 90–100 %, 20–80 % and 0–10 % revealed no significantly different 5-year DSS (respectively, 100.0 %, 100.0 % and 92.0 %) For MMRd tumors, patients with ER 90–100 % and 20–80 % or 0–10 % revealed significantly different 5-year DSS (respectively, 96.0 % vs 80.0 % $P = 0.017$ and 96.0 % vs 71.0 % $P = 0.002$) (Fig. 2C). Within patients with p53mut no significant differences in 5-year DSS were found between the three ER subgroups (Fig. 2D). Within NSMP tumors, patients with ER 0–10 % had an significant worst 5-year DSS of 48.0 % compared to ER 90–100 % (96.0 %, $P < 0.001$) and compared to ER 20–80 % (88.0 %, $P < 0.001$)). (Fig. 2E).

Figure 3A-E shows the 5-year DSS curve of the three-tiered PR risk classification within the entire cohort and the molecular subgroups. In the entire cohort, patients with PR 90–100 % expression showed a significantly better DSS when compared to PR 20–80 % ($P = 0.003$) and PR 0–10 % ($P < 0.001$). Patients with ER 20–80 % had a significant higher 5-year DSS compared to ER 0–10 % ($P < 0.001$) (Fig. 3A). Across all molecular subgroups, patients with PR 90–100 % expression showed the most favorable 5-year DSS and PR 0–10 % the worst (Fig. 3B-E). Within *POLE*mut and MMRd tumors, no significant different 5-year DSS was revealed within the three subgroups of PR expression (Fig. 3B-C). Patients with p53mut EC and PR 90–100 % had a 5-year DSS of 100 %, this was significantly different compared to PR 20–80 % (62.0 %, $P = 0.032$) and PR 0–10 % (48.0 % $P = 0.006$) (Fig. 3D). Within NSMP tumors, patients with PR 90–100 % had an excellent 5-year DSS of 98.0 %, for PR 20–80 % the 5-year DSS was 88.0 % and PR 0–10 % showed the worst 5-year DSS of 56.0 %. All were significantly different from each other (Fig. 3E).

Across all molecular subgroups, PR 0–10 %, p53mut, lympho-vascular space invasion (LVSI) and FIGO stage III-IV remained

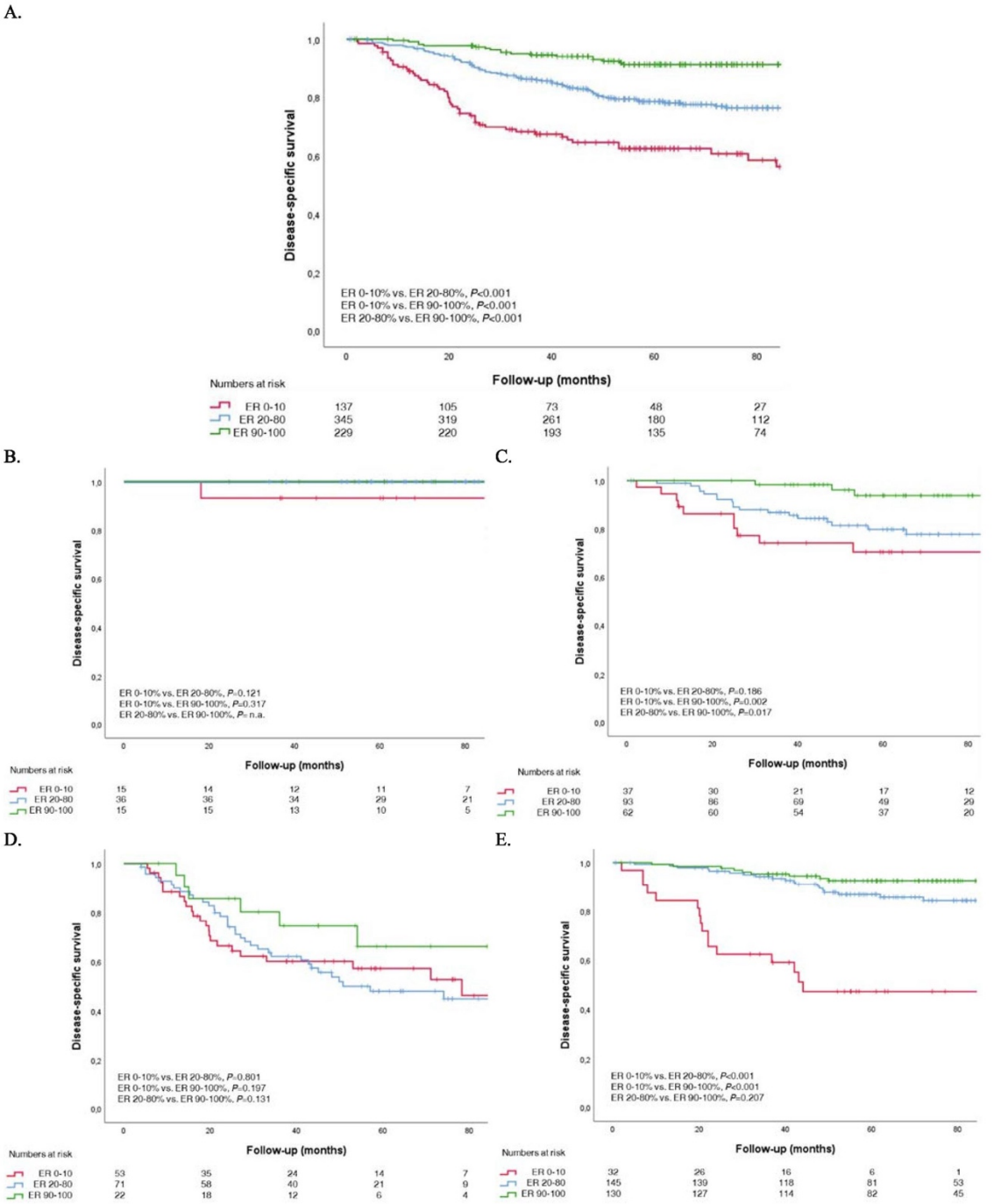

Fig. 2. A. 5-year disease-specific survival (DSS) of the ER three-tiered risk model within the entire cohort. B. 5-year DSS of the ER three-tiered risk model within *POLE*mut patients. C. 5-year DSS of the ER three-tiered risk model within MMRd patients. D. 5-year DSS of the ER three-tiered risk model within p53mut patients. E. 5-year DSS of the ER three-tiered risk model within NSMP patients.
Abbreviations: ER, estrogen receptor; *POLE*, Polymerase epsilon; MMRd, Mismatch repair deficient; *TP53*, p53mut, p53-mutant; NSMP, No-specific molecular profile.

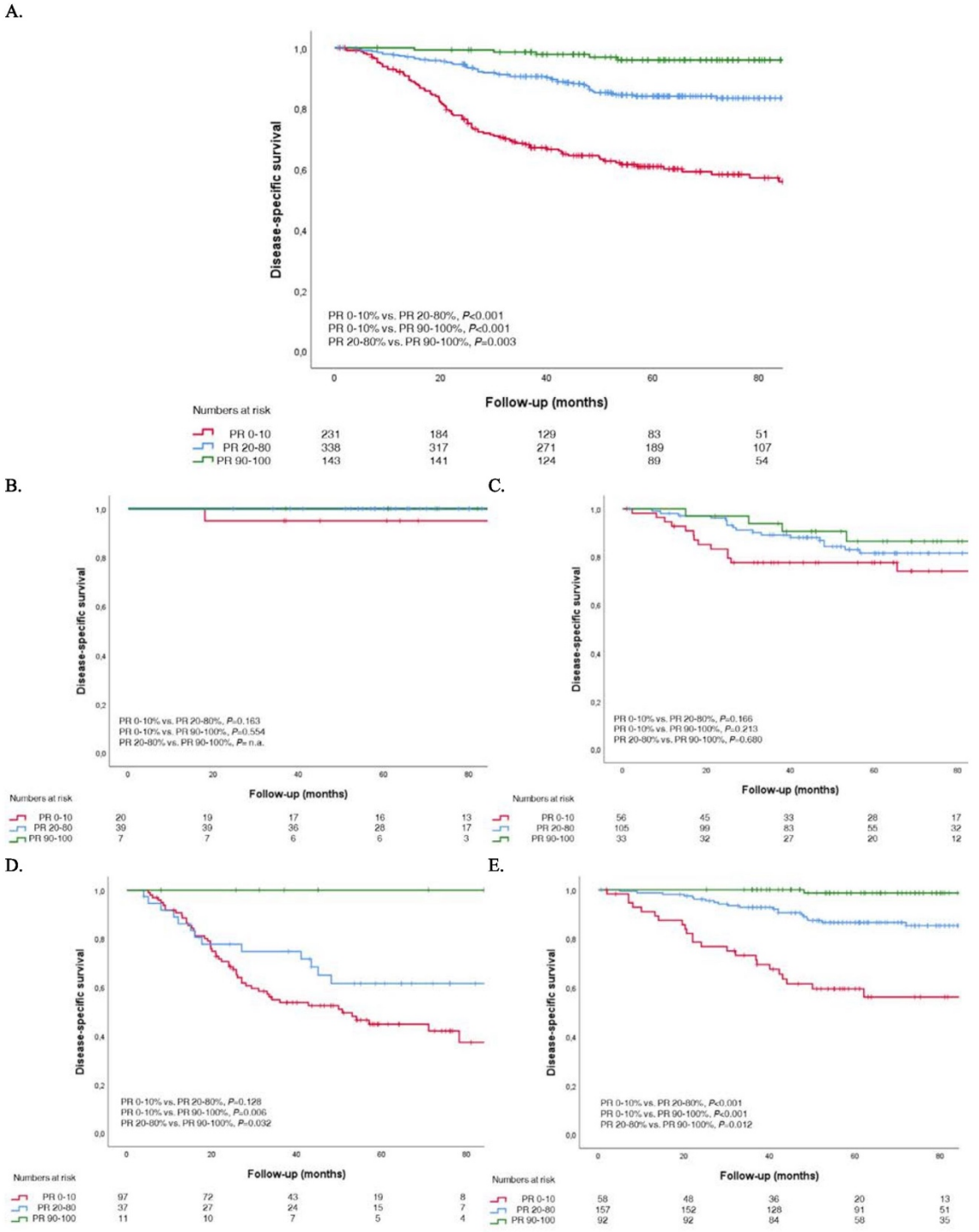

Fig. 3. A. 5-year disease-specific survival (DSS) of the PR three-tiered risk model within the entire cohort. B. 5-year DSS of the PR three-tiered risk model within *POLE*mut patients. C. 5-year DSS of the PR three-tiered risk model within MMRd patients. D. 5-year DSS of the PR three-tiered risk model within p53mut patients. E. 5-year DSS of the PR three-tiered risk model within NSMP patients.
Abbreviations: PR, progesterone receptor; *POLE*, Polymerase epsilon; MMRd, Mismatch repair deficient; *TP53*, p53mut, p53-mutant; NSMP, No-specific molecular profile.

Table 2
Cox regression univariable and multivariable analysis disease-specific survival (DSS).

Variable	Univariable		Multivariable 141 events	
			HR (95 % CI)	*P* value
ER cutoff				
ER 0–10 %	2.16 (1.51–3.08)	<0.001	1.20 (0.80–1.79)	0.378
ER 20–80 %	1		1	
ER 90–100 %	0.41 (0.24–0.69)	<0.001	0.82 (0.48–1.41)	0.463
PR cutoff				
PR 0–10 %	3.09 (2.19–4.35)	<0.001	1.61 (1.04–2.49)	0.030*
PR 20–80 %	1		1	
PR 90–100 %	0.34 (0.16–0.71)	0.005	0.41 (0.17–0.95)	0.039*
Molecular subgroup				
*POLE*mut	0.09 (0.01–0.65)	0.017*	0.06 (0.00–0.50)	0.006*
MMRd	1.37 (0.89–2.10)	0.154	0.79 (0.49–1.26)	0.327
p53mut	4.09 (2.81–5.97)	<0.001*	1.55 (1.00–2.40)	0.046*
NSMP	1		1	
LVSI				
No	1	<0.001*	1	0.025*
Yes	4.14 (2.96–5.78)		1.65 (1.07–2.57)	
FIGO				
Stage I-II	1		1	<0.001*
Stage III-IV	6.17 (4.45–8.55)	<0.001*	2.32 (1.50–3.60)	
Adjuvant treatment				
None	1		1	
Radiotherapy	1.72 (1.04–2.86)	0.035*	1.38 (0.81–2.34)	0.231
Chemotherapy	9.28 (5.65–15.23)	<0.001*	2.25 (1.21–4.17)	0.010*
Chemoradiation	4.27 (2.57–7.09)	<0.001*	1.46 (0.80–2.64)	0.216

Abbreviations: DSS, disease-specific survival; EC, endometrial cancer; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR., progesterone receptor; *POLE*mut, polymerase epsilon mutant; MMRd, mismatch repair deficient; p53, protein 53; NSMP, No specific molecular profile; LVSI, lympho-vascular space invasion; FIGO, Federation International of Gynecology and Obstetrics.

independent prognostic for reduced DSS. Whereas PR 90–100 % and *POLE*mut remained independent prognostic for improved DSS (Table 2).

3.2. Outcome ER + PR expression combined

Supplementary Fig. S2A–E shows the 5-year DSS curve of the three-tiered ER + PR combined risk classification within the entire cohort and the molecular subgroups. The 5-year DSS was significantly different between the three ER + PR risk classification groups (Supplementary Fig. S2A). Patients with p53mut EC and ER + PR 90–100 % had a 5-year DSS of 100 %, and patients with ER + PR 20–80 % and 0–10 % had comparable outcome as ER + PR 0–10 % (respectively, 55.0 % and 44.0 %). The 5-year DSS between ER + PR 0–10 % and 90–100 % was significantly different (Supplementary Fig. S2D). Within NSMP tumors, patients with ER + PR 90–100 % had an excellent 5-year DSS of 98.0 %, for ER + PR 20–80 % the 5-year DSS was 84.0 % and ER + PR 0–10 % showed the worst 5-year DSS of 43.0 %. All were significantly different from each other (Supplementary Fig. S2E). In the entire cohort and within *POLE*mut, MMRd and NSMP subgroup, the patients grouped as 'discordant', showed comparable outcomes as patients with ER + PR 20–80 % expression, Within p53mut EC the outcome was in line with the outcome of ER + PR 0–10 % expression. (Supplementary Fig. S2A–E).

Across all molecular subgroups and ER + PR risk groups, ER + PR 0–10 %, p53mut, lympho-vascular space invasion (LVSI) and FIGO stage III/IV remained independent prognostic factors for reduced DSS. ER + PR 90–100 % and *POLE*mut were independent prognostic factors for improved DSS (*Supplementary Table S2*).

4. Discussion

In this large retrospective multicenter cohort study we confirmed the relevance of using a three-tiered ER/PR risk classification that refined the prognostic relevance across the molecular subgroups. Among all molecular subgroups, patients with ER/PR 90–100 % expression revealed the best 5-year DSS. Interestingly, patients with PR 90–100 % and with p53mut EC revealed an excellent 5-year DSS. In multivariable analyses, PR 0–10 % was an independent prognostic factor for reduced DSS and PR 90–100 % an independent prognostic factor for improved DSS. Combining ER + PR, 0–10 % ER + PR expression was an independent prognostic factor for reduced DSS, while ER + PR 90–100 % for improved DSS.

In EC, numerous studies have already shown the importance of ER and PR expression in relation to predicting LNM and outcome, regardless of risk class [5–7,14,25]. However, no uniform cutoff is applied within EC. In an earlier study, we defined a three-tiered risk classification for ER/PR expression to improve prognostication specifically in patients with EC [10]. The current study confirmed the additional value of using this three-tiered risk classification when compared to the commonly used cutoff of 1 % or 10 %.

The relevance of ER/PR expression within all molecular subgroups was not fully elucidated until this study. Comparable to our data, early studies observed higher PR expression within the NSMP subgroup and low PR expression in p53mut tumors [11,17]. In addition, our study shows the relevance of hormonal biomarkers within the MMRd, p53mut and NSMP subgroups. Vermij et al. confirmed the significance of ER status within the NSMP high-risk EC. Comparable to our study, patients with ER expression <10 % showed the worst outcome compared to ER >10 %. Contrary to our findings, they found no prognostic impact of ER in the other molecular subgroups (especially MMRd) which might be explained by their cut-off of 1–10 % [15]. Jamieson et al. used ER and tumor grade to subclassify the NSMP subgroup. Low-risk NSMP was identified as low-grade EC and ER >1 % with favorable outcome, and high-risk NSMP as high-grade EC and ER <1 % expression with unfavorable outcome [16]. Which confirms the relevance of ER within the NSMP subgroup. Our study revealed also the additional relevance of PR

expression within the NSMP and p53mut subgroup, contrary to the other studies which might again by explained by the use of a three-tiered risk classification [15]. Interestingly, patients with p53mut EC and PR 90–100 % expression showed an excellent 5-year DSS of 100 %, since all these patients had EEC histology, the importance of both morphology and IHC in addition to molecular subgroups within EC is illustrated. Patients within p53mut or NSMP EC and PR 0–10 % show the worst outcome. Early studies indicated that PR <10 % expression was predominantly present in the 'advanced/metastatic' ESGO risk group and predicting disease recurrence in patients and increased risk of death. This is in line with our findings in multivariable regression analysis, were PR expression 0–10 % is more correlated with decreased DSS compared to ER expression 0–10 %. Due to the used cutoffs for ER and PR of 1 % or 10 % the prognostic relevance within the molecular subgroups might have been underestimated when compared with the three-tiered ER/PR risk classification in our study [15,17].

In clinical practice generally both ER and PR IHC expression are determined, therefore, understanding the prognostic relevance of both ER/PR expression within the molecular subgroups is interesting. Early studies indicated that both ER/PR provide additional prognostic information, comparable with our study [5,7,10,17]. Combining ER + PR shows ER + PR 0–10 % as an independent prognostic marker for reduced DSS and ER + PR 90–100 % as an independent prognostic marker for improved DSS. Combining ER + PR expression within the three-tiered risk classification will create a remaining subgroup, in this paper classified as discordant. For clinical practice, when the ER + PR subgroup is discordant in patients with *POLE*mut, MMRd or NSMP EC, the prognosis is in line with an intermediate prognosis. Within p53mut, the prognosis is in line with decreased prognosis (comparable to high risk 0–10 % expression). This decreased prognosis for the discordant group within patients with p53mut, is likely due to more patients with PR 0–10 % (78 %) compared the other molecular subgroups. This is in line with our previous conclusion, showing the relevance of PR expression within the p53mut subgroup.

The strengths of this retrospective study are the large number of included cases from multiple centers, including ER and PR immunohistochemistry and representing all tumor grades and FIGO stages. Second, by including ER/PR expression both and combined these results are highly relevant for clinical practice Furthermore, this is the first study to analyze a three-tried ER/PR risk classification within all molecular subgroups.

Some limitations need to be addressed. First, the mortality rate of *POLE*mut patients is low, possibly hampering interpretation on the impact of ER/PR expression within this specific subgroup. Second, technical allocation of the molecular subgroups differed slightly. However, either full NGS or use of ProMiSe criteria (combination of NGS and IHC) are repeatedly validated as comparable techniques and representative for the daily practice in Europe and Canada [26,27]. Third, a relative high amount of patients were excluded due to unsuccessful molecular profiling, perhaps as an result of using older archival tumor samples for DNA testing, furthermore patients who did the rest of their follow-up within other hospitals ($n = 7$) were included, however exclusion of these 7 cases did not affect results in the multivariable analysis. Fourth, race or ethnicity has not been reported in our study. Although we fully agree that these patients' information might be impact outcome in several diseases [28], within Europe it is not routinely documented in patient files [29]. Fifth, using patients between 1994 and 2019 could have biased the survival because of different treatment strategies over the time. However, the death caused by EC has not been reduced or increased over the 25 years in our study cohort (*data not shown*), therefore we believed this has not biased our results. Finally, according to the ProMiSe criteria the order of molecular subgroup allocation within the Vancouver cohort is different compare to the original TCGA cohort, in which MMRd testing is followed by *POLE* testing [11,13]. The distribution of MMRd that also include *POLE*mut varies, patients with *POLE*mut and MMRd have comparable prognosis to *POLE*mut [30]. Therefore a different allocating order could bias the outcome. However, in the original ProMiSe cohort, no MMRd patients are present with also *POLE*mut. Within the cohorts from the ENITEC centers the order of molecular testing was in line with the original TCGA cohort [10,11,18,19].

This study demonstrates the prognostic importance of ER and PR biomarkers within the era of molecular profiling and the relevance of a three-tiered risk classification. To validate the newly proposed cutoff a large prospective multicenter trial should be performed. Future prospective studies need also focus on response to hormonal treatment within the molecular subgroups, analysis the relevance of ER/PR biomarkers in advanced stage and within adjuvant treatment including patterns of recurrence. Currently, an international randomized control trial has been started to refine the adjuvant treatment in endometrial cancer based on molecular features (RAINBO trial), in which one arm includes patients with NSMP EC (ClinicalTrials.gov Identifier: NCT05255653). Patients with ER positive expression will receive RT and hormonal treatment. However, only the presence of ER expression is part of the inclusion criteria, and the cutoff for positivity is not specified. Furthermore, in order to increase response to hormonal treatment, a different cutoff for ER and PR might be indicated as suggested by a recent paper in which a cutoff of 50 % was suggested [31].

5. Conclusion

Our study demonstrated the prognostic relevance of ER and PR expression within the molecular subgroups of patients with EC and that the use of a three-tiered risk classification refines prognostication. These data support incorporating routine evaluation of ER/PR expression in clinical practice.

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Tweetable statement

Hormonal biomarkers remain prognostically relevant within the molecular subgroups in endometrial cancer.

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Paper presentation information

The findings of this study were presented on a poster presentation session at the IGCS September 2022 Annual Global meeting in New York.

Ethical approcval

ENITEC centers: This study was approved by the Institutional Review Board of the Radboud University Medical Center and the Institutional Review Boards of all participating centers.

Vancouver cohort: Research ethics approval were present for the tissues.

Data was used from previous published studies and, therefore, informed consent was waived for participants.

CRediT authorship contribution statement

Stephanie W. Vrede: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Willem Jan Van Weelden:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **Johan Bulten:** Writing – review & editing, Visualization, Supervision, Resources, Investigation. **C. Blake**

Gilks: Writing – review & editing, Resources, Investigation. **Steven Teerenstra:** Writing – review & editing, Formal analysis. **Jutta Huvila:** Writing – review & editing, Resources. **Xavier Matias-Guiu:** Writing – review & editing, Resources. **Antonio Gil-Moreno:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **Jasmin Asberger:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **Sanne Sweegers:** Writing – review & editing, Investigation. **Louis J.M. van der Putten:** Writing – review & editing, Resources, Investigation. **Heidi V.N. Küsters-Vandevelde:** Writing – review & editing, Resources. **Casper Reijnen:** Writing – review & editing, Visualization, Methodology, Investigation. **Eva Colas:** Writing – review & editing, Resources. **Jitka Hausnerová:** Writing – review & editing, Resources, Investigation. **Vit Weinberger:** Writing – review & editing, Resources. **Marc P.L.M. Snijders:** Writing – review & editing, Visualization, Supervision, Resources. **Petra Vinklerova:** Writing – review & editing, Resources. **Antonella Ravaggi:** Writing – review & editing, Resources, Investigation. **Franco Odicino:** Writing – review & editing, Resources. **Eliana Bignotti:** Writing – review & editing, Resources. **Jessica N. McAlpine:** Writing – review & editing, Resources. **Roy Kruitwagen:** Writing – review & editing, Visualization, Supervision, Methodology. **Johanna M.A. Pijnenborg:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors have declared no conflicts of interest.

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References

[1] J.V. Bokhman, Two pathogenetic types of endometrial carcinoma, Gynecol. Oncol. 15 (1) (1983) 10–17.
[2] J.J. Kim, E. Chapman-Davis, Role of progesterone in endometrial cancer, Semin. Reprod. Med. 28 (1) (2010) 81–90.
[3] M.E. McDonald, D.P. Bender, Endometrial Cancer: obesity, genetics, and targeted agents, Obstet. Gynecol. Clin. N. Am. 46 (1) (2019) 89–105.
[4] A.C. Rodriguez, Z. Blanchard, K.A. Maurer, J. Gertz, Estrogen signaling in endometrial cancer: a key oncogenic pathway with several open questions, Horm Cancer. 10 (2–3) (2019) 51–63.
[5] L.J.M. van der Putten, N.C.M. Visser, K. van de Vijver, M. Santacana, P. Bronsert, J. Bulten, et al., Added value of estrogen receptor, progesterone receptor, and L1 cell adhesion molecule expression to histology-based endometrial carcinoma recurrence prediction models: an ENITEC collaboration study, Int. J. Gynecol. Cancer 28 (3) (2018) 514–523.
[6] J. Trovik, E. Wik, H.M. Werner, C. Krakstad, H. Helland, I. Vandenput, et al., Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial, Eur. J. Cancer 49 (16) (2013) 3431–3441.
[7] S.W. Vrede, W.J. van Weelden, N.C.M. Visser, J. Bulten, L.J.M. van der Putten, K. van de Vijver, et al., Immunohistochemical biomarkers are prognostic relevant in addition to the ESMO-ESGO-ESTRO risk classification in endometrial cancer, Gynecol. Oncol. 161 (3) (2021) 787–794.
[8] M. Yi, L. Huo, K.B. Koenig, E.A. Mittendorf, F. Meric-Bernstam, H.M. Kuerer, et al., Which threshold for ER positivity? A retrospective study based on 9639 patients, Ann. Oncol. 25 (5) (2014) 1004–1011.
[9] K.H. Allison, M.E.H. Hammond, M. Dowsett, S.E. McKernin, L.A. Carey, P.L. Fitzgibbons, et al., Estrogen and progesterone receptor testing in breast Cancer: ASCO/CAP guideline update, J. Clin. Oncol. 38 (12) (2020) 1346–1366.
[10] W.J. van Weelden, C. Reijnen, H.V.N. Küsters-Vandevelde, J. Bulten, P. Bult, S. Leung, et al., The cutoff for estrogen and progesterone receptor expression in endometrial cancer revisited: a European network for individualized treatment of endometrial cancer collaboration study, Hum. Pathol. 109 (2021) 80–91.
[11] C. Kandoth, N. Schultz, A.D. Cherniack, R. Akbani, Y. Liu, H. Shen, et al., Integrated genomic characterization of endometrial carcinoma, Nature 497 (7447) (2013) 67–73.
[12] A. Talhouk, M.K. McConechy, S. Leung, W. Yang, A. Lum, J. Senz, et al., Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer, Cancer 123 (5) (2017) 802–813.
[13] A. Talhouk, M.K. McConechy, S. Leung, H.H. Li-Chang, J.S. Kwon, N. Melnyk, et al., A clinically applicable molecular-based classification for endometrial cancers, Br. J. Cancer 113 (2) (2015) 299–310.
[14] E. Perrone, I. Capasso, F. De Felice, D. Giannarelli, G. Dinoi, A. Petrecca, et al., Back to the future: the impact of oestrogen receptor profile in the era of molecular endometrial cancer classification, Eur. J. Cancer 186 (2023) 98–112.
[15] L. Vermij, J.J. Jobsen, A. León-Castillo, M. Brinkhuis, S. Roothaan, M.E. Powell, et al., Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry, Br. J. Cancer 128 (7) (2023) 1360–1368, https://doi.org/10.1038/s41416-023-02141-0.
[16] A. Jamieson, J. Huvila, D. Chiu, E.F. Thompson, S. Scott, S. Salvador, et al., Grade and estrogen receptor expression identify a subset of no specific molecular profile endometrial carcinomas at a very low risk of disease-specific death, Mod. Pathol. 36 (4) (2023), 100085.
[17] A.N. Karnezis, S. Leung, J. Magrill, M.K. McConechy, W. Yang, C. Chow, et al., Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification, J. Pathol. Clin. Res. 3 (4) (2017) 279–293.
[18] L.J. van der Putten, N.C. Visser, K. van de Vijver, M. Santacana, P. Bronsert, J. Bulten, et al., L1CAM expression in endometrial carcinomas: an ENITEC collaboration study, Br. J. Cancer 115 (6) (2016) 716–724.
[19] A. Ravaggi, D. Capoferri, L. Ardighieri, I. Ghini, F. Ferrari, C. Romani, et al., Integrated biomarker analysis reveals L1CAM as a potential stratification marker for no specific molecular profile high-risk endometrial carcinoma, Cancers (Basel) 14 (21) (2022).
[20] WHO classification of Tumours5th ed, 2020.
[21] S.W. Vrede, J. Kasius, J. Bulten, S. Teerenstra, J. Huvila, E. Colas, et al., Relevance of molecular profiling in patients with low-grade endometrial cancer, JAMA Netw. Open 5 (12) (2022), e2247372.
[22] A. Eijkelenboom, E.J. Kamping, A.W. Kastner-van Raaij, S.J. Hendriks-Cornelissen, K. Neveling, R.P. Kuiper, et al., Reliable next-generation sequencing of formalin-fixed, paraffin-embedded tissue using single molecule tags, J. Mol. Diagn. 18 (6) (2016) 851–863.
[23] E.M.P. Steeghs, L.I. Kroeze, B.B.J. Tops, L.C. van Kempen, A. Ter Elst, A.W.M. Kastner-van Raaij, et al., Comprehensive routine diagnostic screening to identify predictive mutations, gene amplifications, and microsatellite instability in FFPE tumor material, BMC Cancer 20 (1) (2020) 291.
[24] A. León-Castillo, E. Gilvazquez, R. Nout, V.T. Smit, J.N. McAlpine, M. McConechy, et al., Clinicopathological and molecular characterisation of ‘multiple-classifier’ endometrial carcinomas, J. Pathol. 250 (3) (2020) 312–322.
[25] C. Reijnen, E. Gogou, N.C.M. Visser, H. Engerud, J. Ramjith, L.J.M. van der Putten, et al., Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: a development and validation study, PLoS Med. 17 (5) (2020), e1003111.
[26] N. Singh, A.M. Piskorz, T. Bosse, M. Jimenez-Linan, B. Rous, J.D. Brenton, et al., p53 immunohistochemistry is an accurate surrogate for TP53 mutational analysis in endometrial carcinoma biopsies, J. Pathol. 250 (3) (2020) 336–345.
[27] M.K. McConechy, A. Talhouk, H.H. Li-Chang, S. Leung, D.G. Huntsman, C.B. Gilks, et al., Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas, Gynecol. Oncol. 137 (2) (2015) 306–310.
[28] A. Flanagin, T. Frey, S.L. Christiansen, Updated guidance on the reporting of race and ethnicity in medical and science journals, Jama 326 (7) (2021) 621–627.
[29] A. Sheikh, G. Netuveli, J. Kai, S.S. Panesar, Comparison of reporting of ethnicity in US and European randomised controlled trials, Bmj 329 (7457) (2004) 87–88.
[30] A. León-Castillo, H. Britton, M.K. McConechy, J.N. McAlpine, R. Nout, S. Kommoss, et al., Interpretation of somatic POLE mutations in endometrial carcinoma, J. Pathol. 250 (3) (2020) 323–335.
[31] W.J. van Weelden, R.I. Lalisang, J. Bulten, K. Lindemann, H.J. van Beekhuizen, H. Trum, et al., Impact of hormonal biomarkers on response to hormonal therapy in advanced and recurrent endometrial cancer, Am. J. Obstet. Gynecol. 225 (4) (2021) 407.e1-.e16.

4.5 Meijs-Hermanns P, Werner HMJ, Kooreman L, Bretová P, Weinberger V, Vrede S, et al. Improving preoperative binary grading: relevance of p53 and PR expression in grade 2 endometrioid endometrial carcinoma. Int J Gynecol Cancer. 2025;35(4):101682.

As a part of ongoing efforts to identify clinically relevant IHC markers, another multicentric study was conducted to enhance pre-operative risk stratification in patients with grade 2 endometrioid endometrial carcinoma by evaluating the prognostic significance of immunohistochemical expression of p53 and PR. Recognizing the limitations of the current binary grading system, which classifies grade 2 tumors alongside grade 1 as low-grade, the study investigated whether incorporating p53 and PR status could more accurately predict patient outcomes.

The study analyzed data from 1,150 patients across three European cohorts, including the University Hospital Brno, counting 400 with pre-operative grade 2 endometrial EC, 602 with grade 1, and 148 with grade 3. Among the grade 2 group, tumors were further stratified based on p53 and PR expression into two categories: (1) p53 wild-type with PR-positive expression, and (2) p53 aberrant and/or PR-negative expression.

Patients with grade 2 tumors exhibiting p53 wild-type and PR-positive expression had a 7-year DSS rate of 95.8%, comparable to the 97.5% DSS observed in grade 1 patients. Conversely, grade 2 tumors with p53 aberrant and/or PR-negative expression demonstrated a significantly lower 7-year DSS of 83.5%, akin to the 78.1% DSS in grade 3 patients.

Multivariate Cox regression analysis identified p53 aberrant and/or PR-negative status as an independent prognostic factor for both disease-specific and disease-free survival, alongside FIGO stage and lymphovascular space invasion.

These results suggest that assessing p53 and PR expression pre-operatively can effectively distinguish between low- and high-risk grade 2 EECs. Incorporating these biomarkers into the grading system could lead to more tailored surgical and adjuvant treatment strategies, potentially improving patient outcomes.

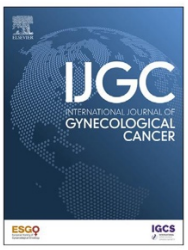
In conclusion, the study supports a modified binary grading approach for grade 2 endometrioid endometrial carcinoma, wherein tumors are classified as low- or high-grade based on combined p53 and PR immunohistochemical profiles. This approach offers a cost-effective and widely accessible means to refine pre-operative risk assessment and guide clinical decision-making.

The study entitled *"Improving preoperative binary grading: relevance of p53 and PR expression in grade 2 endometrioid endometrial carcinoma"* was published in the *International Journal of Gynecologic Oncology* (IF 4.5, Q1) in 2025.

Author's contribution: conceptualization, investigation, data curation, manuscript writing – review and editing.

ORIGINAL RESEARCH

Improving pre-operative binary grading: relevance of p53 and PR expression in grade 2 endometrioid endometrial carcinoma



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ABSTRACT

Objective: This study aimed to evaluate the association between pre-operative progesterone receptor (PR) and p53 expression and prognosis in pre-operative grade 2 endometrioid endometrial carcinoma compared with grade 1 and grade 3 carcinomas.

Methods: Three European endometrial carcinoma cohort studies were included. Patients with pre-operative grade 2 endometrioid carcinoma and known pre-operative PR and p53 status were included ($n = 400$), as were patients with pre-operative grade 1 ($n = 602$) or grade 3 ($n = 148$) endometrioid carcinomas. Kaplan-Meier and Cox regression analyses were performed to analyze disease-specific and disease-free survival.

Results: Patients with pre-operative grade 2 endometrial carcinoma and wild-type p53 plus PR-positive expression showed a similar 7-year disease-specific survival to grade 1 endometrial carcinoma patients (95.8% vs 97.5%, $p = .13$), while the 7-year disease-specific survival of patients with grade 2 endometrial carcinoma with p53 aberrant and/or negative PR expression (83.5%) was significantly lower ($p < .001$). The combination of these markers was an independent prognostic factor in multivariate Cox regression analyses.

Conclusions: The prognostic impact of pre-operative p53 and PR expression in patients with grade 2 endometrioid endometrial carcinoma supports a modified binary grading system in which grade 2 patients should be pre-operatively classified as low- or high-grade depending on p53 and PR expression.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Overall, 14% of grade 2 endometrioid endometrial carcinomas are upgraded post-operatively, putting them at risk of (surgical) undertreatment. Therefore, pre-operative risk stratification can be improved for pre-operative grade 2 endometrial carcinomas.

WHAT THIS STUDY ADDS

Pre-operative grade 2 endometrial carcinomas with wild-type p53 plus positive PR expression showed similar survival to grade 1 endometrial carcinomas, whereas pre-operative grade 2 endometrial carcinomas with aberrant p53 and/or negative PR expression showed survival similar to grade 3 endometrial carcinomas.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

A low-cost, widely available, two-step immunohistochemical approach involving p53 and PR improves risk stratification in pre-operative grade 2 endometrial carcinoma.

Keywords:
Endometrioid Endometrial Carcinoma; p53; Progesterone Receptor; Grade 2

INTRODUCTION

In Europe, endometrial carcinoma is the most common gynecologic malignancy.[1] Endometrial carcinoma is classified into different histologic subtypes, of which the endometrioid subtype accounts for > 75% of cases.[1,2] The extent of surgical treatment for endometrial carcinoma varies from simple hysterectomy to staging procedures, including lymphadenectomy/sentinel lymph node (SLN) biopsy with or without omentectomy.[1] SLN biopsy can be considered in patients with low- or intermediate-risk disease, while lymphadenectomy is recommended in patients who are pre-

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operatively stratified in high-intermediate- or high-risk groups, for which SLN biopsy is an alternative in stage I or II disease.[1]

In this risk stratification, clinical stage, histologic subtype, and grade were considered. As recommendations for surgical procedures are based on this stratification, it is of great importance that they are accurate.[1] Endometrioid endometrial carcinoma is currently graded from 1 to 3, according to the International Federation of Gynecology and Obstetrics (FIGO) grading criteria. All non-endometrioid endometrial carcinomas are considered high grade.[1,3] To simplify and improve the reproducibility of grading, a binary classification was proposed in the most recent international guidelines.[1,4] In this classification, grade 2 endometrioid endometrial carcinomas are lumped together with grade 1 into a low-grade category because their current management is similar.[1,2,4] However, pre- and post-operative agreement in grade 2 endometrioid endometrial carcinomas was only 0.61 (95% CI 0.53 to 0.69, $n = 3027$) in a meta-analysis of Visser and colleagues,[5] with post-operative upgrading in 14%. This issue was not solved using the proposed binary approach and underscores the need for a more accurate pre-operative classification system.

The molecular classification of endometrial carcinomas in the Cancer Genome Atlas was introduced in the most recent guidelines for endometrial carcinoma.[6,7] To date, this molecular classification has been demonstrated to be mainly helpful in high-grade and/or advanced-stage endometrial carcinoma to guide adjuvant treatment in the post-operative setting but not necessarily in the risk stratification of low-grade endometrial carcinomas in the pre-operative setting.[1,4,8-10]

Several immunohistochemical markers may be useful for this purpose. *TP53* mutated endometrial carcinomas have the worst clinical outcomes in the Cancer Genome Atlas molecular classification of endometrial carcinomas and are considered high-risk according to recent guidelines.[1,6,7] Aberrant p53 immunohistochemical staining, a surrogate marker for *TP53* mutations, is known to be associated with the non-endometrioid subtype, unfavorable clinicopathologic factors, and worse outcomes.[6,7,11-18] Hormone receptor expression has been extensively studied in relation to outcomes. Low or absent progesterone receptor (PR) expression is associated with lymph node metastasis and unfavorable outcomes.[19-23] Furthermore, Visser and colleagues[24] demonstrated in a cohort of cases pre-operatively classified as grade 2 endometrioid carcinoma on hematoxylin and eosin staining that post-operative upgrading was reduced by 6% when cases with p53 aberrant and PR-negative immunohistochemical expression were designated as high-grade in the pre-operative setting.

This leads to the question of whether a combination of these markers improves the prognosis of patients with pre-operative grade 2 endometrioid endometrial carcinoma. It has been hypothesized that in patients with grade 2 endometrioid endometrial carcinoma, wild-type p53, and PR-positive expression are associated with improved survival compared to grade 2 endometrioid endometrial carcinoma with a p53 aberrant and/or PR-negative expression profile. Thus, our primary outcome was disease-specific survival in grade 2 endometrioid carcinoma when patients were stratified according to their p53 and PR status. The secondary outcomes were disease-free survival, correlation of clinicopathologic features with p53 and PR status, and differences in post-operative discordance between the 2 pre-operative classification methods.

METHODS

Study Cohort

For this retrospective study, data from 3 European endometrial cancer databases were merged[24-27] (Table S1). Cohort 1 consisted of 763 patients treated between 1995 and 2013 in 10 collaborating centers associated with the European Network for Individualized Treatment of Endometrial Cancer; ethical approval was given in Nijmegen, The Netherlands (Institutional Study Protocol 2015-2101).[25] Cohort 2 consisted of 432 patients who were prospectively included in 9 Dutch hospitals between 2011 and 2013. Ethical approval was provided by the local medical ethical committee, St. Elisabeth Hospital Tilburg, the Netherlands (registered in the Netherlands Trial Register, number NTR3503).[24,27] Cohort 3 consisted of 235 patients treated at the University Hospital of Brno, Czech Republic between 2012 and 2019, ethical approval was granted by the ethics committee of the University Hospital Brno, Czech Republic (approval number 06-151221/EK) (Table S1).[26] All variables in the 3 data sets were recoded when necessary such that the variable names, labels, and label values were identical in all data sets. All patients with pre-operative grade 1 to 3 endometrioid endometrial carcinoma were included in the merged data set. Patients with pre-operative grade 2 disease without known pre-operative PR or p53 status were excluded.

Histologic and Immunohistochemical Analysis

Details of both the pathologic examination and immunohistochemical analyses are provided in the Table S1 and the corresponding articles.[24-27] Examples of immunohistochemical staining are provided in the Figure S1.

Outcome Measures

The biomarkers of interest were the pre-operative grade, p53, and PR status. Patients were grouped into pre-operative grade 1 (irrespective of p53 and PR status), pre-operative grade 2 (stratified by p53 and PR status), or pre-operative grade 3 (irrespective of p53 and PR status). Patients were subsequently stratified into 4 groups ([1] P53 wild-type, PR-positive, [2] P53 aberrant, PR-positive; [3] P53 wild-type, PR-negative, and [4] P53 aberrant, PR-negative), or 2 categories ([1] P53 wild-type, PR-positive and [2] all other).

The primary outcome of interest was disease-specific survival, which was defined as the time between the date of surgery and death due to endometrial carcinoma or censoring. The secondary outcome of interest was disease-free survival, defined as the time between the date of surgery and an event or censoring. Residual disease, local (vaginal vault), regional (regional lymph nodes/pelvis), or distant recurrences were all considered events. Furthermore, the correlations between clinicopathologic features and p53 and PR status were evaluated. Finally, post-operative discordances were compared between the 2 methods of pre-operative classification, considering all patients with pre-operative histologic grade 2 disease as low grade, versus the algorithm of Visser and colleagues,[24] in which patients with pre-operative grade 2 endometrioid endometrial carcinoma with p53 aberrant and PR-negative expression were considered high grade. Because this

algorithm was originally tested in cohort 2, only patients with pre-operative grade 2 disease from cohorts 1 and 3 were used to validate the algorithm.

Statistical Analyses

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 28). The χ^2 or Fisher exact tests with Bonferroni correction were used to compare categorical variables. Analysis of variance or Kruskal-Wallis tests were used for continuous variables. Kaplan-Meier plots were constructed (capped at 7 years), and log-rank tests and Cox regression analyses were performed. Differences were considered statistically significant with a p value of $\leq$.05; significance levels were adjusted accordingly in post hoc comparisons with Bonferroni correction.[28]

In accordance with the journal's guidelines, we will provide our data for independent analysis for the purpose of additional data analysis or for the reproducibility of this study in other centers if requested.

RESULTS

In total, 1150 patients were included: 602 with pre-operative grade 1 (52.3%), 400 with grade 2 (34.8%), and 148 with grade 3 endometrioid endometrial carcinoma (12.9%) (Table 1). Negative prognostic parameters such as advanced FIGO-stage, lymph node metastases, and lymphovascular space invasion were more frequently present when the pre-operative grade increased. Similarly, the recurrence and disease-related mortality increased when the pre-operative grade was higher. Among patients with pre-operative grade 2 endometrioid carcinoma, there were 99 (24.8%) post-operative discordant cases: 52 (52.5%) were reclassified as grade 1, 32 (32.3%) as grade 3, and 15 (15.2%) as non-endometrioid endometrial carcinoma (data not shown). The differences between cohorts are presented in the Supplementary Files (Table S2).

With increasing pre-operative grade, significantly more frequent p53 aberrant and/or negative PR expression were observed ($p <$ .001) (Table 1). Only 1.4% ($n = 8$) of the patients showed a p53 aberrant/negative PR expression profile in the pre-operative grade 1 group, compared to 17.2% ($n = 25$) in the pre-operative grade 3 group (Table 1). There were significantly more distant ($p <$ .001) and total ($p =$ .048) recurrences and disease-related deaths ($p <$ .001) in pre-operative grade 2 with p53 aberrant and/or negative PR expression status than in grade 2 endometrioid endometrial carcinoma with wild-type p53 and positive PR expression (Table 2). In patients with pre-operative grade 2 endometrioid carcinoma who underwent lymphadenectomy ($n = 148$), more lymph node metastases were observed in the p53 wild-type/negative PR (17.6%, $n = 3$) and p53 aberrant/negative PR (33.3%, $n = 1$) subgroups than in the p53 wild-type/PR-positive subgroup (8.7%, $n = 10$). No lymph node metastases in the p53 aberrant/positive PR subgroup. These differences were not statistically significant ($p =$ .19).

Patients with a pre-operative increase in tumor grade had significantly shorter disease-specific survival (Fig. A). Patients with pre-operative grade 1 endometrioid carcinoma ($n = 596$) showed a 7-year disease-specific survival of 97.5%, which was comparable to the disease-specific survival of patients with pre-operative grade 2 endometrial carcinoma with wild-type p53 and PR-positive expression ($n = 312$) of 95.8% ($p =$ .13) (Fig. B). The 7-year disease-specific survival of grade 2 endometrial carcinoma patients with p53 aberrant and/or negative PR expression ($n = 85$) was 83.5%, which was comparable to that of grade 3 endometrioid endometrial carcinoma patients ($n = 146$) with a 7-year disease-specific survival of 78.1% ($p =$ .24) (Fig. B). Both differed significantly from the disease-specific survival of patients with pre-operative grade 1 and grade 2 endometrial carcinoma with wild-type p53 and PR-positive expression ($p <$ .001) (Fig. B). Similarly, the disease-free survival curves showed an overall significant difference ($p <$ .001) (Fig. C). Disease-specific and disease-free survival was significantly shorter in patients with pre-operative grade 2 endometrioid carcinoma with p53 aberrant and/or PR expression profiles (Table 2). Kaplan-Meier disease-specific survival curves for grade 2 tumors divided into 4 subgroups based on p53 and PR expression are shown in the Supplementary Files (Fig. S2).

Subsequently, univariate and multivariate Cox regression analyses were performed to study variables affecting the HRs. The variable "data set" refers to the original cohorts of which cases were extracted and were added in view of observed differences between the original cohorts (Table S2). In both univariate and multivariate analyses of disease-specific survival in pre-operative grade 2 endometrioid carcinoma, FIGO-stage, p53 aberrant and/or negative PR expression, and data set were shown to have an independent prognostic impact (Table 3). Lymphovascular space invasion showed a prognostic impact only in univariate Cox regression analysis. Both p53 aberrant and negative PR status as individual markers affected the HR in univariate disease-specific survival Cox regression analysis, but only PR status remained an independent prognosticator in multivariate regression analyses (data not shown). P53 aberrant and/or negative PR expression status was also an independent prognosticator in Cox regression analyses for disease-free survival, together with FIGO stage and lymphovascular space invasion (Table S4).

In addition to the primary and secondary outcomes, the algorithm proposed by Visser and colleagues was applied to a subgroup of patients with pre-operative grade 2 endometrioid carcinoma in our merged cohort ($n = 304$).[24] The pre-operative and post-operative concordance with the usual binary grading (all pre-operative grade 2 endometrioid carcinomas were considered low-grade, omitting p53 and PR status) was determined, which resulted in 31 post-operative discordant cases. Application of the algorithm of Visser and colleagues (pre-operative grade 2 endometrioid carcinomas with p53 aberrant and negative PR expression were high grade) did not result in a reduction in discordant cases ($n =$ 33) (Table S5).[24]

DISCUSSION

Summary of Main Results

The combination of pre-operative p53 and PR immunohistochemical expression status in pre-operative grade 2 endometrioid endometrial carcinomas is an important prognosticator regarding disease-specific and disease-free survival in both survival and regression analyses.

Results in the Context of Published Literature

Patients with pre-operative grade 2 endometrioid carcinoma, with wild-type p53 plus PR-positive immunohistochemical expression,

Table 1 Patient- and Clinical Characteristics, Divided in Pre-Operative Grade 1 to 3 Endometrioid Endometrial Carcinoma

Variable	Grade 1[a]	Grade 2[a]	Grade 3	*p* Value[b]
Number of patients	602	400	148	
Mean age in years ± SD[c]	64.5 ± 10.1	65.5 ± 9.5	67.4 ± 10.0	**.021**
Median BMI [IQR][d]	29.4 [25.7-34]	31 [26.6-35]	28 [24.7-33.4]	**.005**
Post-menopausal status[e]	56 (9.4)	25 (6.3)	7 (4.7)	.07
Diabetes mellitus[f]	94 (17.3)	97 (24.8)	29 (20.6)	**.020**
Cardiovascular disease[g]	230 (42.4)	203 (51.9)	65 (46.1)	**.016**
Lymph node dissection	217 (36)	148 (37)	82 (55.4)	**< .001**
of which positive	14 (6.5)	14 (9.5)	16 (20.0)	**.002**
Adjuvant treatment[h]	201 (33.4)	188 (47.0)	110 (74.3)	**< .001**
of which radiotherapy	185 (92.0)	164 (87.2)	88 (80)	
of which chemotherapy	6 (3.2)	14 (7.4)	11 (10)	
of which chemoradiation	9 (4.9)	10 (5.3)	11 (10)	
Lymphovascular space invasion[i]	54 (11.5)	51 (14.2)	46 (33.6)	**< .001**
FIGO-stage				**< .001**
Ia	401 (66.6)	219 (54.8)	59 (39.9)	
Ib	150 (24.9)	117 (29.3)	42 (28.4)	
II	23 (3.8)	34 (8.5)	15 (10.1)	
IIIa	8 (1.3)	14 (3.5)	5 (3.4)	
IIIb	4 (0.7)	0 (0)	3 (2)	
IIIc	11 (1.8)	11 (2.8)	16 (10.8)	
IVa	1 (0.2)	0 (0)	1 (0.7)	
IVb	4 (0.7)	5 (1.3)	7 (4.7)	
p53 and PR status[j]				**< .001**
p53 wild-type, PR positive	480 (84.7)	313 (78.3)	67 (46.2)	
p53 aberrant, PR positive	33 (5.8)	43 (10.8)	30 (20.7)	
p53 wild-type, PR negative	46 (8.1)	33 (8.3)	23 (15.9)	
p53 aberrant, PR negative	8 (1.4)	11 (2.8)	25 (17.2)	
Post-operative discordances	199 (33.1)	99 (24.8)	50 (33.8)	**.012**
Median follow-up in mos [IQR][k]	59.8 [49.1-78.5]	58.4 [45.8-71.9]	61.4 [52.1-78.0]	**.028**
Total recurrences[l]	36 (6.0)	59 (14.8)	43 (29.3)	**< .001**
Local	13 (33.3)	26 (43.4)	14 (32.6)	
Regional	5 (12.8)	11 (18.3)	5 (11.6)	
Distant	22 (56.4)	31 (51.7)	35 (81.4)	
Deceased[m]	67 (11.2)	58 (14.6)	54 (36.5)	**< .001**
Endometrial carcinoma-related mortality	17 (25.4)	28 (48.3)	33 (61.1)	

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; IQR, interquartile range; SD, standard deviation. Percentages are calculated from total number of patients in vertical columns. FIGO-stage is according to 2009 FIGO staging system for endometrial cancer. All values are *n* (%) unless otherwise specified.

[a] A total of 4 grade 1 and 2 grade 2 patients had residual disease after surgical treatment.
[b] *p* Value for the ANOVA- or Kruskal-Wallis test for continuous variables and χ^2-test for categorical variables. Bold variables indicate statistical significance.
[c] A total of 291 missing values.
[d] A total of 84 missing values.
[e] A total of 8 missing values.
[f] A total of 75 missing values
[g] A total of 76 missing values.
[h] A total of 1 missing value. One patient received an aromatase inhibitor as adjuvant therapy.
[i] A total of 186 missing values.
[j] A total of 38 missing values (in grade 1 and 3 subgroups).
[k] Median follow-up excluding deceased patients, $n = 961$.
[l] A total of 2 missing values.
[m] A total of 9 missing values regarding variable deceased yes or no. Cause of death is unknown in 27 patients.

Table 2 Tumor- and Patient Characteristics in Pre-Operative Grade 2 Endometrioid Endometrial Carcinoma, Divided in Subgroups According to p53 and PR Status ($N = 400$)

Variable	p53 wild-type, PR positive ($n = 313$)	p53 aberrant, PR positive ($n = 43$)	p53 wild-type, PR negative ($n = 33$)	p53 aberrant, PR negative ($n = 11$)	*p* Value[a]
FIGO-stage					.09
Ia	175 (55.9)	24 (55.8)	16 (48.5)	4 (36.4)	
Ib	93 (29.7)	11 (25.6)	11 (33.3)	2 (18.2)	
II	27 (8.6)	3 (7)	2 (6.1)	2 (18.2)	
IIIa	8 (2.6)	3 (7)	1 (3)	2 (18.2)	
IIIc	8 (2.6)	0 (0)	2 (6.1)	1 (9.1)	
IVb	2 (0.6)	2 (4.7)	1 (3)	0 (0)	
Lymphovascular space invasion[b]	39 (13.7)	5 (13.9)	6 (20)	1 (11.1)	.81
Lymph node metastases[c]	10 (8.7)	0 (0)	3 (17.6)	1 (33.3)	.19
Total recurrences	38 (12.1)	10 (23.3)	8 (24.2)	3 (27.3)	**.048**
Local recurrences[d]	21 (6.7)	0 (0)	3 (9.4)	2 (18.2)	.12
Regional recurrences[e]	6 (1.9)	2 (4.7)	2 (6.3)	1 (9.1)	.22
Distant recurrences[f]	17 (5.4)	7 (16.3)	4 (12.5)	3 (27.3)	**.004**
Endometrial carcinoma-related mortality[g]	14 (4.5)	6 (14.3)	6 (18.8)	2 (18.2)	**< .001**
7-year disease-free survival[h]	273 (87.5)	33 (78.6)	24 (75)	8 (72.7)	**.008**
7-year disease-specific survival[i]	299 (95.8)	36 (85.7)	26 (81.3)	9 (81.8)	**0.001**

Abbreviations: ANOVA, analysis of variance; FIGO, International Federation of Gynecology and Obstetrics; PR, progesterone receptor.
Percentages are calculated from total number of patients in vertical columns unless stated otherwise. FIGO-stage is according to 2009 FIGO staging system for endometrial cancer. All values are *n* (%).

[a] *p* Value for the ANOVA or Kruskal-Wallis test for continuous variables, χ^2-test for categorical variables and log-rank test for survival. Bold variables indicate statistical significance.

[b] 41 missing values.

[c] Percentages are calculated in the subgroups of patients who received lymphadenectomy ($n = 115$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 wild-type, PR positive; $n = 13$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 aberrant, PR positive; $n = 17$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 wild-type, PR negative; $n = 3$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 aberrant, PR negative).

[d] A total of 1 missing value.

[e] A total of 3 missing values.

[f] A total of 1 missing value.

[g] Survival status is unknown in 3 patients. Cause of death is unknown in 7 patients.

[h] A total of 3 missing values.

[i] A total of 3 missing values.

showed similar survival to that of pre-operative grade 1 patients, while the survival of patients with aberrant p53 and/or negative PR expression was comparable to that of patients with grade 3 endometrioid carcinoma. The importance of both p53 and PR as individual prognostic markers is already known from previous studies. The Cancer Genome Atlas reported that prognosis was worse in the copy number-high endometrial carcinoma subgroup, which is considered a high-risk subgroup according to recent guidelines.[1,6,7] In the high copy number group, 92% showed *TP53* mutations, for which p53 aberrant immunohistochemical staining was used as a surrogate marker.[6,7]

Aberrant immunohistochemical p53 expression is an established prognostic biomarker associated with non-endometrioid histologic subtypes, unfavorable clinicopathologic factors, and worse outcomes in many studies.[11-18] In particular, the latter was underscored in this study (eg, patients with pre-operative grade 2 carcinoma showed shorter disease-specific and disease-free survival when there was aberrant p53 expression). Interestingly, although hormone receptor expression profiles have been well-established biomarkers for decades, estrogen receptor (ER) and PR expression are not part of the current molecular classification of endometrial carcinoma. However, several studies have suggested the prognostic value of ER and PR status in endometrial carcinoma molecular subgroups with 'no specific molecular profile.'[7,16,19,20,22,23,29-31] Recently, Huvila and colleagues[21] demonstrated that negative PR expression was an independent risk factor for relapse in patients with stage I and II endometrioid carcinoma. In this study, p53 aberrant expression only significantly affected the HR in univariate analyses, which is similar to our results and underlines the importance of PR as an independent prognostic marker.[21] Similarly, in a study by Trovik and colleagues,[23]

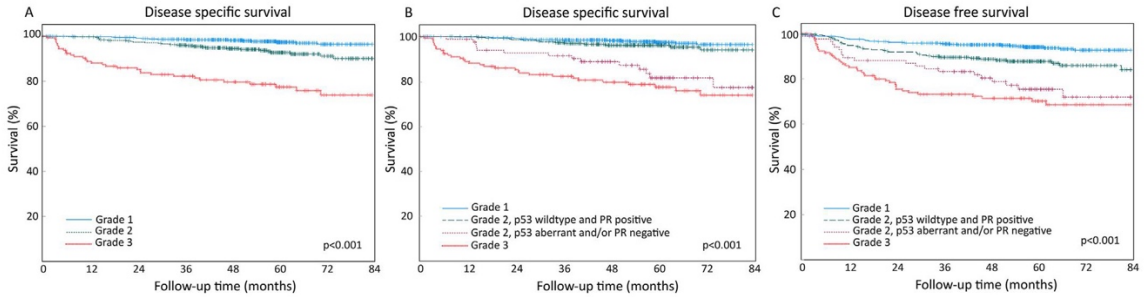

Figure (**A**) Kaplan-Meier plots of 7-year DSS of pre-operative grade 1 to 3 endometrioid endometrial carcinoma; 74 events. (**B**) Kaplan-Meier plots of 7-year DSS of pre-operative grade 1 to 3 endometrioid endometrial carcinoma, with grade 2 further specified regarding PR and p53 status; 74 events. (**C**) Kaplan-Meier plots of 7-year disease-free survival of pre-operative grade 1 to 3 endometrioid endometrial carcinoma, with grade 2 further specified regarding PR and p53 status; 136 events. DSS, disease survival specific; PR, progesterone receptor.

Table 3 Univariate and Multivariate Cox Regression Analyses of Disease-Specific Survival in Pre-Operative Grade 2 Endometrioid Endometrial Carcinoma ($N = 400$)

Variable	Univariate Cox regression			Multivariate Cox regression		
	HR	95% CI	*p* Value	Adjusted HR	95% CI	*p* Value
FIGO-stage[a]	4.5	2.1 to 9.6	**<.001**	3.3	1.3 to 8.2	**.012**
p53 aberrant and/or PR negative[b]	3.8	1.8 to 7.9	**<.001**	3.3	1.5 to 7.5	**.004**
Lymphovascular space invasion[c]	3.7	1.6 to 8.4	**.002**	2.2	0.9 to 5.8	.11
Data set[d]						
Data set 2	2.9	1.3 to 6.8	**.013**	3.0	1.2 to 7.3	**.016**
Data set 3	0.7	0.3 to 2.2	.679	0.9	0.3 to 2.6	.78

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; PR, progesterone receptor.
Events: 25. FIGO-stage is according to 2009 FIGO staging system for endometrial cancer. Bold variables indicate statistical significance.
[a] Reference category is FIGO I; 7 missing values.
[b] Reference category is p53 wild-type and PR positive; 7 missing values.
[c] Reference category is number 48 missing values.
[d] Reference category is data set 1. 7 missing values.

both p53 aberrant expression and loss of ER/PR expression in curettage specimens were associated with shorter disease-specific survival and lymph node metastasis. ER/PR loss was also an independent predictor in multivariate Cox regression.

It is important to note that we specifically explored the combination of PR/p53, based on the broad analysis by Visser and colleagues[24] favoring the use of PR and p53 over ER. The abovementioned studies by Vermij and colleagues[30] and Jamieson and colleagues[29] which evaluated ER as a prognostic marker in the subgroup with 'no specific molecular profile,' likely highlighted a different part of the discussion, demonstrating that ER-positive expression is a favorable marker in this specific molecular class. However, to highlight their relatedness, in the study by Jamieson and colleagues[29] PR was an equally strong marker as ER, albeit only in the univariate analysis. Furthermore, Vermij and colleagues[30] showed that within the ER-positive group, PR-negative expression further identified patients with unfavorable pathologic characteristics (eg, high-grade or non-endometrioid subtypes), underscoring the importance of PR.

The results reported by Visser and colleagues regarding the decrease in post-operative discordance when p53 and PR expression were considered in pre-operative grade 2 patients could not be replicated in the 2 independent cohorts.[24] Although this information could not be retrieved, the pre-operative p53 and/or PR status had already been considered in these 2 other cohorts when pre-operatively assessing the grade or histologic subtype.

STRENGTHS AND WEAKNESSES

The major strengths of this study are the multicenter setting, the large size of the study population, and the focus on the pre-operative setting, analogous to routine clinical practice. Another strength is that even after correcting for data set differences in multivariate analyses, PR and p53 status remained independent prognosticators of disease-specific survival, emphasizing the validity of these markers. The retrospective nature of this study remains a limitation, leading to possible heterogeneity in some variables, including the assessment of lymphovascular space

invasion and uniformity in lymph node dissection indications between the cohorts. However, this was unlikely to have compromised our primary results. Another limitation was the heterogeneity of the 3 cohorts, including differences in staining methods. We evaluated the differences between the 2 scoring methods (staining index vs 10% cutoff value for PR) in 50 cases, which showed 100% concordance. Also, it is known from previous literature that tissue microarrays are a high-quality alternative for whole-slide immunohistochemical analyses in endometrial carcinoma for these markers.[32,33] Furthermore, the use of different antibodies is consistent with daily clinical practice and underscores the robustness of our data. There was a strikingly low post-operative discordance in cohort 3 compared to the other cohorts and the literature. This might be partially explained by the fact that only cases with known pre-operative p53 and PR status were included in this cohort, which was performed only when sufficient tissue was available.

Implications for Practice and Future Research

Despite previously published evidence showing that both p53 and PR are relevant prognostic markers, their exact roles in routine practice remain unclear. We studied their value in the prediction of prognosis in patients with pre-operative grade 2 endometrioid carcinoma and showed that not all morphologic grade 2 carcinomas behave as low-grade tumors. Our data support the hypothesis that pre-operative PR and p53 immunohistochemistry in grade 2 carcinomas improve risk assessment in the pre-operative setting. Although we fully support the binary classification system proposed by the most recent guidelines, based on our data, we would recommend that p53 and PR status should be considered when pre-operatively stratifying morphologic grade 2 endometrioid carcinomas.

Taking this a step further, such an approach may eventually help in the decision regarding the surgical approach and the consideration of whether staging procedures should be performed. This requires further exploration in prospective studies with specific attention to lymph node status and the impact of surgical staging on oncologic outcomes. Furthermore, our results should be interpreted in the increasingly important field of the molecular classification of endometrial carcinomas.[6,7] Our results confirm that p53 is a valuable prognostic marker for grade 2 endometrial carcinoma in the pre-operative setting. In addition, our results underscore the importance of PR expression in pre-operative risk stratification, which is not included in the current molecular classifications. Our proposed, low-cost, and widely available dual p53 and PR immunohistochemical approach may be an attractive alternative to reflex molecular testing for pre-operative morphologic grade 2 endometrioid endometrial carcinomas.[34,35]

CONCLUSION

We demonstrated that the combination of 2 simple immunohistochemical staining methods, p53 and PR, was an important prognosticator regarding survival. Our data support a modified binary classification system in which grade 2 endometrioid endometrial carcinomas are considered low- or high-grade, depending on the p53 and PR expression status.

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REFERENCES

1. Concin N, Matias-Guiu X, Vergote I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer*. 2021; 31(1):12—39. https://doi.org/10.1136/ijgc-2020-002230.
2. Practice bulletin no. 149: endometrial cancer. *Obstet Gynecol*. 2015;125(4): 1006—1026. https://doi.org/10.1097/01.AOG.0000462977.61229.de.
3. Creasman WT, Odicino F, Maisonneuve P, et al. Carcinoma of the corpus uteri. *Int J Gynaecol Obstet*. 2006;95(suppl 1):S105—S143. https://doi.org/10.1016/S0020-7292(06)60031-3.
4. Soslow RA, Tornos C, Park KJ, et al. Endometrial carcinoma diagnosis: use of FIGO grading and genomic subcategories in clinical practice: recommendations of the International Society of Gynecological Pathologists. *Int J Gynecol Pathol*. 2019; 38(suppl 1):S64—S74. https://doi.org/10.1097/PGP.0000000000000518.
5. Visser NCM, Reijnen C, Massuger LFAG, 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—813. https://doi.org/10.1097/AOG.0000000000002261.
6. Cancer Genome Atlas Research Network, Kandoth C, Schultz N. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67—73. https://doi.org/10.1038/nature12113.
7. Talhouk A, McConechy MK, Leung S, et al. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer*. 2015;113(2):299—310. https://doi.org/10.1038/bjc.2015.190.
8. Vrede SW, Kasius J, Bulten J, et al. Relevance of molecular profiling in patients with low-grade endometrial cancer. *JAMA Netw Open*. 2022;5(12):e2247372. https://doi.org/10.1001/jamanetworkopen.2022.47372.
9. Köbel M, Nelson GS, , Letter in response to, McAlpine J, Leon-Castillo A, Bosse T. The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses. *J Pathol*. 2018;245(2):249—250. https://doi.org/10.1002/path.5068. J Pathol 2018; 244: 538-549.
10. McAlpine J, Leon-Castillo A, Bosse T. The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses. *J Pathol*. 2018;244(5): 538—549. https://doi.org/10.1002/path.5034.
11. Abdelrahman AE, Salem A, Al Attar AZ, et al. p53, Pirh2, and L1CAM as promising prognostic biomarkers of endometrial carcinoma: an immunohistochemical and genetic study. *Appl Immunohistochem Mol Morphol*. 2022;30(10):713—725. https://doi.org/10.1097/PAI.0000000000001073.
12. Brett MA, Atenafu EG, Singh N, et al. Equivalent survival of p53 mutated endometrial endometrioid carcinoma grade 3 and endometrial serous carcinoma. *Int J Gynecol Pathol*. 2021;40(2):116—123. https://doi.org/10.1097/PGP.0000000000000674.
13. Garg K, Leitao Jr MM, Wynveen CA, et al. p53 overexpression in morphologically ambiguous endometrial carcinomas correlates with adverse clinical outcomes. *Mod Pathol*. 2010;23(1):80—92. https://doi.org/10.1038/modpathol.2009.153.
14. Ito K, Watanabe K, Nasim S, et al. Prognostic significance of p53 overexpression in endometrial cancer. *Cancer Res*. 1994;54(17):4667—4670.
15. Kalogiannidis I, Bobos M, Papanikolaou A, et al. Immunohistochemical bcl-2 expression, p53 overexpression, PR and ER status in endometrial carcinoma and survival outcomes. *Eur J Gynaecol Oncol*. 2008;29(1):19—25.
16. Karnezis AN, Leung S, Magrill J, et al. Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification. *J Pathol Clin Res*. 2017;3(4):279—293. https://doi.org/10.1002/cjp2.82.
17. Laliscia C, Gadducci A, Coccia N, et al. Lymph-vascular Space Involvement and/or p53 overexpression Correlated with the Clinical Outcome of Early-stage Endometrial Cancer Patients Treated with Adjuvant Vaginal brachytherapy. *Anticancer Res*. 2023; 43(1):149—156. https://doi.org/10.21873/anticanres.16144.
18. Yano M, Ito K, Yabuno A, et al. Impact of TP53 immunohistochemistry on the histological grading system for endometrial endometrioid carcinoma. *Mod Pathol*. 2019;32(7):1023—1031. https://doi.org/10.1038/s41379-019-0220-1.
19. Creasman WT. Prognostic significance of hormone receptors in endometrial cancer. *Cancer*. 1993;71(4):1467—1470. https://doi.org/10.1002/cncr.2820710411. suppl.
20. Gehrig PA, Van Le L. The prognostic value and clinical utility of estrogen and progesterone receptors in endometrial carcinoma. *Prim Care Update Ob Gyns*. 2000; 7(2):55—59. https://doi.org/10.1016/s1068-607x(00)00021-4.
21. Huvila J, Talve L, Carpén O, et al. Progesterone receptor negativity is an independent risk factor for relapse in patients with early stage endometrioid endometrial adenocarcinoma. *Gynecol Oncol*. 2013;130(3):463—469. https://doi.org/10.1016/j.ygyno.2013.06.015.
22. Pijnenborg JM, Romano A, Dam-de Veen GC, et al. Aberrations in the progesterone receptor gene and the risk of recurrent endometrial carcinoma. *J Pathol*. 2005; 205(5):597—605. https://doi.org/10.1002/path.1738.
23. Trovik J, Wik E, Werner HMJ, 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—3441. https://doi.org/10.1016/j.ejca.2013.06.016.
24. Visser NCM, van der Wurff AAM, IntHout J, et al. Improving preoperative diagnosis in endometrial cancer using systematic morphological assessment and a small immunohistochemical panel. *Hum Pathol*. 2021;117:68—78. https://doi.org/10.1016/j.humpath.2021.08.006.
25. Reijnen C, Gogou E, Visser NCM, 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. https://doi.org/10.1371/journal.pmed.1003111.
26. Vinklerová P, Ovesná P, Hausnerová J, et al. External validation study of endometrial cancer preoperative risk stratification model (ENDORISK). *Front Oncol*. 2022;12: 939226. https://doi.org/10.3389/fonc.2022.939226.
27. Visser NCM, Bulten J, van der Wurff AAM, et al. Pipelle Prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study. *BMC Cancer*. 2015;15:487. https://doi.org/10.1186/s12885-015-1487-3.
28. Tripathi A, Pandey A. Post-hoc comparison in survival analysis: an easy approach. *J Biosci Med*. 2017;05:112—119. https://doi.org/10.4236/jbm.2017.53012.
29. Jamieson A, Huvila J, Chiu D, et al. Grade and estrogen receptor expression identify a subset of no specific molecular profile endometrial carcinomas at a very low risk of disease-specific death. *Mod Pathol*. 2023;36(4):100085. https://doi.org/10.1016/j.modpat.2022.100085.
30. Vermij L, Jobsen JJ, León-Castillo A, et al. Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry. *Br J Cancer*. 2023;128(7):1360—1368. https://doi.org/10.1038/s41416-023-02141-0.
31. Ravaggi A, Capoferri D, Ardighieri L, et al. Integrated biomarker analysis reveals L1CAM as a potential stratification marker for no specific molecular profile high-risk endometrial carcinoma. *Cancers*. 2022;14(21):5429. https://doi.org/10.3390/cancers14215429.
32. Visser NCM, van der Wurff AAM, Pijnenborg JMA, et al. Tissue microarray is suitable for scientific biomarkers studies in endometrial cancer. *Virchows Arch*. 2018;472(3): 407—413. https://doi.org/10.1007/s00428-017-2289-6.
33. Fons G, Hasibuan SM, van der Velden J, ten Kate FJW. Validation of tissue microarray technology in endometrioid cancer of the endometrium. *J Clin Pathol*. 2007;60(5): 500—503. https://doi.org/10.1136/jcp.2006.040170.
34. Chen J, Fan W, Gu H, et al. Preoperative MRI and immunohistochemical examination for the prediction of high-risk endometrial cancer. *Gland Surg*. 2021;10(7): 2180—2191. https://doi.org/10.21037/gs-21-38.
35. Weinberger V, Bednarikova M, Hausnerova J, et al. A novel approach to preoperative risk stratification in endometrial cancer: the added value of immunohistochemical markers. *Front Oncol*. 2019;9:265. https://doi.org/10.3389/fonc.2019.00265.

4.6 Bednarikova M, Vinklerova P, Gottwaldova J, Ovesna P, Hausnerova J, Minar L, Felsinger M, Valik D, Cermakova Z, Weinberger V. The Clinical Significance of DJ1 and L1CAM Serum Level Monitoring in Patients with Endometrial Cancer. J Clin Med. 2021 Jun 15;10(12):2640. (IF 4.964, Q2)

In addition to immunohistochemical characteristics, a number of serum markers are potentially applicable in gynecologic oncology, either as prognostic indicators at the time of diagnosis or for monitoring patients after treatment.
However, no serum marker has yet proven sufficiently effective for routine clinical implementation in endometrial carcinoma.

The most frequently studied tumor marker is CA125 (Cancer Antigen 125), while HE4 (Human Epididymis Protein 4) appears to be more sensitive[46,47]. Although both markers are well established in ovarian cancer, their utility in endometrial cancer remains very limited.

In this pilot study, we investigated the clinical utility of new potential serum markers DJ1, also known as Parkinson's disease-associated protein 7 (PARK7), and L1CAM, alongside established tumor markers CA125 and HE4, in patients diagnosed with endometrial cancer. A cohort of 65 patients was monitored, with serum levels assessed at diagnosis and during follow-up periods.

In cases of disease recurrence, serum levels of DJ1, CA125, and HE4 were notably higher than in patients without recurrence. While L1CAM levels were also elevated in recurrent cases, this increase did not reach statistical significance.

DJ1 levels appeared unaffected by patient-specific factors such as age, BMI (body mass index), renal function, or menopausal status. Conversely, L1CAM levels were significantly higher in patients aged ≥60 years, those who were overweight (BMI ≥27 kg/m^2), and postmenopausal women.

No significant correlation was found between DJ1 and L1CAM serum levels and tumor stage, histological type, or recurrence risk at diagnosis.

The study suggests that monitoring serum levels of DJ1 and L1CAM could provide valuable insights into treatment efficacy and early detection of recurrence in EC patients. However, due to the pilot nature of the study and the limited sample size, further research is necessary to validate these biomarkers' clinical applicability.

This pilot study *"The Clinical Significance of DJ1 and L1CAM Serum Level Monitoring in Patients with Endometrial Cancer"* was published in the *Journal of Clinical Medicine* (IF 4.964, Q2) in 2021.

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The Clinical Significance of DJ1 and L1CAM Serum Level Monitoring in Patients with Endometrial Cancer

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Abstract: Circulating tumor markers are not routinely used in patients with endometrial cancer (EC). This pilot study evaluated the role of monitoring new biomarkers DJ1 and L1CAM, in correlation with CA125 and HE4, for the effects of anticancer treatment and preoperative management in EC patients. Serial serum levels of DJ1, L1CAM, CA125 and HE4 were collected in 65 enrolled patients. Serum DJ1, L1CAM, CA125 and HE4 levels were significantly higher at the time of diagnosis compared to those measured during follow-up (FU). In patients with recurrent disease, serum DJ1, CA125 and HE4 levels were significantly higher at the time of recurrence compared to levels in disease-free patients. Serum L1CAM levels were also higher in patients with recurrence but without reaching statistical significance. While DJ1 levels were not affected by any of the observed patient-related characteristics, L1CAM levels were significantly higher in patients with age ≥60 years who were overweight. At the time of EC diagnosis, DJ1 and L1CAM serum levels did not correlate with stage, histological type or risk of recurrence. This is a preliminary description of the potential of serial DJ1 and L1CAM serum level measurement for monitoring the effects of treatment in EC patients.

Keywords: tumor markers; endometrial cancer; DJ1; L1CAM

1. Introduction

The primary clinical use of circulating tumor markers determined in peripheral blood consists of monitoring the course of disease. An increase in serum levels often precedes the clinical manifestation of disease recurrence, and dynamic changes in their levels over time are used to monitor the effects of anticancer treatments. In contrast, circulating markers are not very suitable for cancer screening or primary diagnosis and do not play a significant role in determining the extent of the disease or prognosis, respectively [1,2].

In endometrial cancer (EC) patients, elevated CA125 and HE4 levels have been frequently found [3]. The results of studies investigating the prognostic impact of CA125 in EC have not been unequivocal. While Sood et al. and Reijnen et al. described an association between preoperatively elevated CA125 levels and poor outcomes in EC patients [4,5], other studies did not confirm the correlation between CA125 serum levels and extent of disease at the time of diagnosis [6,7].

In terms of EC detection, marker HE4 has demonstrated higher sensitivity and specificity than CA125, especially in early stages [8]. The prognostic relevance of HE4 has been established in poorly differentiated EC [9]. Neither of the circulating markers have become a standard part of clinical practice [10–13].

Recently, studies of new promising markers DJ1 and L1CAM have been published. The first marker, DJ1, also known as Parkinson's disease-associated protein 7 (PARK7), is a multifunctional protein promoting cell proliferation and playing an important role in cancer pathogenesis and progression by modulating the tumor suppressor PTEN. The results of studies by Italian authors demonstrated not only significantly higher DJ1 serum levels in EC patients compared to healthy controls, but also the association of higher DJ1 levels with high-risk histological type (defined as endometrioid carcinoma grade 3 or non-endometrioid types) in contrast with lower DJ1 levels in low-risk histological types (endometrioid carcinoma grade 1 or 2) [14–16]. The second marker, L1 cell adhesion molecule (L1CAM), is a membrane glycoprotein of the immunoglobulin family, crucially involved in cancer cell migration and adhesion. L1CAM overexpression in EC tissues represents a negative prognostic marker, signaling both more aggressive behavior of the tumor and shorter survival of patients [17,18]. The significance of L1CAM serum level measurement has not been unambiguously established yet [19,20]. All studies concerning circulating DJ1 and L1CAM serum levels assessed merely one-time sampling events without studying the potential significance of time-dependent changes in serial DJ1 or L1CAM serum levels. Therefore, data regarding the importance of DJ1 and L1CAM measurement either during the course of therapy for the monitoring of their effects or during a follow-up after successful primary therapy for the detection of EC recurrence are still lacking.

The aim of our study was to evaluate whether time-dependent changes of serial serum measurements of DJ1, L1CAM, CA125 and HE4 in EC patients correlated with the course of the disease and whether elevated levels at the follow-up signalized recurrence. We wanted to clarify whether the markers' levels were affected by factors associated with patients' health conditions. We also investigated if marker levels at the time of diagnosis correlated with clinico-pathological features of the tumor.

2. Materials and Methods

2.1. Patients

Patients undergoing surgical treatment from May 2016 to April 2019 for histologically proven EC in the Oncogynecological Center of University Hospital (UH) Brno, Czech Republic, were consecutively involved in this single-institutional, prospective, observational study. The patients with other malignancies or neuro-degenerative diseases were excluded. All subjects gave their written informed consent to participate in the study.

2.2. Clinical Management

Diagnosis of EC was made after histopathological examination of a tumor biopsy obtained from hysteroscopy or dilatation and curettage. Each patient with newly diagnosed EC underwent a clinical examination, CT of the chest/abdomen and an expert ultrasound (US) examination, according to local guidelines [21]. The blood samples for CA125, HE4, DJ1 and L1CAM serum level assessment as well as for basic biochemical and hematological laboratory tests were performed at the time of diagnosis. Subsequently, patients were divided into groups with a low or high risk of recurrence according to histology, grade and clinical staging. The low-risk group was defined as endometrioid carcinoma grade 1 TNM stage cT1a or cT1b and/or endometrioid carcinoma grade 2 TNM stage cT1a, all of them without clinical or imaging evidence of lymphadenopathy (cN0) or distant metastases (cM0) [22]. Patients who did not meet criteria for a low-risk group were classified as a high-risk group. The staging surgical procedure consisting of a total hysterectomy with bilateral salpingo-oophorectomy was performed in all patients. Whereas the sentinel node biopsy was not a standard at the time of the study in our department, systematic pelvic and paraaortic lymphadenectomies were performed in the high-risk group. In the case of

serous endometrial carcinoma, carcinosarcoma and undifferentiated carcinoma, a staging infracolic omentectomy was added to the surgical procedure [23].

The definitive histopathological classification of a tumor, containing data about histotype, grade, lymphovascular space involvement (LVSI) and surgical stage was made by one of two pathologists experienced in gynecological malignancies according to the FIGO, 2014, and the World Health Organization, 2014 [24,25]. Based on final histopathological findings, patients were once again stratified into low- or high-risk groups and thereafter, decisions regarding adjuvant treatment, considering all the relevant factors, were made by the multidisciplinary board according to local guidelines.

After the completion of primary therapy, patients were transferred to a follow-up (FU) program consisting of an outpatient visit three or four times per year for the first 2 years, from years 3 to 5 on a six-month basis, and then once per year. The gynecological examination and pelvic US were an obligatory part of each visit. Blood sampling for marker assessment was performed once or twice during the follow-up and always when EC recurrence was suspected. The diagnosis of EC recurrence was confirmed either histologically or radiologically.

2.3. Clinical Data

The following data were prospectively recorded in the clinical database: age at the time of diagnosis, menopausal status, weight, height, body mass index (BMI), body surface area (BSA), renal functions (serum creatinine and glomerular filtration rate, calculated according to a chronic kidney disease epidemiology collaboration (CKD-EPI) equation), treatment data and date of the last follow-up visit or death (EC related/non-related).

2.4. Serum CA125, HE4, DJ1 and L1CAM Level Measurement

The sampling of the peripheral blood was performed under the standard procedure from the cubital vein, using 7.5 mL tubes of S-Monovette® Serum Gel (Sarstedt) preoperatively, once or twice during the follow-up period and always when recurrence was suspected. The samples were transported to the Department of Clinical Biochemistry UH Brno, where the serum was separated by centrifugation, and the samples were analyzed either immediately (CA125, HE4) or stored frozen at −80 °C until analysis (DJ1 and L1CAM).

The quantitative assessments of L1CAM and DJ1 levels were performed by enzyme-linked immunosorbent assay (ELISA) using ELISA reader iMARK (Bio-Rad). For L1CAM, kit CN MBS 2023094 (MYBioSource, USA) was used. DJ1 serum levels were measured using kit CN CY-9050V2 (CircuLex MBL, UK). The serum concentrations of HE4 and CA125 were determined using quantitative, chemiluminescent microparticle immunoassay (CMIA) on the analyzer Architect i2000 (Abbott, Abbott Laboratories, USA). For CA125 measurement, the diagnostic set ARCHITECT Ca125 II (CN 2K45-24, Abbott) was used. HE4 serum level assessments were performed using the diagnostic set ARCHITECT HE4 (CN 2P51-25, Abbott).

2.5. Statistical Analysis

Categorical variables were summarized as absolute and relative frequencies and continuous variables as median, interquartile range (IQR), or range. Linear mixed-effects models were applied to evaluate the profiles of marker levels over time, and the impact of disease and patient characteristics on the levels. Models included the patient's identification number as a random effect because one patient had more than one measurement. Original values were log-transformed due to their log-normal distribution for the purpose of the model. All tests were performed as two-sided at the significance level of alpha = 0.05. Analyses were done in R software.

3. Results

3.1. Patient Characteristics

A total of 65 patients with a median age of 65 years (30–65 years) and median BMI of 31.2 (17.3–45.7) were enrolled in the study. The majority of patients were diagnosed with an

early stage disease (FIGO I–II, n = 58; 89%), while lymph nodes or distant metastases were diagnosed in 7 (11%) patients. In terms of histology, patients with low-grade endometrioid carcinoma predominated (n = 51; 79%). All the patients underwent a staging surgical procedure (n = 65; 100%), adjuvant radiotherapy was performed in 23 patients (35%) and chemotherapy in 7 (11%) patients. The median time between the preoperative blood sample and the first FU visit's collection was 6.3 months (2.6–20.7 months), whereas between the first and second FU samples, 4.55 months (1.7–14.7 months). Detailed patients' characteristics are shown in Table 1.

Table 1. Characteristics of endometrial cancer patients.

Age (Years) Median (Min; Max)		**65 (30; 85)**
Menopausal status (n, %)	Pre-/perimenopausal	11 (17%)
	Postmenopausal	54 (83%)
Biometric data Median (min; max)	Weight (kg)	82 (50; 121)
	Height (cm)	165 (146; 176)
	BMI (kg/m^2)	31.3 (17.3; 45.7)
	BSA (m^2)	1.89 (1.5; 2.33)
Renal function, n = 64 Median (min; max)	Creat/S (umol/L)	69 (52; 134)
	CKD-EPI (mL/s)	1.33 (0.52; 1.81)
FIGO stage [25] (n; %)	I	50 (77%)
	II	8 (12%)
	III	5 (8%)
	IV	2 (3%)
Myometrial invasion (n; %)	<50%	48 (74%)
	≥50%	17 (26%)
Histology (n; %)	E G1-2	51 (79%)
	E G3, non-E	14 (21%)
Treatment (n; %)	HY and AE	65 (100%)
	PLN +/− PALN	20 (31%)
	RT	23 (35%)
	CHT	7 (11%)
Recurrence (n; %)	Local	2 (3%)
	Distant	3 (5%)
	No	60 (92%)
Time (months) Median (min; max) Surgery—FU1 FU1–FU2, n = 56 Dg–last FU		6.3 (2.6; 20.7)
		4.6 (1.7; 14.7)
		29.5 (13.7; 46.5)
Status at the end of FU (n; %)	Alive	58 (89%)
	Died of EC	4 (6%)
	Died (another cause)	3 (5%)

Abbreviations: n = number of patients, BMI = Body Mass Index, BSA = Body Surface Area, creat/S = creatinine serum level, CKD-EPI = glomerular filtration rate calculated according to chronic kidney disease epidemiology collaboration (CKD-EPI) equation, E = endometrioid, G = grading, HY = hysterectomy, AE = adnexectomy, PLN = pelvic lymphadenectomy, PALN = paraaortic lymphadenectomy, RT = radiotherapy, CHT = chemotherapy, FU = follow-up, FU1 = first follow-up blood sample, FU2 = second follow-up blood sample.

After a median follow-up time of 29.5 months, a total of five patients (8%) developed recurrent disease, with the time to progression between 7 and 16 months. One patient with initial FIGO stage II developed local recurrence, three had distant metastases (one of them initially staged FIGO IA, and two of them FIGO IVB) and one patient with initial FIGO

stage IA developed both local recurrence and metastatic disease (Table 2). All four patients who developed distant metastases died from their disease (Table 1).

Table 2. Characteristics of patients with recurrent endometrial cancer.

Pts	Age	Histology	G	HY+AE	PLN/ PALN	RT	CHT	FIGO	Relapse	TTP (Months)
I	75	E	2	yes	no	yes	yes	IVB	Distant	8
II	71	E	3	yes	yes	yes	no	II	Local	16
III	72	E	2	yes	no	no	no	IA	Local, Distant	11
IV	55	E	2	yes	no	yes	yes	IVB	Distant	13
V	66	Non-E	3	yes	yes	no	yes	IA	Distant	7

Abbreviations: Pts = patients, E = endometrioid, non-E = non-endometrioid, HY = hysterectomy, AE = adnexectomy, PLN = pelvic lymphadenectomy, PALN = paraaortic lymphadenectomy, RT = radiotherapy, CHT = chemotherapy, TTP = time to progression.

3.2. DJ1, L1CAM, CA125 and HE4 Serial Serum Levels in Correlation with Disease Status

In all enrolled patients, median DJ-1, L1CAM, CA125 and HE4 serum levels fell after initial treatment and remained low for both subsequent follow-ups. Median serum levels of each marker were significantly higher at the time of diagnosis than afterwards in FU ($p < 0.001$ for DJ-1, L1CAM, CA125 and HE4, respectively). The serum levels of DJ-1, L1CAM, CA125 and HE4 at the time of diagnosis and during the follow-ups are shown in Table 3. Serial measurements are graphically illustrated in Figure 1.

Table 3. The measured DJ1, L1CAM, HE4 and CA125 levels in patients with EC.

		Time of Collection			*p* Value
		Preoperative	FU1	FU2	
DJ1 (ng/mL)	Valid *n*	64	65	50	<0.001
	Median (IQR)	78 (38.4–139)	23.5 (15.9–31.9)	25 (18.4–40.1)	
L1CAM (pg/mL)	Valid *n*	64	65	50	<0.001
	Median (IQR)	1919 (1519–2387)	1690 (1229–2087)	1602.5 (1195–2105)	
HE4 (pmol/L)	Valid *n*	49	65	56	<0.001
	Median (IQR)	68.2 (55.8–98)	54.5 (44.8–70.5)	54.7 (44.2–76.7)	
CA125 (kU/L)	Valid *n*	60	65	56	<0.001
	Median (IQR)	16.3 (11.4–24.2)	9.4 (7.1–15.2)	11.3 (7.5–15.4)	

Abbreviations: *n* = number of patients, IQR = interquartile range, FU1 = first follow-up blood sample, FU2 = second follow-up blood sample.

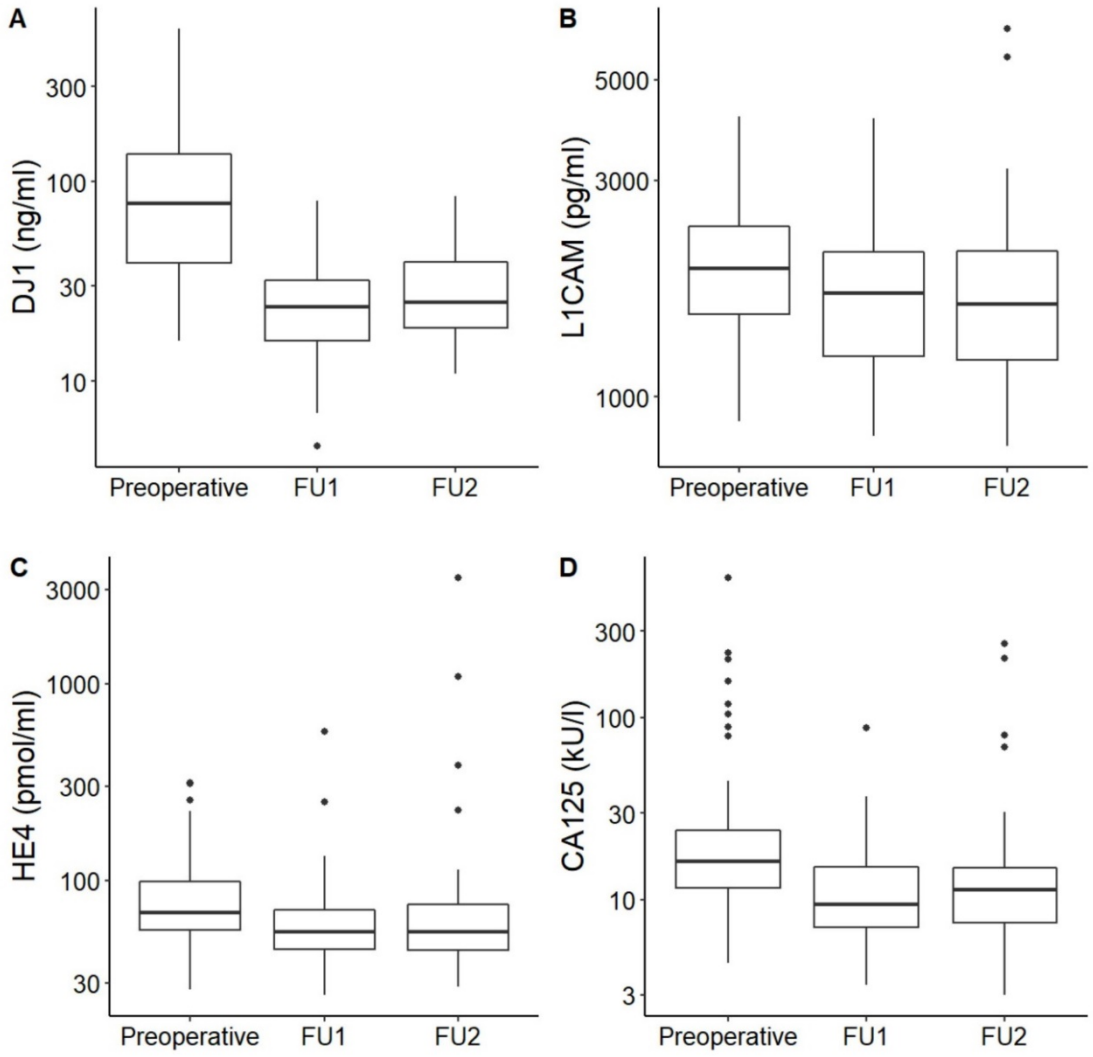

Figure 1. DJ-1 (**A**), L1CAM (**B**), HE4 (**C**) and CA125 (**D**) levels at three different time points: before surgery (preoperative), first follow-up collection (FU1) and second follow-up collection (FU2).

In patients with recurrent disease, serum DJ1, CA125 and HE4 levels were significantly higher at the time of recurrence compared to levels in disease-free patients ($p = 0.035$ for DJ1, $p < 0.001$ for CA125 and HE4, respectively) (Table 4, Figure 2A,C,D). Serum L1CAM levels were also higher in patients with recurrence; however, the patients' profiles with or without recurrence did not differ significantly ($p = 0.353$) (Table 4, Figure 2B).

Table 4. DJ1, L1CAM, HE4 and CA125 follow-up serum levels in patients with or without recurrence of endometrial cancer.

		Serum Levels at Follow-Up		*p* Value
		Remission	Recurrence	
DJ1 (ng/mL)	Valid *n*	110	5	0.035
	Median (IQR)	23.9 (16.8–32.8)	60.1 (32.7–61)	
L1CAM (pg/mL)	Valid *n*	110	5	0.353
	Median (IQR)	1650.5 (1203–2050)	2630 (2474–2740)	
HE4 (pmol/L)	Valid *n*	116	5	<0.001
	Median (IQR)	53.5 (44.5–71.2)	572 (110–1083)	
CA125 (kU/L)	Valid *n*	116	5	<0.001
	Median (IQR)	10 (7.5–14.8)	34.4 (12.8–69.2)	

Abbreviations: *n* = number of measurements, IQR = interquartile range.

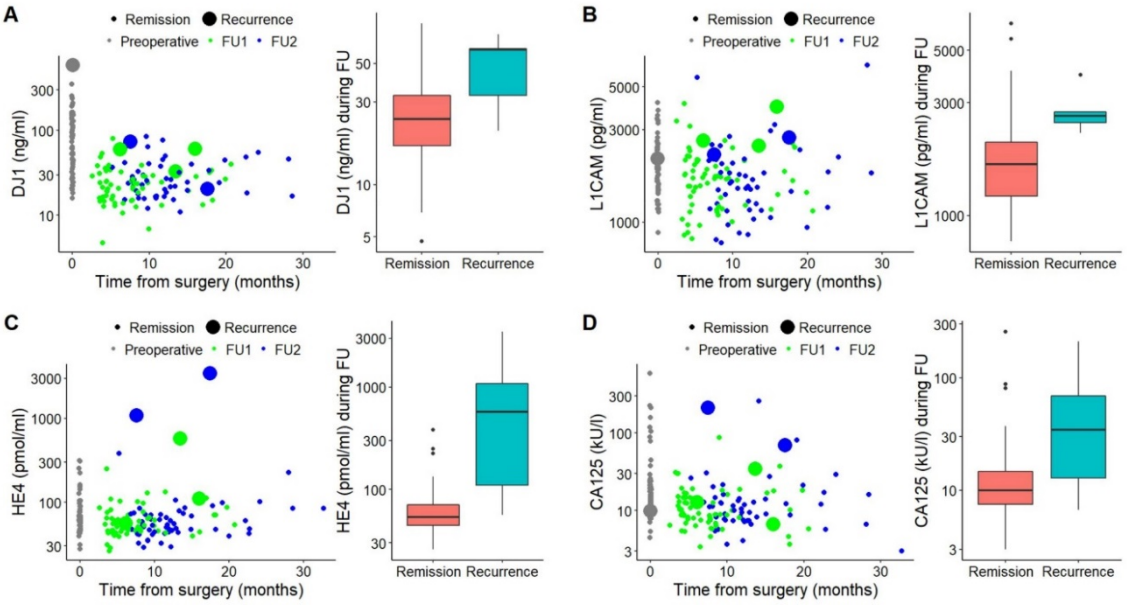

Figure 2. Charts displaying comparison of DJ-1 (**A**), L1CAM (**B**), HE4 (**C**) and CA125 (**D**) levels at the times of remission and recurrence. Scatter charts (left) display marker levels at the time of diagnosis (gray) and from the collections during follow-ups (first collection, green; second collection, blue). Large spots mean samples at the time of recurrence. Box plots (right) demonstrate marker levels at the times of remission and recurrence, respectively.

3.3. DJ1, L1CAM, CA125 and HE4 Serum Levels in Correlation with Patient-Related Characteristics

The correlation of DJ-1, L1CAM, CA125 and HE4 serum levels with age, weight, renal function and menopausal status are shown in Table 5. Median DJ1 serum levels were not affected by any of the observed patient-related characteristics. In contrast, median L1CAM levels were significantly higher in patients with age ≥60 years ($p = 0.004$), overweight (BMI ≥ 27 kg/m^2, $p = 0.002$) and post-menopause ($p = 0.010$), respectively. None of the monitored patient-related parameters were related to either median CA125 or HE4 serum levels.

Table 5. DJ1, L1CAM, HE4 and CA125 preoperative serum levels according to age, weight, renal function and menopausal status.

			Valid *n*	Median (IQR)	*p* Value
DJ1 (ng/mL)	Age	<60 years	20	53.4 (31.4–103)	0.152
		≥60 years	44	88 (43–151.8)	
	Weight	BMI < 27 kg/m^2	18	52.9 (25.5–121.9)	0.181
		BMI ≥ 27 kg/m^2	48	86.2 (44.4–141.7)	
	Renal function	CKD-EPI ≥ 1 mL/s	56	83.4 (40.6–139)	0.604
		CKD-EPI < 1 mL/s	7	74.1 (24.7–352)	
	Menopausal status	Pre/perimenopausal	11	45.4 (24.4–142.4)	0.393
		Postmenopausal	53	84.9 (41.4–135.5)	
L1CAM (pg/mL)	Age	<60 years	20	1546.5 (1273.5–1872.5)	0.004
		≥60 years	44	2070 (1703.5–2447)	
	Weight	BMI < 27 kg/m^2	16	1519.3 (1331.3–1804)	0.002
		BMI ≥ 27 kg/m^2	48	2028 (1676.8–2507)	
	Renal function	CKD-EPI ≥ 1 mL/s	56	1889.5 (1503.5–2350.3)	0.105
		CKD-EPI < 1 mL/s	7	2247 (1878–3343.5)	
	Menopausal status	Pre/perimenopausal	11	1528 (1173–1928)	0.010
		Postmenopausal	53	2000 (1565–2424.5)	
HE4 (pmol/L)	Age	<60 years	15	65.7 (57.7–105)	0.765
		≥60 years	34	68.4 (53.1–98)	
	Weight	BMI < 27 kg/m^2	13	65.7 (59.1–104.3)	0.570
		BMI ≥ 27 kg/m^2	36	68.7 (53.6–94)	
	Renal function	CKD-EPI ≥ 1 mL/s	47	67.1 (53.1–98)	0.196
		CKD-EPI < 1 mL/s	1	161 (161–161)	
	Menopausal status	Pre/perimenopausal	8	63.4 (58.9–90.6)	0.468
		Postmenopausal	41	68.6 (53.1–98)	
CA125 (kU/L)	Age	<60 years	17	17.6 (9.5–24.9)	0.873
		≥60 years	43	15.8 (11.9–23.7)	
	Weight	BMI < 27 kg/m^2	16	17.8 (13–24.8)	0.481
		BMI ≥ 27 kg/m^2	44	15.4 (11.2–23.8)	
	Renal function	CKD-EPI ≥ 1 mL/s	54	17.3 (12.4–24.6)	0.128
		CKD-EPI < 1 mL/s	5	12.5 (9.9–15.8)	
	Menopausal status	Pre/perimenopausal	9	17.6 (15.1–24.9)	0.873
		Postmenopausal	51	15.8 (10.8–23.8)	

Abbreviations: *n* = number of patients, IQR = interquartile range, BMI = Body Mass Index, CKD-EPI = glomerular filtration rate calculated according to the chronic kidney disease epidemiology collaboration (CKD-EPI) equation.

3.4. DJ1, L1CAM, CA125 and HE4 Serum Levels in Correlation with Tumor Clinicopathological Characteristics

In our study, DJ1 and L1CAM serum levels did not correlate with stage, histological type or risk of recurrence. Serum HE4 levels were statistically significantly higher in tumors with myometrial invasion ≥50% ($p = 0.002$), with lymph node involvement ($p = 0.033$), distant metastases ($p = 0.021$) and high risk of recurrence based on definitive histopathological findings ($p = 0.02$). There were also statistically significant different CA125 serum levels depending on the degree of myometrial invasion (lower levels in patients with invasion <50%, $p = 0.009$) and lymph node involvement (higher in case of lymph node metastases, $p = 0.010$). The detailed correlation of marker levels with clinicopathological features are shown in Table 6.

Table 6. DJ1, L1CAM, HE4 and CA125 serum levels in correlation with both clinical and pathological features of endometrial cancer.

			Valid *n*	Median (IQR)	*p* Value
DJ1 (ng/mL)	Histological type	E G1-2	50	83.4 (41.4–151.5)	0.191
		E G3, non-E	14	69 (22.1–94.5)	
	Myometrial invasion	<50%	47	63.9 (35.4–135.5)	0.331
		≥50%	17	91.1 (51.9–151.5)	
	LN involvement	No	60	78 (38.4–145.2)	0.688
		Yes	4	73.2 (36.4–111.3)	
	Distant metastasis	No	62	83.4 (37.1–142.4)	0.714
		Yes	2	56.7 (53.1–60.3)	
	Definitive risk	Low [1]	34	57.8 (39.7–147.9)	0.941
		High [2]	30	86.2 (37.1–128)	
L1CAM (pg/mL)	Histological type	E G1-2	50	1889.5 (1523.5–2418)	0.620
		E G3, non-E	14	2132.5 (1460–2345.5)	
	Myometrial invasion	<50%	47	1969 (1528–2355)	0.721
		≥50%	17	1680 (1515–2418)	
	LN involvement	No	60	1919 (1519.3–2389.8)	0.945
		Yes	4	2055.5 (1586.5–2381.8)	
	Distant metastasis	No	62	1919 (1523.5–2355)	0.772
		Yes	2	2200.5 (1515–2886)	
	Definitive risk	Low [1]	34	1948.5 (1580–2355)	0.687
		High [2]	30	1837.75 (1469–2418)	
HE4 (pmol/mL)	Histological type	E G1-2	38	65 (51.4–98)	0.276
		E G3, non-E	11	76.2 (60.2–153.7)	
	Myometrial invasion	<50%	39	63.7 (51.4–79.2)	0.002
		≥50%	10	148.4 (68.6–255)	
	LN involvement	No	45	65.7 (53.1–89.6)	0.033
		Yes	4	148.4 (105.8–189.4)	
	Distant metastasis	No	47	67.1 (53.1–90)	0.021
		Yes	2	284.5 (255–314)	
	Definitive risk	Low [1]	28	61.9 (48.3–79.9)	0.020
		High [2]	21	76.2 (61.1–153.7)	
CA125 (kU/L)	Histological type	E G1-2	47	15.8 (12.4–23.8)	0.837
		E G3, non-E	13	17.9 (10.8–24.7)	
	Myometrial invasion	<50%	45	14.9 (10.5–20)	0.009
		≥50%	15	23.8 (16.9–119)	
	LN involvement	No	56	15.8 (10.7–23.6)	0.010
		Yes	4	192.4 (88.2–407.4)	
	Distant metastasis	No	58	16 (10.8–23.8)	0.104
		Yes	2	116.7 (23.4–210)	
	Definitive risk	Low [1]	32	15.3 (10.2–19.8)	0.135
		High [2]	28	19 (12.2–41.4)	

Abbreviations: *n* = number of patients, IQR = interquartile range. [1] Low definitive risk = endometrial carcinoma G1-2 AND myometrial invasion <50% AND LVSI negative (i.e., adjuvant treatment not recommended) [2] High definitive risk = criteria for low risk not met (i.e., adjuvant treatment recommended).

4. Discussion

Circulating tumor marker serum level determinations provide interpretable results that can significantly help to monitor treatment efficacy or early detection of recurrence in a broad array of solid tumors [26]. In EC patients, clear evidence of clinical benefit from circulating marker assessment has still been lacking and therefore, none of the circulating markers have become an integral part of EC patient management in clinical practice [23,27,28]. Studies that have been published so far have failed to demonstrate a higher proportion of recurrences diagnosed in asymptomatic patients, even when extensive FU was used consisting of not only gynecological examination, but also of serial CA125 measurements and examinations by imaging methods. The proportion of symptomatic recurrence remains 41–83% [29]. In general, there is still no consensus about FU regimens of EC patients after successful primary treatment [23,27,28]. Undoubtedly, the identification of a circulating marker detecting EC recurrence before the onset of symptoms, when lower tumor burden enables using a wider spectrum of therapeutic options, would have a significant impact on EC patient management.

In this pilot study, we aimed to evaluate the significance of the new circulating markers DJ1 and L1CAM in correlation with markers CA125 and HE4 in EC patients. We demonstrated that DJ1 and L1CAM serum levels were significantly higher at the time of EC diagnosis than levels collected after the initial treatment in disease-free patients ($p < 0.001$ for both markers, Figure 1A,B). Therefore, the dynamics of serial DJ1 and L1CAM serum levels correlate with disease status. The essential condition thus has been met for the use of DJ1 and L1CAM in monitoring of anticancer treatment efficacy. In concordance with previously published studies, we showed that CA125 and HE4 serum levels generally decreased after initial treatment as well ($p < 0.001$ for differences between levels collected preoperatively and during follow-up; Figure 1C,D) [12,13,30,31].

Despite the generally favorable prognosis of EC, 13–17% patients will develop recurrence, in most cases within three years after initial treatment [32]. While three-year survival following vaginal recurrence is ~73%, in case of pelvic or distant recurrence, it drops to less than 15% [33]. Moreover, patients with symptomatic recurrence survive for a significantly shorter time compared to patients whose recurrence is diagnosed in asymptomatic status [34]. In our study, a total of 5 patients (8%) developed recurrence after the median follow-up of 29.5 months. Three patients developed distant metastases despite initial stage FIGO I or II (Table 3). DJ1, CA125 and HE4 levels increased significantly at the time of recurrence ($p = 0.035$ for DJ1 and $p < 0.001$ for CA125 and HE4, respectively) (see Table 4 and Figure 2A,C,D). L1CAM serum levels at the time of recurrence increased as well, but statistical significance was not achieved ($p = 0.353$, Figure 2B and Table 4). The probable explanation for this fact could be a small number of patients with recurrent disease in our cohort. Furthermore, Tangen et al. demonstrated a correlation of L1CAM serum levels, with L1CAM tumor overexpression assessed immunohistochemically [20]. Similarly, Fogel et al. showed elevated L1CAM serum levels in patients with LCAM-positive EC in contrast to significantly lower levels in both healthy controls and patients with L1CAM-negative tumors [17]. In our study, there were only 10 patients with L1CAM-immunohistochemically positive tumors, and just one of them developed recurrent disease (unpublished data). Our results on CA125 and HE4 serum levels correlate with previously published studies providing evidence for the significance of serial monitoring of CA125 and HE4 serum levels for the detection of EC recurrence [12,13,35].

Undoubtedly, the identification of patient-related factors associated with health status that could affect marker serum levels is crucial for the use of circulating markers in clinical practice. In our study, DJ1 serum levels were affected only by the status of the disease (i.e., whether the samples were taken at the time of EC diagnosis, after treatment or at the time of recurrence). On the contrary, L1CAM serum levels also depended on age, weight and menopausal status (Table 5). Any association of CA125 or HE4 serum levels with any of the monitored parameters (Table 5) was not observed. The possible explanation of an inconsistency between our results and the previously described dependence of HE4 serum levels on renal function [36,37] might be a composition of the study cohort. Only

one patient in a cohort assessable for HE4 had renal insufficiency, whereas the number of patients evaluable for L1CAM, DJ1 and CA125 having renal insufficiency was higher (see Table 5).

With respect to the comparison of DJ1 and L1CAM serum levels in relation to histological type, lymph node involvement, presence of distant metastases or risk of recurrence at the time of diagnosis, we did not demonstrate any statistically significant correlation (Table 6). Regarding DJ1, our data were consistent with the results of a study by Benati et al. Although this study observed higher DJ1 levels in EC patients ($n = 45$) compared to healthy controls ($n = 29$, $p < 0.0001$), the differences in serum levels between patients with an early stage (FIGO I, II; $n = 38$) or advanced disease (FIGO III, IV; $n = 7$) failed to reach statistical significance ($p = 0.86$) [16]. We did not confirm results of the study published by Di Cello et al. that demonstrated significantly higher DJ1 levels ($p \leq 0.05$) in patients with a high-risk histological type (endometrioid carcinoma grade 3 or non-endometrioid carcinoma) than in patients with a low-risk histotype (endometrioid carcinoma grade 1 or 2) [15]. This discrepancy could be explained by the fact that the proportion of patients in our study with high-risk histotype was too small to reach statistical significance ($n = 14$; 22%) (see Table 6).

With respect to the correlation of L1CAM serum levels with tumor clinicopathological features, two studies have been published with conflicting results. Our results are consistent with the previous study published by Wojciechowski et al. ($n = 35$). Although the authors observed different L1CAM serum levels in EC patients compared to individuals with benign gynecological conditions, they were unable to demonstrate correlation among L1CAM serum levels and stage, histological type or tumor grading [19]. On the contrary, we did not confirm the correlation of L1CAM serum levels with the lymph node involvement demonstrated by Tangen et al. ($n = 372$; $p = 0.048$) [20]. L1CAM serum levels apparently depend on many patient-related variables, as we show in Table 5. In addition, the relation between L1CAM serum levels and L1CAM percentage tumor positivity (if any) has not been clearly defined yet [17]. Correlations of CA125 and HE4 serum levels with known prognostic factors in EC patients shown in our study (Table 6) are consistent with previously published data [10,12,13,38].

Preoperative knowledge of marker serum levels is only one part of the clinical complexity determining the risk of recurrence in EC patients [39]. Recently, the integrated genomic analyses performed by The Cancer Genome Atlas Research network (TCGA) proposed dividing EC into four groups with different clinical behaviors and prognoses [40–42]. This novel, molecular-based classification dramatically changed risk stratification and clinical management of EC patients [43–45]. Moreover, there are other promising methods that might be able to predict the behavior and pathological characteristics of EC such as metabolomics [46,47]. In this context, analyses of larger cohorts of patients taking into consideration other factors affecting EC prognosis such as the presence of POLE and p53 mutation, mismatch repair (MMR) status, the levels of L1CAM and estrogen/progesterone receptors' positivity in the tumor, etc., need to be done for more specific assessment of the significance of circulating marker serum levels.

The strengths of this study include the fact that this is a cohort of fully staged EC patients from a real clinical practice with prospective data collection. To our best knowledge, this study is the first to analyze the dynamic changes of serial DJ1 and L1CAM serum levels in EC patients. In the case of DJ1, these are the first data supporting its potential role as a serum marker for the detection of recurrence in EC patients during the follow-up period after successful primary treatment. To date, none of the published studies on DJ1 and L1CAM serum levels have addressed this issue. A limitation of our study was that this was a relatively small cohort of EC patients with a shorter follow-up period and therefore a low recurrence rate, as it was a pilot study aimed at finding a reasonable preliminary evidence for future research on DJ1 and L1CAM as serum circulation tumor biomarkers in EC patients.

5. Conclusions

We demonstrated that DJ1 and L1CAM serum levels correlated with disease status in EC patients; they may therefore be potentially useful in clinical practice for monitoring the effects of treatment. Unlike the L1CAM marker, DJ1 serum levels were not affected by other factors associated with the health status of patients such as age or BMI. Further studies with longer follow-ups will be needed to definitively assess the benefit from monitoring of DJ1, L1CAM, CA125 and HE4 serum levels for the diagnosis of asymptomatic EC recurrence. At the time of EC diagnosis and in contrast to CA125 and HE4, DJ1 and L1CAM serum levels did not correlate with disease stage, histological type or risk of disease recurrence, leaving us unable to assess their prognostic significance in patients with endometrial cancer based on the limited pilot study cohort.

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References

1. Bidart, J.-M.; Thuillier, F.; Augereau, C.; Chalas, J.; Daver, A.; Jacob, N.; Labrousse, F.; Voitot, H. Kinetics of Serum Tumor Marker Concentrations and Usefulness in Clinical Monitoring. *Clin. Chem.* **1999**, *45*, 1695–1707. [CrossRef]
2. Ayhan, A.; Reed, N.; Gultekin, M.; Dursun, P. (Eds.) *Textbook of Gynaecological Oncology*; Günes Publishing: Ankara, Turkey, 2017; ISBN 978-975-277-682-1.
3. Bast, J.R.; Xu, F.-J.; Yu, Y.-H.; Barnhill, S.; Zhang, Z.; Mills, G. CA 125: The past and the Future. *Int. J. Biol. Mark.* **1998**, *13*, 179–187. [CrossRef]
4. Sood, A.K.; Buller, R.E.; Burger, R.A.; Dawson, J.D.; Sorosky, J.I.; Berman, M. Value of Preoperative CA 125 Level in the Management of Uterine Cancer and Prediction of Clinical Outcome. *Obstet. Gynecol.* **1997**, *90*, 441–447. [CrossRef]
5. Reijnen, C.; Visser, N.C.; Kasius, J.C.; Boll, D.; Geomini, P.M.; Ngo, H.; Van Hamont, D.; Pijlman, B.M.; Vos, M.C.; Bulten, J.; et al. Improved preoperative risk stratification with CA-125 in low-grade endometrial cancer: A multicenter prospective cohort study. *J. Gynecol. Oncol.* **2019**, *30*, e70. [CrossRef]
6. Fischerova, D. Ultrasound scanning of the pelvis and abdomen for staging of gynecological tumors: A review. *Ultrasound Obstet. Gynecol.* **2011**, *38*, 246–266. [CrossRef]
7. Kalogera, E.; Scholler, N.; Powless, C.; Weaver, A.; Drapkin, R.; Li, J.; Jiang, S.-W.; Podratz, K.; Urban, N.; Dowdy, S.C. Correlation of serum HE4 with tumor size and myometrial invasion in endometrial cancer. *Gynecol. Oncol.* **2012**, *124*, 270–275. [CrossRef]
8. Moore, R.G.; Brown, A.K.; Miller, M.C.; Badgwell, D.; Lu, Z.; Allard, W.J.; Granai, C.; Bast, R.; Lu, K. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. *Gynecol. Oncol.* **2008**, *110*, 196–201. [CrossRef]
9. Lo, S.; Cheng, D.; Ng, T.; Wong, L.; Ngan, H. Prognostic Significance of Tumour Markers in Endometrial Cancer. *Tumor Biol.* **1997**, *18*, 241–249. [CrossRef]
10. Bignotti, E.; Ragnoli, M.; Zanotti, L.; Calza, S.; Falchetti, M.L.; Lonardi, S.; Bergamelli, S.; Bandiera, E.; Tassi, R.A.; Romani, C.; et al. Diagnostic and prognostic impact of serum HE4 detection in endometrial carcinoma patients. *Br. J. Cancer* **2011**, *104*, 1418–1425. [CrossRef] [PubMed]

11. Zanotti, L.; Bignotti, E.; Calza, S.; Bandiera, E.; Ruggeri, G.; Galli, C.; Tognon, G.; Ragnoli, M.; Romani, C.; Tassi, R.A.; et al. Human epididymis protein 4 as a serum marker for diagnosis of endometrial carcinoma and prediction of clinical outcome. *Clin. Chem. Lab. Med.* **2012**, *50*, 2189–2198. [CrossRef] [PubMed]
12. Brennan, D.J.; Hackethal, A.; Mann, K.P.; Mutz-Dehbalaie, I.; Fiegl, H.; Marth, C.; Obermair, A. Serum HE4 detects recurrent endometrial cancer in patients undergoing routine clinical surveillance. *BMC Cancer* **2015**, *15*, 33. [CrossRef] [PubMed]
13. Abbink, K.; Zusterzeel, P.L.; Geurts-Moespot, A.J.; van Herwaarden, A.E.; Pijnenborg, J.M.; Sweep, F.; Massuger, L.F. HE4 is superior to CA125 in the detection of recurrent disease in high-risk endometrial cancer patients. *Tumor Biol.* **2018**, *40*, 101042831875710. [CrossRef] [PubMed]
14. Morelli, M.; Scumaci, D.; Di Cello, A.; Venturella, R.; Donato, G.; Faniello, M.C.; Quaresima, B.; Cuda, G.; Zullo, F.; Costanzo, F.S. DJ-1 in Endometrial Cancer: A Possible Biomarker to Improve Differential Diagnosis Between Subtypes. *Int. J. Gynecol. Cancer* **2014**, *24*, 649–658. [CrossRef]
15. Di Cello, A.; Di Sanzo, M.; Perrone, F.M.; Santamaria, G.; Rania, E.; Angotti, E.; Venturella, R.; Mancuso, S.; Zullo, F.; Cuda, G.; et al. DJ-1 is a reliable serum biomarker for discriminating high-risk endometrial cancer. *Tumor Biol.* **2017**, *39*, 101042831770574. [CrossRef]
16. Benati, M.; Montagnana, M.; Danese, E.; Paviati, E.; Giudici, S.; Ruzzenente, O.; Franchi, M.; Lippi, G. The clinical significance of DJ-1 and HE4 in patients with endometrial cancer. *J. Clin. Lab. Anal.* **2018**, *32*, e22223. [CrossRef]
17. Fogel, M.; Gutwein, P.; Mechtersheimer, S.; Riedle, S.; Stoeck, A.; Smirnov, A.; Edler, L.; Ben-Arie, A.; Huszar, M.; Altevogt, P. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. *Lancet* **2003**, *362*, 869–875. [CrossRef]
18. Van Gool, I.C.; Stelloo, E.; Nout, R.A.; Nijman, H.W.; Edmondson, R.; Church, D.N.; Mackay, H.J.; Leary, A.; Powell, M.E.; Mileshkin, L.; et al. Prognostic significance of L1CAM expression and its association with mutant p53 expression in high-risk endometrial cancer. *Mod. Pathol.* **2016**, *29*, 174–181. [CrossRef]
19. Wojciechowski, M.; Głowacka, E.; Wilczyński, M.; Pękala-Wojciechowska, A.; Malinowski, A. The sL1CAM in sera of patients with endometrial and ovarian cancers. *Arch. Gynecol. Obstet.* **2016**, *295*, 225–232. [CrossRef]
20. Tangen, I.L.; Kopperud, R.K.; Visser, N.C.; Staff, A.C.; Tingulstad, S.; Marcickiewicz, J.; Amant, F.; Bjørge, L.; Pijnenborg, J.M.; Salvesen, H.B.; et al. Expression of L1CAM in curettage or high L1CAM level in preoperative blood samples predicts lymph node metastases and poor outcome in endometrial cancer patients. *Br. J. Cancer* **2017**, *117*, 840–847. [CrossRef]
21. Frühauf, F.; Dvořák, M.; Haaková, L.; Hašlík, L.; Herboltová, P.; Chaloupková, B.; Kožnarová, J.; Kubešová, B.; Lukáčová, I.; Marek, R.; et al. Ultrasound staging of endometrial cancer—Recommended methodology of examination. *Ceska Gynekol.* **2014**, *79*, 466–476.
22. Sobin, L.H.; Gospodarowicz, M.K.; Wittekind, C. *TNM Classification of Malignant Tumours*; Wiley: Somerset, UK, 2011; ISBN 978-1-4443-5896-4.
23. Colombo, N.; Creutzberg, C.; Amant, F.; Bosse, T.; González-Martín, A.; Ledermann, J.; Marth, C.; Nout, R.; Querleu, D.; Mirza, M.R.; et al. ESMO–ESGO–ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. *Radiother. Oncol.* **2015**, *117*, 559–581. [CrossRef] [PubMed]
24. Kurman, R.J.; International Agency for Research on Cancer; World Health Organization. (Eds.) *WHO Classification of Tumours of Female Reproductive Organs*, 4th ed.; World Health Organization classification of tumours; International Agency for Research on Cancer: Lyon, France, 2014; ISBN 978-92-832-2435-8.
25. FIGO Committee on Gynecologic Oncology. FIGO staging for carcinoma of the vulva, cervix, and corpus uteri. *Int. J. Gynecol. Obstet.* **2014**, *125*, 97–98. [CrossRef]
26. Diamandis, E.P. *Tumor Markers: Physiology, Pathobiology, Technology and Clinical Application*; AACC Press: Washington, WA, USA, 2002.
27. Burke, W.M.; Orr, J.; Leitao, M.; Salom, E.; Gehrig, P.; Olawaiye, A.B.; Brewer, M.; Boruta, D.; Villella, J.; Herzog, T.; et al. Endometrial cancer: A review and current management strategies: Part I. *Gynecol. Oncol.* **2014**, *134*, 385–392. [CrossRef] [PubMed]
28. Burke, W.M.; Orr, J.; Leitao, M.; Salom, E.; Gehrig, P.; Olawaiye, A.B.; Brewer, M.; Boruta, D.; Herzog, T.J.; Abu Shahin, F. Endometrial cancer: A review and current management strategies: Part II. *Gynecol. Oncol.* **2014**, *134*, 393–402. [CrossRef]
29. Salani, R.; Backes, F.J.; Fung, M.F.K.; Holschneider, C.H.; Parker, L.P.; Bristow, R.E.; Goff, B.A. Posttreatment surveillance and diagnosis of recurrence in women with gynecologic malignancies: Society of Gynecologic Oncologists recommendations. *Am. J. Obstet. Gynecol.* **2011**, *204*, 466–478. [CrossRef]
30. Patsner, B.; Orr, J.W.; Mann, W.J. Use of serum CA 125 measurement in posttreatment surveillance of early-stage endometrial carcinoma. *Am. J. Obstet. Gynecol.* **1990**, *162*, 427–429. [CrossRef]
31. Rose, P.G.; Sommers, R.M.; Reale, F.R.; Hunter, R.E.; Fournier, L.; Nelson, B.E. Serial serum CA 125 measurements for evaluation of recurrence in patients with endometrial carcinoma. *Obstet. Gynecol.* **1994**, *84*, 12–16.
32. Fung-Kee-Fung, M.; Dodge, J.; Elit, L.; Lukka, H.; Chambers, A.; Oliver, T. Follow-up after primary therapy for endometrial cancer: A systematic review. *Gynecol. Oncol.* **2006**, *101*, 520–529. [CrossRef] [PubMed]
33. Creutzberg, C.L.; van Putten, W.L.; Koper, P.C.; Lybeert, M.L.; Jobsen, J.J.; Wárlám-Rodenhuis, C.C.; De Winter, K.A.; Lutgens, L.C.; Bergh, A.C.V.D.; van der Steen-Banasik, E.; et al. Survival after relapse in patients with endometrial cancer: Results from a randomized trial. *Gynecol. Oncol.* **2003**, *89*, 201–209. [CrossRef]

34. Smith, C.J.; Heeren, M.; Nicklin, J.L.; Perrin, L.; Land, R.; Crandon, A.J.; Obermair, A. Efficacy of routine follow-up in patients with recurrent uterine cancer. *Gynecol. Oncol.* **2007**, *107*, 124–129. [CrossRef]
35. Angioli, R.; Capriglione, S.; Scaletta, G.; Aloisi, A.; Miranda, A.; Nardone, C.D.C.; Terranova, C.; Plotti, F. The role of HE4 in endometrial cancer recurrence: How to choose the optimal follow-up program. *Tumor Biol.* **2015**, *37*, 4973–4978. [CrossRef] [PubMed]
36. Coppolino, G.; Bolignano, D.; Rivoli, L.; Mazza, G.; Presta, P.; Fuiano, G. Tumour Markers and Kidney Function: A Systematic Review. *BioMed Res. Int.* **2014**, *2014*, 1–9. [CrossRef]
37. Chovanec, J.; Selingerova, I.; Greplova, K.; Antonsen, S.L.; Nalezinska, M.; Høgdall, C.; Høgdall, E.; Søgaard-Andersen, E.; Jochumsen, K.; Fabian, P.; et al. Adjustment of serum HE4 to reduced glomerular filtration and its use in biomarker-based prediction of deep myometrial invasion in endometrial cancer. *Oncotarget* **2017**, *8*, 108213–108222. [CrossRef] [PubMed]
38. Saarelainen, S.K.; Peltonen, N.; Lehtimäki, T.; Perheentupa, A.; Vuento, M.H.; Mäenpää, J.U. Predictive value of serum human epididymis protein 4 and cancer antigen 125 concentrations in endometrial carcinoma. *Am. J. Obstet. Gynecol.* **2013**, *209*, 142.e1–142.e6. [CrossRef]
39. Reijnen, C.; Gogou, E.; Visser, N.C.M.; Engerud, H.; Ramjith, J.; Van Der Putten, L.J.M.; Van De Vijver, K.; Santacana, M.; Bronsert, P.; Bulten, J.; et al. Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: A development and validation study. *PLoS Med.* **2020**, *17*, e1003111. [CrossRef]
40. Kandoth, C.; Schultz, N.; Cherniack, A.D.; Akbani, R.; Liu, Y.; Shen, H.; Robertson, A.G.; Pashtan, I.; Shen, R.; Benz, C.C.; et al. Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature* **2013**, *497*, 67–73. [CrossRef]
41. Murali, R.; Delair, D.F.; Bean, S.M.; Abu-Rustum, N.R.; Soslow, R.A. Evolving Roles of Histologic Evaluation and Molecular/Genomic Profiling in the Management of Endometrial Cancer. *J. Natl. Compr. Cancer Netw.* **2018**, *16*, 201–209. [CrossRef]
42. Urick, M.E.; Bell, D.W. Clinical actionability of molecular targets in endometrial cancer. *Nat. Rev. Cancer* **2019**, *19*, 510–521. [CrossRef]
43. Concin, N.; Matias-Guiu, X.; Vergote, I.; Cibula, D.; Mirza, M.R.; Marnitz, S.; Ledermann, J.; Bosse, T.; Chargari, C.; Fagotti, A.; et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int. J. Gynecol. Cancer* **2021**, *31*, 12–39. [CrossRef] [PubMed]
44. Raffone, A.; Travaglino, A.; Gabrielli, O.; Micheli, M.; Zuccalà, V.; Bitonti, G.; Camastra, C.; Gargiulo, V.; Insabato, L.; Zullo, F. Clinical features of ProMisE groups identify different phenotypes of patients with endometrial cancer. *Arch. Gynecol. Obstet.* **2021**, *303*, 1393–1400. [CrossRef]
45. Santoro, A.; Angelico, G.; Travaglino, A.; Inzani, F.; Arciuolo, D.; Valente, M.; D'Alessandris, N.; Scaglione, G.; Fiorentino, V.; Raffone, A.; et al. New Pathological and Clinical Insights in Endometrial Cancer in View of the Updated ESGO/ESTRO/ESP Guidelines. *Cancers* **2021**, *13*, 2623. [CrossRef] [PubMed]
46. Troisi, J.; Raffone, A.; Travaglino, A.; Belli, G.; Belli, C.; Anand, S.; Giugliano, L.; Cavallo, P.; Scala, G.; Symes, S.; et al. Development and Validation of a Serum Metabolomic Signature for Endometrial Cancer Screening in Postmenopausal Women. *JAMA Netw. Open* **2020**, *3*, e2018327. [CrossRef] [PubMed]
47. Raffone, A.; Troisi, J.; Boccia, D.; Travaglino, A.; Capuano, G.; Insabato, L.; Mollo, A.; Guida, M.; Zullo, F. Metabolomics in endometrial cancer diagnosis: A systematic review. *Acta Obstet. Gynecol. Scand.* **2020**, *99*, 1135–1146. [CrossRef] [PubMed]

5 Lymph Node Staging

The status of regional lymph nodes (pelvic and para-aortic) represents one of the key markers for accurate disease staging and the indication of appropriate adjuvant therapy. Even among low-risk patients, there is a 4–6% risk of nodal metastases[48,49]. The initial assumption that patients would therapeutically benefit from systematic aortopelvic lymphadenectomy has not been confirmed[50,51]; moreover, this procedure is associated with a high rate of intraoperative and postoperative morbidity, particularly the development of lymphedema, lymphoceles, and lymphatic ascites[52].

As a result, SNB (sentinel node biopsy) has been incorporated into the diagnostic and therapeutic algorithm. This method is associated with a significantly lower incidence of postoperative complications[53]. Initially recommended only for low-risk tumors, SNB was later accepted as an alternative for high-risk cases as well. It is now the predominant staging method for all types of endometrial carcinomas that are preoperatively believed to be confined to the uterus[3].

A major advancement in the accuracy of sentinel lymph node detection has been achieved with the use of ICG (indocyanine green), which complements the traditionally used tracers—Tc99 (technetium-99m) and blue dyes (patent blue, isosulfan blue, or methylene blue). In one of the early studies, the bilateral detection rate using ICG reached 78%, compared to only 31% with blue dye[54]. Similar findings have been confirmed in subsequent studies. A potential alternative with comparable success to ICG is the combined use of Tc99 and blue dye[55].

The cervix has become the most commonly used site for ICG administration. Using this technique, sensitivity for detecting nodal metastases can reach up to 97%[56]. The bilateral detection rate with intracervical injection of ICG ranges from 78% to 85%[49,54,55,57]. Although hysteroscopic peritumoral ICG injection under direct visual control yields higher sensitivity for detecting para-aortic sentinel nodes[58], this approach has not been widely adopted in clinical practice.

Another important advantage of SNB is the potential for more detailed lymph node evaluation through ultrastaging, compared to conventional histopathological

examination of multiple nodes[59]. This approach increases the detection rate of nodal involvement, including MIC (micrometastases; 0.2–2 mm) and ITC (isolated tumor cells; <0.2 mm)[60]. Micrometastases are associated with poorer survival outcomes and lead to FIGO stage IIIC classification, thereby indicating the need for adjuvant therapy. In contrast, current evidence does not suggest that ITCs have a prognostic impact, and such patients typically do not receive adjuvant treatment[61].

A question remains whether the current trend of abandoning systematic lymphadenectomy is suitable for all subtypes of endometrial carcinoma. A possible future resurgence of lymphadenectomy cannot be ruled out, particularly for tumors with specific molecular characteristics—e.g., increased risk of nodal involvement in preoperatively low-stage p53-abnormal or MMR-deficient tumors. Nonetheless, ongoing expert discussions do not support such a reversal. On the contrary, with the publication of updated European guidelines[45], sentinel lymph node biopsy has become the dominant staging method for all types of endometrial carcinoma.

5.1 Vinklerová P, Minář L, Weinberger V, Felsinger M, Koblížková M. Změna trendu operační léčby a stagingu lymfatických uzlin u karcinomu endometria – výsledky Onkogynekologického centra Gynekologicko-porodnické kliniky FN Brno a LF MU 2012–2021. Čes. Gynek., 2022; 87(5): 308-316.

Following international trends, surgical treatment and lymph node staging in endometrial cancer have undergone significant changes in recent years. This study aims to highlight these developments, with particular emphasis on the growing use of minimally invasive techniques, by analyzing surgical outcomes at the Gynecological Oncological Center of the Department of Gynecology and Obstetrics, University Hospital Brno, from 2012 to 2021. The results are evaluated in the context of national and international guidelines and compared with ESGO quality indicators of surgical treatment.

Our findings demonstrate a clear and sustained shift toward MIS (minimally invasive surgery), with total laparoscopic hysterectomy emerging as the predominant surgical approach for early-stage EC by the end of the study period. Conversely, the use of laparotomy has steadily declined. This trend mirrors international developments in gynecologic oncology and reflects both improved availability of MIS technologies and enhanced surgical proficiency among gynecologic oncologists in the Czech Republic.

We also emphasize the positive impact of healthcare centralization and adherence to evidence-based guidelines, which have contributed to better clinical outcomes. The increased utilization of MIS was associated with reduced intraoperative morbidity, shorter hospitalization, and oncologic outcomes comparable to those achieved through open surgery. Our department met all selected ESGO quality indicators, confirming the high standard of surgical care provided.

In conclusion, our analysis demonstrates that our department has effectively embraced the global trend of transitioning to minimally invasive surgical management of endometrial carcinoma. We recommend continued investment in specialized

training, institutional centralization, and systematic outcome monitoring to further enhance the quality and safety of surgical care.

The retrospective analysis *"Change in the trend of surgical treatment and staging of lymph nodes in endometrial cancer – results of the Oncogynecology Center, Department of Gynecology and Obstetrics, University Hospital Brno and Masaryk University in the years 2012–2021"* was published in *Česká gynekologie* (IF 0.4, Q4) in 2022.

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Změna trendu operační léčby a stagingu lymfatických uzlin u karcinomu endometria – výsledky Onkogynekologického centra Gynekologicko-porodnické kliniky FN Brno a LF MU v letech 2012–2021

Change in the trend of surgical treatment and staging of lymph nodes in endometrial cancer – results of the Oncogynecology Center, Department of Gynecology and Obstetrics, University Hospital Brno and Masaryk University in the years 2012–2021

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Souhrn: **Úvod:** Pohled na karcinom endometria se v poslední dekádě dramaticky proměnil, došlo k výrazné změně v operačním přístupu a stagingu lymfatických uzlin. Tyto změny prezentujeme na výsledcích Onkogynekologického centra Gynekologicko-porodnické kliniky FN Brno v letech 2012–2021 v kontextu aktuálních národních i evropských doporučení. **Metodika:** Retrospektivní unicentrická observační studie, přehled národních a evropských doporučených postupů. **Výsledky:** Ve sledovaném období bylo na klinice léčeno 715 pacientek s karcinomem endometria, operační léčbu podstoupilo 636 z nich (89 %). V rámci stagingu lymfatických uzlin je v první polovině hodnoceného období patrný trend rozšiřování lymfadenektomie o paraaortální oblast, v letech 2018–2019 pak zavádění detekce sentinelové uzliny, nakonec přechod k této metodě jako k hlavnímu stagingovému výkonu v roce 2021, kdy bylo vyšetření provedeno u 73 % operací, a to i u high risk karcinomů splňujících kritéria pro provedení biopsie sentinelové uzliny, tj. nádory omezené na dělohu. S rozšířením biopsie sentinelové uzliny je patrný postupný pokles laparotomických výkonů (maximum 41 % v roce 2016, 18 % v roce 2021), snížení krevní ztráty (2012–2019 medián 100 ml, s poklesem na 50 ml v letech 2020–2021). Délka hospitalizace se ustálila na mediánu 5–6 dnů. **Závěr:** Operační léčba karcinomu endometria se stala pro většinu pacientek výkonem minimálně invazivním, snížila se průměrná krevní ztráta, délka hospitalizace. Biopsie sentinelové uzliny se stala preferovanou metodou stagingu lymfatických uzlin.

Klíčová slova: endometriální karcinom – lymfadenektomie – sentinelová uzlina – totální laparoskopická hysterektomie – děložní manipulátor

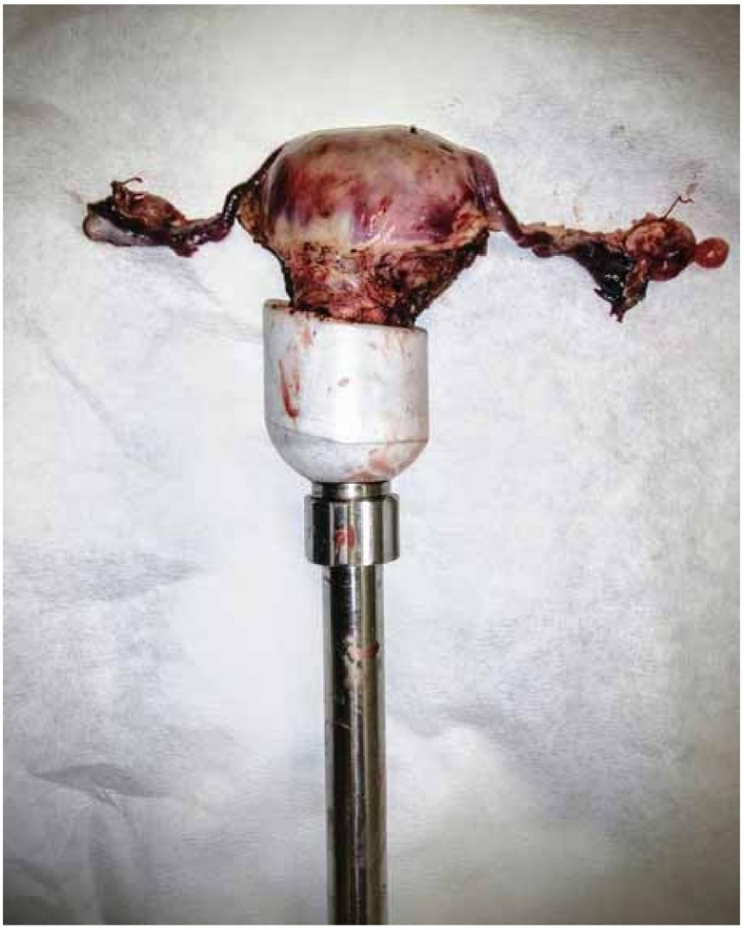
Summary: **Introduction:** In the last decade, the view of endometrial cancer has shifted enormously, and the surgical approach or lymph node staging has changed significantly. We are presenting these changes with the University Hospital Brno Oncogynecology center's results in the years 2012–2021 in the actual national and European guidelines context. **Methods:** The retrospective unicentric observational study, national and European guidelines review. **Results:** In the observation period, 715 endometrial cancer patients were treated in our clinic, and 636 of them underwent surgical treatment (89%). Concerning lymph node staging, firstly, there is a clear trend of expanding lymphadenectomy to the paraaortic area, followed by the sentinel node biopsy introduction in the years 2018–2019, and finally, the complete transition to this method as the main staging procedure in 2021, when this examination was performed in 73% of surgeries, even with high-risk cancers limited to the uterus. Within the sentinel node biopsy expansion, a gradual decrease in laparotomy approach (maximum 41% in 2016, 18% in 2021), and blood loss (2012–2019 median 100 mL, with a decrease to 50 mL in 2020–2021) was evident. A hospitalization length stabilized at a median of 5–6 days. **Conclusions:** Surgical treatment of endometrial cancer has become a minimally invasive procedure for the majority of patients, the average blood loss and hospitalization length have decreased. Sentinel node biopsy has become the preferred lymph node staging method.

Key words: endometrial cancer – lymphadenectomy – sentinel lymph node – total laparoscopic hysterectomy – uterine manipulator

Úvod

Zhoubný nádor těla dělohy je v ČR nejčastější gynekologickou malignitou (karcinom prsu nepočítaje) s incidencí 35/100 000 žen v roce 2018 [1]. Karcinom endometria (EC – endometrial carcinoma) je pak jeho nejčastější histologickou variantou. Vzhledem k časné symptomatologii je většina onemocnění (67 %) diagnostikována ve stadiu I dle FIGO (the International Federation of Gynecology and Obstetrics) klasifikace s vynikajícím 5letým přežitím blížícím se 90 % [2]. Výrazně se však zhoršuje u vyšších stadií a s horšími prognostickými vlastnostmi nádoru.

Pohled na EC se v poslední dekádě dramaticky proměnil. Největší změny se udály na poli histopatologického členění a ve stagingu lymfatických uzlin. Tradiční dělení karcinomu do dvou typů (typ I: 80–90 %, estrogen dependentní, endometroidní/mucinózní typ, vznik v terénu hyperplastického endometria; typ II: 10–20 %, na estrogenu nezávislé, non-endometroidní typy, vznik v terénu atrofického endometria) [3] je dnes zcela nedostačující a je doporučeno používat čtyři nové molekulární podskupiny, které lépe vystihují biologické chování nádorů a reálnou prognózu pacientek [4].



Obr. 1. Děložní manipulátor.
Fig. 1. Uterine manipulator.

Druhým důležitým milníkem pak byla právě změna trendu operační léčby a stagingu lymfatických uzlin. Původně poměrně hojně indikovaná systematická pánevní a paraaortální lymfadenektomie je dnes nahrazována minimálně invazivní biopsií sentinelové uzliny (SNB), a to (při splnění podmínek) i u high-intermediate a high risk tumorů [4].

Cílem práce je prezentovat tyto změny na výsledcích Onkogynekologického centra Gynekologicko-porodnické kliniky FN Brno a LF MU v letech 2012–2021 v kontextu aktuálních národních i evropských doporučení. Vedlejším cílem je porovnat naše výsledky z roku 2021 s vybranými ESGO (European Society of Gynecologic Oncology) indikátory kvality operační léčby, které byly publikovány v témže roce [5].

Metodika

Jedná se o retrospektivní analýzu souboru pacientek s EC léčených na Gynekologicko-porodnické klinice FN Brno a LF MU v letech 2012–2021. Z databáze klinických dat jsme vyhledali následující údaje:

- typ primární léčby (chirurgická/neoperační);
- operační přístup (laparotomický, laparoskopický, vaginální);
- typ výkonu na lymfatických uzlinách (pelvická ± paraaortální lymfadenektomie, biopsie sentinelové uzliny);
- délka operačního výkonu;
- krevní ztráta;
- peroperační komplikace (dle ESGO kritérií poranění orgánů – močového měchýře, střeva, velkých cév, nervů);
- délka hospitalizace.

V případě minimálně invazivního výkonu se jednalo o laparoskopicky asistovanou vaginální hysterektomii (LAVH) nebo totální laparoskopickou hysterektomii (TLH), s použitím děložního manipulátoru (obr. 1), kde je celý výkon vč. sutury poševního pahýlu proveden laparoskopicky (obr. 2).

Indikace k lymfadenektomii se řídila dle aktuálně platných doporučení, která se v průběhu sledovaného období změnila a jsou podrobněji popsána v diskuzi. Pacientky indikované k systematické lymfadenektomii podstoupily odstranění tukově-lymfatické tkáně z pánevní a paraaortální oblasti až po úroveň renálních žil (obr. 3).

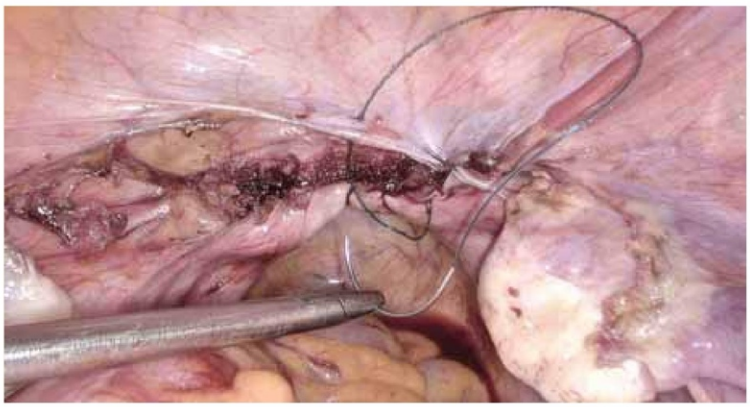
Pokud byla jako stagingová metoda zvolena detekce sentinelových uzlin (SLN – sentinel lymph node), aplikovali jsme indocyanovou zeleň intracervikálně (na čísle 3 a 9) v hloubce 5 a 20 mm, následovala detekce SLN v malé pánvi speciální kamerou pro snímání fluorescenčního záření (Novadaq Pinpoint) 15–20 min od aplikace (obr. 4). Při histopatologickém ultrastagingu byly všechny sentinelové uzliny fixovány v 10% pufrovaném formalínu, poté změřeny ve třech rozměrech a zpracovány v celém rozsahu ve 2mm lamelách. Z každé lamely byl zhotoven jeden řez o tloušťce 4 µm obarvený hematoxylinem a eozinem (HE), následoval 4 µm tlustý řez obarvený imunohistochemicky protilátkou proti cytokeratinům AE1/AE3 a poté následovaly tři řezy HE vždy po odkrojení 200 µm. Tento postup byl opakován až do úplného spotřebování každé lamely zhotovené ze SLN.

Data z posledního sledovaného roku byla podrobněji analyzována a porovnána s indikátory kvality operační léčby dle doporučení ESGO. Z 29 parametrů bylo vybráno 15, které přímo souvisí s primární operační léčbou a stagingem lymfatických uzlin.

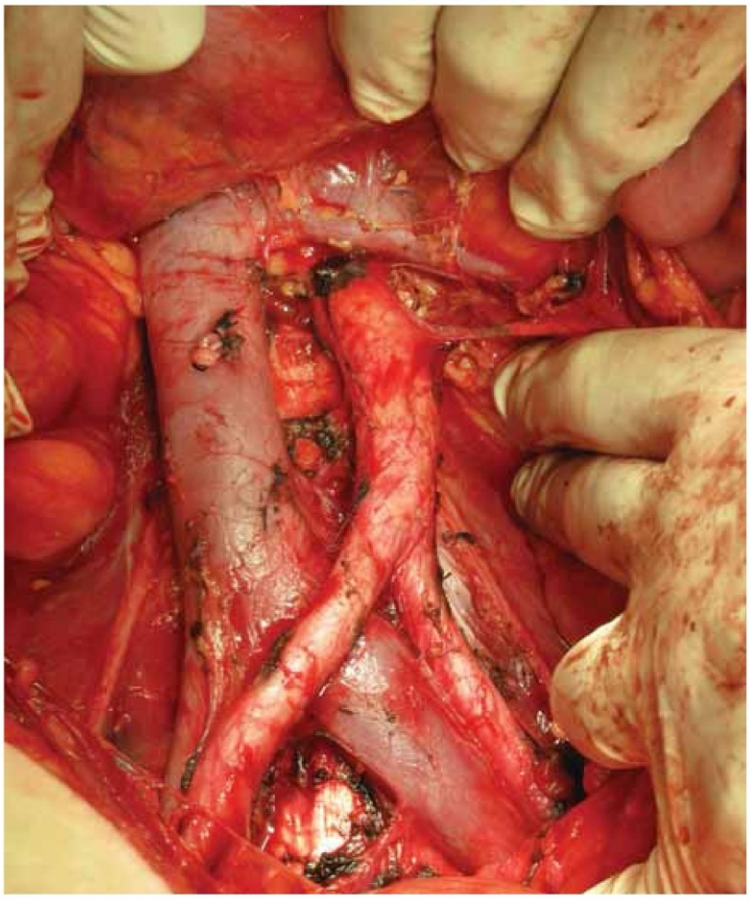
V rámci diskuze popisujeme nejdůležitější body národních a evropských guidelines týkající se chirurgické léčby a stagingu lymfatických uzlin u karcinomu endometria.

Výsledky

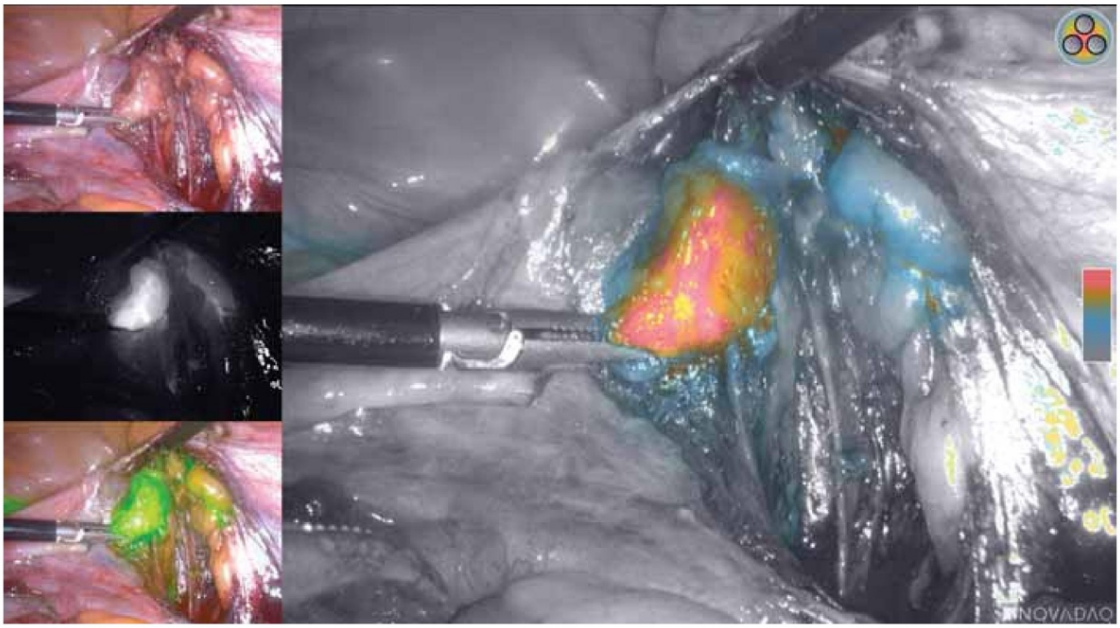
V letech 2012–2021 bylo na Gynekologicko-porodnické klinice FN Brno a LF MU diagnostikováno a léčeno 715 pacientek s karcinomem endometria (vč. karcinosarkomu), primárně operační léčbu



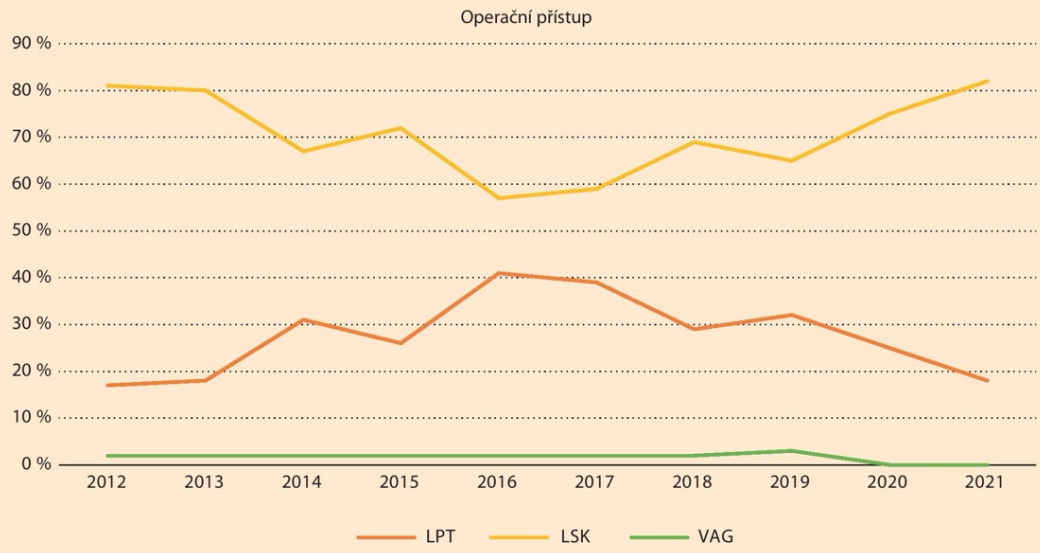
Obr. 2. Sutura poševního pahýlu laparoskopicky s použitím V-lock stehu.
Fig. 2. V-lock suture of the vaginal cuff.



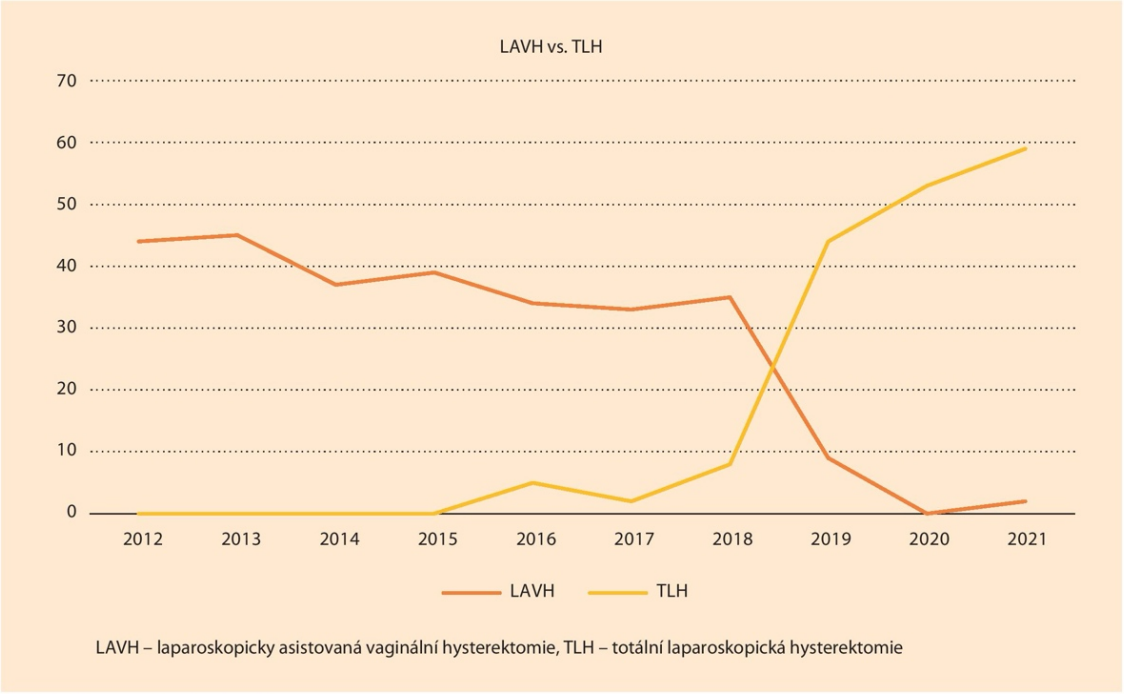
Obr. 3. Retroperitoneum po provedené pánevní a paraaortální lymfadenektomii po úroveň levé renální žíly.
Fig. 3. Retroperitoneal space after pelvic and paraaortic lymphadenectomy to the level of the left renal vein.



Obr. 4. Detekce pánevní sentinelové uzliny pomocí kamery Novadaq Pinpoint.
Fig. 4. Pelvic sentinel node detection using the Novadaq Pinpoint camera.



Graf 1. Typy operačních přístupů.
Graph 1. Surgical approaches.



LAVH – laparoskopicky asistovaná vaginální hysterektomie, TLH – totální laparoskopická hysterektomie

Graf 2. Miniinvazivní techniky.
Graph 2. Mini-invasive techniques.

podstoupilo 636 z nich (89 %). Trend přechodu laparotomického přístupu v laparoskopický ukazuje graf 1, vaginální přístup se vyskytuje po celou dobu jen v ojedinělých případech. Ve sledovaném období došlo k zavedení metody TLH, která postupně zcela nahradila LAVH (graf 2). Ve stagingu lymfatických uzlin nejprve dominuje samostatná pánevní lymfadenektomie, ke které se záhy přidává paraaortální, až jsou konečně vytlačeny metodou detekce sentinelové uzliny v roce 2020–2021 (graf 3). V případě systematické aortopelvické lymfadenektomie bylo průměrně odstraněno 62 uzlin.

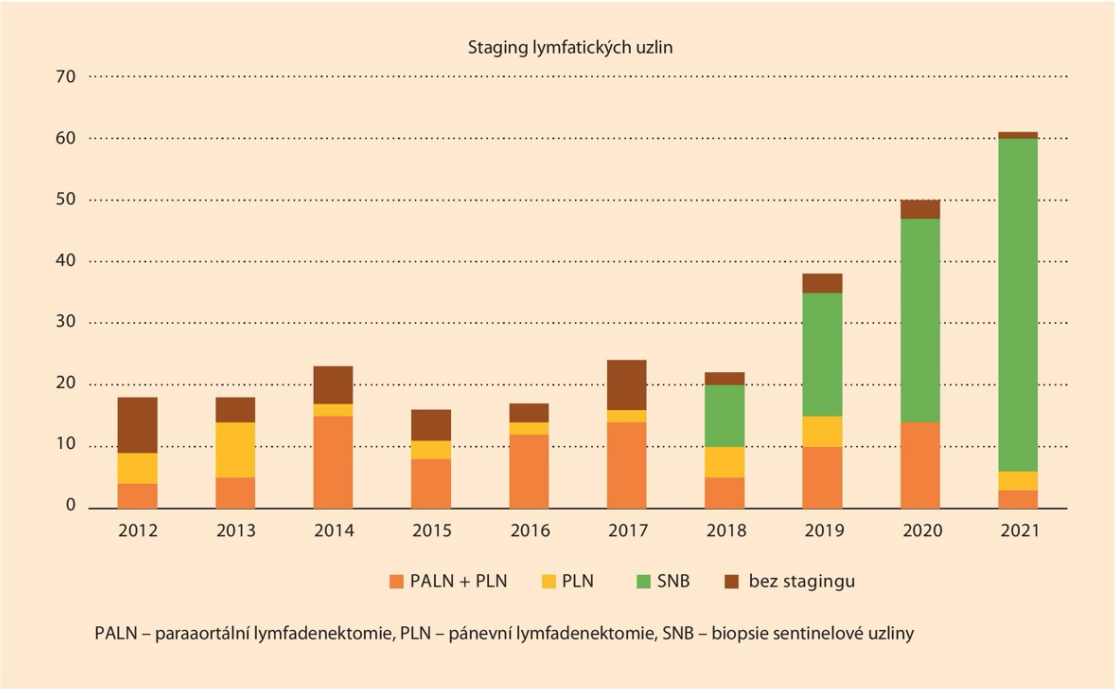
Medián krevní ztráty se v letech 2012–2019 drží na 100 ml, s poklesem na 50 ml v posledních 2 letech. Délka operačního výkonu se prodloužila z mediánu 90 min na počátku sledovaného období na 115 min v roce 2020. Délka hospitalizace se z původních 7–8 dnů zkrátila na 5–6.

Peroperační komplikace (ve smyslu poranění orgánů) se v první půlce sledovaného období vyskytly v 5–6 % případů, od roku 2017 je to < 3 %.

Tab. 1 porovnává vybrané ESGO indikátory kvality operační léčby karcinomu endometria s výsledky našeho Onkogynekologického centra z roku 2021.

Diskuze

Pohled na operační léčbu a staging lymfatických uzlin u karcinomu endometria se v posledních letech výrazně proměnil. V roce 2013 byl Onkogynekologickou sekcí ČGPS (Česká gynekologická a porodnická společnost) vydán aktualizovaný doporučený postup Guidelines gynekologických zhoubných nádorů: Standard – Komplexní léčba zhoubných nádorů endometria [6]. U pacientek s operabilním karcinomem endometria se za základní chirurgický výkon považovala extrafasciální hysterektomie s bilaterální adnexektomií, která byla dostačující u low risk nádorů (endometroidní/mucinózní histologický typ stadium I, grade 1 bez ohledu na invazi do myometria; endometroidní/mucinózní typ stadium I, grade 2, invaze zasahující < 1/2 myometria), v této skupině byly kromě abdominálního přístupu doporučeny i miniinvazivní techniky (LAVH, TLH). U pacientek s předpokládaným vysokým rizikem postižení lymfatických uzlin (high risk) byl doporučen kompletní chirurgický staging v rozsahu pánevní a paraaortální lymfadenektomie až do úrovně levostranné renální žíly. U pacientek s invazí tumoru do děložního hrdla bylo doporučeno provést radikální hysterektomii v rozsahu radikality typu B. Techniky detekce sentinelové uzliny byly v té době určeny pouze pro standardně definované studie v rámci klinického hodnocení. Peroperačně bylo doporučeno provést histologické vyšetření dělohy k potvrzení zařazení do rizikové skupiny. V případě změny na high risk byl výkon extendován o systematickou aortopelvickou lymfadenektomii.



Graf 3. Typ operačního výkonu u pacientek indikovaných ke stagingu lymfatických uzlin.
Graph 3. Type of surgical procedure in patients indicated for lymph node staging.

Další aktualizace již Onkogynekologická sekce nevydává a řídíme se evropskými guidelines, která vydávají v roce 2016 společnosti ESMO (European Society for Medical Oncology), ESGO a ESTRO (European SocieTy for Radiotherapy and Oncology) [7]. Standardním chirurgickým výkonem zůstává extrafasciální hysterektomie s bilaterální adnexektomií bez poševní manžety. Radikální hysterektomie u nádorů postihujících stroma děložního hrdla není nadále doporučována. Odstranění parametrií připadá v úvahu při jejich postižení tumorem ve snaze o dosažení volného resekčního okraje, a tedy nulového pooperačního rezidua. Minimálně invazivní výkony jsou doporučeny u low a intermediate risk karcinomů, mohou být zváženy i u high risk tumorů. Pokud je prováděna lymfadenektomie, je rovněž doporučeno systematické odstranění uzlin pánevních a paraaortální až do úrovně renálních žil. Biopsie sentinelové uzliny je stále považována za experimentální metodu, nicméně s upozorněním na rozsáhlé studie, které ukazují na použitelnost u karcinomu endometria [8,9]. Díky této technice a ultrastagingu uzlin roste záchyt mikrometastáz a izolovaných nádorových buněk, jejich význam je ovšem zatím nejasný. Lymfadenektomie není indikována u pacientek s nízkým rizikem postižení uzlin (grade 1/2, invaze do myometria < 50 %), zvažována je jako stagingová metoda u intermediate risk tumorů (grade 1/2 s invazí do myometria > 50 % nebo grade 3 s invazí do myometria < 50 %), doporučena je pak u high risk karcinomů (grade 3, s invazí do myometria > 50 %), u klinického/peroperačního stadia II a non-endometroidních typů.

Nejnovější guidelines pak vydává ESGO/ESTRO/ESP (European Society of Pathology) v roce 2020 [4]. Zdůrazněna je role miniinvazivních technik i u high risk tumorů (mimo případy s metastázami mimo dělohu a čípek). Nově je metoda SNB považována za adekvátní stagingový výkon u low-/intermediate risk karcinomů, kde nemusí být provedena při absenci invaze do myometria. Dále může být i alternativou systematické lymfadenektomie u high-intermediate risk nebo high risk tumorů stadia I a II. Lymfadenektomie je prováděna v rámci debulkingu u makroskopicky zvětšených uzlin, případně při peroperační pozitivitě SNL, kdy při její lokalizaci v oblasti pánevní je doporučeno odstranit pouze klinicky zvětšené uzliny v oblasti pánve a výkon doplnit o systematickou suprapelvickou lymfadenektomii. Preferovanou metodou je intracervikální injekce (ICG – indocyanin green) a následný patologický ultrastaging SLN. Detekce mikrometastáz (< 2 mm) je považována za metastatické postižení spolu s makrometastázami (> 2 mm), význam nálezu ITC (izolované nádorové buňky, < 0,2 mm) je stále nejasný.

Tab. 1. Vybrané indikátory kvality operační léčby dle ESGO kritérií.
Tab. 1. Selected indicators of the quality of surgical treatment according to ESGO criteria.

Indikátor kvality	Cíl dle ESGO	Realita GPK FN Brno 2021
Počet nově diagnostikovaných a léčených případů karcinomu endometria za rok	optimálně ≥ 90 minimálně ≥ 50	86
Počet primárně chirurgicky léčených případů karcinomu endometria (počáteční i pokročilé)	optimálně ≥ 80 minimálně ≥ 50	74
Operace provedené specialistou v onkogynekologii/trénovaným chirurgem v onkogynekologické operativě	≥ 95 %	97 %
Procento pacientek s počátečním stádiem, které podstoupí úspěšně mini invazivní operační výkon	optimálně ≥ 80 % minimálně 60 %	87 %
Procento pacientek s BMI > 35 s úspěšným mini invazivním operačním výkonem	> 60 %	88 %[a]
Procento konverzí mini invazivních operací na laparotomie	< 10 %	1,50 %
Peroperační poranění orgánů	< 2 %	2,70 %
Provedení stagingu lymfatických uzlin u pacientek s předpokladem počátečních stadií (I–II) s high-intermediate nebo high risk charakteristikami	> 85 %	80 %[b]
Procento biopsií sentinelových uzlin u pacientek podstupujících staging lymfatických uzlin	90 %	91 %
Počet biopsií sentinelové uzliny provedených (nebo supervize) onkogynekologem za rok	≥ 20	22–35
Procento případů s intracervikální aplikací indocyanové zeleně	≥ 95 %	100 %
Proporce high-intermediate/high risk pacientek se stranově specifickou dokončenou pánevní lymfadenektomií v případě selhání detekce sentinelové uzliny	> 90 %	100 %[c]
Procento případů vyšetřených metodou ultrastagingu sentinelové uzliny	≥ 99 %	100 %
Úspěšnost bilaterální detekce sentinelové uzliny	≥ 75 %	78 %
Procento kompletní makroskopické resekce s kurativním záměrem u pacientek s primárně pokročilým karcinomem endometria (stadium III–IV)	≥ 75 %	75 %[d]

[a] 27 pacientek S BMI > 35
[b] 10 pacientek stadia I–II s high-intermediate/high risk charakteristikami předoperačně
[c] pouze jedna pacientka předoperačně high-intermediate/high risk, kde selhala SNB
[d] čtyři pacientky ve stadiu III–IV s primárně kurativním záměrem operačního výkonu
BMI – body mass index, GPK FN – Gynekologicko-porodnická klinika Fakultní nemocnice, ESGO – European Society of Gynecologic Oncology, SNB – biopsie sentinelové uzliny

Z analýzy výsledků desetiletého období na naší klinice jasně vyplývá vzestupný trend laparoskopických operačních technik doprovázený menší krevní ztrátou, snížením peroperačních komplikací a zkrácením doby hospitalizace. Nejnovější evropská doporučení jednoznačně tento trend propagují. Dle ESGO kritérií indikátorů kvality operační léčby karcinomu endometria je vyžadováno minimálně 60 %, ale optimálně > 80 % operací provést miniinvazivně [5]. Důležité je úspěšné zvládnutí těchto technik i u obézních pacientek, kde může tento faktor výrazně ovlivnit pooperační hojení rány a morbiditu. V roce 2021 jsme operačně léčili 27 pacientek s BMI > 35 kg/m^2, z nich 88 % podstoupilo úspěšně laparoskopický výkon.

Aktuálně preferovaným výkonem je SNB, doporučuje se provádět až u 90 % pacientek podstupujících staging lymfatických uzlin [5]. Metodou volby je intracervikální aplikace ICG. S nástupem této techniky a s navýšením počtu stagingů lymfatických uzlin došlo celkově k prodloužení průměrného času operačního výkonu. Nicméně SNB jako alternativa systematické aortopelvické lymfadenektomie je výkon výrazně kratší.

Se vzrůstající oblibou miniinvazivního operování se do popředí výkonů na naší klinice dostala technika TLH s využitím děložního manipulátoru a s následnou laparoskopickou suturou poševního pahýlu. Byť je laparoskopické operování v evropských guidelines silně podporováno, diskuze ohledně onkologické bezpečnosti při použití děložního manipulátoru u karcinomu endometria zde chybí, publikované retrospektivní studie mají někdy i protichůdné závěry. Ucella et al [10] prezentovali multicentrickou retrospektivní studii, v níž u 951 pacientek neprokázali zvýšené riziko recidivy ve sledovaném období (medián 46 měsíců) v případech s manipulátorem (13,5 %) a bez něj (11,6 %). Také celkové přežití (OS – overall survival), specifické přežití (DSS – disease specific survival) a přežití bez onemocnění (DFS – disease free survival) byla srovnatelná. Tyto výsledky podporují i závěry metaanalýzy publiko-

vané v roce 2020, která zahrnuje 11 studií a ukazuje, že užití děložního manipulátoru není asociováno s pozitivní peritoneální cytologií, vyšším výskytem lymfangioinvaze (LVSI – lymphovascular space invasion) nebo recidivy u pacientek s EC [11].

Překvapivé výsledky přináší retrospektivní multicentrická studie zahrnující 2 661 žen z 15 center ve Španělsku [12]. Výskyt recidivy u pacientek s použitím manipulátoru (11,7 %) a bez něj (7,4 %) byl signifikantní ($p < 0,001$), autoři doporučují prospektivní studie k potvrzení výsledků.

Naopak nejnovější prospektivní randomizovaná studie z Itálie, kde bylo zařazeno 154 pacientek v počátečním stadiu EC, opět podporuje názory na onkologickou bezpečnost při použití manipulátoru [13]. V obou sledovaných skupinách nebyl rozdíl ve výskytu recidivy (7,9 vs. 5,2 %; $p = 0,486$).

V každém případě je s výhodou u karcinomu endometria využívat manipulátor s uchycením čípku na bázi amerických kleští, bez vrutu, který by mechanicky hmoždil děložní hrdlo. Případně lze použít samostatně keramickou část manipulátoru k distenzi poševní klenby.

U karcinomu endometria došlo v posledních letech k dramatickému odklonu od provádění systematické pánevní a paraaortální lymfadenektomie a jako stagingová metoda je nahrazována SNB. Kurativní význam lymfadenektomie je kontroverzní, týká se zejména debulkingu při makroskopicky zvětšených uzlinách. Se zaváděním molekulární klasifikace však dochází k retrospektivním re-analýzám velkých kohort pacientek s jasně definovanou pooperační léčbou a přežitím a jejich překlasifikování do rizikových skupin. Zde by mohl být prostor pro znovunalezení významu u některého subtypu. Nebo je tento výkon u EC již zcela překonán? Otázkou též zůstává, jaká bude při současném trendu kompetence mladé generace onkogynekologů stran operování v retroperitoneu a s tím související řešení komplikací v této oblasti.

Měli bychom (vzhledem k miniinvazivitě výkonu, malému množství peroperačních komplikací a naopak velkému informačnímu přínosu) provádět detekci a biopsii sentinelových uzlin u všech pacientek, nebo jen od určitého rizika? Vzhledem k elevaci počtu ultrastagingem vyšetřených sentinelových uzlin by mohl vzniknout již dostatečně velký soubor případů s ITC a my se snad díky multicentrickým retrospektivním studiím dozvíme prognostických význam tohoto nálezu, který je u EC zatím neznámý.

Výsledky Onkogynekologického centra Gynekologicko-porodnické kliniky FN Brno splňují požadavky ESGO kritérií indikátorů kvality operační léčby, které jsou komplexním nástrojem k hodnocení evropských onkogynekologických center. Dle aktuálních poznatků lze operaci pacientky s karcinomem endometria doporučit jen v onkogynekologických centrech s dostupností metody detekce sentinelové uzliny s jednoznačnou preferencí využití intracervikální aplikace ICG.

Závěr

Přístup k operační léčbě a stagingu lymfatických uzlin u karcinomu endometria se za poslední dekádu výrazně proměnil. Lymfadenektomie ztratila svůj kurativní význam a jako stagingová metoda byla vytlačena detekcí sentinelové uzliny. Důraz je aktuálně kladen na miniinvazivitu výkonu, která je považována za důležitý indikátor kvality operační léčby. Snížila se průměrná krevní ztráta, délka hospitalizace a operační komplikace na úkor prodloužení operačního času při SNB u těch případů, kde by v minulosti staging uzlin proveden nebyl.

Literatura

1. Dušek L, Mužík J, Kubásek M, et al. Epidemiologie zhoubných nádorů v České republice. Masarykova univerzita. 2007 [online]. Dostupné z: http://www.svod.cz.
2. Creasman WT, Odicino F, Maisonneuve P et al. Carcinoma of the corpus uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet 2006; 95(Suppl 1): S105–S143. doi: 10.1016/S0020-7292(06)60031-3.
3. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15(1): 10–17. doi: 10.1016/0090-8258(83)90111-7.
4. Concin N, Matias-Guiu X, Vergote I et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer 2021; 31(1): 12–39. doi: 10.1136/ijgc-2020-002230.
5. Concin N, Planchamp F, Abu-Rustum NR et al. European Society of Gynaecological Oncology quality indicators for the surgical treatment of endometrial carcinoma. Int J Gynecol Cancer 2021; 31(12): 1508–1529. doi: 10.1136/ijgc-2021-003178.
6. Chovanec J, Feranec R, Mouková L et al. Guideline gynekologických zhoubných nádorů: standard – komplexní léčba zhoubných nádorů endometria. 2022 [online]. Dostupné z: http://www.onkogynekologie.com/?page_id=80.
7. Colombo N, Creutzberg C, Amant F et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up. Int J Gynecol Cancer 2016; 26(1): 2–30. doi: 10.1097/IGC.0000000000000609.

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8. Ballester M, Dubernard G, Lécuru F 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(5): 469–476. doi: 10.1016/S1470-2045(11)70070-5.
9. Abu-Rustum NR. Sentinel lymph node mapping for endometrial cancer: a modern approach to surgical staging. J Natl Compr Cancer Netw 2014; 12(2): 288–297. doi: 10.6004/jnccn.2014.0026.
10. Uccella S, Bonzini M, Malzoni M et al. The effect of a uterine manipulator on the recurrence and mortality of endometrial cancer: a multi-centric study by the Italian Society of Gynecological Endoscopy. Am J Obstet Gynecol 2017; 216(6): 592.e1–592.e11. doi: 10.1016/j.ajog.2017.01.027.
11. Meng Y, Liu Y, Lin S et al. The effects of uterine manipulators in minimally invasive hysterectomy for endometrial cancer: a systematic review and meta-analysis. Eur J Surg Oncol 2020; 46(7): 1225–1232. doi: 10.1016/j.ejso.2020.03.213.
12. Padilla-Iserte P, Lago V, Tauste C et al. Impact of uterine manipulator on oncological outcome in endometrial cancer surgery. Am J Obstet Gynecol 2021; 224(1): 65.e1–65.e11. doi: 10.1016/j.ajog.2020.07.025.
13. Gueli Alletti S, Perrone E, Fedele C et al. A multicentric randomized trial to evaluate the role of uterine manipulator on laparoscopic/robotic hysterectomy for the treatment of early-stage endometrial cancer: the RO-MANHY trial. Front Oncol 2021; 11: 720894. doi: 10.3389/fonc.2021.720894.

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5.2 Bretová P, Minář L, Ovesná P, Weinberger V, Felsinger M, Koblížková M, Hausnerová J, Jandáková E, Stupková T. Predictors for sentinel lymph node mapping failure using indocyanine green injection in apparent early stages of endometrial cancer: A single-center prospective study. International Journal of Gynecology & Obstetrics. 2025; 2025;170:1214–1224.

Immediately after introducing sentinel node biopsy into clinical practice in our department, we conducted a prospective single-center study to identify predictive factors associated with the failure of sentinel lymph node mapping using intracervical injection of ICG in patients with apparently early-stage endometrial cancer.

Between June 2019 and August 2023, we prospectively enrolled 225 patients with histologically confirmed EC classified as FIGO stage I–II. All participants underwent SLN mapping as part of surgical staging, strictly following ESGO/ESTRO/ESP guidelines. The technique involved standardized intracervical injection of ICG at 3 and 9 o'clock positions, with sentinel nodes identified using near-infrared fluorescence imaging and assessed via ultrastaging. We excluded patients with advanced disease or enlarged lymph nodes, thus focusing exclusively on patients eligible for SNB per current European recommendations.

The overall detection rate was 90%, with bilateral mapping success achieved in 79% of cases. Bilateral or unilateral mapping failure was observed in 21% of patients. Our multivariable logistic regression analysis revealed that higher BMI and the presence of uterine myomas were independent predictors of overall mapping failure. When focusing on bilateral mapping failure specifically, both higher BMI and older age were confirmed as significant independent risk factors.

Importantly, histopathological features (including tumor type, grade, molecular classification, and lymphovascular space invasion) did not significantly influence mapping success. Furthermore, prior pelvic surgery and adhesiolysis were not associated with failure in our cohort. Notably, all sentinel nodes containing macrometastases were successfully identified and resected, confirming the technique's reliability in appropriately selected cases.

Our study underlines the importance of meticulous patient selection, precise ICG application techniques, and preoperative exclusion of advanced disease to maximize the success rate of sentinel node mapping. In conclusion, age, BMI, and uterine myomas should be considered in surgical planning, and further multicenter research is warranted to validate these findings and refine patient stratification strategies.

The study entitled *"Predictors for Sentinel Lymph Node Mapping Failure Using Indocyanine Green Injection in Apparent Early Stages of Endometrial Cancer: A Single-center Prospective Study"* was published in the *International Journal of Gynecology & Obstetrics* (IF 2.6, Q2) in 2025.

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CLINICAL ARTICLE

Gynecology

Predictors for sentinel lymph node mapping failure using indocyanine green injection in apparent early stages of endometrial cancer: A single-center prospective study

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Abstract

Objective: The current study aimed to analyze predictive factors of sentinel lymph node mapping failure in apparently early stages of endometrial cancer using intracervical indocyanine green injection.

Methods: A single-center prospective study was conducted between June 2019 and August 2023 at the Department of Gynecology and Obstetrics, University Hospital Brno, Czech Republic. All patients with apparently early stage (I or II according to FIGO [International Federation of Gynecology & Obstetrics] 2009) endometrial cancer, who were indicated for sentinel node biopsy were consecutively included. The injection of 4–6 mL of indocyanine green was applied superficially and deeply into cervical tissue at the 3- and 9-o'clock positions. Patients' clinical data, surgical characteristics, and histopathological information were recorded. Univariable and multivariable regression analyses were applied.

Results: A total of 225 patients were eligible during the study period. Considering bilateral and unilateral failed mapping together, the only statistically significant factors for risk of failure in univariable analysis were body mass index (BMI; $P=0.036$), FIGO 2009 stage ($P=0.019$), and the presence of a myoma ($P=0.017$). Nevertheless, when the multivariable logistic regression analysis was applied, all factors became statistically insignificant except for myoma ($P=0.031$). Regarding only bilateral mapping failure, in univariable analysis, BMI ($P=0.021$) and FIGO 2009 stage ($P=0.046$) were significant predictors of failure. Interestingly, multivariable logistic regression analysis revealed that in addition to BMI ($P=0.007$), age ($P=0.004$) was also an independent predictor of bilateral failure.

Conclusions: Higher BMI and age were statistically significant independent factors for bilateral sentinel node mapping failure in early-stage endometrial cancer.

KEYWORDS
biopsy, endometrial cancer, myomas, obesity, sentinel lymph node

1 | INTRODUCTION

Sentinel node biopsy (SNB) has become the leading lymph node surgical staging method in endometrial cancer (EC) in recent years. Initially, only low-risk and intermediate-risk patients underwent SNB; however, according to the guidelines of the European Society of Gynecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP), SNB is now an acceptable alternative to systematic lymphadenectomy, even for women with high-intermediate and high-risk early stage (I or II) EC.[1] Furthermore, according to ESGO quality indicators for the surgical treatment of EC, SNBs should be performed in 90% of patients undergoing lymph node staging.[2] Side-specific pelvic lymphadenectomy should be provided in cases of SNB failure, and debulking of enlarged lymph nodes is performed regardless of mapping.[1]

In addition to reducing postoperative morbidity associated with systematic lymphadenectomy,[3] SNB allows more precise pathological assessment and detection of low-volume metastases: micrometastasis and isolated tumor cells (ITCs), which could be missed by standard histological evaluation of multiple lymph nodes.[4]

The use of indocyanine green (ICG) has significantly improved the accuracy of sentinel lymph node (SLN) detection, supplementing the traditionally used technetium (Tc99) and blue dyes. Bilateral detection using intracervical injection of ICG achieves a 78%–85% success rate.[5–8]

The importance of successful bilateral SLN mapping is indisputable; however, there are still only a few studies focusing on risk factors of SNB failure in EC using intracervical ICG injection. Understanding these factors is crucial for surgerical planning strategies. A recent meta-analysis by Raffone et al. included only six studies.[9] They identified ICG <3 mL, FIGO (International Federation of Gynecology & Obstetrics) stage III or IV, enlarged lymph nodes, and lymph node involvement as significant predictive factors of SLN mapping failure.

As SNB is now the leading surgical staging method, we believe it is important to understand the main limitations of the procedure. Our study aimed to analyze predictive factors of SLN mapping failure in apparently early stages of EC using intracervical ICG injection while excluding already known risk factors, which could be eliminated (enlarged lymph nodes, advanced disease, ICG <3 mL). We analyzed both bilateral and unilateral mapping failure to determine whether there are factors that might have a local or systemic impact on SLN detection success.

2 | MATERIALS AND METHODS

2.1 | Patient cohort and data collection

The prospective study took place at the Department of Gynecology and Obstetrics, University Hospital Brno between June 2019 and August 2023. All patients with apparently early stage of EC (FIGO stage I or II) indicated by the Multidisciplinary Oncogynecologic Tumor Board of University Hospital Brno for surgical treatment with SNB (according to ESGO/ESTRO/ESP[1] recommendations) were consecutively included. Patients with suspicion of advanced disease (FIGO stage III or IV), cancer duplicity, and those deemed unsuitable for surgical treatment were excluded (Figure 1). Data were collected from an institutional database and surgical and histological reports.

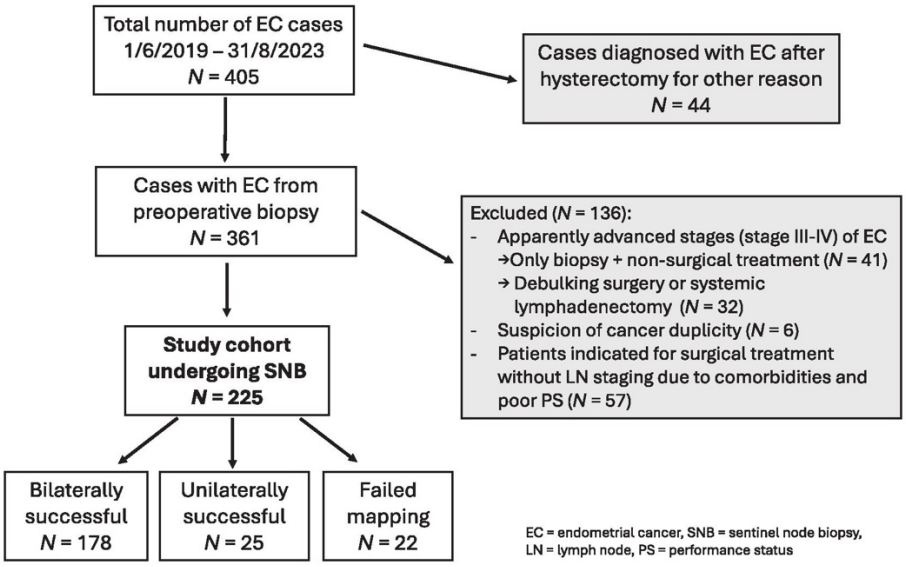

FIGURE 1 Flowchart describing the study population. EC, endometrial cancer; LN, lymph node; PS, performance status; SNB, sentinel node biopsy.

TABLE 1 Clinical and histopathological characteristics.

Characteristic	Overall, N = 225	Bilateral success, N = 178	Failure, N = 47
Age (years)			
Mean (SD)	65 (10)	64 (10)	66 (9)
Median (IQR)	65 (58–72)	65 (58–72)	65 (61–73)
BMI			
Mean (SD)	32 (6)	31 (6)	34 (7)
Median (IQR)	32 (28–36)	31 (27–36)	33 (29–37)
Cone biopsy	13 (100.0%)	11 (84.6%)	2 (15.4%)
Myomectomy	2 (100.0%)	2 (100.0%)	0 (0.0%)
Cesarean section	24 (100.0%)	17 (70.8%)	7 (29.2%)
Adnexal surgery	18 (100.0%)	14 (77.8%)	4 (22.2%)
Retroperitoneal surgery	0 (NA)	0 (NA%)	0 (NA)
Previous pelvic radiotherapy	0 (NA)	0 (NA%)	0 (NA)
Previous chemotherapy	3 (100.0%)	2 (66.7%)	1 (33.3%)
Histotype			
Endometrioid (including mucinous)	211 (100.0%)	168 (79.6%)	43 (20.4%)
Nonendometrioid	14 (100.0%)	10 (71.4%)	4 (28.6%)
Grade			
Low grade (grade 1 or 2)	189 (100.0%)	149 (78.8%)	40 (21.2%)
High grade (grade 3)	36 (100.0%)	29 (80.6%)	7 (19.4%)
Myometrial infiltration			
<50%	173 (100.0%)	136 (78.6%)	37 (21.4%)
≥50%	52 (100.0%)	42 (80.8%)	10 (19.2%)
Cervical involvement	32 (100.0%)	27 (84.4%)	5 (15.6%)
Tumor size			
<2 cm	115 (100.0%)	105 (91.3%)	10 (8.7%)
≥2 cm	110 (100.0%)	98 (89.1%)	12 (10.9%)
Tumor localization			
Uterine fundus	192 (100.0%)	176 (91.7%)	16 (8.3%)
Lower uterine segment	16 (100.0%)	14 (87.5%)	2 (12.5%)
Uterine fundus + lower segment	17 (100.0%)	13 (76.5%)	4 (23.5%)
FIGO 2009 stage			
IA	153 (100.0%)	119 (77.8%)	34 (22.2%)
IB	23 (100.0%)	18 (78.3%)	5 (21.7%)
II	19 (100.0%)	16 (84.2%)	3 (15.8%)
IIIA	8 (100.0%)	5 (62.5%)	3 (37.5%)
IIIB	3 (100.0%)	1 (33.3%)	2 (66.7%)
IIIC	19 (100.0%)	19 (100.0%)	0 (0.0%)
FIGO 2009 stage			
Local disease (IA to II)	195 (100.0%)	153 (78.5%)	42 (21.5%)
Advanced disease (IIIA to IIIC)	30 (100.0%)	25 (83.3%)	5 (16.7%)
LVSI			
No	144 (100.0%)	112 (77.8%)	32 (22.2%)
Focal	45 (100.0%)	35 (77.8%)	10 (22.2%)
Substantial	36 (100.0%)	31 (86.1%)	5 (13.9%)
MELF	33 (100.0%)	26 (78.8%)	7 (21.2%)

TABLE 1 (Continued)

Characteristic	Overall, N = 225	Bilateral success, N = 178	Failure, N = 47
Uterine length			
Mean (SD)	84 (18)	84 (17)	82 (23)
Median (IQR)	80 (70–90)	80 (70–90)	80 (70–90)
Myoma	104 (100.0%)	75 (72.1%)	29 (27.9%)
Adenomyosis	50 (100.0%)	36 (72.0%)	14 (28.0%)
Molecular classification			
Unknown	66 (100.0%)	46 (69.7%)	20 (30.3%)
POLEmut	2 (100.0%)	2 (100.0%)	0 (0.0%)
MMRd	54 (100.0%)	45 (83.3%)	9 (16.7%)
NSMP	89 (100.0%)	76 (85.4%)	13 (14.6%)
p53mut	14 (100.0%)	9 (64.3%)	5 (35.7%)
Prognostic risk group			
Low	126 (100.0%)	99 (78.6%)	27 (21.4%)
Intermediate	31 (100.0%)	24 (77.4%)	7 (22.6%)
High-intermediate	30 (100.0%)	24 (80.0%)	6 (20.0%)
High	38 (100.0%)	31 (81.6%)	7 (18.4%)

Note: Values are expressed as number (percentage) unless otherwise indicated.

Abbreviations: BMI, body mass index; FIGO, International Federation of Gynecology & Obstetrics; IQR, interquartile range; LVSI, lymphovascular space invasion; MELF, microcystic, elongated, and fragmented; MMRd, mismatch repair deficient; NSMP, nonspecific molecular profile; p53mut, p53 mutated; POLEmut, polymerase-ε-mutated/ultramutated; SD, standard deviation.

Informed consent to use their clinical and histopathological data was obtained from all patients included in the study. The study was approved by the University Hospital Brno Ethics Committee (approval number 02-190122/EK).

Patient characteristics included age, body mass index (BMI), previous cervical and pelvic surgery (cone biopsy, myomectomy, cesarean section, adnexal surgery, retroperitoneal surgery), previous pelvic radiotherapy, and previous chemotherapy. Surgical characteristics recorded were approach (laparoscopic/open), adhesiolysis, length of surgery, blood loss, SLN mapping success (bilateral/unilateral/failure), and surgical and postoperative complications (perioperative bleeding, large vessels/nervous/ureter injury, postoperative lymphocyst/lymphoedema). Histopathological data reported were tumor histotype and grade, molecular classification, myometrial infiltration, cervical involvement, tumor localization and size, FIGO 2009 stage, lymphovascular space invasion (LVSI) status (absence/focal/substantial), microcystic, elongated, and fragmented (MELF) type of invasion, uterine length, presence of myomas/adenomyosis, number of SLN removed, and SLN status (negative/macrometastasis/micrometastasis/ITC).

2.2 | Preoperative staging

The preoperative staging procedure in histologically proven EC contained a gynecological clinical examination, transvaginal and transabdominal gynecologic ultrasound performed by expert ultrasonographers, and a chest-abdominal-pelvis computed tomography scan. Preoperative procedures focused on both the local spread of the disease and discovering lymph node metastases or distant metastases, which would be a criterion for the patient's exclusion from the study.

2.3 | Surgical procedure

Surgeries were performed by two groups of surgeons with different levels of experience. Advanced surgeons were defined as certified gynecologic oncologists with years of surgical experience, while trainees were fellows in gynecologic oncology working under the supervision of advanced surgeons. All of the surgeons followed the same protocol: 4–6 mL of ICG (1.25 mg/mL) were injected intracervically immediately before surgery in an operating theater. We used the technique of superficial (3–5 mm) and deep (10–20 mm) application into cervical tissue at the 3- and 9-o'clock positions. In the situation of no cervical involvement, a uterine manipulator was inserted. Sentinel nodes were searched in the retroperitoneum using the Pinpoint Fluorescence Imaging System (Novadaq) endoscopically or by portable handheld imager in case of primary open surgery or conversion into laparotomy. Removed SLNs were sent to definitive histopathological ultrastaging without perioperative frozen section. Reinjection of ICG was performed in cases of fluorescent signal absence in the retroperitoneal space. The surgery continued with standard extrafascial hysterectomy and bilateral salpingo-oophorectomy. Any macroscopically enlarged nodes (>2 cm) were removed as part of debulking regardless of mapping. Complete or side-specific pelvic lymph node dissection was added in cases of mapping failure,

TABLE 2 Univariable analysis: Bilateral or unilateral failure.

Characteristic	No. of patients	No. of failures	OR	95% CI	*P* value
Surgeon's experience					
Advanced surgeon	179	39	–	–	0.506
Trainee	46	8	0.76	0.31–1.68	
Age (years)	225	47	1.03	0.99–1.06	0.141
BMI	225	47	1.06	1.00–1.11	**0.036**
Cone biopsy					
No	212	45	–	–	0.603
Yes	13	2	0.67	0.10–2.63	
Myomectomy					
No	223	47	–	–	0.332
Yes	2	0	0.00		
Cesarean section					
No	201	40	–	–	0.309
Yes	24	7	1.66	0.61–4.13	
Adnexal surgery					
No	207	43	–	–	0.885
Yes	18	4	1.09	0.30–3.22	
Previous chemotherapy					
No	222	46	–	–	0.614
Yes	3	1	1.91	0.09–20.4	
Histotype					
Endometrioid (including mucinous)	211	43	–	–	0.481
Nonendometrioid	14	4	1.56	0.41–4.92	
Grade					
Low grade (grade 1 or 2)	189	40	–	–	0.815
High grade (grade 3)	36	7	0.90	0.34–2.10	
Myometrium infiltration					
<50%	173	37	–	–	0.735
≥50%	52	10	0.88	0.38–1.85	
Cervical involvement					
No	193	42	–	–	0.415
Yes	32	5	0.67	0.22–1.70	
Tumor size					
<2 cm	115	22	–	–	0.507
≥2 cm	110	25	1.24	0.65–2.38	
Tumor localization					
Uterine fundus	192	36	–	–	0.118
Lower uterine segment	16	4	1.44	0.39–4.42	
Uterine fundus + lower segment	17	7	3.03	1.04–8.45	
FIGO 2009 stage					
IA	153	34	–	–	**0.019**
IB	23	5	0.97	0.30–2.65	
II	19	3	0.66	0.15–2.12	
IIIA	8	3	2.10	0.41–9.00	

TABLE 2 (Continued)

Characteristic	No. of patients	No. of failures	OR	95% CI	*P* value
IIIB	3	2	7.00	0.65–153	
IIIC	19	0	0.00		
FIGO 2009 stage					
Local disease (IA to II)	195	42	–	–	0.532
Advanced disease (IIIA to IIIC)	30	5	0.73	0.23–1.88	
LVSI					
No	144	32	–	–	0.503
Focal	45	10	1.00	0.43–2.18	
Substantial	36	5	0.56	0.18–1.46	
MELF					
No	192	40	–	–	0.961
Yes	33	7	1.02	0.39–2.42	
Uterine length	225	47	1.0	0.98–1.01	0.566
Myoma					
No	121	18	–	–	**0.017**
Yes	104	29	2.21	1.15–4.34	
Adenomyosis					
No	175	33	–	–	0.171
Yes	50	14	1.67	0.79–3.41	
Molecular classification					
NSMP	89	13	–	–	0.267
MMRd	54	9	1.17	0.45–2.93	
p53mut	14	5	3.25	0.88–11.1	
POLEmut	2	0	0.00		
Prognostic risk group					
Low	126	27	–	–	0.972
Intermediate	31	7	1.07	0.39–2.65	
High-intermediate	30	6	0.92	0.31–2.35	
High	38	7	0.83	0.31–2.00	
Approach					
Laparoscopy	202	39	–	–	0.102
Laparotomy	23	8	2.23	0.85–5.52	
Adhesiolysis					
No	163	32	–	–	0.457
Yes	62	15	1.31	0.64–2.59	

Note: Bold values represent significance of $p < 0.05$.
Abbreviations: BMI, body mass index; CI, confidence interval; FIGO, International Federation of Gynecology & Obstetrics; LVSI, lymphovascular space invasion; MELF, microcystic, elongated, and fragmented; MMRd, mismatch repair deficient; NSMP, nonspecific molecular profile; OR, odds ratio; p53mut, p53 mutated; POLEmut, polymerase-ε-mutated/ultramutated.

providing the patient had preoperatively high-intermediate or high-risk disease according to ESGO/ESTRO/ESP guidelines.[1]

2.4 | Histopathological evaluation

All SLNs were fixed in 10% buffered formalin, sliced at 2-mm lamellas, embedded in paraffin, and further examined by ultrastaging protocol. This protocol consists of 4-μm-thick consecutive sections: two stained with hematoxylin–eosin and cytokeratins (AE1/AE3), followed by two additional sections stained with hematoxylin–eosin obtained at regular 200-μm intervals. This sequence of sections continued until there was no lymph node tissue left. We classified lymph nodes as follows: negative, ITCs (≤0.2 mm or single cells/clusters of cells ≤200 cells in a single SLN cross-section), micrometastasis (0.2–2 mm), and macrometastasis

(>2 mm). Histopathological examination of tumor tissue and molecular testing were performed following national and international guidelines.[1,10]

2.5 | Statistical analysis

Standard descriptive statistics were used to describe the cohort: mean, standard deviation (SD), median, and interquartile range (IQR) for continuous variables, and absolute and relative frequencies for categorical variables. Comparisons of variables between the two groups (bilateral success vs. any failure, and bilateral failure vs. any success, respectively) were performed using the Mann–Whitney test and Fisher test, respectively.

Univariable and then multivariable logistic models were created to estimate the risk of SNB mapping failure. Any of the covariates that were statistically significant in the univariable model ($P<0.10$) and clinically important factors were initially considered for inclusion in the multivariable model. Variable selection was performed using a backward stepwise approach based on Akaike Information Criterion minimization to optimize model fit while avoiding overfitting. The risk of failure was quantified by odds ratios (ORs) with 95% confidence intervals (CIs). Any failure (bilateral or unilateral) and exclusively bilateral failure were modeled. Analyses were performed in R software version 4.3.2 (R Foundation for Statistical Computing), models were conducted using the lme4 package. The significance level was set at 5%.

3 | RESULTS

During the study period, 405 patients were diagnosed with EC at our institution. Ultimately, a total of 225 patients met the eligibility criteria (Figure 1). The median patient age was 65 years, and BMI was 32 kg/m^2 (Table 1).

Most of the surgeries were laparoscopic (90%) and performed by advanced surgeons (80%). The median surgery length was 115 min and blood loss was 50 mL. Adhesiolysis was necessary in 28% of cases. Severe perioperative complications occurred in <2% of cases (major vessel injury, excessive bleeding requiring conversion to laparotomy, nerve injury, and ureter injury). Postoperative complications in <1%, with two cases of lymphocyst—one following SNB and the other after side-specific pelvic lymphadenectomy.

The overall detection rate was 90%. Bilateral success was achieved in 178 cases (79%), while unilateral success was observed in 25 cases (11%). Mapping failure was observed in 22 cases (10%).

The median number of removed SLNs in bilaterally successful procedures was three nodes. The final lymph node status was as follows: 170 negative, 14 isolated tumor cells, 12 micrometastases, and seven macrometastases. Five side-specific pelvic lymphadenectomies were oerformed in preoperatively high-intermediate and high-risk cases with SNB failure. Overall, 22 cases lacked lymph node staging.

3.1 | Bilateral or unilateral failure

Considering both bilateral and unilateral failed mapping together ($N=47$, at least unilateral failures), higher BMI ($P=0.036$), advanced FIGO 2009 stage ($P=0.019$), and the presence of a myoma ($P=0.017$) were the only statistically significant factors for mapping failure in univariable analysis. Other clinical and histopathological characteristics had minimal or no influence (Table 2).

However, when multivariable logistic regression analysis was applied, only the presence of a myoma retained statistical significance (OR, 2.08 [95% CI, 1.08–4.11], $P=0.031$), while the others became statistically insignificant (Table 3).

3.2 | Bilateral failure only

Regarding bilateral SNB failures ($N=22$), univariable analysis identified BMI ($P=0.021$) and FIGO 2009 stage ($P=0.046$) as significant factors affecting success (Table 4). Interestingly, multivariable logistic regression analysis revealed that, in addition to BMI (OR, 1.11 [95% CI, 1.03–1.2], $P=0.007$), age (OR, 1.09 [95% CI, 1.03–1.17], $P=0.008$) was also an independent predictor of bilateral SNB failure (Table 5).

4 | DISCUSSION

Currently, SLN biopsy using intracervical ICG injection is a standard procedure in EC surgical staging. However, the reasons for failed mapping are still not satisfactorily defined, because of the heterogeneity of the published studies' design and results. The most significant risk factors for bilateral failure in our analysis of 225 surgeries were BMI and age.

Our overall (90%) and bilateral (79%) detection rate was similar to other studies, despite including all possible failure cases (failed ICG migration, diffuse smearing of the ICG, and no evidence of nodal tissue on final pathology) and not excluding initial SNB attempts as part of the learning curve of each surgeon. Some of these were omitted in other studies, which could have affected their success rate.[8,11]

Obesity is one of the most debated potential risk factors for SNB failure. Eriksson et al. studied 472 patients undergoing robotic surgery using ICG or blue dye and demonstrated that successful mapping decreases with increasing BMI (irrespective of the dye used).[8] However,

TABLE 3 Multivariable analysis: Bilateral or unilateral failure.

Characteristic	OR	95% CI	*P* value
BMI	1.05	1.00–1.11	0.067
Myoma			
No	–	–	0.031
Yes	2.08	1.08–4.11	

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

TABLE 4 Univariable analysis: Bilateral failure only.

Characteristic	No. of patients	No. of bilateral failures	OR	95% CI	*P* value
Surgeon's experience					
Advanced surgeon	179	20	–	–	0.131
Trainee	46	2	0.36	0.06–1.30	
Age (years)	225	22	1.05	1.00–1.10	0.064
BMI	225	22	1.08	1.01–1.17	**0.021**
Cone biopsy					
No	212	22	–	–	0.097
Yes	13	0	0.00		
Myomectomy					
No	223	22	–	–	0.520
Yes	2	0	0.00		
Cesarean section					
No	201	18	–	–	0.264
Yes	24	4	2.03	0.55–6.12	
Adnexal surgery					
No	207	21	–	–	0.500
Yes	18	1	0.52	0.03–2.75	
Previous chemotherapy					
No	222	21	–	–	0.260
Yes	3	1	4.79	0.22–52.0	
Histotype					
Endometrioid (incl. mucinous)	211	21	–	–	0.721
Non-endometrioid	14	1	0.70	0.04–3.77	
Grade					
Low grade (grade 1 or 2)	189	18	–	–	0.772
High grade (grade 3)	36	4	1.19	0.33–3.44	
Myometrial infiltration					
<50%	173	18	–	–	0.554
≥50%	52	4	0.72	0.20–2.03	
Cervical involvement					
No	193	20	–	–	0.444
Yes	32	2	0.58	0.09–2.12	
Tumor size					
<2 cm	115	10	–	–	0.576
≥2 cm	110	12	1.29	0.53–3.17	
Tumor localization					
Uterine fundus	192	16	–	–	0.190
Lower uterine segment	16	2	1.57	0.23–6.32	
Uterine fundus + lower segment	17	4	3.38	0.88–10.9	
FIGO 2009 stage					
IA	153	16	–	–	**0.046**
IB	23	4	1.80	0.48–5.55	
II	19	0	0.00		
IIIA	8	2	2.85	0.40–13.6	
IIIB	3	0	0.00		
IIIC	19	0	0.00		

TABLE 4 (Continued)

Characteristic	No. of patients	No. of bilateral failures	OR	95% CI	*P* value
FIGO 2009 stage					
Local disease (IA or II)	195	20	–	–	0.519
Advanced disease (IIIA or IIIC)	30	2	0.63	0.10–2.31	
LVSI					
No	144	16	–	–	0.632
Focal	45	3	0.57	0.13–1.82	
Substantial	36	3	0.73	0.16–2.35	
MELF					
No	192	18	–	–	0.633
Yes	33	4	1.33	0.37–3.89	
Uterine length	225	22	1.01	0.99–1.04	0.226
Myoma					
No	121	9	–	–	0.203
Yes	104	13	1.78	0.73–4.49	
Adenomyosis					
No	175	14	–	–	0.111
Yes	50	8	2.19	0.83–5.47	
Molecular classification					
NSMP	89	6	–	–	0.646
MMRd	54	6	1.73	0.51–5.82	
P53mut	14	2	2.31	0.31–11.4	
POLEmut	2	0	0.00		
Prognostic risk group					
Low	126	12	–	–	0.921
Intermediate	31	4	1.41	0.37–4.41	
High-intermediate	30	3	1.06	0.23–3.61	
High	38	3	0.81	0.18–2.74	
Approach					
Laparoscopy	202	18	–	–	0.231
Laprotomy	23	4	2.15	0.58–6.51	
Adhesiolysis					
No	163	15	–	–	0.642
Yes	62	7	1.26	0.46–3.15	

Note: Bold values represent significance of $p < 0.05$.

Abbreviations: BMI, body mass index; CI, confidence interval; FIGO, International Federation of Gynecology & Obstetrics; LVSI, lymphovascular space invasion; MELF, microcystic, elongated, and fragmented; MMRd, mismatch repair deficient; NSMP, nonspecific molecular profile; OR, odds ratio; p53mut, p53 mutated; POLEmut, Polymerase-ε-mutated/ultramutated.

they did not identify a critical BMI cutoff at which the mapping rate changes abruptly. Nevertheless, this finding has not been confirmed in other series.[11,12] In our study, the higher BMI significantly affected only bilateral mapping success in both univariable and multivariable analyses. This may support the theory that increasing BMI could modify the physiological pelvic lymphatic drainage by increasing vascular permeability and causing interstitial edema[13] or simply make it more difficult to visualize lymphatic tissue due to the increased visceral adipose tissue.[8]

A similar hypothesis of increasing vascular permeability is considered regarding older age.[12] In our cohort, age was a significant risk factor for bilateral mapping failure in multivariable analysis. Every additional year was associated with a 10% higher risk of failure. However, together with unilateral failure, age became statistically insignificant. This suggests that age may affect lymphatic drainage systematically. Nonetheless, other authors have not confirmed age as a significant factor.[11,12,14]

Considering possible local factors influencing mapping success, the presence of a myoma ($n = 104$, 46% of cases) was an interesting finding in our study. Using univariable analysis, it was a significant predictor ($P = 0.017$) of overall failure and remained significant in multiple regression analysis ($P = 0.031$). Similar results were

TABLE 5 Multivariable analysis: Bilateral failure only.

Characteristic	OR	95% CI	*P* value
Age (years)	1.09	1.03–1.17	**0.008**
BMI	1.11	1.03–1.20	**0.007**

Note: Bold values represent significance of $p < 0.05$.
Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

published by Sozzi et al.[15] Depending on the localization and size of the myoma, it may influence the physiological lymphatic uterine drainage; however, further investigations are necessary to confirm this theory. Histopathological characteristics (histology type, grade, LVSI, molecular classification) had minimal or no influence in our cases, in contrast with the study mentioned above. Using multivariate analysis, the study by Sozzi et al. identified LVSI (OR, 2.4 [95% CI, 1.04–1.12], $P = 0.003$) and nonendometrioid histology (OR, 3.0 [95% CI, 1.43–6.29], $P = 0.004$) as independent predictors of mapping failure.[15] Nevertheless, other studies have confirmed our results and report these factors as insignificant.[11,12,14,16]

Although larger tumors, those with deep myometrial invasion, or tumors extending into the lower uterine segment or cervical stroma could potentially alter lymphatic drainage patterns, leading to atypical sentinel node localization or mapping failure, this was not observed in our study, nor in others.[11,12,14] On the other hand, in a prospective study in which different methods of sentinel node mapping (ultrasound-guided myometrial injection of radiotracer) were applied, a tumor size <2 cm was associated with a higher SLN preoperative detection rate.[17] Other studies focusing on factors that affect sentinel node localization are needed to explain this theory in more detail.

Advanced FIGO stage (III or IV) and enlarged lymph nodes are the most important risk factors for SNL mapping failure, as previously reported.[11,15,16] A possible explanation for this is blocked lymphatic flow and the presence of tumorous thrombi in cases with high-volume metastases.[11,14] Nevertheless, we did not prove this in our current study, as we focused on preoperative staging to exclude all cases with bulky lymph nodes and distant metastases, fully adhering to the European guidelines for SNB indications.[1] As a result, there was no association between positive lymph node status and mapping failure. Even in the case of macrometastasis, all seven were successfully identified and removed. Additionally, there was no FIGO IV stage in the final pathological statement. In conclusion, ICG sentinel node mapping works well even in nodes with macrometastases under conditions where enlarged lymph nodes are detected preoperatively and excluded.

Previous pelvic surgeries (adnexal surgery: $n = 18$ [8%]; myomectomy: $n = 2$ [1%]; cesarean section: $n = 24$ [11%]) had no impact on SLN mapping in our cohort as well as in other series.[11,12] However, the main reason could be the absence of previous retroperitoneal surgeries in our cases, in which the surgical dissection of the parametria could damage the physiological lymphatic drainage.[12] This might be an important factor in the future given the increasing number of patients undergoing excessive surgeries due to deep infiltrative endometriosis. Another potential risk factor not proven in our study was lysis of adhesions. To eliminate the potential adverse effect of adhesiolysis, this procedure should be performed at the very beginning of surgery, before ICG injection.[11] Consequently, the patient's medical history is an integral part of surgery planning—not only the anamnesis of previous pelvic operations but also any history of previous abdominal inflammatory diseases are crucial factors.

Regarding the surgical procedure, we used cervical grasping by uterine manipulator immediately after the injection of ICG, except in cases with suspicious cervical tumor infiltration. According to a previous study, this practice has no impact on SLN detection.[17] Additionally, we avoided another risk factor by using an ICG dose of 4–6 mL and performing reinjection in cases of failed ICG migration. It has been shown that an ICG dose <3 mL is associated with a higher risk of mapping failure.[9]

We consider the strengths of our study to be its prospective design and uniform surgical procedure. All surgeons followed the same application protocol to minimize the surgeon's influence, which was the only significant parameter for failure described by Ianieri et al.[12] Nevertheless, those authors acknowledged that, although all surgeons were experts in the field of gynecologic oncology, the ICG procedure was not always performed by the same surgeon, but rather by several gynecologic oncology fellows.

The study's limitations include its single-center design and nonrandomized allocation of cases to surgeons, which followed clinical practice. Presuming that more difficult surgeries (especially those involving patients who were morbidly obese or with a history of repeated surgical interventions) were predominantly assigned to more experienced surgeons, this resulted in a higher detection rate in the trainees' group, although this difference was not statistically significant.

5 | CONCLUSIONS

In our single-institution study, we showed that higher BMI and age are statistically significant factors for bilateral mapping failure. Additionally, the presence of a myoma was a significant local risk factor for overall (unilateral or bilateral) failure. Precise preoperative staging with the exclusion of inappropriate cases (bulky lymphadenopathy, distant metastases) is a crucial factor for SNB success. For surgeons, it is important to eliminate potentially influenceable factors by proper application technique (ICG >3 mL), adequate timing of individual surgical steps (adhesiolysis, opening retroperitoneal spaces), and careful visual or gentle palpation control of removed tissue. Other studies, ideally multicentric, are needed to validate our findings and help understand other potential risk factors for failure, which could be eliminated and could further improve bilateral SLN mapping outcomes.

AUTHOR CONTRIBUTIONS

Petra Bretová: Conceptualization, data curation, project administration, writing – original draft. Luboš Minář: Conceptualization, methodology, supervision, writing – original draft. Petra Ovesná: Data

curation, formal analysis, software, writing – review and editing. Vít Weinberger: Methodology, supervision, writing – review and editing. Michal Felsinger: Methodology, supervision, writing – review and editing. Michaela Koblížková: Data curation, writing – review and editing. Jitka Hausnerová: Investigation, methodology, writing – review and editing. Eva Jandáková: Investigation, methodology, writing – review and editing. Tatiana Stupková: Investigation, methodology, writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Concin N, Matias-Guiu X, Vergote I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer*. 2021;31(1):12-39. doi:10.1136/ijgc-2020-002230
2. Concin N, Planchamp F, Abu-Rustum NR, et al. European Society of Gynaecological Oncology quality indicators for the surgical treatment of endometrial carcinoma. *Int J Gynecol Cancer*. 2021;31(12):1508-1529.
3. Accorsi GS, Paiva LL, Schmidt R, Vieira M, Reis R, Andrade C. Sentinel lymph node mapping vs systematic lymphadenectomy for endometrial cancer: surgical morbidity and lymphatic complications. *J Minim Invasive Gynecol*. 2020;27(4):938-945.
4. Backes FJ, Cohen D, Salani R, et al. Prospective clinical trial of robotic sentinel lymph node assessment with isosulfane blue (ISB) and indocyanine green (ICG) in endometrial cancer and the impact of ultrastaging (NCT01818739). *Gynecol Oncol*. 2019;153(3):496-499.
5. Buda A, Di Martino G, Restaino S, et al. The impact on survival of two different staging strategies in apparent early stage endometrial cancer comparing sentinel lymph nodes mapping algorithm and selective lymphadenectomy: an Italian retrospective analysis of two reference centers. *Gynecol Oncol*. 2017;147(3):528-534.
6. Frumovitz M, Plante M, Lee PS, et al. Near-infrared fluorescence for detection of sentinel lymph nodes in women with cervical and uterine cancers (FILM): a randomised, phase 3, multicentre, non-inferiority trial. *Lancet Oncol*. 2018;19(10):1394-1403.
7. Papadia A, Zapardiel I, Bussi B, et al. Sentinel lymph node mapping in patients with stage I endometrial carcinoma: a focus on bilateral mapping identification by comparing radiotracer Tc99m with blue dye versus indocyanine green fluorescent dye. *J Cancer Res Clin Oncol*. 2017;143(3):475-480. doi:10.1007/s00432-016-2297-y
8. Eriksson AG, Beavis A, Soslow RA, et al. A comparison of the detection of sentinel lymph nodes using Indocyanine green and near-infrared fluorescence imaging versus blue dye during robotic surgery in uterine cancer. *Int J Gynecol Cancer*. 2017;27(4):743-747. doi:10.1097/IGC.0000000000000959
9. Raffone A, Fanfani F, Raimondo D, et al. Predictive factors of sentinel lymph node failed mapping in endometrial carcinoma patients: a systematic review and meta-analysis. *Int J Gynecol Cancer*. 2023;33(6):853-859. doi:10.1136/ijgc-2022-004014
10. Dundr P, Cibula D, Doležel M, et al. Molecular testing in endometrial carcinoma (joint recommendation of Czech Oncological Society, Oncogynecological Section of the Czech Gynecological and Obstetrical Society, Society of Radiation Oncology, Biology and Physics, and the Society of Czech Pathologists). *Cesk Patol*. 2021;57(3):181-187.
11. Tortorella L, Casarin J, Multinu F, et al. Sentinel lymph node biopsy with cervical injection of indocyanine green in apparent early-stage endometrial cancer: predictors of unsuccessful mapping. *Gynecol Oncol*. 2019;155(1):34-38.
12. Ianieri MM, Puppo A, Novelli A, et al. Sentinel lymph node biopsy in the treatment of endometrial cancer: why we fail? Results of a prospective multicenter study on the factors associated with failure of node mapping with Indocyanine green. *Gynecol Obstet Investig*. 2019;84(4):383-389.
13. Heppell C, Roose T, Richardson G. A model for interstitial drainage through a sliding lymphatic valve. *Bull Math Biol*. 2015;77(6):1101-1131.
14. Taşkın S, Sarı ME, Altın D, et al. Risk factors for failure of sentinel lymph node mapping using indocyanine green/near-infrared fluorescent imaging in endometrial cancer. *Arch Gynecol Obstet*. 2019;299(6):1667-1672.
15. Sozzi G, Fanfani F, Berretta R, et al. Laparoscopic sentinel node mapping with intracervical indocyanine green injection for endometrial cancer: the SENTIFAIL study – a multicentric analysis of predictors of failed mapping. *Int J Gynecol Cancer*. 2020;30(11):1713-1718. doi:10.1136/ijgc-2020-001724
16. Body N, Grégoire J, Renaud MC, Sebastianelli A, Grondin K, Plante M. Tips and tricks to improve sentinel lymph node mapping with Indocyanin green in endometrial cancer. *Gynecol Oncol*. 2018;150(2):267-273.
17. Angeles MA, Migliorelli F, Ramírez LFL, et al. Predictive factors of preoperative sentinel lymph node detection in intermediate and high-risk endometrial cancer. *Q J Nucl Med Mol Imaging*. 2023;67(1):37-45. doi:10.23736/S1824-4785.20.03246-X

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5.3 Boria F, Chacón E, Iyer RR, Fanfani F, Falcone F, Bretová P, et al. Sentinel SENECA risk factors for unsuccessful bilateral sentinel lymph node mapping in endometrial cancer. Int J Gynecol Cancer. 2025;35(5):101771.

As members of the previously mentioned SENECA study (*see Chapter 4.2*), we also contributed our data to a subanalysis focused on risk factors associated with sentinel lymph node detection failure. However, it is important to note that this was a retrospective subanalysis of a dataset originally compiled for different primary objectives, which may have significantly influenced the study's outcomes.

The SENECA study represents the most extensive dataset currently available for investigating surgical and technical parameters influencing SLN detection. Detailed clinical, surgical, histological, and molecular data were collected and analyzed using univariate and multivariate methods.

Bilateral SLN detection was achieved in 82.7% of cases, with unilateral detection observed in 97.3%. Five independent risk factors were identified as significantly associated with unsuccessful bilateral SLN mapping: high-grade histology, deep myometrial invasion, inexperienced surgeon (<20 cases/year), open surgical approach, and use of non-ICG tracers.

In contrast to some previous studies, BMI and tracer volume were not associated with mapping failure in our cohort. Notably, the use of ICG and minimally invasive surgery were clearly linked to improved detection rates and reduced perioperative morbidity.

Furthermore, we confirmed that combining SLN biopsy with systematic pelvic or paraaortic lymphadenectomy resulted in significantly higher complication rates, longer operative time, and prolonged hospital stays. These findings underscore the importance of using SLN biopsy as a standalone staging technique in eligible patients.

Our results support current guidelines emphasizing the use of ICG for SLN mapping and recommend that procedures be conducted or supervised by surgeons performing at least 20 endometrial cancer surgeries per year. These data also advocate for structured training and centralization of care to ensure optimal outcomes.

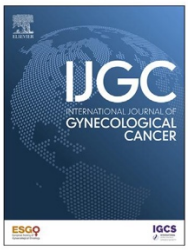
In conclusion, this study contributes important evidence to refine the implementation of SLN biopsy in endometrial cancer, particularly in the context of surgical quality assurance and guideline adherence. Further prospective and standardized studies are needed to validate these findings and enhance surgical performance across diverse clinical settings.

The study *"Sentinel SENECA risk factors for unsuccessful bilateral sentinel lymph node mapping in endometrial cancer"* was published in the *International Journal of Gynecological Cancer* (IF 4.5, Q1) in 2025.

The author's contribution: data curation, manuscript writing – review and editing.

ORIGINAL RESEARCH

Sentinel SENECA risk factors for unsuccessful bi-lateral sentinel lymph node mapping in endometrial cancer



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ABSTRACT

Objective: Our study aims to assess the risk factors associated with bi-lateral sentinel lymph node (SLN) mapping failure in endometrial cancer.

Methods: The SENECA study was a retrospective multi-center international observational study that reviewed data from 2139 women with clinical stage I-to-II endometrial cancer across 64 centers in 17 countries. Between January 2021 and December 2022, patients underwent surgical treatment with SLN assessment, following the guidelines of the European Society of Gynaecological Oncology. Risk factors associated with the absence of bi-lateral mapping were analyzed using χ^2 and *t* tests. All factors that showed statistical associations were included in a multi-variate regression analysis.

Results: Among the 2139 patients, the bi-lateral lymph node detection rate was 82.7%, whereas the unilateral detection rate was 97.3%. In multi-variate analysis, 5 risk factors remained statistically associated with unsuccessful bi-lateral lymph node mapping: high-grade histology (OR 1.35, 95% CI 1.02 to 1.79, $p=.03$), myometrial invasion $>50\%$ (OR 1.37, 95% CI 1.07 to 1.75, $p=.012$), low-volume surgeon <20 cases/year (OR 2.11, 95% CI 1.55 to 2.89, $p<.01$), open surgical approach (OR 1.72, 95% CI 1.06 to 2.78 , $p=.03$), and non-indocyanine green tracer (OR 4.59, 95% CI 2.64 to 7.99, $p<.01$). The addition of bi-lateral pelvic lymphadenectomy and/or paraaortic lymphadenectomy to SLN biopsy caused an increased rate of intra-operative complications (2% vs 8.4%, $p<.01$) and all-grade post-operative complications (4.1% vs 11.2%, $p<.01$).

Conclusions: Our study identifies 5 risk factors associated with unsuccessful lymph node mapping in endometrial cancer. Efforts should be made to perform this technique with indocyanine green, through minimally invasive surgery, and performed or supervised by an experienced surgeon with ≥ 20 endometrial cancer cases per year.

WHAT IS ALREADY KNOWN ON THIS TOPIC

High detection rates and accurate staging are achieved when sentinel lymph node biopsy is performed using a standardized protocol. Previous studies have identified risk factors for failed sentinel lymph node mapping, but the findings have varied widely owing to differences in study designs and methods.

WHAT THIS STUDY ADDS

This study represents the largest cohort of patients published to date studying factors associated with failed bi-lateral lymph node mapping in early-stage endometrial cancer, and identifies 5 key factors: high-grade histology, myometrial invasion $>50\%$, surgeons performing <20 cases per year, open surgical approach, and use of tracers other than indocyanine green (ICG).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

Future studies should focus on optimizing sentinel lymph node biopsy techniques and standardizing surgical protocols to improve success rates in bi-lateral lymph node mapping. Efforts should be made to perform this technique with ICG, through minimally invasive surgery, and performed or supervised by an experienced surgeon with ≥ 20 endometrial cancer cases per year.

Keywords:
Endometrial Neoplasms; Sentinel Lymph Node

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INTRODUCTION

Endometrial cancer is the most common gynecologic malignancy in high-income countries, with an estimated number of 420,242 new cases and 97,704 deaths worldwide in 2022.[1] Lymph node involvement is a crucial prognostic indicator in patients with endometrial carcinoma, influencing both the selection of therapeutic strategies and patient outcomes. In recent years, the technique of sentinel lymph node biopsy has gained increasing popularity as a minimally invasive alternative to systematic lymphadenectomy, aiming to provide precise information about lymphatic spread while reducing surgical morbidity. Prospective clinical trials have confirmed the high sensitivity to detect lymph node metastasis and the high negative predictive value using a standardized sentinel lymph node (SLN) algorithm even in high-risk endometrial cancer.[2-4]

Although SLN biopsy holds promise, it is not infallible and can occasionally fail to identify the SLN accurately. Understanding the predictive factors associated with failed SLN mapping in patients with endometrial carcinoma is imperative for refining the application and efficacy of this technique. Several studies have examined these factors, but much heterogeneity and disparity can be observed among the results published in the literature.[5-10]

In 2023, to assess the rate of SLN involvement according to the different molecular subtypes in patients with stage I-to-II endometrial cancer, the SENECA study[11] was established. With 2139 patients treated with hysterectomy and SLN biopsy and >200 variables related to surgery and lymph nodes assessment, it represents the largest database available to study factors associated with SLN mapping nowadays.

The main objective of this study was to assess the risk factors associated with the failure of lymph node mapping in a large cohort of patients with early-stage endometrial cancer. The secondary objective was to evaluate the impact of avoiding bi-lateral pelvic and/or paraaortic lymphadenectomy.

METHODS

The retrospective multi-centric international observational study reviewed data of patients diagnosed with early-stage (International Federation of Gynecology and Obstetrics [FIGO] stage I-II) endometrial cancer who underwent surgery with lymph node evaluation between January 2021 and December 2022, both included. All factors previously described as possible risk factors for non-sentinel mapping and factors related to SLN technique were included in the SENECA database and were analyzed and retrieved. High volume per surgeon was defined as >20 cases per year following the European Society of Gynaecological Oncology (ESGO) recommendations for quality indicators in endometrial cancer.[12] The study protocol was reviewed by the institutional review board (IRB) of Clinica Universidad de Navarra (CUN 2022.195). Each institution was responsible for obtaining its own IRB/European Commission approval.

Patients were deemed eligible if all the following criteria were met: aged ≥18 years; histologic confirmation of endometrial cancer with endometrioid histology or high-risk histology (serous, clear cell, carcinosarcoma, and mixed histologies); pre-operative FIGO stage I or II by magnetic resonance imaging (MRI) or ultrasound; and pre-operative computed tomography (CT) scan or positron emission tomography-CT without evidence of local or distant disease (could be omitted in low-risk and intermediate-risk endometrial carcinoma with low-grade histology according to ESGO guidelines). In addition, a detailed SLN study protocol had to be accredited, either by ultra-staging or one-step nucleic acid amplification. Molecular analysis had to be performed on the pre-operative biopsy or hysterectomy specimen (polymerase epsilon mutation analysis could be omitted in low-risk and intermediate-risk endometrial carcinoma with low-grade histology according to ESGO guidelines).

Patients were excluded if they were pregnant; if they underwent previous hysterectomy and/or previous pelvic/paraaortic lymphadenectomy; and in cases of presence of extra-uterine disease (peritoneal, visceral, or suspicious lymph node metastasis) or medical history of any invasive tumor, previous abdominal, or pelvic radiotherapy of any type (including brachytherapy), and history of pre-operative neoadjuvant chemotherapy.

Risk factors associated with the absence of bi-lateral mapping (unilateral detection or no detection) were analyzed using χ^2 and *t* tests, as appropriate. A receiver-operating characteristic (ROC) curve was obtained with body mass index (BMI) to try to predict the best cut-off point for failed bi-lateral mapping. All factors that showed statistical associations were included in a multi-variate regression analysis. A $p<.05$ was considered to indicate statistical significance. Statistical analysis was performed using SPSS 27.0 (IBM SPSS Statistics).

In accordance with the journal's guidelines, we will provide our data for independent analysis by a team selected by the Editorial Team for the purposes of additional data analysis or for the reproducibility of this study in other centers if such is requested.

RESULTS

A total of 2139 patients were included in the study. The rate of bi-lateral lymph node detection was 82.7%, whereas the rate of unilateral detection was 97.3%. Mean age was 64.5 years (standard deviation [SD] 10.80). Mean BMI was 30.2 kg/m^2 (SD 6.65). Clinical characteristics are listed in Table 1. The median surgical time was 144 minutes (SD 68). The injection of the tracer for SLN biopsy was most frequently performed by a senior surgeon, using indocyanine green (ICG) as the tracer with a concentration of 1.25 mg/mL, in a volume of 4 mL, and injected both deeply and superficially at the 3 o'clock and 9 o'clock positions. All the details regarding the SLN biopsy technique are listed in Table 2.

A total of 319 patients (14.9%) underwent bi-lateral pelvic lymphadenectomy and/or paraaortic lymphadenectomy in addition to the SLN biopsy. When any of these procedures were added to SLN biopsy, intra-operative complications and all-grade post-operative complications increased from 2% to 8.4% ($p<.01$) and from 4.1% to 11.2% ($p<.01$). Length of stay and duration of surgery also increased from 2.6 to 3.8 days ($p>.01$) and from 133 to 207 minutes ($p<.01$).

All factors associated with failed bi-lateral detection in univariate analysis are listed in Table 3. Data on BMI were available in 600 patients (28.1%). BMI was not associated as a continuous variable to bi-lateral detection. A ROC curve was designed to determine the best cut-off point for predicting bi-lateral detection. The area under

Table 1 Baseline Characteristics of Study Participants

Baseline characteristics	*N* = 2139
Age (y) (mean/SD)	64.55 (10.80)
Body mass index (kg/m^2) (mean/SD)	30.24 (6.65)
Surgery duration (min) (mean/SD)	144 (69.7)
Surgical approach *n* (%)	
Laparoscopic	1432 (66.9)
Robotic	594 (27.8)
Open	113 (5.3)
Previous pelvic surgery *n* (%)	
Yes	301 (14.1)
No	1482 (69.3)
Not reported	356 (16.6)
Uterine manipulator *n* (%)	
Yes	1147 (53.6)
No	957 (44.7)
Not reported	35 (1.6)
Histology *n* (%)	
Endometrioid	1866 (87.2)
Serous	129 (6.0)
Mixed histology	63 (2.9)
Carcinosarcoma	42 (2.0)
Clear cell	30 (1.4)
Not reported	9 (0.4)
Grade *n* (%)	
Low grade	1655 (77.4)
High grade	432 (20.2)
Not reported	52 (2.4)
Myometrial invasion *n* (%)	
No	1649 (77.1)
Yes	479 (22.4)
Not reported	11 (0.5)
Cervical invasion *n* (%)	
Yes	181 (8.5)
No	1951 (91.2)
Not reported	7 (0.3)
FIGO stage *n* (%)	
IA	1278 (59.7)
IB	523 (24.5)
II	152 (7.1)
IIIA	41 (1.9)
IIIB	8 (0.4)
IIIC1	126 (5.9)
IIIC2	4 (0.2)
IV	7 (0.3)
Molecular profile *n* (%)	
POLE-mut	95 (4.4)
MMR-d	581 (27.2)
NSMP	1191 (55.7)
p53-abn	272 (12.7)

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; MMR-d, mismatch repair deficiency; NSMP, no specific molecular profile; POLE, polymerase epsilon; POLE-mut, polymerase epsilon-mutated; SD, standard deviation.

the curve was 0.46, and the most predictive cut-off point was 30 kg/m^2. BMI >30 kg/m^2 was not associated with failed bi-lateral detection either.

Table 2 SLN Procedure Characteristics

SLNB characteristics	*N* = 2139
Center caseload >20 cases/surg/y *n* (%)	
<20	461 (21.6)
>20	1544 (72.2)
Not reported	134 (6.3)
Nodes Approach *n* (%)	
SLNB	1686 (78.8)
SLNB + PLND (only 1 pelvic side)	131 (6.1)
SLNB + PLND (both pelvic sides)	188 (8.8)
SLNB + PLND (1 side) + PALND	7 (0.3)
SLNB + PLND (both sides) + PALND	115 (5.4)
SLNB + PALND	12 (0.6)
Tracer *n* (%)	
ICG	1865 (87.2)
Radiocolloid and ICG	189 (8.8)
Blue dye	74 (3.5)
Blue dye and ICG	5 (0.2)
Radiocolloid and blue dye	2 (0.1)
Not reported	4 (0.2)
Tracer volume *n* (%)	
4 cm^3	1544 (72.2)
2 cm^3	371 (17.3)
1 cm^3	90 (4.2)
Not reported	134 (6.3)
ICG dilution *n* (%)	
0.5 mg/mL	386 (18)
1.25 mg/mL	878 (41)
2.5 mg/mL	622 (29.1)
Not reported	253 (4.3)
Injection technique *n* (%)	
Superficial 3-9	159 (7.4)
Superficial and deep 3-9	1720 (80.4)
Superficial 3-6-9-12	25 (1.2)
Superficial and deep 3-6-9-12	185 (8.6)
Not reported	50 (2.3)
Re-injection of ICG if failed *n* (%)	
Yes	1489 (69.6)
No	650 (30.4)
Dissection of pre-sacral space if failed *n* (%)	
Yes	1042 (48.7)
No	1097 (51.3)
Injecter *n* (%)	
Attending	1208 (56.5)
Fellow	527 (24.6)
Resident	311 (14.5)
Not reported	93 (4.3)
SLN total number (median)	2.8
SLN distribution *n* (%)	
Both pelvic sides	1729 (80.8)
Right pelvic side	152 (7.1)
Left pelvic side	150 (7.0)
Both pelvic sides + aortic area	41 (1.9)
Left pelvic side + aortic area	8 (0.4)
Right pelvic side + aortic area	1 (0.05)
Aortic area	1 (0.05)
Not any SLN identified	57 (2.7)
SLN involvement *n* (%)	205 (9.6)
Isolated tumor cells	51 (24.9)

(*continued on next page*)

Table 2 (*continued*)

SLNB characteristics	*N* = 2139
Micro-metastases	58 (28.3)
Macro-metastases	56 (27.3)
Not reported	40 (24.9)

Abbreviations: ICG, indocyanine green; PALND, paraaortic lymph node dissection; PLND, pelvic lymph node dissection; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy; surg, surgeries.

All factors associated with failed bi-lateral detection in univariate analysis were included in a multi-variate analysis (Table 4). Only 5 risk factors remained statistically associated with unsuccessful bi-lateral lymph node mapping in the multi-variate analysis: high-grade histology (OR 1.35, 95% CI 1.02 to 1.79, $p=.039$), myometrial invasion >50% (OR 1.37, 95% CI 1.07 to 1.75, $p=.012$), low-volume surgeon <20 cases per year (OR 2.11, 95% CI 1.55 to 2.89; $p<.01$), open surgical approach (OR 1.72, 95% CI 1.06 to 2.78, $p=.03$), and non-ICG tracer (OR 4.59, 95% CI 2.64 to 7.99, $p<.01$).

DISCUSSION

Summary

Our study found that the factors associated with failed lymph node mapping included high-grade histology, myometrial invasion >50%, surgeons performing <20 cases per year, an open surgical approach, and the use of a non-ICG tracer. SLN biopsy should be performed using ICG, through minimally invasive surgery, and conducted or supervised by a surgeon who manages ≥20 procedures annually.

Results in the Context

SLN biopsy is now deemed the standard of care in the treatment of endometrial cancer. It has been associated with less morbidity (as shown in our study) and even higher detection rates of nodal metastases than in traditional lymphadenectomy.[2-4,13] One of the

Table 3 Univariate Analysis of Factors Associated With Failed Bi-Lateral Detection

Univariate analysis					
Variable	**Bi-lateral detection rate**				***p*-value**
	Yes		**No**		
FIGO stage III-IV	83.3%		82.7%		.82
Nodal positivity	85.9%		82.4%		.21
SLN frozen	80.5%		83.2%		.34
Uterine manipulator	84%		81.7%		.08
Previous pelvic surgery	81.1%		83%		.4
Injection performed by resident	80.4%		83%		.26
Endometrioid histology	79.9%		83.5%		.17
BMI >30	82.5%		79.7%		.37
Volume tracer >3 mL	84.1%		81.3%		.17
High grade	79.2%		83.5%		***.03***
Cervical invasion	77.3%		83.2%		***.04***
Myometrial invasion	79.7%		84.2%		***.01***
ICG as tracer	83.9%		53%		***<.01***
MIS approach	83.5%		69%		***<.01***
>20 cases/surg/year	85.5%		81.2%		***<.01***
Re-injection if failed	84.9%		78%		***<.01***
Pre-sacral dissection if failed	85.3%		80.4%		***<.01***
Injection technique	**SD 3-9**	**SD 3-9**	**SD 3-6-9-12**	**SD 3-6-9-12**	***.03***
	83.4%	84.9%	75.5%	72%	
Molecular profile	**POLE**	**p53**	**NSMP**	**MMR-d**	.75
	87.3%	80.5%	82.7%	83.4%	
Dilution of ICG	**0.5 mg/mL**	**1.25 mg/mL**	**2.5 mg/mL**		.08
	85%	83%	82.6%		
Age (mean, y)	58.8		62.1		.65
BMI (mean, kg/m^2)	31.1		32.1		.55

Abbreviations: BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; ICG, indocyanine green; MIS, minimally invasive surgery; MMR-d, mismatch repair deficiency; NSMP, no specific molecular profile; POLE, polymerase epsilon; SD, standard deviation; SLN, sentinel lymph node; surg, surgeries.
Boldface and italic if statistically significant.

Table 4 Multi-Variate Analysis of Factors Associated With Failed Bi-Lateral Detection

Multi-variate analysis			
Variable	**OR (no detection)**	**95% CI**	***p*-value**
Myometrial invasion >50%	**1.37**	**1.07 to 1.75**	**.01**
High grade	**1.35**	**1.02 to 1.79**	**.04**
Non-ICG tracer	**4.59**	**2.64 to 7.99**	**<.01**
Open surgery	**1.72**	**1.06 to 2.78**	**.03**
Low volume surgeon (<20/y)	**2.11**	**1.56 to 2.89**	**<.01**
Injection technique	1.1	0.91 to 1.33	.32
Pre-sacral dissection	0.88	0.68 to 1.13	.31
Re-injection if failure	0.86	0.66 to 1.12	.27
Cervical invasion	1.45	0.9 to 2.15	.06

Abbreviation: ICG, indocyanine green.
Boldface if statistically significant ($p < 0.05$).

main challenges with SLN biopsy is the heterogeneity in techniques. In our study, we observed significant variation in the techniques used, although none of these differences seemed to affect detection rates. In 2021, to address these disparities, Moloney and colleagues[14] published an assessment tool that we highly recommend to the reader.

Several studies have indicated the superior performance of ICG to that of blue dye or radiocolloid.[2,5,10,15-18] In our study, the use of any other tracer was associated with nearly a 5-fold higher likelihood of failure to detect the SLN bi-laterally. The rate of bi-lateral detection improves when the surgery is performed or supervised by a surgeon with >20 cases per year. This threshold was established by ESGO in their quality indicators. Although this benchmark might seem arbitrary, it highlights the importance of surgical volume in endometrial cancer, as shown in the existing literature. In a study published by Gedgaudaite and colleagues,[19] 190 patients were prospectively evaluated to assess the learning curve for SLN biopsy in endometrial cancer. The authors found that ≥30 procedures using ICG-traced laparoscopic SLN biopsy were required to achieve an acceptable level of competence, with a bi-lateral SLN detection rate of ≥75%. This finding aligns with the only prospective study analyzing risk factors in the literature. Ianieri and colleagues[20] prospectively analyzed 110 patients and found that the only factor significantly associated with successful bi-lateral lymph node mapping was the surgeon.

All previous studies analyzing these factors were primarily conducted using minimally invasive surgery. In endometrial cancer surgery, SLN biopsy is typically performed through minimally invasive surgery. In our study, 5% of the patients underwent open surgery. However, on the basis of our findings, it is crucial to consider that when choosing to operate through an open approach, the rates of bi-lateral lymph node detection are likely to decrease. The association between open surgery and lower SLN detection rates may be due to increased tissue manipulation, anatomical distortion, or differences in tracer dispersion. Similarly, aggressive histologic subtypes and myometrial invasion might have altered lymphatic architecture, reducing tracer migration. This finding aligns with previous studies.[21]

Pre-operative molecular profiling and advanced imaging (MRI or specialized ultrasound) could help identify patients at higher risk for failed mapping, guiding decisions on comprehensive lymphadenectomy. Furthermore, our results emphasize the importance of adhering to the Memorial Sloan Kettering SLN algorithm for surgical staging in patients with endometrial cancer, particularly in high-risk cases.[22]

Enlarged lymph nodes on pre-operative imaging, BMI, and the use of a tracer volume <3 mL have previously been described as factors associated with failed lymph node mapping. Regarding enlarged lymph nodes, we must note that our study included only patients with pre-operative stage I-to-II endometrial cancer, so these cases were excluded. BMI was not found to be associated with failed mapping, either as a continuous variable or with a BMI >30 kg/m^2. The literature on this topic is inconsistent, with some studies suggesting that higher BMI is associated with failed detection[21,23,24] whereas others suggest the opposite.[6,8,9] In our data, it is important to acknowledge that BMI information was available for 600 patients (28% of the cohort), which may have biased our results.

A recent meta-analysis reported that using >3 mL of tracer is associated with higher rates of bi-lateral lymph node mapping. However, although the meta-analysis[6] included 1345 cases, the volume of tracer was analyzed in only 325 patients. In our study, with data on tracer volume for 2005 patients, we did not observe any differences in bi-lateral detection rates. This is consistent with a more recent study by Mauro and colleagues,[5] which specifically analyzed tracer volume in 352 patients and found no differences in detection rates between 2 mL and 4 mL.

Strengths and Weaknesses

To the best of our knowledge, this is the largest cohort study in endometrial cancer analyzing factors associated with failed lymph node mapping. With up to 22 variables potentially influencing bi-lateral lymph node mapping, we consider our multi-variate analysis to be robust and reliable. Our cohort also shows a high rate of bi-lateral lymph node mapping, minimizing potential surgical biases related to suboptimal SLN biopsy technique.

The primary limitation of our study is its retrospective nature. It is also important to note that BMI data were available for only 28% of cases, which may have influenced our findings regarding this variable. Although ICG is well-established for its superior detection performance, only 76 cases (3.6%) did not receive ICG, which could represent a potential bias to the study.

CONCLUSION

Our study identifies 5 risk factors associated with unsuccessful lymph node mapping. SLN biopsy is a safe technique that improves surgical morbidity with high rates of bi-lateral detection. Efforts must be made to perform this technique with ICG, by minimally invasive surgery, and conducted or supervised by an experienced surgeon with ≥20 endometrial cancer cases per year.

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Author Contributions FB, EC, JAM and LC had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: FB, EC, JAM and LC. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: FB. Critical revision of the manuscript for important intellectual content: EC, LC. Statistical analysis: FB. Supervision: LC.

Declaration of Competing Interests None declared.

REFERENCES

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–263. https://doi.org/10.3322/CAAC.21834.
2. Persson J, Salehi S, Bollino MLönnerfors C, Falconer H, Geppert B. Pelvic Sentinel lymph node detection in High-Risk Endometrial Cancer (SHREC-trial)-the final step towards a paradigm shift in surgical staging. *Eur J Cancer*. 2019;116:77–85. https://doi.org/10.1016/J.EJCA.2019.04.025.
3. Cusimano MC, Vicus D, Pulman K, et al. Assessment of sentinel lymph node biopsy vs lymphadenectomy for intermediate- and high-grade endometrial cancer staging. *JAMA Surg*. 2021;156(2):157–164. https://doi.org/10.1001/JAMASURG.2020.5060.
4. Rossi EC, Kowalski LD, Scalici J, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. *Lancet Oncol*. 2017;18(3):384–392. https://doi.org/10.1016/S1470-2045(17)30068-2.
5. Mauro J, Raimondo D, Di Martino G, et al. Assessment of sentinel lymph node mapping with different volumes of Indocyanine green in early-stage ENdometrial cancer: the ALIEN study. *Int J Gynecol Cancer*. 2024;34(6):824–829. https://doi.org/10.1136/IJGC-2023-005100.
6. Raffone A, Fanfani F, Raimondo D, et al. Predictive factors of sentinel lymph node failed mapping in endometrial carcinoma patients: a systematic review and meta-analysis. *Int J Gynecol Cancer*. 2023;33(6):853–859. https://doi.org/10.1136/IJGC-2022-004014.
7. Touhami O, Trinh XB, Gregoire J, et al. Predictors of non-sentinel lymph node (non-SLN) metastasis in patients with sentinel lymph node (SLN) metastasis in endometrial cancer. *Gynecol Oncol*. 2015;138(1):41–45. https://doi.org/10.1016/J.YGYNO.2015.04.008.
8. Taşkõn S, Sarõ ME, Altõn D, et al. Risk factors for failure of sentinel lymph node mapping using indocyanine green/near-infrared fluorescent imaging in endometrial cancer. *Arch Gynecol Obstet*. 2019;299(6):1667–1672. https://doi.org/10.1007/S00404-019-05137-5.
9. Tortorella L, Casarin J, Multinu F, et al. Sentinel lymph node biopsy with cervical injection of indocyanine green in apparent early-stage endometrial cancer: predictors of unsuccessful mapping. *Gynecol Oncol*. 2019;155(1):34–38. https://doi.org/10.1016/J.YGYNO.2019.08.008.
10. Body N, Grégoire J, Renaud MC, Sebastianelli A, Grondin K, Plante M. Tips and tricks to improve sentinel lymph node mapping with Indocyanin green in endometrial cancer. *Gynecol Oncol*. 2018;150(2):267–273. https://doi.org/10.1016/J.YGYNO.2018.06.001.
11. Chacon E, Boria F, Lyer RR, et al. SENECA study: staging endometrial cancer based on molecular classification. *Int J Gynecol Cancer*. 2024;34(9):1313–1321. https://doi.org/10.1136/IJGC-2024-005711.
12. Concin N, Matias-Guiu X, Vergote I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer*. 2021;31(1):12–39. https://doi.org/10.1136/IJGC-2020-002230.
13. Soliman PT, Westin SN, Dioun S, et al. A prospective validation study of sentinel lymph node mapping for high-risk endometrial cancer. *Gynecol Oncol*. 2017;146(2):234–239. https://doi.org/10.1016/J.YGYNO.2017.05.016.
14. Moloney K, Janda M, Frumovitz M, et al. Development of a surgical competency assessment tool for sentinel lymph node dissection by minimally invasive surgery for endometrial cancer. *Int J Gynecol Cancer*. 2021;31(5):647–655. https://doi.org/10.1136/IJGC-2020-002315.
15. How J, Gotlieb WH, Press JZ, et al. Comparing indocyanine green, technetium, and blue dye for sentinel lymph node mapping in endometrial cancer. *Gynecol Oncol*. 2015;137(3):436–442. https://doi.org/10.1016/J.YGYNO.2015.04.004.
16. Maramai M, Achilarre MT, Aloisi A, et al. Cervical re-injection of indocyanine green to improve sentinel lymph node detection in endometrial cancer. *Gynecol Oncol*. 2021;162(1):38–42. https://doi.org/10.1016/J.YGYNO.2021.04.026.
17. Ruscito I, Gasparri ML, Braicu EI, et al. Sentinel node mapping in cervical and endometrial cancer: indocyanine Green versus Other conventional dyes-A meta-analysis. *Ann Surg Oncol*. 2016;23(11):3749–3756. https://doi.org/10.1245/S10434-016-5236-X.
18. Nagar H, Wietek N, Goodall RJ, Hughes W, Schmidt-Hansen M, Morrison J. Sentinel node biopsy for diagnosis of lymph node involvement in endometrial cancer. *Cochrane Database Syst Rev*. 2021;6(6):CD013021. https://doi.org/10.1002/14651858.CD013021.pub2.
19. Gedgaudaite M, Paskauskas S, Bartusevicius A, et al. Laparoscopic sentinel lymph node mapping with indocyanine green in endometrial cancer: surgeon's learning curve (cumulative sum analysis). *Int J Gynecol Cancer*. 2023;33(4):521–527. https://doi.org/10.1136/IJGC-2022-004033.
20. Ianieri MM, Puppo A, Novelli A, et al. Sentinel lymph node biopsy in the treatment of endometrial cancer: why we fail? Results of a prospective multicenter study on the factors associated with failure of node mapping with indocyanine green. *Gynecol Obstet Invest*. 2019;84(4):383–389. https://doi.org/10.1159/000496699.
21. Garrett AA, Wield A, Mumford B, et al. Clinical factors associated with failed sentinel lymph node mapping in endometrial cancer. *Gynecol Oncol Rep*. 2022;44:101080. https://doi.org/10.1016/J.GORE.2022.101080.
22. Barlin JN, Khoury-Collado F, Kim CH, et al. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: beyond removal of blue nodes. *Gynecol Oncol*. 2012;125(3):531–535. https://doi.org/10.1016/J.YGYNO.2012.02.021.
23. Restaino S, Buda A, Puppo A, et al. Anatomical distribution of sentinel lymph nodes in patients with endometrial cancer: a multicenter study. *Int J Gynecol Cancer*. 2022;32(4):517–524. https://doi.org/10.1136/IJGC-2021-003253.
24. Eriksson AGZ, Montovano M, Beavis A, et al. Impact of obesity on sentinel lymph node mapping in patients with newly diagnosed uterine cancer undergoing robotic surgery. *Ann Surg Oncol*. 2016;23(8):2522–2528. https://doi.org/10.1245/S10434-016-5134-2.

5.4 Münzová D, Bretová P, Hausnerová J, Bednaříková M, Minář L, Weinberger V. Nízkoobjemové postižení regionálních lymfatických uzlin u karcinomu endometria – 2024 update. Čes. Gynek. 2025;90(2): 158-162.

One key advantage of sentinel lymph node detection and biopsy is the ability to perform detailed ultrastaging, which significantly improves the detection of metastatic involvement, especially low-volume metastases such as micrometastases and isolated tumor cells. This approach offers greater diagnostic sensitivity than conventional histopathological methods in the context of systematic lymphadenectomy.

This comprehensive review article provides an updated synthesis of the literature (January 2019–September 2024) concerning LVM (low-volume metastasis) in regional lymph nodes in endometrial carcinoma, with specific focus on sentinel lymph node ultrastaging, prevalence, prognostic implications, and associations with molecular subtypes.

Reported LVM prevalence ranges from 1.7% to 9.0%, varying with tumor histotype, myometrial invasion depth, and LVSI. MICs are more frequent in non-endometrioid and high-risk tumors, whereas ITCs are more often found in endometrioid histologies.

MICs are classified as metastatic disease under FIGO 2023 (stage IIIC1/2i) and warrant adjuvant therapy. ITCs, in contrast, are not classified as nodal metastases (pN0[i+]) and do not routinely alter treatment recommendations due to inconsistent evidence of prognostic significance. Nevertheless, some studies suggest a potentially reduced DFS in ITC-positive patients, indicating a need for further prospective studies.

LVM appears more frequent in MMRd and p53mut tumors, with no clear enrichment in *POLE*mut or NSMP subtypes. Genomic studies have yet to identify consistent genetic predictors of LVM beyond p53 status.
In conclusion, while MICs are treated as metastases due to associated recurrence risk, the clinical role of ITCs remains uncertain. Current guidelines support routine SLN

mapping with ultrastaging for appropriate risk groups. The integration of molecular classification refines risk stratification, though further prospective data are needed to optimize management strategies for patients with low-volume nodal disease.

The comprehensive review entitled *"Low-volume regional lymph node metastasis in endometrial cancer – 2024 update"* was published in *Česká gynekologie* (IF 0.5, Q4) in 2025.

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Nízkoobjemové postižení regionálních lymfatických uzlin u karcinomu endometria – 2024 update

Low-volume regional lymph node metastasis in endometrial cancer – 2024 update

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Souhrn: Prevalence detekce izolovaných nádorových buněk a mikrometastáz v sentinelové uzlině stoupá díky jejich podrobnému zpracování formou ultrastagingu. Předkládaná práce poskytuje aktuální přehled literatury od ledna roku 2019 do září roku 2024 se zaměřením na nízkoobjemové postižení regionálních uzlin, jeho prevalenci, prognózu a souvislost s molekulární klasifikací. Přítomnost mikrometastáz je aktuálně považována za metastatické postižení lymfatických uzlin, avšak s lepší prognózou než makrometastázy, dle toho je volen i terapeutický postup společný pro obě tyto kategorie uzlinového postižení. Naopak přítomnost izolovaných nádorových buněk není v rámci Mezinárodní federace gynekologie a porodnictví (FIGO) 2023 stagingu považována za uzlinové postižení a neovlivňuje doporučený terapeutický postup, protože doposud nebyly prokázány signifikantní prognostické důsledky jejich přítomnosti.

Klíčová slova: nízkoobjemové postižení uzlin – karcinom endometria – ultrastaging – izolované nádorové buňky – mikrometastázy – sentinelová uzlina

Summary: Due to the implementation of sentinel lymph node ultrastaging, the prevalence of isolated tumor cells and micrometastases have increased. This literature review comprises of articles published between January 2019 and September 2024 aiming at low-volume metastases in regional lymph nodes, their prognosis, and links to molecular classification. Micrometastases are currently considered as having metastatic lymph node involvement; however, they have a better prognosis than macrometastases. Accordingly, therapy is tailored. In contrast, isolated tumor cell presence is not considered metastatic involvement according to International Federation of Gynecology and Obstetrics (FIGO) 2023 staging and does not affect the therapeutic procedure because their significant prognostic importance has not been proven so far.

Key words: low-volume metastasis – endometrial cancer – ultrastaging – isolated tumor cells – micrometastases – sentinel lymph node

Úvod

Karcinom endometria je nejčastější gynekologickou malignitou v ČR s incidencí 37/100 000 žen v roce 2022, v absolutním počtu to bylo 2 005 pacientek [1]. V posledních letech se velmi změnil pohled na toto onemocnění, a to nejen díky zavedení molekulární klasifikace, změně stagingu lymfatických uzlin, ale i díky novinkám v adjuvantní terapii (imunoterapie, antiangiogenní léčba). Dle aktuálních guidelines ESGO/ESTRO/ESP (European Society of Gynecological Oncology / European SocieTy for Radiotherapy and Oncology / European Society of Pathology) z roku 2021 je staging lymfatických uzlin prováděn preferenčně pomocí detekce sentinelových uzlin na obou pánevních stěnách namísto provedení systematické aortopelvické lymfadenektomie [2]. Tato minimálně invazivní metoda je doporučena u všech pacientek zařazených do skupiny nízkého nebo středního rizika recidivy s výjimkou případů s absencí myometrální invaze, kde může být chirurgický staging uzlin zcela vynechán. U pacientek ve skupině středně vysokého nebo vysokého rizika ve stadiu I–II je oboustranná biopsie sentinelové

Tab. 1. Přehled publikací zabývající se prevalencí nízkoobjemového postižení uzlin u karcinomu endometria.
Tab. 1. Overview of publications dealing with the prevalence of low-volume metastases in endometrial carcinoma.

Název	Rok	Země	Typ studie	Počet pacientek	Prevalence ITC	Prevalence mikrometastáz
Mueller et al. [12]	2020	USA	retrospektivní	1 044	6,0 %	2,0–9,0 % (mikro + makro)
De Vitis et al. [13]	2024	USA, Itálie	retrospektivní	1 570	5,3 %	3,2 %
Buda et al. [14]	2023	Itálie	retrospektivní	1 428	1, 9 %	3,6 %
Lavecchia et al. [15]	2023	Kanada	retrospektivní	1 012	1,1 %	0,6 %
Matsuo et al. [16]	2024	USA	retrospektivní	56 527	2,6 %	3,5 %
García Pineda et al. [17]	2020	Španělsko	retrospektivní	270	2,6 %	

ITC – izolované nádorové buňky/isolated tumor cells

uzliny (SLN – sentinel lymph node) plnohodnotnou alternativou ke klasické systematické lymfadenektomii, kterou je však nezbytné doplnit v případě selhání biopsie [2]. Dle ESGO indikátorů kvality operační léčby karcinomu endometria má být staging lymfatických uzlin až v 90 % případů proveden právě pomocí detekce sentinelové uzliny [3].

Vzhledem k odstranění pouze malého množství uzlin, ve srovnání s lymfadenektomií, je možné sentinelové uzliny vyšetřit podrobnější metodou, nejčastěji ultrastagingem [4]. V ČR neexistuje obecně platný protokol pro patologické ultrastagingové vyšetření sentinelových uzlin u karcinomu endometria, vyšetření je tedy prováděno dle zvyklostí pracoviště. Na Ústavu patologie FN Brno je každá sentinelová uzlina zpracována rozdělením na 2 mm silné lamely, které jsou zablokovány. V případě negativity prvního řezu barveného hematoxylinem eosinem je každá lamela prokrájena v několika úrovních ve vzdálenosti 200 µm, přičemž jsou mezi hematoxylin eosinová skla pravidelně vkládány řezy pro imunohistochemický průkaz cytokeratinů. Tímto způsobem je zpracována uzlina beze zbytku. Prostřednictvím ultrastagingu je možné spolehlivěji detekovat nízkoobjemové postižení lymfatických uzlin (LVM – low-volume metastasis), tedy mikrometastázy (MIC – micrometastases) a izolované nádorové buňky (ITC – isolated tumor cells) [4]. Mikrometastázy jsou definovány jako ložiska nádorových buněk o velikosti 0,2–2 mm a/nebo > 200 buněk, izolované nádorové buňky jsou shluky buněk < 0,2 mm a/nebo < 200 buněk. Makrometastázy jsou definovány velikostí > 2 mm [5]. Další možnou metodou vyšetření sentinelových uzlin je OSNA (one-step nucleic acid amplification), kdy je detekováno množství mRNA kódující cytokeratin-19, který je produkován několika typy solidních nádorů, avšak ne zdravou lymfatickou tkání. V porovnání s ultrastagingem je tato metoda snadněji automatizovatelná a reprodukovatelná [6–8], avšak v reálné klinické praxi méně užívaná [9]. Konkordance metody OSNA a ultrastagingu je 86,0 %, metodou OSNA bylo diagnostikováno více mikrometastáz a o 20,7 % více pacientek bylo zařazeno do stadia III dle Mezinárodní federace gynekologie a porodnictví (FIGO) 2023 [6]. Studie provedená Kosťunem et al. v letech 2016–2018 sledovala recidivu onemocnění u pacientek s karcinomem endometria, jejichž uzliny vyšetřené ultrastagingem byly negativní, zatímco uzliny vyšetřené metodou OSNA pozitivní, přičemž u těchto pacientek došlo k recidivě onemocnění ve 25,0 % [10].

V roce 2019 vyšel v časopise Česká gynekologie přehledový článek „Nízkoobjemové postižení uzlin u karcinomu endometria“ [11], cílem našeho literárního review je volně na něj navázat a přinést aktualizované informace z posledních 5 let, kdy vešla v platnost nová evropská doporučení a nový FIGO staging.

Metodika

Jedná se o přehledovou práci. K vyhledání publikací byly použity databáze PubMed, Embase a Web of Science za užití klíčových hesel pro vyhledávání: endometrial AND (cancer OR carcinoma OR malignancy) AND („low volume metastasis“ OR micrometastas* OR ITC OR „isolated tumor cells“). Použity byly původní práce (retrospektivní a prospektivní studie) a metaanalýzy publikované od ledna 2019 do září 2024.

Literární rešerše

Prevalence nízkoobjemového postižení uzlin

Prevalence nízkoobjemového postižení uzlin (LVM) uzlin (zahrnující MIC a ITC) se u karcinomu endometria pohybuje mezi 1,7 a 9,0 % (tab. 1) [12–17]. Liší se v závislosti na histologickém typu karcinomu, hloubce invaze do myometria nebo lymfangioinvazi (LVSI – lymphovascular space invasion). U pacientek s FIGO grade 1 endometroidním karcinomem a myoinvazí maximálně do poloviny tloušťky myometria je přítomnost ITC v SLN nízká (0,5 %), naopak u pacientek ve stejné skupině s invazí karcinomu přes polovinu síly myometria byl pozitivní nález ITC v SLN u 31 % pacientek [12]. Přítomnost LVM také souvisí s histologickým typem nádoru – byl

pozorován signifikantní rozdíl v přítomnosti MIC v SLN u pacientek s low-grade endometroidním karcinomem (2,8 %) na rozdíl od pacientek s non-endometroidními typy (6,0 %) [13]. Avšak přítomnost ITC v rámci stejné studie byla častěji zaznamenána u pacientek s endometroidním typem karcinomu (5,3 %) ve srovnání s karcinomem serózním (1,7 %), které mají naopak sigfnifikatně vyšší prevalenci sentinelových makrometastáz (11,0 vs. 2,9 %) [13].

Prognóza nízkoobjemového postižení uzlin a adjuvantní terapie

Stran prognózy LVM u karcinomu endometria je rozdíl mezi MIC a ITC. Nález MIC je považován za postižení lymfatických uzlin a stejně jako u nálezu makrometastáz vede k upstagingu onemocnění [3]. Nález ITC k zařazení do vyššího stadia onemocnění dle FIGO 2023 nevede, v literatuře je místy uváděn možný odlišný biologický potenciál ITC, které jsou považovány za dispergované nádorové buňky neschopné další proliferace, bez přítomnosti mitóz a vaskulární invaze [18,19].

Přítomnost MIC snižuje dobu přežití bez přítomnosti onemocnění (DFS – disease free survival), která však může být prodloužena podáním adjuvantní léčby (radioterapie a/nebo chemoterapie) [20]. Některé studie naopak ukazují, že DFS není ovlivněn podáním adjuvantní léčby u pacientek s přítomností ITC v SLN v časném stadiu karcinomu bez jiných rizikových děložních faktorů (LVSI) [21,22]. Doba přežití bez onemocnění u pacientek s předoperačně časným stadiem (stadium I–II dle FIGO 2009) karcinomu endometria ve 3letém intervalu byla 90,6 % u pacientek bez uzlinového postižení a 84,3 % u pacientek s LVM, recidiva onemocnění byla častěji zaznamenána u pacientek s LVM (14,5 %) ve srovnání s pacientkami s negativními uzlinami (5,9 %) [14]. Nicméně nutno podotknout, že se jednalo o velmi heterogenní skupinu karcinomů endometria zahrnující všechny histologické typy (vyloučeny byly pouze metastázy z jiných lokalit a sarkomy).

Co se týče prognózy pacientek s ITC, neposkytuje zatím dostupná literatura jednoznačný závěr. U pacientek s ITC s low-grade endometroidním karcinomem bez lymfangioinvaze a invaze děložní serózy nebyl zaznamenán rozdíl v DFS při podání adjuvantní terapie (radioterapie a/nebo chemoterapie) ve srovnání se skupinou, která adjuvantní terapii nepodstoupila, limitací těchto studií je však poměrně krátká doba follow-up (medián 31 měsíců) [21,23]. Existují však i studie popisující signifikantně kratší dobu přežití bez recidivy u nízkorizikových pacientek s ITC ve srovnání s pacientkami bez postižení uzlin, přičemž doba follow-up byla obdobná jako u výše zmiňovaných studií [14,24]. Podle Matsuo et al. je pacientkám s nálezem ITC v SLN častěji podána nějaká forma adjuvantní terapie, avšak ve většině dostupných studií je podání, či nepodání adjuvantní terapie ovlivněno především přítomností dalších rizikových faktorů (high-grade histologie, podstatná LVSI, hluboká myometrální invaze) [16].

Nízkoobjemové postižení uzlin a molekulární klasifikace

Retrospektivní multicentrická studie, která zahrnovala 101 pacientek s LVM, neprokázala specifické zastoupení v rámci molekulární klasifikace v porovnání s pacientkami bez nálezu LVM, avšak p53 mutace byla stanovena jako významný faktor pro riziko recidivy onemocnění [25]. Ani v případě přítomnosti pouze ITC nebyly zjištěny žádné rozdíly v rámci molekulárního profilu v retrospektivní studii zahrnující 1 214 pacientek [26]. V rámci recentní retrospektivní multicentrické studie SENECA (Staging ENdomEtrial CAncer based on molecular classification) s 2 139 pacientkami byla popsána větší pravděpodobnost metastatického postižení uzlin u pacientek s karcinomy mismatch repair deficientními (MMRd) a s mutací proteinu p53 (p53mut) ve srovnání s dalšími molekulárními typy [9]. V rámci provedení genomického profilu karcinomu endometria byly studovány i další geny, jejichž mutace by mohly vést k větší pravděpodobnosti nízkoobjemového uzlinového postižení – konkrétně geny *PTEN* a *PIK3CA*, avšak žádná souvislost nebyla v tomto souboru 42 pacientek prokázána [27]. Ve studii se 100 pacientkami provedené Watanabe et al. byly nalezeny mutace v genu *FBWX7*, které byly spojeny se signifikantně vyšší přítomností uzlinového postižení [28].

Nízkoobjemové postižení uzlin v rámci FIGO 2023 stagingu

V rámci FIGO 2023 stagingu karcinomu endometria při pozitivním nálezu makrometastáz i mikrometastáz je onemocnění zařazeno do stadia IIIC1, případně IIIC2 v závislosti na lokalizaci uzliny (pánevní, resp. paraaortální oblast). Vzhledem k lepší prognóze pacientek s MIC oproti makrometastázám bylo nutné tuto skutečnost uvést v rámci nové klasifikace – pomocí malého písmene „i“ na konci označíme mikrometastázy (IIIC1i, IIIC2i) nebo makrometastázy (IIIC1ii, IIIC2ii). Přítomnost ITC není považována za metastatické postižení uzliny, ale měla by být v dokumentaci zaznamenána jako pN0(i+). Dle ESGO/ESTRO/ESP nejsou stanovena kritéria podání adjuvantní léčby při nálezu ITC [2].

Závěr

Přítomnost mikrometastáz je aktuálně považována za metastatické postižení lymfatických uzlin, avšak s lepší prognózou než makrometastázy, nicméně terapeutický postup je společný pro obě tyto kategorie uzlinového postižení. Naopak přítomnost izolovaných nádorových buněk není v rámci FIGO 2023 považována za uzlinové postižení a neovlivňuje doporučený terapeutický postup, protože doposud nebyly prokázány žádné

signifikantní prognostické důsledky přítomnosti ITC. Možnou příčinou je nedostatečná doba follow-up, protože např. přítomnost ITC v SLN u karcinomu prsu významně zkracovala DFS až při sledování po dobu 5 let [29]. Z doposud publikovaných výsledků týkajících se prognostického vlivu ITC nelze stanovit jednoznačný konsenzus stran dalšího postupu, a to především s ohledem na podání adjuvantní terapie. Je tedy s jistotou zapotřebí dalších, zejména prospektivních studií k získání dostatečně robustního objemu informací týkajících se této problematiky.

Literatura

1. Krejčí D, Mužík J, Šnábl I et al. Portál epidemiologie novotvarů v ČR. Masarykova univerzita, Brno. 2024 [online]. Dostupné z: https://www.svod.cz.
2. Concin N, Matias-Guiu X, Vergote I et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer 2021; 31(1): 12–39. doi: 10.1136/ijgc-2020-002230.
3. Concin N, Planchamp F, Abu-Rustum NR et al. European Society of Gynaecological Oncology quality indicators for the surgical treatment of endometrial carcinoma. Int J Gynecol Cancer 2021; 31(12): 1508–1529. doi: 10.1136/ijgc-2021-003178.
4. Burg LC, Hengeveld EM, In't Hout J et al. Ultrastaging methods of sentinel lymph nodes in endometrial cancer – a systematic review. Int J Gynecol Cancer 2021; 31(5): 744–753. doi: 10.1136/ijgc-2020-001964.
5. Berek JS, Matias-Guiu X, Creutzberg C et al; Endometrial Cancer Staging Subcommittee, FIGO Women's Cancer Committee. FIGO staging of endometrial cancer: 2023. Int J Gynaecol Obstet 2023; 162(2): 383–394. doi: 10.1002/ijgo.14923.
6. Kosťun J, Pešta M, Sláma J et al. One-step nucleic acid amplification vs ultrastaging in the detection of sentinel lymph node metastasis in endometrial cancer patients. J Surg Oncol 2019; 119(3): 361–369. doi: 10.1002/jso.25322.
7. Cuadras M, Planas J, Celma A et al. Application of One-Step Nucleic Acid amplification (OSNA) in different cancer entities and usefulness in prostate cancer: a systematic review. BMC Cancer 2022; 22(1): 357. doi: 10.1186/s12885-022-09355-0.
8. Diestro MD, Berjón A, Zapardiel I et al. One-Step Nucleic Acid amplification (OSNA) of sentinel lymph node in Early-Stage Endometrial cancer: spanish multicenter study (ENDOOSNA). Cancers (Basel) 2021; 13(17): 4465. doi: 10.3390/cancers13174465.
9. Chacon E, Boria F, Lyer RR et al. SENECA study: staging endometrial cancer based on molecular classification. Int J Gynecol Cancer 2024; 34: 1313–1321. doi: 10.1136/ijgc-2024-005711.
10. Kosťun J, Ismail KM, Pešta M et al. One-Step Nucleic Acid amplification analysis of sentinel nodes in endometrial cancer versus ultrastaging: first long-term follow-up data of discordant cases. Cancer Rep (Hoboken) 2024; 7(12): e70082. doi: 10.1002/cnr2.70082.
11. Hambálek J, Maděrka M, Kolečková M et al. Nízkoobjemové postižení uzlin u karcinomu endometria. Ceska gynekol 2019; 84(6): 458–462.
12. Mueller JJ, Pedra Nobre S, Braxton K et al. Incidence of pelvic lymph node metastasis using modern FIGO staging and sentinel lymph node mapping with ultrastaging in surgically staged patients with endometrioid and serous endometrial carcinoma. Gynecol Oncol 2020; 157(3): 619–623. doi: 10.1016/j.ygyno.2020.03.025.
13. De Vitis LA, Fumagalli D, Schivardi G et al. Incidence of sentinel lymph node metastases in apparent early-stage endometrial cancer: a multicenter observational study. Int J Gynecol Cancer 2024; 34(5): 689–696. doi: 10.1136/ijgc-2023-005173.
14. Buda A, Paniga C, Taskin S et al. The risk of recurrence in endometrial cancer patients with low-volume metastasis in the sentinel lymph nodes: a retrospective multi-institutional study. Cancers (Basel) 2023; 15(7): 2052. doi: 10.3390/cancers15072052.
15. Lavecchia M, Jang JH, Lee HJ et al. Sentinel lymph node biopsy in endometrial cancer: the new norm – a multicentre, international experience. Surg Oncol 2023; 48: 101922. doi: 10.1016/j.suronc.2023.101922.
16. Matsuo K, Chen L, Neuman MK et al. Lymph node isolated tumor cells in patients with endometrial cancer. JAMA Netw Open 2024; 7(3): e240988. doi: 10.1001/jamanetworkopen.2024.0988.
17. García Pineda V, Hernández Gutiérrez A, Gracia Segovia M et al. Low-volume nodal metastasis in endometrial cancer: risk factors and prognostic significance. J Clin Med 2020; 9(6): 1999. doi: 10.3390/jcm9061999.
18. Carretta A. Clinical value of nodal micrometastases in patients with non-small cell lung cancer: time for reconsideration? J Thorac Dis 2016; 8(12): E1755–E1758. doi: 10.21037/jtd.2016.12.83.
19. Sloothaak DA, Sahami S, van der Zaag-Loonen HJ et al. The prognostic value of micrometastases and isolated tumour cells in histologically negative lymph nodes of patients with colorectal cancer: a systematic review and meta-analysis. Eur J Surg Oncol 2014; 40(3): 263–269. doi: 10.1016/j.ejso.2013.12.002.
20. Ignatov A, Lebius C, Ignatov T et al. Lymph node micrometastases and outcome of endometrial cancer. Gynecol Oncol 2019; 154(3): 475–479. doi: 10.1016/j.ygyno.2019.07.018.
21. Ghoniem K, Larish AM, Dinoi G et al. Oncologic outcomes of endometrial cancer in patients with low-volume metastasis in the sentinel lymph nodes: an international multi-institutional study. Gynecol Oncol 2021; 162(3):590–598.doi:10.1016/j.ygyno.2021.06.031.
22. Bogani G, Mariani A, Paolini B et al. Low-volume disease in endometrial cancer: the role of micrometastasis and isolated tumor cells. Gynecol Oncol 2019; 153(3): 670–675. doi: 10.1016/j.ygyno.2019.02.027.
23. Backes FJ, Felix AS, Plante M et al. Sentinel lymph node (SLN) isolated tumor cells (ITCs) in otherwise stage I/II endometrioid endometrial cancer: to treat or not to treat? Gynecol Oncol 2021; 161(2): 347–352. doi: 10.1016/j.ygyno.2021.02.017.
24. Cucinella G, Schivardi G, Zhou XC et al. Prognostic value of isolated tumor cells in sentinel lymph nodes in low risk endometrial cancer: results from an international multi-institutional study. Int J Gynecol Cancer 2024; 34(2): 179–187. doi: 10.1136/ijgc-2023-005032.
25. Schivardi G, Cucinella G, Mariani A et al. 2022-RA-987-ESGO Molecular characterization of endometrial cancer with low vol-

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ume metastasis in the sentinel lymph node: a multicentric international study. Int J Gynecol Cancer 2022; 32(Suppl 2): A124.2. doi: 10.1136/ijgc-2022-ESGO.268.
26. Rios-Doria E, Abu-Rustum NR, Alektiar KM et al. Prognosis of isolated tumor cells and use of molecular classification in early stage endometrioid endometrial cancer. Int J Gynecol Cancer 2024; 34(9): 1373–1381. doi: 10.1136/ijgc-2024-005522.
27. Sullivan SA, Hawkins G, Zhao X et al. Genomic profiling of endometrial cancer and relationship with volume of endometrial cancer disease spread. Gynecol Oncol Rep 2021; 36: 100720. doi: 10.1016/j.gore.2021.100720.
28. Watanabe T, Nanamiya H, Kojima M et al. Clinical relevance of oncogenic driver mutations identified in endometrial carcinoma. Transl Oncol 2021; 14(3): 101010. doi: 10.1016/j.tranon.2021.101010.
29. de Boer M, van Deurzen CH, van Dijck JA et al. Micrometastases or isolated tumor cells and the outcome of breast cancer. N Engl J Med 2009; 361(7): 653–663. doi: 10.1056/NEJMoa0904832.

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6 Predictive Models

Predictive models have become an integral component of precision medicine, offering clinicians a means to estimate individual patient risk, guide therapeutic decision-making, and optimize oncologic outcomes. In gynecologic oncology, particularly in managing endometrial carcinoma, predictive models are employed to assess the likelihood of lymph node metastasis, disease recurrence, treatment response, and overall survival. To generate individualized risk estimates, these models integrate diverse data sources, including clinical parameters, histopathological features, molecular profiles, and imaging findings.

Depending on the methodological approach, predictive models may take the form of nomograms, decision trees, Bayesian networks, or machine learning algorithms etc. Their application supports a shift from traditional one-size-fits-all strategies to a more tailored and evidence-based approach in the individualized care of patients with endometrial cancer. For example:

- **Nomogram**

A nomogram is a graphical representation of a multivariable statistical model, typically derived from logistic or Cox regression. It allows clinicians to calculate an individual patient's probability of a specific clinical outcome by aligning values for multiple risk factors on a visual scale[62]. Nomograms are favored in oncology for their interpretability and ease of bedside application.

Example in EC: A nomogram developed and validated by Wang et al. predicts the risk of lymph node metastasis in patients with endometrial cancer[63]. The model integrates seven clinical and pathological variables, demonstrating high predictive accuracy (AUC > 0.8). Such tools are particularly useful in guiding the decision to perform lymphadenectomy or sentinel lymph node biopsy in early-stage disease.

- **Decision Tree**

A decision tree is a non-parametric supervised learning algorithm that stratifies data into subsets based on a series of binary decisions made on explanatory variables. Each internal node represents a test on a feature, each branch denotes the outcome of the

test, and each leaf node provides a classification or prediction[64]. Decision trees are intuitive and can be easily interpreted, but are prone to overfitting if not properly pruned.

Example in EC: Another study applied interpretable decision tree models to molecular and clinical datasets of endometrial cancer. The authors demonstrated how the branching logic of the model revealed novel associations between p53-mut tumors and specific clinical features, thus facilitating hypothesis generation for future studies[65].

- **Bayesian Network**

A Bayesian network is a probabilistic graphical model that represents a set of variables and their conditional dependencies via a directed acyclic graph. This model incorporates prior knowledge and can update predictions as new evidence is introduced, making it especially suitable for managing uncertainty in complex clinical scenarios[66].

Example in EC: The ENDORISK (ENDOmetrial cancer preoperative RISK stratification) model, based on a Bayesian network framework, predicts the likelihood of lymph node metastasis and five-year disease-specific survival in endometrial carcinoma. The model integrates preoperative clinical, histological, and molecular features (limited to p53 abnormality). External validation has shown a high discriminatory capacity (AUC = 0.82), underlining its potential utility in pre-surgical risk stratification[67].

These predictive tools exemplify the convergence of statistical modeling and personalized medicine in gynecologic oncology. Their integration into routine practice requires careful validation, transparent reporting, and continuous refinement based on evolving clinical evidence.

6.1 Weinberger V, Bednaříková M, Hausnerová J, Ovesná P, Vinklerová P, Minář L, Felsinger M, Jandáková E, Číhalová M, Zikán M. A Novel Approach to Preoperative Risk Stratification in Endometrial Cancer: The Added Value of Immunohistochemical Markers. Front. Oncol. 2019, 9:265.

The first study of this chapter demonstrates an example of a decision tree, which aimed to enhance the preoperative risk stratification of endometrial cancer patients by incorporating immunohistochemical markers into the existing diagnostic model, which was based on traditional histopathological examination, clinical, and imaging findings. It is important to note that this study was conducted before the era of molecular classification introduction into clinical practice.

Our study sought to evaluate whether the addition of specific IHC markers—ER, PR, L1CAM, and p53—could improve the sensitivity and specificity of preoperative risk assessments in EC patients.

We conducted a prospective analysis involving patients diagnosed with endometrial carcinoma. Preoperative biopsies were assessed for the expression levels of ER, PR, L1CAM, and p53 using standardized IHC techniques. These findings were then integrated into the existing diagnostic model, which included clinical and imaging data, to assess any improvements in risk stratification accuracy.

Incorporating IHC markers into the diagnostic model significantly improved the sensitivity for identifying high-risk EC patients from 48.4% to 75.8% ($p < 0.001$). The presence of p53 mutations was associated with a PPV (positive predictive value) of 94% and an NPV (negative predictive value) of 61%, indicating its potential as a significant prognostic marker. We established specific cut-off values for high-risk tumors: ER expression below 78%, PR below 88%, and L1CAM expression equal to or exceeding 4%.

In conclusion, our findings demonstrate that the integration of IHC markers into preoperative diagnostic models substantially enhances the accuracy of risk stratification in endometrial cancer patients. This approach allows for more precise

identification of high-risk individuals, potentially leading to better-informed surgical decisions and personalized treatment plans.

The study *"A Novel Approach to Preoperative Risk Stratification in Endometrial Cancer: The Added Value of Immunohistochemical Markers"* was published in *Frontiers in Oncology* (IF 4.848, Q2) in 2019.

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A Novel Approach to Preoperative Risk Stratification in Endometrial Cancer: The Added Value of Immunohistochemical Markers

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Background: The current model used to preoperatively stratify endometrial cancer (EC) patients into low- and high-risk groups is based on histotype, grade, and imaging method and is not optimal. Our study aims to prove whether a new model incorporating immunohistochemical markers, L1CAM, ER, PR, p53, obtained from preoperative biopsy could help refine stratification and thus the choice of adequate surgical extent and appropriate adjuvant treatment.

Materials and Methods: The following data were prospectively collected from patients operated for EC from January 2016 through August 2018: age, pre- and post-operative histology, grade, lymphovascular space invasion, L1CAM, ER, PR, p53, imaging parameters obtained from ultrasound, CT chest/abdomen, final FIGO stage, and current decision model (based on histology, grade, imaging method).

Results: In total, 132 patients were enrolled. The current model revealed 48% sensitivity and 89% specificity for high-risk group determination. In myometrial invasion >50%, lower levels of ER ($p = 0.024$), PR (0.048), and higher levels of L1CAM ($p = 0.001$) were observed; in cervical involvement a higher expression of L1CAM ($p = 0.001$), lower PR ($p = 0.014$); in tumors with positive LVSI, higher L1CAM ($p = 0.014$); in cases with positive LN, lower expression of ER/PR ($p < 0.001$), higher L1CAM ($p = 0.002$) and frequent mutation of p53 ($p = 0.008$).

Cut-offs for determination of high-risk tumors were established: ER <78% ($p = 0.001$), PR <88% ($p = 0.008$), and L1CAM ≥4% ($p < 0.001$). The positive predictive values (PPV) for ER, PR, and L1CAM were 87% (60.8–96.5%), 63% (52.1–72.8%), 83% (70.5–90.8%); the negative predictive values (NPV) for each marker were as follows: 59% (54.5–63.4%), 65% (55.6–74.0%), and 77% (67.3–84.2%). Mutation of p53 revealed PPV 94% (67.4–99.1%) and NPV 61% (56.1–66.3%). When immunohistochemical markers were included into the current diagnostic model, sensitivity improved (48.4 vs. 75.8%, $p < 0.001$). PPV was similar for both methods, while NPV (i.e., the probability of extremely low risk in negative test cases) was improved (66 vs. 78.9%, $p < 0.001$).

Conclusion: We proved superiority of new proposed model using immunohistochemical markers over standard clinical practice and that new proposed model increases accuracy of prognosis prediction. We propose wider implementation and validation of the proposed model.

Keywords: endometrial cancer, ER, imaging method, L1CAM, PR, preoperative biopsy, p53, risk stratification

INTRODUCTION

Endometrial carcinoma (EC) is one of the most common female cancers. It predominantly has a favorable prognosis, due to the early onset of signs and symptoms such as postmenopausal bleeding or spotting, which lead to early-stage diagnosis in most patients and five-year overall survival rates of up to 85% (1). However, 20% of those EC patients who are estimated to be at low risk of recurrence will nevertheless recur while up to 50% of those designated "high-risk" will not (2, 3). It is clear the prognostic markers currently used (FIGO stage, tumor subtype, and histological grade) are far from optimal in terms of preoperative stratification of patients into low- or high-risk groups regarding surgical planning and adjuvant treatment.

One of the currently used prognostic markers is FIGO stage. This is obligatory and determined by transvaginal ultrasound of the pelvis (US). Computed tomography (CT) of the chest and abdomen is an imaging method of choice and is routinely used to exclude retroperitoneal lymphadenopathy and metastases in parenchymal organs. The other prognostic markers, histotype, and grade of tumor differentiation, are assessed from a biopsy obtained either by dilatation and curettage of the uterus or by hysteroscopy. Based on the established FIGO stage, histotype, and tumor grade, patients are divided into two groups regarding the recurrence risk. Low-risk patients are treated with surgery alone, consisting of hysterectomy and bilateral salpingo-oophorectomy, while high-risk patients undergo more aggressive surgical treatment, including pelvic (PLN) and/or paraaortic lymphadenectomy (PALN) with or without adjuvant radiotherapy or chemotherapy. An aggressive therapeutic approach is associated with significantly higher side effects, such as increased blood loss, risk of thrombosis, infection, lymphoceles, lymphatic ascites, and lymphedema (4, 5).

The discovery of new histotype-specific and prognostic biomarkers for better stratification into high- or low-risk EC seems to be urgently needed in order to avoid over- or undertreatment of EC patients. The results of studies on potential new biomarkers assessed immunohistochemically (IHC), related to EC patient prognosis, were recently published. L1 cell adhesion molecule (L1CAM) overexpression and the loss of estrogen receptors (ER) and/or progesterone receptors (PR) are associated with poor prognosis and a high risk of relapse and death (6–9). Mutations of the tumor protein p53 are associated with L1CAM expression, but not universally (10). However, to our best knowledge, neither the significance of L1CAM, ER, and PR expression nor knowledge of their relevant cut-offs together with determination of p53 mutation status in preoperative biopsies for pretreatment stratification into low- or high-risk have been established yet. No IHC biomarkers from preoperative biopsy are currently routinely used in the decision-making process for EC management.

The purpose of this study was to evaluate the clinical usefulness and added value of preoperatively assessed IHC biomarkers L1CAM, ER, PR, and p53 in differentiation between low- and high-risk EC patients through comparison of the current clinical practice model with a proposed model that includes immunohistochemical markers. The secondary objective of our study was to evaluate the correlation of IHC biomarkers with specific clinical (according to preoperative ultrasound and CT chest/abdomen) and pathological parameters.

PATIENTS AND METHODS

Patients

Patients undergoing surgical treatment for histologically proven or suspicious EC in the oncogynecological center of University Hospital Brno, Czech Republic, from January 2016 to August 2018 were consecutively included. The study was approved by the Institutional Ethical Board as was a version of written informed consent regarding tissue and clinical data use for scientific purposes obtained from each eligible patient.

Preoperative Imaging

All patients underwent a clinical examination, preoperative ultrasound staging examination, and CT of the chest/abdomen according to the local guidelines (11, 12). Each patient underwent both a transabdominal and a transvaginal US scan within 14 days before a board discussion led by one of the two oncogynecologists experienced in the field of US diagnostics in gynecologic oncology. Each US examination was immediately described in a written report; these reports were used for study analysis. Descriptions and examination reports were based on the standards applied by our center (13). During US staging examination of the uterine cavity, myometrium and cervix and pelvic lymph nodes were carefully assessed in every patient to describe the local extent of the tumor (14, 15).

Each patient underwent a CT scan of the chest, abdomen, and pelvis within 14 days before board discussion and admission to the operating theater. CT was performed with oral and intravenous contrast in order to exclude bowel wall implants, parenchymatous metastasis, and pathological lymphadenopathy. When lymph nodes measured >1 cm in the shorter axis or morphological changes as a rounded shape or necrosis were observed, tumor involvement was marked as suspicious.

Risk Stratification and Clinical Management

The extent of the surgery was determined by the multidisciplinary board after dividing patients into the low- or high-risk group based on clinical staging and the preoperative histopathological examination and determination of the histotype and grading. The low-risk group was defined as endometrioid or mucinous carcinoma TNM stage cT1a or cT1b, grade 1 and/or endometrioid or mucinous carcinoma TNM stage cT1a, grade 2, all without clinical or imaging evidence of lymphadenopathy (cN0) or distant metastases (cM0). Patients were defined as high-risk unless these low-risk criteria were met. Type A radical hysterectomy with bilateral salpingo-ophorectomy was performed in all patients (16). Systematic pelvic and paraaortic lymphadenectomy was performed in the high-risk group only; in high-grade serous uterine cancer cases, total omentectomy and appendectomy were added to the staging procedure. The definitive histopathological examination was provided by one of three pathologists with experience in gynecological malignancies and contained data about stage, histotype, and grade, lymphovascular space involvement (LVSI), and measures of the IHC expression of markers L1CAM, ER, PR, and p53. Based on final histopathological findings, the patients were once again stratified into low- or high-risk groups based on the same preoperative criteria (i.e., irrespective of known IHC status of ER, PR, L1CAM, and p53) and, thereafter, decisions regarding adjuvant treatment and follow-up were made by the multidisciplinary board.

Clinical Data

Age, results of US and CT scan with respect to depth of myometrial invasion, cervical involvement, lymphadenopathy, parenchymal organ involvement, and pathological data from biopsies (histotype, grading, IHC status of L1CAM, ER, PR, p53) were recorded.

Tissue and Immunohistochemistry Analysis

All hematoxylin and eosin-stained slides were read by one of three experienced gynecological histopathologist to confirm histological subtype, grade, and (definitive excision specimen) stage and the presence or absence of LVSI. The evaluator was blinded to patient characteristics. All specimens were assessed according to the WHO Classification of Tumors of Female Reproductive Organs, 2014 (17). No additional later review of the slides was performed for the purpose of this study because it would not copy our real clinical practice. Immunohistochemical staining was performed on formalin-fixed and paraffin-embedded (FFPE) tissue sections. Immunohistochemistry for ER (clone SP1, product no. RBK 018-05, Zytomed, dilution 1:300), PR (clone 16, product no. NCL-L-PGR-312, Novocastra, dilution 1:80), L1CAM/CD171 (clone 14.10, product no. 826701, BioLegend, dilution 1:100), and p53 (clone DO-7, product no. M7001, DAKO, dilution 1:300) were performed using an automatic immunostainer (BenchMark Ultra, Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer's instructions. For ER, PR, and p53, only nuclear staining was scored as positive. Positivity of L1CAM was defined as distinct membrane staining. For ER, PR, and L1CAM, the percentage of positive tumor cells was assessed. p53 was classified into wild type or mutant (excessive = strong diffuse overexpression in more than 90% of tumor cells or completely negative) phenotypes. Representative microphotographs of the expression of estrogen receptor (ER), progesterone receptor (PR), L1CAM and p53 in serous (high risk) and grade 1 endometrioid (low risk) carcinoma are shown in **Figures 1A–H**.

Statistical Analysis

Categorical data were summarized using absolute and relative frequencies and compared by Fisher's exact test. Continuous variables were summarized as median with 10 and 90^{th} percentile and tested by the Mann-Whitney *U*-test.

A model for the best classification of final risk was built using the CHAID growing method with crossover validation. Misclassification cost for wrongly determining high-risk patients as low-risk was set twice higher because of the preference for the correct high-risk group EC patient determination.

The success of risk-group classification was evaluated using four standard measures: (i) *sensitivity* is the ability of the test to correctly identify those with an occurrence of the assessed marker (true positive rate), whereas (ii) *specificity* is the ability of the test to correctly identify those without an occurrence of the assessed marker (true negative rate), (iii) *positive predictive value* (PPV) is the probability that the marker is present when the test is positive, whereas (iv) *negative predictive value* (NPV) is the probability that the marker is not present when the test is negative. All these statistics were accompanied by 95% confidence intervals (CI).

The comparison of sensitivities and specificities of the two binary diagnostic tests in a paired study design was performed using McNemar's test with continuity correction. Differences in (positive and negative) predictive values of two binary diagnostic tests were tested using a generalized score statistic proposed by Leisenring, Alonzo, and Pepe (18). All tests were performed as two-sided at the significance level 0.05. Analyses were done in IBM SPSS Statistics and R.

RESULTS

Clinical and Histopathological Characteristics

From January 2016 to August 2018, 132 patients underwent surgical treatment for EC in the oncogynecological center of University Hospital Brno, Czech Republic, and have been consecutively enrolled in the study. The median age was 66 years. According to ultrasound and CT staging, before operation 95 patients (72%) were evaluated as FIGO stage IA, while 25 (19%) were stage IB, 5 (4%) stage II, 3 (2%) stage III, and 4 (3%) were at an unknown stage. Preoperative biopsy was available for all 132 patients; 102 patients had endometrioid cancer, of whom 50 (49%) had endometrioid or mucinous carcinoma grade 1 (EG1), 45 (44%) grade 2 (EG2), 6 (6%) grade 3 (EG3), and one had a non-diagnostic grade. Seventeen (13%) patients were diagnosed with non-endometrioid carcinoma (NEC). Furthermore, there were

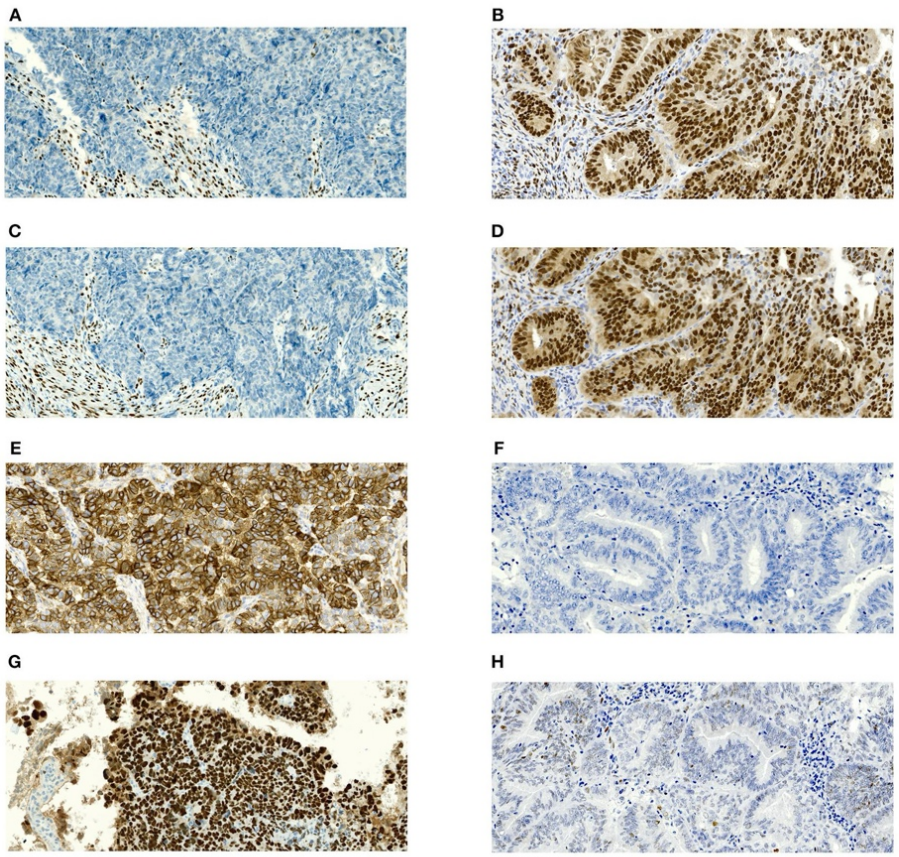

FIGURE 1 | Microphotographs showing representative examples of immunohistochemical expression of estrogen receptors (ER), progesterone receptor (PR), L1CAM and p53 in tissue specimens of endometrial carcinomas. Magnification 100x. **(A)** Complete negativity of ER expression in serous carcinoma with 0% cells positive; **(B)** Complete negativity of PR expression in serous carcinoma with 0% cells positive; **(C)** Strong diffuse membranous positivity of L1CAM expression in serous carcinoma with 100% cells positive; **(D)** p53 nuclear overexpression (mutant pattern) in serous carcinoma; **(E)** Nuclear positivity of ER expression in grade 1 endometrioid carcinoma with almost 100% cells positive; **(F)** nuclear positivity of PR expression in grade 1 endometrioid carcinoma with almost 100% cells positive; **(G)** complete negativity of L1CAM expression in grade 1 endometrioid carcinoma with 0% cells positive; **(H)** p53 wildtype immunohistochemical pattern in grade 1 endometrioid carcinoma.

eight cases (6%) of endometrial intraepithelial neoplasia (EIN) in preoperative biopsy (**Table 1**). Based on both histological and clinical findings, 94 (71%) patients were preoperatively classified as low-risk and 38 (29%) as high-risk by current model and, consequently, the recommendation for the extent of surgery was issued. In the high-risk group, PLN and PALN were performed for 26 (20%) patients, apart from hysterectomy and bilateral salpingo-oophorectomy. After the surgical procedure and definitive histopathological examination, 82 (62%) patients were at FIGO stage IA, 17 (13%) at FIGO stage IB, 19 (14%) FIGO stage II, 12 (9%) FIGO stage III and two (2%) with FIGO stage IV. Regarding endometrioid or mucinous carcinoma grade, 30 (28%) were at EG1, 69 (65 %) at EG2, 8 (7 %) EG3, 20 (15%) NEC, and 2 (2%) EIN. In contrast to the preoperative risk determination, the final post-operative stratification in risk groups was as follows: 70 (53%) low-risk patients and 62 (47%) high-risk patients (**Table 1**).

Immunohistochemical Characteristics

The expression of markers ER, PR, L1CAM, and p53 status were immunohistochemically evaluated from the specimen obtained both by diagnostic procedure and definitive surgery. The correlation of IHC markers between preoperative examination and definitive histopathological findings was statistically significant for all of the evaluated markers ($p < 0.005$). In the preoperative specimen, the expression was evaluable in 98 patients for ER and PR, 97 for L1CAM, and 98 for p53 mutational status; results are listed in **Table 2**.

TABLE 1 | Patients' clinical and histopathological characteristics.

Age at diagnosis	**66 (50–78)**		***p*-value**
Histology	**Preoperative (biopsy or imaging)**	**Final specimen**	
Endometrioid (incl. mucinous)	102 (77%)	107 (81%)	0.216
Non-endometrioid	17 (13%)	20 (15%)	
Serous	6 (35%)	2 (10%)	
Clear cell	4 (24%)	2 (10%)	
Carcinosarcoma	1 (6%)	2 (10%)	
Undifferentiated carcinoma	2 (11%)	3 (15%)	
Mixed carcinoma	4 (24%)	11 (55%)	
EIN	8 (6%)	2 (2%)	
Non-diagnostic	5 (4%)	3 (2%)	
GRADE (ONLY ENDOMETRIOID)			
G1	50 (49%)	30 (28%)	0.006
G2	45 (44%)	69 (65%)	
G3	6 (6%)	8 (7%)	
Non-diagnostic	1 (1%)		
MYOMETRIAL INVASION			
<50%	96 (73%)	93 (70%)	0.557
≥50%	33 (25%)	39 (30%)	
Unknown	3 (2%)		
CERVICAL INVASION			
Yes	9 (7%)	24 (18%)	0.015
No	120 (91%)	108 (82%)	
Unknown	3 (2%)		
LYMPHADENOPATHY			
Yes	3 (2%)	9 (7%)	0.070
No	129 (98%)	123 (93%)	
TUMOR BOARD DECISION			
Low-risk EC	94 (71%)	70 (53%)	<0.001
High-risk EC	38 (29%)	62 (47%)	

values denote median (10-90th percentile) or n (%); p-values of chi-square or McNemar's test; G, grade; EC, endometrial cancer.

Correlation of IHC Markers With Disease Extent (FIGO Staging)

The correlation was assessed between IHC markers in preoperative tissue samples and the final histopathological findings (e.g., myometrial invasion, cervical, and lymph node involvement). Moreover, the correlation with LVSI was evaluated because LVSI is one of the important markers for adjuvant treatment strategy decisions. There were statistically significant lower levels of ER ($p = 0.024$) and PR (0.048) and higher levels of L1CAM ($p = 0.001$) in tumors with myometrial invasion >50%. In tumors with cervical involvement, a significantly higher expression of L1CAM was observed ($p = 0.001$), while differences among levels of ER ($p = 0.236$) and PR ($p = 0.108$) did not reach statistical significance. PR were significantly lower ($p = 0.014$) and L1CAM higher ($p = 0.014$) in tumors with positive LVSI. In patients with positive LN, levels of ER and PR were lower ($p = 0.001$ and $p < 0.001$, respectively); on the other hand, levels of L1CAM were higher ($p = 0.002$) and the mutation of p53 more frequent ($p = 0.008$), see **Table 3**.

The Precision of EC Risk Stratification Based on Markers Currently Used in Clinical Praxis

Concerning depth of myometrial invasion, we classified all the patients in whom invasion reached ≥50% as high-risk ($n = 36$) and the patients with invasion <50% as low-risk ($n = 96$). Our approach to preoperative risk stratification of EC patients revealed a sensitivity of 48% and specificity of 89% in terms of high-risk group determination. Taking all current standard prognostic markers together, it can be concluded that, whereas low-risk EC patients are preoperatively classified with relatively high accuracy (62/70, 82%), the determination of high risk is far from optimal, since more than half of EC patients with actual high-risk disease were established as low-risk. See **Table 4**.

The Accuracy of EC Risk Stratification by Using IHC Markers

IHC markers were assessed in a preoperative tumor sample, and optimal cut-offs for continuous markers (obtained preoperatively) were designed using ROC analyses. At a cut-off for ER <78% ($p = 0.001$), PR <88% ($p = 0.008$), and L1CAM ≥4% ($p < 0.001$), high-risk tumors were determined with a sensitivity of 28% for ER (15.6–42.6%), 62% for PR (46.4–75.5%), and 72% for L1CAM (57.4–84.4%). Specificity was 96% (86.5–99.5%), 68% (52.1–79.2%), and 86% (73.3–94.2%), respectively. The PPV was 87% for ER (60.8–96.5%), 63% for PR (52.1–72.8%), and 83% for L1CAM (70.5–90.8%); the NPV for each marker were as follows: 59% (54.5–63.4%), 65% (55.6–74.0%), and 77% (67.3–84.2%), respectively. The sensitivity of p53 mutated status ($p < 0.001$) for high-risk detection was low (34%), but the specificity was high (98.0%, CI 88.7–99.9%), which represents PPV 94% (67.4–99.1%) and NPV 61% (56.1–66.3%), respectively. If p53 mutated, there was a high probability the patient fit into the high-risk group (15 out of 16 patients in our series). As far as accuracy of high-risk determination in the largest number of patients was concerned, the marker L1CAM seemed to be the most robust: 34 from 41 patients who had L1CAM values ≥4% were classified as high-risk (**Table 5**, **Figure 2**).

The Added Value of IHC Markers for Improvement of EC Risk Stratification

To evaluate whether IHC markers would contribute to more accurate stratification of EC patients into risk groups, a model consisting of parameters obtained by imaging methods (myometrial invasion, cervical involvement, lymph node involvement), histology (endometrioid or mucinous vs. non-endometrioid), grade and IHC markers (ER, PR, L1CAM, p53) was introduced. According to risk stratification, the following parameters have been shown as statistically significant and crucial for the model: L1CAM, PR, and myometrial invasion.

TABLE 2 | Immunohistochemical biomarkers in preoperative biopsies.

IHC markers	Overall values ($n = 98$)	EG1 ($n = 36$)	EG2 ($n = 37$)	EG3 ($n = 9$)	EIN ($n = 3$)	NEC ($n = 13$)
ER (%), $n = 98$	86 (27)	95 (15)	96 (8)	71 (34)	100 (0)	44 (42)
	99 (40–100)	100 (90–100)	99 (85–100)	80 (0–100)	100 (100–100)	30 (0–100)
PR (%), $n = 98$	73 (34)	88 (21)	79 (28)	48 (39)	87 (23)	28 (33)
	90 (5–100)	99 (60–100)	95 (30–100)	70 (0–95)	100 (60–100)	20 (0–85)
L1CAM (%), $n = 97$	16 (29)	2 (4)	7 (13)	33 (35)	0 (1)	72 (33)
	3 (0–70)	1 (0–8)	3 (0–15)	30 (0–100)	0 (0–1)	85 (15–100)
p53, $n = 98$						
Mut	16 (16.3%)	1 (2.8%)	2 (5.4%)	2 (22.2%)	0 (0%)	11 (84.6%)
Wt	75 (76.5%)	35 (97.2%)	32 (86.5%)	4 (44.4%)	2 (66.7%)	2 (15.4%)
Non-specific	7 (7.1%)	0 (0%)	3 (8.1%)	3 (33.3%)	1 (33.3%)	0 (0%)

Values denote mean (SD) and median (10–90th percentile) or n (%); means and SD are shown only for exploratory purpose since data are not normally distributed; IHC, immunohistochemical markers; ER, estrogen receptors; PR, progesterone receptors; L1CAM, L1cell adhesion molecule; mut, mutated; wt, wild type; EG1, endometrioid or mucinous cancer, grade 1; EG2, endometrioid or mucinous cancer, grade 2; EG3, endometrioid or mucinous cancer, grade 3; EIN, endometrioid intraepithelial neoplasia; NEC, non-endometrioid cancer.

TABLE 3 | Correlation of IHC markers from preoperative biopsy with staging after surgery.

	ER (%) ($N = 98$)	PR (%) ($N = 98$)	L1CAM (%) ($N = 97$)	p53 mut ($N = 16$)	P53 wt ($N = 75$)
Myometrial invasion	$p = 0.024$	$p = 0.048$	$p = 0.001$	$p = 0.382$	
<50% ($N = 66$)	88 (26) 100 (60–100)	77 (31) 95 (20–100)	14 (29) 1 (0–70)	9 (14.8%)	52 (85.2%)
≥50% ($N = 32$)	83 (30) 98 (40–100)	65 (38) 82.5 (0–100)	20 (29) 5 (1–70)	7 (23.3%)	23 (76.7%)
Cervical involvement	$p = 0.236$	$p = 0.108$	$p = 0.001$	$p = 0.070$	
Yes ($N = 19$)	82 (31) 95 (0–100)	60 (39) 70 (0–100)	32 (38) 8 (1–95)	6 (35.3%)	11 (64.7%)
No ($N = 79$)	88 (26) 99 (40–100)	76 (32) 90 (15–100)	12 (26) 2 (0–50)	10 (13.5%)	64 (86.5%)
LN (lymph node) metastases	$p = 0.001$	$p < 0.001$	$p = 0.002$	$p = 0.008$	
Yes ($N = 6$)	43 (47) 35 (0–95)	11 (20) 0.5 (0–50)	60 (36) 70 (4–100)	4 (66.7%)	2 (33.3%)
No ($N = 92$)	89 (23) 99 (70–100)	77 (31) 92.5 (20–100)	13 (27) 2 (0–50)	12 (14.1%)	73 (85.9%)
LVSI	$p = 0.111$	$p = 0.014$	$p = 0.014$	$p = 0.260$	
Yes ($N = 14$)	74 (41) 95 (0–100)	48 (43) 45 (0–100)	23 (32) 6 (2–70)	4 (22.2%)	10 (55.6%)
No ($N = 84$)	89 (24) 99 (60–100)	77 (31) 93 (20–100)	15 (29) 2 (0–70)	12 (13.0%)	65 (70.7%)

Values denote mean (SD) and median (10-90th percentile) or n (%), p-value of Mann-Whitney U-test or Fisher's exact test; means and SD are shown only for exploratory purpose since data are not normally distributed; LVSI, lymphovascular space involvemen; mut, mutated; wt, wild type.

The procedure of classification is shown in **Figure 3**. The overall EC risk stratification success was 78% for this model. Successful group inclusion was observed for 80% of the low-risk patients (56/70), and for 76% of high-risk patients (47/62 patients).

New Model in Comparison to Current Practice

Immunohistochemical markers included in the current diagnostic practice would significantly improve sensitivity (48.4 vs. 75.8%, $p < 0.001$) associated with a slightly, statistically non-significant decrease in specificity (to 80%, $p = 0.238$). Positive predictive values were similar for both methods, while negative predictive value (i.e., the probability of extremely low risk in negative test cases) was significantly improved (66 vs. 78.9%, $p < 0.001$) (**Table 6**, **Figure 4**).

DISCUSSION

The determination of an appropriate surgery and its adequate extent is a crucial part of treatment in newly diagnosed EC patients and significantly differs between the high- and low-risk groups. Existing models determining patient risk are based on the

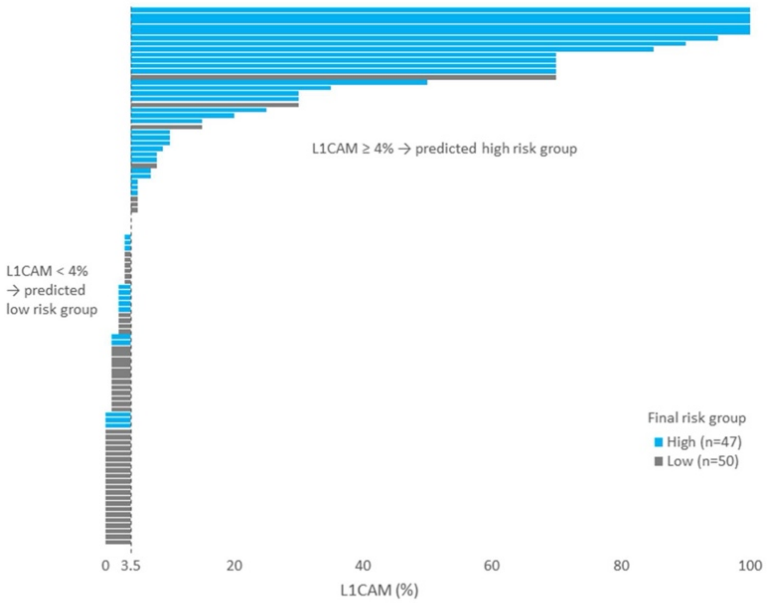

FIGURE 2 | Each patient is represented by one bar. Patients on the left part of the figure (i.e., with L1CAM<4) was predicted as low risk. Blue bars represent wrongly predicted patients here. On the contrary, gray bars on the right side of the figure represent patients wrongly predicted as high risk.

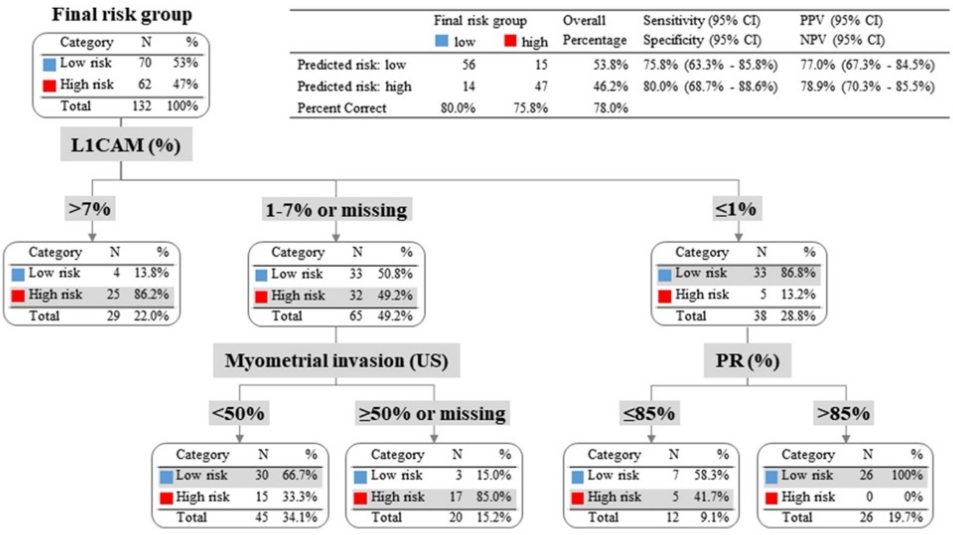

FIGURE 3 | Patients with L1CAM positivity >4% were identified as high risk; in fact 86% of them were high-risk indeed. Patients with L1CAM value from 1 to 4% were further divided according to myometrial invasion. In case of myometrial invasion <50% they were stratified as low-risk, on the contrary in case of myometrial invasion >50% or unknown they were classified as high-risk. Patients with L1CAM <1% were further disaggregated by PR value. In case of PR >85% they were identified as low-risk (with success rate of 100%), on the other hand in case of PR ≤85% they were stratified as high-risk according to clinical preference for more precise high-risk group determination even with the expectation of higher false positivity.

TABLE 4 | Accuracy of low-/high-risk group classification according to current practice.

	Final risk				
	Low-risk	**High-risk**	**Total *N***	**Sensitivity (95% CI) Specificity (95% CI)**	**PPV (95% CI) NPV (95% CI)**
Current model: low-risk	62	32	94	48.4% (35.5–61.4%)	78.9% (65.0–88.3%)
Current model: high-risk	8	**30**	38	88.6% (78.7–94.9%)	66.0% (60.0–71.4%)
Total *N*	70	62	132		

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value. Bold values are significant.

TABLE 5 | Correlation of IHC with final EC risk stratification.

	Final risk					
	Low-risk	**High-risk**	**Total *N***	***p*-value**	**Sensitivity (95% CI) Specificity (95% CI)**	**PPV (95% CI) NPV (95% CI)**
ER <78 (high-risk)	2	**13**	15	0.001	27.7% (15.6–42.6%)	86.7% (60.8–96.5%)
ER 78+ (low-risk)	49	34	83		96.1% (86.5–99.5%)	59.0% (54.5–63.4%)
Total *N*	51	47	98			
PR <88 (high-risk)	17	**29**	46	0.008	61.7% (46.4–75.5%)	63.0% (52.1–72.8%)
PR 88+ (low-risk)	34	18	52		66.7% (52.1–79.2%)	65.4% (55.6–74.0%)
Total *N*	51	47	98			
L1CAM <4 (low-risk)	43	13	56	<0.001	72.3% (57.4–84.4%)	82.9% (70.5–90.8%)
L1CAM 4+ (high-risk)	7	**34**	41		86.0% (73.3–94.2%)	76.8% (67.3–84.2%)
Total *N*	50	47	97			
p53 mut (high-risk)	1	**15**	16	<0.001	34.1% (20.5–49.9%)	93.8% (67.4–99.1%)
P53 wt (low-risk)	46	29	75		97.9% (88.7–99.9%)	61.3% (56.1–66.3%)
Total *N*	47	44	91			

p-value of Fisher's exact test; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; mut, mutated; wt, wild type. Bold values are significant.

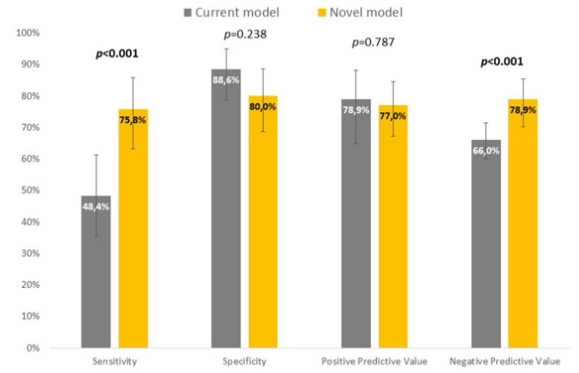

FIGURE 4 | Comparison of current and novel model for prediction of risk tumor type.

synthesis of information obtained from the result of preoperative biopsy (histotype, grading) and imaging methods. Based on the results, patients are included in a risk group before the surgery and so only hysterectomy and salpingo-oophorectomy are indicated, or the procedure is extended by pelvic and para-aortic lymphadenectomy. Proper preoperative inclusion of a

TABLE 6 | Comparison of standard and new diagnostic approaches.

		Final risk: Low			Final risk: High		
		Current model: low-risk	**Current model: high-risk**	**Total**	**Current model: low risk**	**Current model: high risk**	**Total**
Novel model (IMG+IHC)	Low	50 (71.4%)	**6 (8.6%)**	56 (80%)	15 (24.2%)	**0 (0%)**	15 (24,2%)
	High	**12 (17.1%)**	2 (2.9%)	14 (20%)	**17 (27.4%)**	30 (48.4%)	47 (75,8%)
Total		62 (88.6%)	8 (11.4%)	70 (100%)	32 (51.6%)	30 (48.4%)	62 (100%)

IMG, imaging method; IHC, immunohistochemical markers; current model, histology, grading, imaging method. Bold values are significant.

patient in the risk group is crucial for her treatment and overall survival and is a clinically crucial question.

Other approaches to assess the biological behavior of endometrial cancers are under development. Several research groups have defined immunohistochemical and/or mutation profiles to allow distinguishing endometrial cancer subtypes. The Cancer Genome Atlas (TCGA) project provided the most comprehensive molecular study on endometrial cancer so far. They identified four group with distinct molecular changes that correlate with progression free survival – *POLE* (Polymerase Epsilon subunit) ultramutated, MSI (microsatellite instability) hypermutated, copy-number low, and copy-number high (19). This approach allows objective categorization of endometrial cancers, however, methodologically remains costly, complex and unsuitable for wider clinical application.

Others introduced a concept of sentinel lymph node detection in endometrial cancer patients. The large prospective study led by Rossi (20) showed very high sensitivity (97.2%) and low false negativity rate (3%) for sentinel lymph node (SLN) detection. SLN detection concept is based on low risk of paraaortic lymph nodes involvement in patients with negative pelvic lymph nodes (21). However, controversy regarding sentinel lymph node detection in high-risk disease and management of low volume nodal disease on ultrastaging still remains.

In many centers, frozen section of uterus is still standard-of-care in terms to confirm or to more specify type and grade of the tumor. Accuracy of frozen section histopathological evaluation is, however, comparable to imaging methods and interobserver agreement regarding both the categories, type and grade, is poor (22–26).

The current development of risk prediction model is mainly focused on combination of imaging and molecular predictors, as our study does. In 2014, Van Holsbeke et al. published a study that externally validated two mathematical models of preoperative risk group prediction in a particular patient (27). The models were based on histology, grading, and the preoperative sonographic evaluation of tumor invasion into the myometrium and cervix. Both models achieved sensitivity of 78–83% and specificity of 68–72% in the detection of high-risk EC patients. In our study, the current clinical model reliably determined low-risk patients (correctly in 83% of cases), while only 8 (11%) patients in the study were false positives included in the high-risk group. The current model preoperatively stratified patients to high-risk with sensitivity of only 48% (35.5–61.4%) and specificity 89% (78.7–94.9%), NPV 66% (60.0–71.4%), and PPV 79% (65.0–88.3%) (**Table 4**).

A number of ultrasound studies have been published for the assessment of individual staging parameters to determine the depth of tumor invasion into the myometrium, with ultrasound sensitivity from 61 to 93% and specificity from 71 to 92%, when performed by an expert sonography specialist (28–32). The sensitivity reported in the evaluation of tumor invasion in cervical stroma was lower, from 25 to 93%, and specificity from 85 to 99% (30, 33, 34). In a study utilizing expert sonography in a specialized center, Fruhauf et al. reported a PPV of 67.6% and NPV of 83.3% for the detection of deep myometrial invasion and PPV of 60.0% and NPV of 88.1% in the detection of tumor affection of the uterine cervix. According to a recent meta-analysis of 18 studies, CT sensitivity is 47% and specificity 93%; as for ultrasound, sensitivity is 55% and specificity up to 85% for the detection of malignant lymphadenopathy (35). In our group of 132 female patients, the invasion of the tumor to half the thickness of the myometrium was determined correctly in 90%; a false-negative result in the high-risk group of patients was reported in 39 cases. CT did not detect pathological lymphadenopathy in six cases out of nine. A total of nine patients were classified as false positives on the basis of US and CT as validated by the definitive histology.

Studies showing the discrepancy between histology obtained from preoperative curettage or hysteroscopy and definitive histological findings have been published (36–38). On the other hand, there are studies showing good concordance between histology, grade, and immunohistochemical staining in curettage and hysterectomy samples (39, 40). We confirmed that the preoperatively determined histological type, grade, and immunohistochemical biomarkers L1CAM, ER, PR, p53 correlated with the final preparation.

In our study, 70 (70/132) patients were classified as low-risk and 62 (62/132) as high-risk. According to the pre-operative staging, PLN and PALN were performed in 26 patients (20%) in our cohort. However, if the definitive risk were known, staging lymphadenectomy would be performed in all 62 patients in the high-risk group (47%). Due to an inappropriate staging surgery, patients underwent repeated surgery or adjuvant radiotherapy, which may have been avoided if a complete surgical staging with negative histological findings of the presence of the tumor in the lymph nodes had been performed. On the contrary, there are onco-gynecological centers which report extensive PLN + PALN in EC patients, thereby increasing post-operative morbidity without an oncology safety increase (41). We focused on currently promising prognostic IHC markers ER, PR, L1CAM, and p53 mutation to determine whether these markers can help

to refine the preoperative stratification of patients into high- and low-risk categories to assist the gynecological oncology surgeon selecting the adequate surgical extent.

A study published by van der Putten et al. revealed that L1CAM expression in curettage specimens is associated with features of aggressive endometrial cancer disease and poor survival of EC patients (42). van der Putten et al. (42) stated that L1CAM, ER, PR were associated with advanced stage, high-grade, non-endometrioid histology, lymphovascular space invasion (LVSI), and reduced disease-free survival (42). Trovik et al. (43) reported that combined ER/PR loss is a significant predictor of nodal affection and overall poor prognosis of patients. ER and PR are prospectively investigated in an ongoing study where the decision of whether to perform or not to perform lymphadenectomy is based on the pre-operational condition of hormone receptors (44). Prospective studies PIPENDO and PORTEC 4 are currently underway. The first study examines the use of molecular risk markers to identify high-risk patients requiring extensive surgery and/or adjuvant therapy (8). PORTEC 4 uses molecular risk factors for the stratification and indication of adjuvant radiotherapy (45).

In our study, ER, PR, L1CAM and p53 values from preoperative histology were related to definitive histology and grading. In endometroid carcinoma, there was a greater percentage of ER, PR receptors and no or ultimately low percentages of L1CAM mutations. The opposite ratio was seen in the occurrence of markers in non-endometroid ECs; p53 was mutated dominantly in endometrial grade 3 and non-endometroid carcinoma. The correlation of IHC markers with the extent of disease shows a decrease in ER and PR expression in higher stages of the disease. Furthermore, an increase in L1CAM expression can be observed when compared with early stages. Similarly, the p53 mutation was more common. Our results are in line with the published data in larger patient cohorts (43, 46). The correlation of markers with the presence of distant metastases could not be assessed as there were only two patients with distant metastases at the time of diagnosis in our study group.

To our best and honest knowledge, this is the first study to evaluate the added value of L1CAM, ER, PR and p53 markers in low- and high-risk EC preoperative diagnostics. This is the first study attempting to determine the cut-off of the individual markers for this classification.

We focused on the correlation of IHC markers with the determination of high-risk EC and the assessment of optimal cut-offs for continuous markers using ROC analyses. At the cut-off for ER <78% ($p = 0.001$), PR <88% ($p = 0.008$), and L1CAM ≥4% ($p < 0.001$), high-risk tumors were determined with a sensitivity of 28, 62, and 72%, respectively, and with respective specificity of 96, 72, and 86%. The PPV for ER, PR, and L1CAM were 87, 63, 83; the NPV for each marker were as follows: 59%, 65%, and 77%. The sensitivity of p53 mutated status ($p < 0.001$) for high-risk detection was low (34%), but the specificity was high (98%), which represents PPV 94% and NPV 61%, respectively.

A cut-off of 10% for positive L1CAM staining has been reported (6, 40, 47). van Gool et al. reported that when using a cut-off of 10% for positive staining, tumors in the study were classified as L1CAM-positive, with no significant association between L1CAM positivity and the rate of distant metastasis ($p = 0.195$). However, increasing the threshold for L1CAM positivity to 50% resulted in a reduction of the frequency of L1CAM-positive tumors and a significant association with the rate of distant metastasis ($p = 0.018$) (10). Estrogen receptors and PR are considered lost when expression is seen in <10% of the tumor cells. This cut-off used with breast cancer management in the prediction of hormone resistance was also evaluated in EC for prognosis prediction and published (42, 43, 48). In our cohort, we determined optimal cut-offs to distinguish low- and high-risk EC for ER <78% ($p = 0.001$), PR <88% ($p = 0.008$), and L1CAM ≥4% ($p < 0.001$). These cut-offs were established in a prospectively assessed cohort of consecutively included patients. In contrast to the cut-offs we defined, the published values are determined in retrospective cohorts of women with recurrence during follow-up, or with an adverse course of their disease with metastatic spread in parenchymatous organs and retroperitoneal lymph nodes. The incidence of L1CAM positivity and loss of ER, PR in these patients in retrospective cohorts may be significantly higher as it is an already pre-selected group of patients. The explanation may be based on the fact that patients who do not exceed the published cut-off values (10% for L1CAM, ER, and PR or 50% for L1CAM) but exceed the cut-offs set for the high-risk group in our study cannot be traced back in the retrospective studies, as they had not been radically treated with combined surgical and ± adjuvant therapy (radiotherapy, chemotherapy) and there was no recurrence during the long follow-up. Our results were confronted with 10 and 50% cut-offs for L1CAM; this setting led to good differentiation (high specificity) but at a very low sensitivity and good specificity.

To evaluate whether IHC markers would contribute to more accurate stratification of EC risk stratification, a new model was established. Parameters were obtained by imaging methods (myometrial invasion, cervical involvement, lymph node involvement), histology (endometrioid or mucinous vs. non-endometrioid), grade and IHC markers (ER, PR, L1CAM, p53). As deciding for risk stratification, the following parameters have been shown as crucial: L1CAM, PR, and myometrial invasion. The procedure of classification is shown in **Figure 3**. EC risk stratification's overall success was 78% for this model. Successful inclusion into a low-risk group was observed in 80% (56/70 patients), while for high-risk it was 76% (47/62 patients). We provided the comparison of current procedure represented by a model based on histotype, grading, and imaging methods with a new model consisting of imaging examination along with markers (IMG+IHC) (**Table 6**). IHC markers included in the current diagnostic would significantly improve sensitivity (48.4 vs. 75.8%, $p < 0.001$) associated with a slightly, statistically non-significant decrease in specificity to 80% ($p = 0.238$). Positive predictive value was similar for both methods, while negative predictive value (i.e., the probability of being true negative if the test is negative) was significantly improved (66 vs. 78.9%, $p < 0.001$), (**Figure 4**). Using our model, a significantly higher proportion of patients would be properly determined as high-risk.

When comparing the accuracy of the parameters used in the old model with the definite histology, we find discrepancies, especially in the grade and cervical invasion category (**Table 1**). At the present level of knowledge, no significant improvement in preoperative diagnostic accuracy can be expected by, for example, using imaging methods. Therefore, we are introducing a new model using molecular markers that are not dependent on imaging or other methods of clinical examination.

The strength of our study is that it is a cohort from a real clinical practice with prospective data collection and complete knowledge of preoperative and postoperative data. This is the first study to deal with the real implementation of new IHC markers in the pre-operational decision model. Our study design represents daily routine practice.

We acknowledge the study also has weaknesses. This is a relatively small cohort of EC patients, where all stages are not adequately represented; only two female patients in the FIGO IV stage were present. Considering the excellent correlation between preoperative and postoperative histology and grading, the weakness of the model is in its imaging method, which in the case of ultrasound is dependent on the expert skills of a particular sonographer or imaging specialist.

CONCLUSION

We have demonstrated in our cohort that incorporating IHC markers into preoperative practice in endometrial cancer patients increases prognosis prediction accuracy and allows for the development of a new model for more accurate patient clinical management. Should we prefer a higher specificity model, then the most accurate classification is based on L1CAM values, myometrial invasion, and the condition of PR receptors. However, for wider implementation and the validation of proposed model, additional study is needed. Ideally, a prospective randomized trial would evaluate the role of IHC markers L1CAM, ER, PR, and p53 in a preoperative setting together with imaging method and histology/grade. To further improve the new model, it would be interesting to focus on general weaknesses in the accuracy of preoperative imaging methods and in the quality of preoperatively obtained samples with a full IHC examination on a routine daily basis. Incorporating IHC markers seems to be the best way to treat EC patients more accurately.

ETHICS STATEMENT

The text of informed consent, information for patients and the study protocol was approved by Institutional Ethical Board of University Hospital Brno.

AUTHOR CONTRIBUTIONS

VW and MZ: idea of research project, overseeing, coordination of analyses; MB, PV, LM, and MF: collection and preparation of clinical data; JH, EJ, and MC: immunohistochemistry and interpretation of pathological data; PO: statistics; VW, MB, JH, PO, PV, and MZ: writing manuscript. All authors: final reading and approval of manuscript.

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REFERENCES

1. Kurman RJ, International Agency for Research on Cancer, World Health Organization (eds.). *WHO Classification of Tumours of Female Reproductive Organs*. Lyon: International Agency for Research on Cancer (2014).
2. Salvesen HB, Haldorsen IS, Trovik J. Markers for individualised therapy in endometrial carcinoma. *Lancet Oncol.* (2012) 13:e353–61. doi: 10.1016/S1470-2045(12)70213-9
3. Dedes KJ, Wetterskog D, Ashworth A, Kaye SB, Reis-Filho JS. Emerging therapeutic targets in endometrial cancer. *Nat Rev Clin Oncol.* (2011) 8:261–71. doi: 10.1038/nrclinonc.2010.216
4. Abu-Rustum NR, Barakat RR. Observations on the role of circumflex iliac node resection and the etiology of lower extremity lymphedema following pelvic lymphadenectomy for gynecologic malignancy. *Gynecol Oncol.* (2007) 106:4–5. doi: 10.1016/j.ygyno.2007.03.026
5. Weinberger V, Cibula D, Zikan M. Lymphocele: prevalence and management in gynecological malignancies. *Expert Rev Anticancer Ther.* (2014) 14:307–17. doi: 10.1586/14737140.2014.866043
6. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Azim SA, et al. L1CAM in early-stage type I endometrial cancer: results of a large multicenter evaluation. *J Natl Cancer Inst.* (2013) 105:1142–50. doi: 10.1093/jnci/djt144
7. van der Putten LJ, Visser NC, van de Vijver K, Santacana M, Bronsert P, Bulten J, et al. L1CAM expression in endometrial carcinomas: an ENITEC collaboration study. *Br J Cancer.* (2016) 115:716–24. doi: 10.1038/bjc.2016.235
8. Visser NCM, Bulten J, van der Wurff AAM, Boss EA, Bronkhorst CM, Feijen HWH, et al. PIpelle Prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study. *BMC Cancer.* (2015) 15:487. doi: 10.1186/s12885-015-1487-3
9. Hua T, Liu S, Xin X, Jin Z, Liu Q, Chi S, et al. Prognostic significance of L1 cell adhesion molecule in cancer patients: A systematic review and meta-analysis. *Oncotarget.* (2016) 7:85196–207. doi: 10.18632/oncotarget.13236
10. Van Gool IC, Stelloo E, Nout RA, Nijman HW, Edmondson RJ, Church DN, et al. Prognostic significance of L1CAM expression and its association with mutant p53 expression in high-risk endometrial cancer. *Mod Pathol.* (2016) 29:174–81. doi: 10.1038/modpathol.2015.147
11. Lin MY, Dobrotwir A, McNally O, Abu-Rustum NR, Narayan K. Role of imaging in the routine management of endometrial cancer. *Int J Gynaecol Obstet.* (2018) 143 (Suppl. 2):109–17. doi: 10.1002/ijgo.12618
12. Frühauf F, Dvorák M, Haaková L, Hašlík L, Herboltová P, Chaloupková B, et al. [Ultrasound staging of endometrial cancer - recommended methodology of examination]. *Ceska Gynekol.* (2014) 79:466–76.
13. Fischerova D. Ultrasound scanning of the pelvis and abdomen for staging of gynecological tumors: a review. *Ultrasound Obstet Gynecol.* (2011) 38:246–66. doi: 10.1002/uog.10054

14. Fischerova D, Frühauf F, Zikan M, Pinkavova I, Kocián R, Dundr P, et al. Factors affecting sonographic preoperative local staging of endometrial cancer. *Ultrasound Obstet Gynecol.* (2014) 43:575–85. doi: 10.1002/uog.13248
15. Eriksson LSE, Lindqvist PG, Flöter Rådestad A, Dueholm M, Fischerova D, Franchi D, et al. Transvaginal ultrasound assessment of myometrial and cervical stromal invasion in women with endometrial cancer: interobserver reproducibility among ultrasound experts and gynecologists. *Ultrasound Obstet Gynecol.* (2015) 45:476–82. doi: 10.1002/uog.14645
16. Querleu D, Morrow CP. Classification of radical hysterectomy. *Lancet Oncol.* (2008) 9:297–303. doi: 10.1016/S1470-2045(08)70074-3
17. FIGO Committee on Gynecologic Oncology. FIGO staging for carcinoma of the vulva, cervix, and corpus uteri. *Int J Gynaecol Obstet.* (2014) 125:97–8. doi: 10.1016/j.ijgo.2014.02.003
18. Leisenring W, Alonzo T, Pepe MS. Comparisons of predictive values of binary medical diagnostic tests for paired designs. *Biometrics.* (2000) 56:345–51. doi: 10.1111/j.0006-341X.2000.00345.x
19. 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) 497:67–73. doi: 10.1038/nature12113
20. Rossi EC, Kowalski LD, Scalici J, Cantrell L, Schuler K, Hanna RK, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): A multicentre, prospective, cohort study. *Lancet Oncol.* (2017) 18:384–92. doi: 10.1016/S1470-2045(17)30068-2
21. Abu-Rustum NR, Gomez JD, Alektiar KM, Soslow RA, Hensley ML, Leitao MM Jr., et al. The incidence of isolated paraaortic nodal metastasis in surgically staged endometrial cancer patients with negative pelvic lymph nodes. *Gynecol Oncol.* (2009) 115:236–8. doi: 10.1016/j.ygyno.2009.07.016
22. Gilks CB, Oliva E, Soslow RA. Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. *Am J Surg Pathol.* (2013) 37:874–81. doi: 10.1097/PAS.0b013e31827f576a
23. Clarke BA, Gilks CB. Endometrial carcinoma: controversies in histopathological assessment of grade and tumour cell type. *J Clin Pathol.* (2010) 63:410–5. doi: 10.1136/jcp.2009.071225
24. Hussein YR, Broaddus R, Weigelt B, Levine DA, Soslow RA. The genomic heterogeneity of FIGO grade 3 endometrioid carcinoma impacts diagnostic accuracy and reproducibility. *Int J Gynecol Pathol.* (2016) 35:16–24. doi: 10.1097/PGP.0000000000000212
25. Han G, Sidhu D, Duggan MA, Arseneau J, Cesari M, Clement PB, et al. Reproducibility of histological cell type in high-grade endometrial carcinoma. *Mod Pathol.* (2013) 26:1594–604. doi: 10.1038/modpathol.2013.102
26. Alkushi A, Kobel M, Kalloger SE, Gilks CB. High-grade endometrial carcinoma: serous and grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. *Int J Gynecol Pathol.* (2010) 29:343–50. doi: 10.1097/PGP.0b013e3181cd6552
27. Van Holsbeke C, Ameye L, Testa AC, Mascilini F, Lindqvist P, Fischerova D, et al. Development and external validation of new ultrasound-based mathematical models for preoperative prediction of high-risk endometrial cancer. *Ultrasound Obstet Gynecol.* (2014) 43:586–95. doi: 10.1002/uog.13216
28. Antonsen SL, Jensen LN, Loft A, Berthelsen AK, Costa J, Tabor A, et al. MRI, PET/CT and ultrasound in the preoperative staging of endometrial cancer - a multicenter prospective comparative study. *Gynecol Oncol.* (2013) 128:300–8. doi: 10.1016/j.ygyno.2012.11.025
29. Savelli L, Ceccarini M, Ludovisi M, Fruscella E, De Iaco PA, Salizzoni E, et al. Preoperative local staging of endometrial cancer: transvaginal sonography vs. magnetic resonance imaging. *Ultrasound Obstet Gynecol.* (2008) 31:560–6. doi: 10.1002/uog.5295
30. Akbayir O, Corbacioglu A, Numanoglu C, Guleroglu FY, Ulker V, Akyol A, et al. Preoperative assessment of myometrial and cervical invasion in endometrial carcinoma by transvaginal ultrasound. *Gynecol Oncol.* (2011) 122:600–3. doi: 10.1016/j.ygyno.2011.05.041
31. Sawicki W, Spiewankiewicz B, Stelmachów J, Cendrowski K. The value of ultrasonography in preoperative assessment of selected prognostic factors in endometrial cancer. *Eur J Gynaecol Oncol.* (2003) 24:293–8. doi: 10.1002/uog.14905
32. Alcazar JL, Pineda L, Martinez-Astorquiza Corral T, Orozco R, Utrilla-Layna J, Juez L, et al. Transvaginal/transrectal ultrasound for assessing myometrial invasion in endometrial cancer: a comparison of six different approaches. *J Gynecol Oncol.* (2015) 26:201–7. doi: 10.3802/jgo.2015.26.3.201
33. Mascilini F, Testa AC, Van Holsbeke C, Ameye L, Timmerman D, Epstein E. Evaluating myometrial and cervical invasion in women with endometrial cancer: comparing subjective assessment with objective measurement techniques. *Ultrasound Obstet Gynecol.* (2013) 42:353–8. doi: 10.1002/uog.12499
34. Frühauf F, Zikan M, Semeradova I, Dundr P, Nemejcova K, Dusek L, et al. The diagnostic accuracy of ultrasound in assessment of myometrial invasion in endometrial cancer: subjective assessment versus objective techniques. *Biomed Res Int.* (2017) 2017:1318203. doi: 10.1155/2017/1318203
35. Gong Y, Wang Q, Dong L, Jia Y, Hua C, Mi F, et al. Different imaging techniques for the detection of pelvic lymph nodes metastasis from gynecological malignancies: a systematic review and meta-analysis. *Oncotarget.* (2016) 8:14107–25. doi: 10.18632/oncotarget.12959
36. Lampe B, Kürzl R, Hantschmann P. Reliability of tumor typing of endometrial carcinoma in prehysterectomy curettage. *Int J Gynecol Pathol.* (1995) 14:2–6.
37. Frumovitz M, Singh DK, Meyer L, Smith DH, Wertheim I, Resnik E, et al. Predictors of final histology in patients with endometrial cancer. *Gynecol Oncol.* (2004) 95:463–8. doi: 10.1016/j.ygyno.2004.07.016
38. Leitao MM, Kehoe S, Barakat RR, Alektiar K, Gattoc LP, Rabbitt C, et al. Accuracy of preoperative endometrial sampling diagnosis of FIGO grade 1 endometrial adenocarcinoma. *Gynecol Oncol.* (2008) 111:244–8. doi: 10.1016/j.ygyno.2008.07.033
39. Stelloo E, Nout RA, Naves LCLM, Ter Haar NT, Creutzberg CL, Smit VTHBM, et al. High concordance of molecular tumor alterations between pre-operative curettage and hysterectomy specimens in patients with endometrial carcinoma. *Gynecol Oncol.* (2014) 133:197–204. doi: 10.1016/j.ygyno.2014.02.012
40. Bosse T, Nout RA, Stelloo E, Dreef E, Nijman HW, Jürgenliemk-Schulz IM, et al. L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: pooled PORTEC trial results. *Eur J Cancer.* (2014) 50:2602–10. doi: 10.1016/j.ejca.2014.07.014
41. Amant F, Mirza MR, Koskas M, Creutzberg CL. Cancer of the corpus uteri. *Int J Gynecol Obstetr.* (2018) 143:37–50. doi: 10.1002/ijgo.12612
42. van der Putten LJM, Visser NCM, van de Vijver K, Santacana M, Bronsert P, Bulten J, et al. Added value of estrogen receptor, progesterone receptor, and L1 cell adhesion molecule expression to histology-based endometrial carcinoma recurrence prediction models: an ENITEC collaboration study. *Int J Gynecol Cancer.* (2018) 28:514–23. doi: 10.1097/IGC.0000000000001187
43. Trovik J, Wik E, Werner HMJ, 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) 49:3431–41. doi: 10.1016/j.ejca.2013.06.016
44. *Biomarker Guided Treatment in Gynaecological Cancer - Full Text View - ClinicalTrials.gov*. Available online at: https://clinicaltrials.gov/ct2/show/NCT02543710 (accessed March 28, 2017).
45. PORTEC-4a. *Molecular Profile-based Versus Standard Adjuvant Radiotherapy in Endometrial Cancer - Full Text View - ClinicalTrials.gov*. Available online at: https://clinicaltrials.gov/ct2/show/NCT03469674 (accessed March 19, 2018).
46. Tangen IL, Kopperud RK, Visser NC, Staff AC, Tingulstad S, Marcickiewicz J, et al. Expression of L1CAM in curettage or high L1CAM level in preoperative blood samples predicts lymph node metastases and poor outcome in

endometrial cancer patients. *Br J Cancer.* (2017) 117:840–7. doi: 10.1038/bjc.2017.235

47. Geels YP, Pijnenborg JMA, Gordon BBM, Fogel M, Altevogt P, Masadah R, et al. L1CAM expression is related to non-endometrioid histology, and prognostic for poor outcome in endometrioid endometrial carcinoma. *Pathol Oncol Res.* (2016) 22:863–8. doi: 10.1007/s12253-016-0047-8
48. Jongen V, Briët J, de Jong R, ten Hoor K, Boezen M, van der Zee A, et al. Expression of estrogen receptor-alpha and -beta and progesterone receptor-A and -B in a large cohort of patients with endometrioid endometrial cancer. *Gynecol Oncol.* (2009) 112:537–42. doi: 10.1016/j.ygyno.2008.10.032

Conflict of Interest Statement: 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.

6.2 Vinklerová P, Ovesná P, Hausnerová J, Pijnenborg JMA, Lucas PJF, Reijnen C, Vrede S, Weinberger V. External Validation Study of Endometrial Cancer Preoperative Risk Stratification Model (ENDORISK). Front. Oncol. 2022; 12:939226.

The ENITEC group published one of the most advanced models in endometrial cancer called ENDORISK, a machine learning-based Bayesian network design to predict lymph node metastasis and 5-year DSS[67].

Our study aimed to externally validate the predictive performance of the ENDORISK model in a cohort of EC patients from the University Hospital Brno, Czech Republic. We also sought to assess its applicability in the context of sentinel lymph node biopsy and its accuracy in advanced-stage disease.

We retrospectively analyzed 425 EC patients treated between January 2006 and May 2021. For LNM (lymph node metastasis) prediction, 226 patients with known lymph node status were included, while 299 patients with available follow-up data were analyzed for DSS prediction. Patients underwent either pelvic and paraaortic lymph node dissection or sentinel node biopsy. The ENDORISK model's predictions were compared to actual outcomes, and its performance was evaluated using the AUC (area under the curve) and calibration plots.

The ENDORISK model demonstrated strong predictive capabilities for LNM (AUC of 0.84 (95% CI: 0.77–0.90)) and 5-year DSS (AUC of 0.86 (95% CI: 0.79–0.93)). Calibration plots indicated excellent predictive accuracy for low-risk tumors (grades 1–2). However, the model tended to underestimate risk in high-grade tumors (grade 3) and advanced FIGO stages, particularly concerning DSS.

Our findings confirm the ENDORISK model's robust performance in preoperative risk stratification for EC, especially among low-risk patients. Nonetheless, its predictive accuracy diminishes in high-risk and advanced-stage cases, suggesting the need for further refinement and training of the model to enhance its applicability across all risk categories. We suggest incorporating preoperative imaging results into the model to better stratify advanced stages.

The *"External Validation Study of Endometrial Cancer Preoperative Risk Stratification Model (ENDORISK)"* was published in *Frontiers in Oncology* (IF 4.7, Q2) in 2022.

Author's contribution: first author, conceptualization, investigation, methodology, data curation, manuscript writing – original draft.

Based on our findings and recommendations, an improved version of the model—ENDORISK-2—was developed, which now incorporates imaging results as part of the preoperative assessment. The manuscript is currently under review in a reputable peer-reviewed journal.

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External validation study of endometrial cancer preoperative risk stratification model (ENDORISK)

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Introduction: Among industrialized countries, endometrial cancer is a common malignancy with generally an excellent outcome. To personalize medicine, we ideally compile as much information as possible concerning patient prognosis prior to effecting an appropriate treatment decision. Endometrial cancer preoperative risk stratification (ENDORISK) is a machine learning–based computational Bayesian networks model that predicts lymph node metastasis and 5-year disease-specific survival potential with percentual probability. Our objective included validating ENDORISK effectiveness in our patient cohort, assessing its application in the current use of sentinel node biopsy, and verifying its accuracy in advanced stages.

Methods: The ENDORISK model was evaluated with a retrospective cohort of 425 patients from the University Hospital Brno, Czech Republic. Two hundred ninety-nine patients were involved in our disease-specific survival analysis; 226 cases with known lymph node status were available for lymph node metastasis analysis. Patients were included undergoing either pelvic lymph node dissection (N = 84) or sentinel node biopsy (N =70) to explore the accuracy of both staging procedures.

Results: The area under the curve was 0.84 (95% confidence interval [CI], 0.77–0.9) for lymph node metastasis analysis and 0.86 (95% CI, 0.79–0.93) for 5-year disease-specific survival evaluation, indicating quite positive concordance between prediction and reality. Calibration plots to visualize results demonstrated an outstanding predictive value for low-risk cancers (grades 1–2), whereas outcomes were underestimated among high-risk patients (grade 3), especially in disease-specific survival. This phenomenon was even more obvious when patients were subclassified according to FIGO clinical stages.

Conclusions: Our data confirmed ENDORISK model's laudable predictive ability, particularly among patients with a low risk of lymph node metastasis and expected favorable survival. For high-risk and/or advanced stages, the ENDORISK network needs to be additionally trained/improved.

KEYWORDS

Bayesian networks model, disease-specific survival, endometrial cancer, prognosis, risk stratification, sentinel node biopsy, lymph node metastasis

Introduction

In industrialized countries, endometrial cancer (EC) is a common malignancy with generally an excellent outcome and 5-year relative survival rate of 76% among European women (1). Despite its overall favorable prognosis, up to 15% of patients classified as low-risk will experience recurrence and may profit from adjuvant treatment (2). Conversely, a substantial number of patients classified as high-risk surprisingly evidence no disease recurrence many years after treatment. Respecting the current emphasis on personalized medicine, we ideally seek as much information as possible concerning a patient's prognosis prior to determine the most effective therapeutic approach, avoid overtreatment, and prevent treatment-related morbidity. Current European guidelines classify patients into five prognostic risk groups based on final tumor stage and histological characteristics (3). However, in the preoperative setting, risk stratification can be challenging owing to the lack of certain essential definitive histology information such as lymphovascular space invasion (LVSI) and myometrial invasion.

Lymph node (LN) involvement is an important issue that impacts treatment approach and is related to poor prognosis. Two large randomized trials (4, 5) renounced the curative significance of lymphadenectomy. Nowadays, pelvic and para-aortic lymphadenectomy (PLN and PALN) are mainly considered as staging tools with substantial morbidity (6). According to the recent European guidelines, sentinel node biopsy (SNB) is an alternative to full lymphadenectomy in low/intermediate-risk stage I/II EC and can also be considered in high-intermediate and high-risk stage I/II groups (3).

In order to identify preoperatively which patients are at risk for lymph node metastasis (LNM), the endometrial cancer preoperative risk stratification (ENDORISK) was constructed within the ENITEC network (European Network of Individal Treatment in Endometrial Cancer) (7). This is a machine learning–based computational Bayesian networks model, which predicts the probability of LNM and 5-year disease specific survival (DSS) in EC cases. This ENDORISK model has been validated forthwith using two multicentric cohorts: MoMaTEC (the Molecular Markers in Treatment in Endometrial Cancer) (8) and PIPENDO (the PIpelle Prospective ENDOmetrial carcinoma) study (9). The diagnostic accuracy was 0.82 and 0.84, respectively. Input data contains preoperative clinical and histological characteristics. Since the original model consisted of a notably heterogeneous patient group from many countries with possible treatment decision divergencies, we were questioning how this model would perform within our patient cohort with very well-structured and collected preoperative clinical/histological data, adjuvant treatment, and follow-up.

Our aim was to validate the ENDORISK model's accuracy and the applicability within the current SNB staging era. Since the model was constructed based on full lymphadenectomy, our further objective was to evaluate the model's potential accuracy bias by introducing the SNB method. Additionally, we wanted to verify the model's performance within advanced EC stages. Our study points out the weaknesses and strengths of the original ENDORISK model and proposes certain modifications in order to utilize the model within the actual and real clinical practice worldwide.

Methods

Patient cohort

We evaluated the ENDORISK model in our retrospectively collected study cohort including 425 patients treated at the University Hospital Brno, Czech Republic. Our cohort evolved from an EC database of 835 patients treated between January 2006 and May 2021. Cases that were incorporated in the original ENDORISK model (N = 150) and those without the minimally required data for using ENDORISK (N = 240) were excluded.

We assessed clinical and histological characteristics from the EC database and patients' medical records: age, BMI, follow-up length, preoperative tumor grade/histotype, estrogen receptor (ER), progesterone receptor (PR), L1 Cell Adhesion Molecule

(L1CAM), p53 expression, cancer antigen (Ca) 125 serum level, platelet count, preoperative cervical cytology result, lymphadenopathy according to imaging methods, myometrial/cervical invasion, LVSI, clinical/surgical staging, LN staging method, LNM, and adjuvant treatment.

All patients underwent preoperative biopsy *via* hysteroscopy or dilatation and curettage, imaging staging procedures with expert ultrasound and computed tomography (CT) scan to detect local or distant disease spread, and retroperitoneal lymphadenopathy. Patients were allocated to the clinical FIGO (International Federation of Gynecology and Obstetrics) (2009) stages. Subsequently, patients were classified into low- and high-risk groups. The low-risk group was defined as endometrioid/mucinous carcinoma, clinically FIGO stage 1A or 1B, grade 1; and endometrioid/mucinous cancer clinically FIGO 1A, grade 2, all without clinical or imaging evidence of lymphadenopathy or distant metastases. When the low-risk criteria were not met, patients were considered high risk.

Surgical treatment

Hysterectomy with bilateral salpingo-oophorectomy as basic surgical treatment was performed with an abdominal or laparoscopic approach. In addition, high-risk patients underwent systematic para-aortic/pelvic lymphadenectomy (historically pelvic lymphadenectomy only)—at least five LNs from each hemipelvis and 10 from the para-aortic region were removed. Since 2019, systematic lymphadenectomy has been replaced by SNB in all EC patients regardless of their preoperative risk group. Currently, lymphadenectomy is limited to patients experiencing bulky LNs on preoperative imaging or perioperative finding.

Sentinel node ultrastaging

Regarding sentinel node methodology, we used intracervical indocyanine green injections and searched for the nodes with an endoscopic fluorescence imaging camera (Novadaq Pinpoint).

All sentinel LNs were fixed in 10% buffered formalin, sliced at 2-mm lamellas, embedded in paraffin, and further examined by ultrastaging protocol. This protocol consists of two consecutive 4-µm thick sections obtained in regular 200-µm intervals, which are cut from each paraffin block. The first section was stained with hematoxylin and eosin, and the second section was examined with cytokeratins (AE1/3). We classified micrometastasis (0.2–2 mm) together with macrometastases (>2 mm) as LN positive, whereas isolated tumor cells (≤0.2 mm or single cells/clusters of cells ≤200 cells in a single LN cross-section) were considered LN negative.

Immunohistochemical analysis

The experienced gynecological histopathologist (J. H.) examined all hematoxylin and eosin-stained slides to confirm preoperative histological subtype and grade. Immunohistochemical staining was effected on formalin-fixed and paraffin-embedded tissue sections. L1CAM positivity was defined as distinct membrane staining in ≥10% of tumor cells. ER and PR were considered positive when there were ≥10% of tumor cells with nuclear staining. p53 was classified into wild type or mutant (strong diffuse overexpression in more than 90% of tumor cells or completely negative) phenotypes.

Statistical analysis

Following the original ENDORISK model validation, we used preoperative tumor grade, at least three IHC markers (ER, PR, p53, or L1CAM) and at least one of the clinical preoperative markers (CA 125 serum level, LN status according to imaging method, platelet count, or pap smear result) as the minimal input data. A five-year follow-up (in the 5-year DSS group) and LN staging procedure (in the LNM group) were available in all included cases.

Probabilities of LNM and 5-year DSS were calculated for each patient and compared with observed reality. (i) Discrimination testing was assessed using a receiver operating characteristic (ROC) curve generated by plotting sensitivity against 1-specificity. Discriminating performance was quantified based on the AUC (area under the curve). (ii) The model's overall performance was quantified by the Brier score, which is the mean squared difference between each predicted probability and the observed outcome; a lower Brier score indicates better accuracy of probabilistic predictions. (iii) Calibration was visualized using a calibration plot, in which the predicted outcome was plotted against the observed outcome. To quantify model calibration, the predicted number of events (i.e., sum of each predicted probability) was compared with the observed number. (iv) Concordance between the ENDORISK model, our data, and recent DSS prediction was undertaken by using U.K. Uterine cancer survival data for different FIGO stages (10). Sensitivity analysis was accomplished by omitting patients with only SNB. Analyses were achieved in R (4.1.1) with the bnlearn (4.7), pROC (1.18.0), DescTools (0.99.44), and caret (6.0–90) packages.

Ethics approval

Our study was approved by the University Hospital Brno Ethics Committee, Approval Number 06-151221/EK. All

patients signed informed consent for histology sample storage, scientific use, and publication purposes.

Results

Among the 835 patients in our EC database treated between January 2006 and May 2021, 299 patients were involved in our DSS analysis; 226 cases were available for LNM analysis (Figure 1). Table 1 summarizes clinical data, histological characteristics, and adjuvant treatment.

LNM analysis

A total of 226 patients were included in our LNM analysis: 84 (37%) PLN, 72 (32%) PLN+PALN, and 70 (31%) SNB. Forty-one patients had at least one LNM (18%): 24 (59%) in pelvic, three (7%) in para-aortic, and 14 (34%) in both localizations. A median of 27 and 22 LNs were removed during PLN and PALN, respectively.

The AUC (0.84) and Brier score (0.11) indicated good concordance between prediction and reality (Table 2, Figure 2). Predicted/observed ratio displayed non-significant underestimation (0.76; 95% CI 0.49–1.03). Results from sensitivity analysis, where cases with SNB were excluded, were comparable (Supplementary Material: Table 1, **Figure 1**), indicating that involvement in the main analysis did not alter the accuracy of ENDORISK.

Figure 3 shows LNM prediction and reality for the different clinical FIGO stages (Supplementary Figure 2 complements surgical stages).

DSS analysis

Only patients with at least 5 years of follow-up or who died from EC were included (N = 299). The AUC was 0.86 (95% CI,

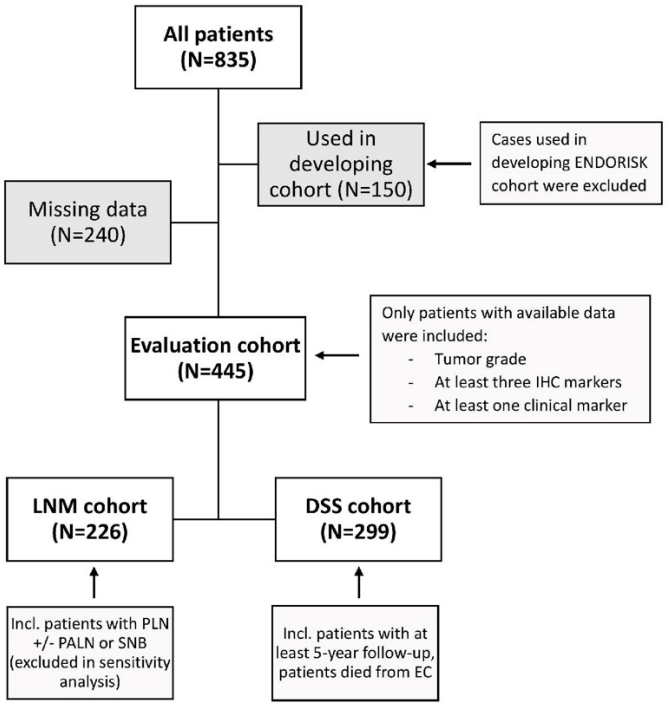

FIGURE 1
Cohort development. The evaluation cohort was developed using all patients from our clinical database treated between January 2006 and May 2021 with available data and lymph node staging (LNM cohort) and/or 5-year follow-up (DSS cohort). IHC, immunohistochemical; LNM, lymph node metastasis; DSS, disease specific survival; PLN, pelvic lymphadenectomy; PALN, para-aortal lymphadenectomy; SNB, sentinel node biopsy; EC, endometrial cancer.

TABLE 1 Clinical and histological characteristics.

Variable		LNM cohort	5- year DSS cohort
Total N		N = 226*	N = 299*
Age (years)		64.5 (59.0 to 68.8)	65.0 (59.0 to 72.0)
BMI (kg/m^2)		30.0 (26.0 to 34.0)	32.0 (27.0 to 36.0)
Follow up length (month)		35.2 (13.0 to 90.6)	91.8 (64.5 to 122.2)
Preoperative tumor grade	1	38 (16.8)	62 (20.7)
	2	103 (45.6)	171 (57.2)
	3	85 (37.6)	66 (22.1)
ER expression	Negative	28 (12.4)	23 (7.7)
	Positive	198 (87.6)	276 (92.3)
PR expression	Negative	42 (18.6)	38 (12.7)
	Positive	184 (81.4)	261 (87.3)
L1CAM expression	Negative	175 (77.4)	258 (86.3)
	Positive	48 (21.2)	40 (13.4)
	Unknown	3 (1.3)	1 (0.3)
p53 expression	Wild type	185 (81.9)	244 (81.6)
	Muttated	37 (16.4)	37 (12.4)
	Missing	4 (1.8)	18 (6.0)
Ca-125	Negative (<35)	167 (73.9)	195 (65.2)
	Positive (35+)	47 (20.8)	63 (21.1)
	Unknown	12 (5.3)	41 (13.7)
Trombocytosis	No	215 (95.1)	279 (93.3)
	Yes	7 (3.1)	11 (3.7)
	Unknown	4 (1.8)	9 (3.0)
Imaging results	No lymphadenopathy	210 (92.9)	271 (90.6)
	Lymphadenopathy	11 (4.9)	10 (3.3)
	Unknown	5 (2.2)	18 (6.0)
Cervical cytology	Normal	143 (63.3)	200 (66.9)
	Abnormal	7 (3.1)	4 (1.3)
	Unknown	76 (33.6)	95 (31.8)
Histological subtype	Endometrioid	186 (82.3)	275 (92.0)
	Non-endometrioid	40 (17.7)	24 (8.0)
Myometrial invasion	less then 50%	129 (57.1)	200 (66.9)
	more then 50%	97 (42.9)	99 (33.1)
Cervical invasion	No	193 (85.4)	266 (89.0)
	Yes	33 (14.6)	33 (11.0)
FIGO stage (surgical)	IA	108 (47.8)	180 (60.2)
	IB	41 (18.1)	58 (19.4)
	II	29 (12.8)	30 (10.0)
	IIIA	5 (2.2)	9 (3.0)
	IIIB	1 (0.4)	1 (0.3)
	IIIC	38 (16.8)	16 (5.4)
	IV	4 (1.8)	5 (1.7)
LVSI	No	170 (75.2)	271 (90.6)
	Yes	53 (23.5)	24 (8.0)
	Unknown	3 (1.3)	4 (1.3)
Type of lymphadenectomy	PLN	84 (37.2)	68 (22.7)
	PLN+PALN	72 (31.9)	31 (10.4)
	SNB	70 (31.0)	1 (0.3)
	Unknown	0 (0.0)	199 (66.6)

(Continued)

TABLE 1 Continued

Variable		LNM cohort	5- year DSS cohort
Lymph nodes	Negative	185 (81.9)	87 (29.1)
	Positive	41 (18.1)	17 (5.7)
	Unknown	0 (0.0)	195 (65.2)
SNB	Negative	65 (28.8)	1 (0.3)
	Positive	5 (2.2)	0 (0.0)
	Unknown	156 (69.0)	298 (99.7)
Adjuvant treatment	None	84 (37.2)	163 (54.5)
	RT	94 (41.6)	106 (35.5)
	CHT	17 (7.5)	14 (4.7)
	CHRT	27 (11.9)	9 (3.0)
	Unknown	4 (1.8)	7 (2.3)

*n (%); Median (IQR).
DSS, disease-specific survival; LNM, lymph node metastasis; BMI, Body Mass Index; ER, Estrogen receptor; PR, Progesterone receptor; L1CAM, L1 cell adhesion molecule; LVSI, lymphovascular space invasion; PLN, pelvic lymphadenectomy; PALN, para-aortic lymphadenectomy; SNB, sentinel node biopsy; RT, radiotherapy; CHT, chemotherapy; CHRT, chemoradiotherapy.

0.79–0.93), Brier score 0.09. Five-year DSS prediction was well calibrated with a trend toward overestimating survival among the lower predicted survival rates (Figure 4 and Table 2).

Figure 5 displays the 5-year DSS prediction compared with reality and expected survival according to previously published probability (10) in different clinical FIGO stages (Supplementary Figure 3 expands on surgical stages).

Discussion

In the era of personalized medicine, we aim to have optimal information concerning a prognosis to facilitate adequate shared decision-making with the patient and define the most appropriate treatment decision. The ENDORISK model definitively contributes to the preoperative knowledge on risk of LNM and DSS.

Several EC predictive models have been published and focus on discriminating patients pre- and postoperatively into risk groups with predicting LNM or outcome. Previous models used traditional clinicopathological characteristics including LVSI, myometrial invasion, histotype, grade, age, and/or BMI (11, 12). So far, results were only moderate and, currently, additional immunohistochemical markers are already frequently used in the clinic: ER, PR, L1CAM, p53, Ki67 (13, 14). Some authors also included imaging information such as tumor diameter, myometrial/cervical invasion, or lymphadenopathy (15, 16).

The original multivariate analysis is based on a simple graphic calculating tool called nomogram. Jiang et al. published an LNM prediction model based on histological and IHC markers with a sensitivity of 82.8% and specificity of 82.7% (AUC 0.9) (14). However, this model cannot be applied when certain data are missing. Moreover, information on LVSI is required, which limits the use of the model in a preoperative setting. Similar results were presented with an effort to predict 3-year recurrence-free survival (sensitivity 76.5%, specificity 86.7%, AUC 0.82) with comparable limitations (including LVSI, all data required) (13).

With the development of computer technology, a Bayesian network has become more accessible, used for determining

TABLE 2 Model concordance statistics.

	LNM	5-year DSS
AUC (95% CI)	0.84 (0.77-0.9)	0.86 (0.79-0.93)
Brier score	0.11	0.09
Predicted no. of events	31.2	271.7
Observed no. of events	41	262
Predicted/observed ratio (95% CI)	0.76 (0.49-1.03)	1.04 (0.91-1.16)

Both AUC and Brier score substantiate very good concordance between prediction and reality in general across the dataset. Discriminative performance was quantified based on AUC (a higher AUC indicates better performance). Overall model performance was quantified by the Brier score (a lower Brier score characterizes better accuracy of the probabilistic predictions). The predicted/observed ratio <1 denotes a lower prediction than reality, whereas a ratio >1 signals overestimation compared with reality. If 95% CI includes value 1, the difference is non-significant.
AUC, area under the curve; CI, confidence interval; DSS, disease specific survival; LNM, lymph node metastasis.

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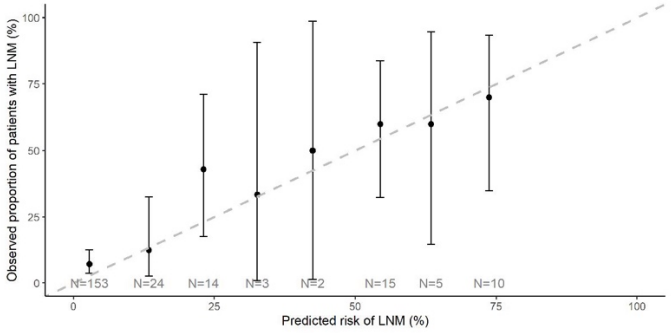

FIGURE 2
Lymph node metastasis calibration plot of observed versus predicted events. A dashed line displays the predicted value, and black dots represent the observed LNM. Ideally, all black marks are lying on the dashed line. LNM, lymph node metastasis.

probable relationships and causalities based on expert knowledge with machine learning. An enormous advantage is that it can be applied, even when some patient characteristics are absent, which often occurs in clinical practice. The ENDORISK model was established with a variety of pre- and postoperative information, yet it could be applied exclusively with preoperative data. Minimally required data to work properly include (1) preoperative tumor grade, (2) minimally three of four IHC markers (ER, PR, p53, or L1CAM), and (3) at least one clinical biomarker (CA 125 serum level, LN status according to imaging method, platelet count, or pap smear result) (7).

The original model was created cognizant of histologic results from pelvic and para-aortic LN staging. Nowadays, complete lymphadenectomy is not the standard practice with all patients, and less invasive SNB is recommended with a low/intermediate-risk disease (3). Certain authors prefer this method even in high-risk cases (17). Isolated para-aortic nodal metastasis (notwithstanding negative pelvic nodes) occurs in approximately 1% of surgically staged cases (18). Consequently, we decided to also include patients with only pelvic dissection or SNB, reflecting current diagnostic practice. Sensitivity analysis, excluding SNB cases, presented comparable results, supporting the results of the complete study cohort (Supplementary Figure 1).

Historically, knowing the potential preoperative risk of LNM guided whether or not para-aortic-pelvic lymphadenectomy was indicated. Currently, SNB is preferred not only in low- but also in high-risk EC and might reduce the benefit of preoperative risk stratification. Yet, based on the very low risk in EC patients without myometrial invasion, LN staging could be omitted in these cases (3). If patients with truly low risk of LNM (<5%)

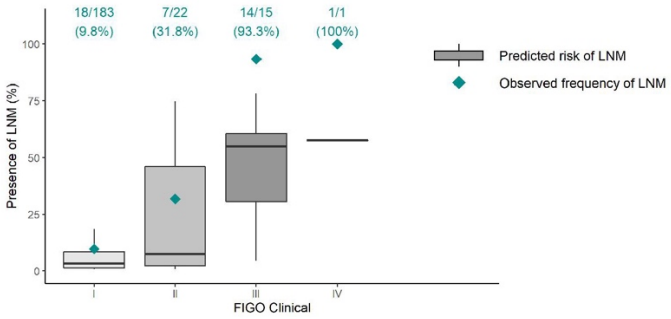

FIGURE 3
Lymph node metastasis prediction versus reality in different clinical FIGO stages. Gray boxes represent the model's prediction; green rhombuses indicate the real LNM frequency. Ideally, all green rhombuses lie in gray boxes. In clinical FIGO III–IV stages, ENDORISK predicts fewer cases of LNM than reality. LNM, lymph node metastasis; FIGO, International Federation of Gynecology and Obstetrics.

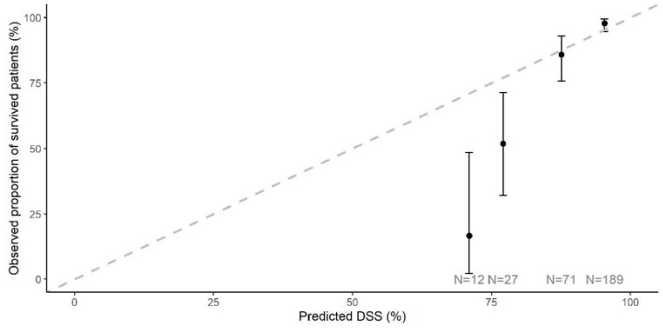

FIGURE 4
Five-year disease-specific survival calibration plot of observed versus predicted events. A dashed line displays the predicted value; black dots represent observed DSS. Ideally, all black marks are lying on the dashed line. There is a trend toward overestimating survival in the lower predicted survival rates. DSS, disease-specific survival.

could be properly identified preoperatively by using the ENDORISK model, SNB could be safely omitted in those hospitals where this technique is not available. An interesting question is whether it is necessary to provide LN staging in all EC types or if, according to other preoperative markers, we could abandon it. In an era of SNB staging practice, the ENDORISK model for LNM prediction could be used in hospitals, where this method is not available. Additionally, it could be supportive if SNB fails and side-specific lymphadenectomy is considered, especially in obese and fragile patients.

Our LNM prediction results were comparable with validation on MoMaTEC cohort: AUC 0.84 versus 0.82, Brier score 0.11 versus 0.09. The model very precisely predicts LNM in early stages, albeit underestimates clinically advanced carcinomas (Figure 3). For example, in patients with preoperative suspicion of LNM according to imaging methods, the ENDORISK model estimated an average probability of only 51% (25–78%). In fact, all were finally LNM positive. This might be explained by the low number of advanced cases; however, the model should be able to predict even worse stages.

ENDORISK model validation for 5-year DSS with our cohort displayed very similar results with previous cohorts MoMaTEC and PIPENDO, evaluated as well adjusted according to AUC (0.82, 0.84) and Brier score (0.12, 0.10) (7). Nevertheless, when using

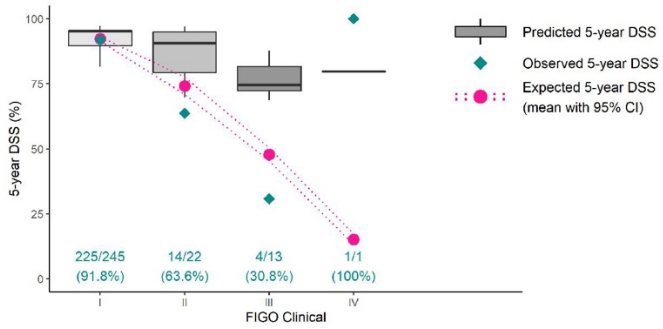

FIGURE 5
Five-year disease-specific survival prediction versus reality versus expectation in different clinical FIGO stages. Gray boxes represent the model's prediction, green rhombuses indicate the real 5-year DSS, and pink dots denote expected 5-year survival according to surgical FIGO stages (10). Ideally, all green rhombuses lie in gray boxes. In clinical FIGO II–III stages, ENDORISK predicts much better survival than reality. Only one patient was preoperatively categorized into FIGO IV stadium—the survival result implies her misclassification. DSS, disease-specific survival; FIGO, International Federation of Gynecology and Obstetrics.

calibration plots to visualize the results, predictive value was obviously outstanding only for low-risk patients and significantly overrated for high-risk patients. This phenomenon was even more evident when patients were classified according to clinical FIGO stages (Figure 5). Definitely, the most accurate results were achieved, when the final surgical stage was applied (Supplementary Figure 3); nevertheless, this information is unknown preoperatively.

The FIGO stage is an important independent factor affecting survival, even during molecular classification times. The average 5-year survival is declining from 92% in stage I, 74% within stage II, and 48% in stage III to only 15% in stage IV (10). The ENDORISK model, currently, does not include information about the clinical stage disease (except for "enlarged lymph nodes on imaging"), even though, there are other possibilities for attaining these data. An expert oncogynecologic ultrasound or magnetic resonance imaging (MRI) is suitable for myometrial and cervical invasion detection; a CT scan can identify distant metastasis (19). Although myometrial invasion <50% of >50% is incorporated in the ENDORISK network, it is currently based on final histology, yet might be a very valuable addition to the model when determined preoperatively by either ultrasound or MRI. In addition, ultrasound-measured tumor-free distance from the tumor to the uterine serosa is another promising marker for predicting deep myometrial invasion and poor prognosis (20), which might be incorporated in an updated version of the network.

Even when we situate the worst clinical and histological characteristics into the model, the lowest survival prediction was 66%. This seems not in line with the published survival data of only 48%/15% in stage III/IV (10). Nevertheless, the number of cases with advanced stage in our cohort was limited and, hence, validation in larger cohorts is needed.

ENDORISK is one of the most complex risk stratification models so far. The authors imperiously searched the literature for potential relevant risk factors and assigned them statistically significant prognostic values. Unlike other models, ENDORISK could be applied even with strictly preoperative and incomplete information. However, as we ascertained, there is a need for further improvement before introduction into clinical practice. Clinical FIGO stage extension would definitively increase the model's accuracy. Additionally, the incorporation of molecular classification would be highly relevant and is currently prepared in the ENDORISK 2.0.

Forthwith, we present the first unicentric ENDORISK model validation study, indicating a capacity for consistent treatment decisions and high-quality follow-up data. Innovatively, we have confirmed its application with SNB cases. Furthermore, we have suggested certain ancillary improvements to achieve better results among advanced cases that need to be considered when updating the ENDORISK network.

Conclusions

ENDORISK is one of the best and most complex preoperative risk stratification models promulgated at this point in time. Nevertheless, there is still a place for improvement, particularly with survival prediction. Including clinical FIGO staging would increase model accuracy in advanced disease cases. In this SNB era, preoperative LNM predictive importance is waning; however, since SNB is not yet standard procedure in all countries, ENDORISK could be a helpful factor in decision-making regarding lymphadenectomy. With molecular classification's inclusion into clinical practice, the ENDORISK model's authors should consider its incorporation as well.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee, University Hospital Brno, Brno, Czech Republic. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, PV, PO, and VW; study design, PV, PO, VW, and JP; statistical analysis, PO and PL; pathological examination, JH; writing – original draft preparation, PV, VW, and PO; writing – review and editing, JP, JH, PL, SV, and CR; supervision, VW and JP. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.939226/full#supplementary-material

References

1. Sant M, Lopez MDC, Agresti R, Sánchez Pérez MJ, Holleczek B, Bielska-Lasota M, et al. Survival of women with cancers of breast and genital organs in Europe 1999–2007: Results of the EUROCARE-5 study. *Eur J Cancer*. (2015) 51 (15):2191–205. doi: 10.1016/j.ejca.2015.07.022

2. Salani R, Khanna N, Frimer M, Bristow RE, Chen LM. An update on post-treatment surveillance and diagnosis of recurrence in women with gynecologic malignancies: Society of gynecologic oncology (SGO) recommendations. *Gynecol Oncol* (2017) 146(1):3–10. doi: 10.1016/j.ygyno.2017.03.022

3. 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

4. ASTEC study group, Kitchener H, Swart AMC, Qian Q, Amos C, Parmar MKB. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet Lond Engl* (2009) 373(9658):125–36. doi: 10.1016/S0140-6736(08)61766-3

5. Benedetti Panici P, Basile S, Maneschi F, Lissoni AA, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. *J Natl Cancer Inst* (2008) 100(23):1707–16. doi: 10.1093/jnci/djn397

6. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the management of endometrial cancer. *Cochrane Database Syst Rev* (2017) 10(10): CD007585. doi: 10.1002/14651858.CD007585.pub4

7. Reijnen C, Gogou E, Visser NCM, Engerud H, Ramijith 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

8. Trovik J, Wik E, Werner HMJ, 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

9. Visser NCM, Bulten J, van der Wurff AAM, Boss EA, Bronkhorst CM, Feijen HWH, et al. PIpelle prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study. *BMC Cancer* (2015) 15:487. doi: 10.1186/s12885-015-1487-3

10. Cancer survival in England - adults diagnosed - office for national statistics. Available at: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/datasets/cancersurvivalratescancersurvivalinenglandadultsdiagnosed (Accessed February 28, 2022).

11. Cong R, Kong F, Ma J, Li Q, Wu Q, Ma X. Combination of preoperative neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and monocyte-lymphocyte ratio: a superior prognostic factor of endometrial cancer. *BMC Cancer* (2020) 20 (1):464. doi: 10.1186/s12885-020-06953-8

12. Dong Y, Cheng Y, Tian W, Zhang H, Wang Z, Li X, et al. An externally validated nomogram for predicting lymph node metastasis of presumed stage I and II endometrial cancer. *Front Oncol* (2019) 9:1218. doi: 10.3389/fonc.2019.01218.

13. Jiang P, Jia M, Hu J, Huang Z, Deng Y, Hu Z. A nomogram model involving immunohistochemical markers for predicting the recurrence of stage i-ii endometrial cancer (2021) (Accessed April 9, 2022).

14. 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.

15. Zheng T, Yang L, Du J, Dong Y, Wu S, Shi Q, et al. Combination analysis of a radiomics-based predictive model with clinical indicators for the preoperative assessment of histological grade in endometrial carcinoma. *Front Oncol* (2021) 11:582495. doi: 10.3389/fonc.2021.582495.

16. Weinberger V, Bednarikova M, Hausnerova J, Ovesna P, Vinklerova P, Minar L, et al. A novel approach to preoperative risk stratification in endometrial cancer: the added value of immunohistochemical markers. *Front Oncol* (2019) 9:265. doi: 10.3389/fonc.2019.00265

17. Pineda VG, Zapardiel I, Gracia M, Siegrist J, Diestro MD, Alonso M, et al. Avoiding full lymphadenectomies in intermediate- and high-risk endometrial cancer by sentinel lymph node biopsy implementation. *Front Oncol* (2021) 11:654285. doi: 10.3389/fonc.2021.654285

18. Abu-Rustum NR, Gomez JD, Alektiar KM, Soslow RA, Hensley ML, Leitao MM Jr., et al. The incidence of isolated paraaortic nodal metastasis in surgically staged endometrial cancer patients with negative pelvic lymph nodes. *Gynecol Oncol* (2009) 115(2):236–8. doi: 10.1016/j.ygyno.2009.07.016

19. Faria SC, Devine CE, Rao B, Sagebiel T, Bhosale P. Imaging and staging of endometrial cancer. *Semin Ultrasound CT MR*. (2019) 40(4):287–94. doi: 10.1053/j.sult.2019.04.001

20. Liro M, Śniadecki M, Wycinka E, Wojtylak S, Brzeziński M, Stańczak A, et al. Ultrasound measurement of tumor-free distance from the serosal surface as the alternative to measuring the depth of myometrial invasion in predicting lymph node metastases in endometrial cancer. *Diagn Basel Switz* (2021) 11(8):1472. doi: 10.3390/diagnostics11081472

7 Conclusions

This habilitation thesis is presented as a collection of previously published scientific articles focused on advancing individualized care in patients with endometrial cancer. The author's work reflects a consistent effort to move beyond conventional treatment paradigms by incorporating molecular classification, expert ultrasonography, risk stratification tools, and precision surgical approaches into routine clinical practice. The research was primarily conducted at the Department of Gynecology and Obstetrics, University Hospital Brno, in collaboration with international centers, especially under the umbrella of ENITEC, of which the author is an active member.

The thesis is divided into four core sections: diagnostic and screening strategies, molecular classification and prognostic markers, lymph node staging, and predictive modeling.

- **Diagnostic and Screening:**

The author's early publications address the challenge of overdiagnosis in asymptomatic women with incidental ultrasound findings. Studies demonstrated that ultrasound-detected endometrial polyps or hyperplasia, in the absence of clinical symptoms, do not independently justify invasive biopsy. Instead, individualized decision-making—based on patient age, BMI, symptomatology, and sonographic risk features—is advocated. Furthermore, a large cohort study found no significant difference in overall or disease-specific survival between asymptomatic and symptomatic patients diagnosed with EC, reinforcing the notion that early diagnosis in asymptomatic women does not necessarily translate to better outcomes. These findings support a more nuanced, risk-adapted approach to endometrial assessment rather than a blanket biopsy policy.

- **Molecular Classification and Prognostic Markers:**

A key element of the thesis is the integration of the TCGA-based molecular classification into clinical decision-making. The author's contributions include real-world implementation data showing that incorporation of *POLE* mutation analysis and immunohistochemistry for MMR proteins and p53 is feasible and does not delay treatment planning. Importantly, the introduction of molecular classification altered

risk stratification in a significant proportion of patients, potentially changing adjuvant therapy and surgical recommendations.

Moreover, the author participated in multicenter studies demonstrating that traditional biomarkers, such as estrogen and progesterone receptor status, retain independent prognostic value within molecular subgroups. For instance, in p53-abnormal tumors, high progesterone receptor expression was associated with markedly improved disease-specific survival, underscoring the continued relevance of hormonal profiling in EC. Similarly, the evaluation of serum biomarkers such as DJ1 and L1CAM was explored for potential use in post-treatment surveillance, although further validation is required.

- **Lymph Node Staging:**

Another major theme of the thesis is the evolution of lymph node staging. The author contributed significantly to the adoption and refinement of SNB in endometrial cancer. Multiple prospective and retrospective studies highlighted the safety and efficacy of SLN mapping using CG, particularly in early-stage disease. Risk factors for unsuccessful bilateral mapping—such as high BMI, older age, and uterine myomas—were identified to guide surgical planning.

The international SENECA study, to which the author contributed, analyzed SLN involvement across molecular subtypes. p53mut and MMRd tumors exhibited the highest rates of nodal metastasis, but overall, molecular classification did not outperform histopathologic parameters in predicting nodal disease. These findings support the continued use of surgical staging algorithms while incorporating molecular data into broader risk assessment.

- **Predictive Models:**

The final section of the thesis presents a forward-looking view of individualized care through predictive modeling. The author contributed to both the development and validation of models such as ENDORISK, a Bayesian network designed to predict lymph node metastasis and survival using preoperative clinical, histological, and molecular data. The model demonstrated high accuracy, especially in low-risk patients, but tended to underestimate risk in advanced-stage disease. In another original study, the inclusion of immunohistochemical markers—such as p53, PR, ER, and L1CAM—

significantly improved preoperative risk stratification accuracy, supporting their integration into routine diagnostics.

The body of work presented in this habilitation thesis demonstrates a comprehensive and multifaceted approach to individualizing care for patients with endometrial cancer. The integration of molecular diagnostics, targeted biomarker assessment, refined surgical techniques, and predictive analytics provides a robust framework for personalized treatment planning. The findings support a paradigm shift toward biology-driven, evidence-based decision-making in endometrial cancer, ultimately aiming to optimize outcomes while minimizing overtreatment. This work highlights the critical importance of cross-disciplinary collaboration, adherence to evolving international guidelines, and the need for continuous innovation in gynecologic oncology.

8 Commentary

The habilitation thesis entitled *"Individualized Approach to Patients with Endometrial Cancer"* is presented as a thematically integrated collection of original scientific papers that collectively document the author's systematic and long-term contribution to advancing personalized care in gynecologic oncology. With a focus on endometrial carcinoma, the thesis addresses key areas of modern patient management, including early diagnostics, molecular profiling, individualized surgical staging, and predictive modeling. The central aim of the work is to improve risk-adapted clinical decision-making by implementing biologically and clinically informed strategies that enhance diagnostic precision, optimize therapeutic planning, and ultimately contribute to better patient outcomes.

The methodologies employed across the included studies are diverse and appropriately selected for the posed research questions. They encompass both prospective and retrospective cohort designs, largely conducted at the Department of Obstetrics and Gynecology, University Hospital Brno, often in collaboration with leading European research centers, especially under the auspices of the ENITEC (European Network for Individualized Treatment in Endometrial Cancer) consortium. The author utilizes advanced ultrasound diagnostics, immunohistochemical and molecular-genetic analysis (including mismatch repair protein expression, *POLE* and *TP53* mutation testing), and statistical modeling approaches such as Bayesian networks and decision trees. Multicenter collaboration and large patient cohorts further strengthen the robustness and clinical relevance of the findings.

Among the most significant outcomes of the thesis is the clarification of the clinical role of ultrasound and endometrial biopsy in asymptomatic patients. The author's work demonstrates that in the absence of bleeding or other risk features, the detection of polyps or hyperplasia on ultrasound should not be considered an automatic indication for biopsy. Furthermore, her data show that early detection of endometrial cancer in asymptomatic women does not translate into improved survival when adjusted for tumor stage and biology, supporting a more individualized, risk-adapted diagnostic approach.

In the area of molecular classification, the author demonstrates that routine application of immunohistochemistry for mismatch repair proteins and p53,

supplemented by *POLE* sequencing where indicated, is both feasible and clinically impactful. Implementation of molecular stratification led to a reclassification of risk categories in a substantial number of patients and thus influenced recommendations for surgical procedure and adjuvant therapy. The clinical relevance of traditional biomarkers such as estrogen and progesterone receptors is also revisited, with new evidence confirming their independent prognostic value even within molecular subtypes. Pilot study on serum biomarkers such as DJ1 and L1CAM suggests potential for treatment monitoring and early recurrence detection, although further validation is needed.

A particularly strong part of the thesis lies in its contribution to refining lymph node staging. The author has conducted both single-center and multicenter studies on sentinel lymph node biopsy using indocyanine green, identifying key predictors of mapping failure and supporting its adoption as the standard staging procedure. These findings are corroborated by her contribution to the international SENECA study, which examined the correlation between sentinel node involvement and molecular subtypes. Although p53-abnormal and MMR-deficient tumors had higher nodal positivity rates, molecular classification did not yet surpass histopathological criteria in predictive accuracy, emphasizing the complementary rather than substitutive role of molecular data in surgical planning.

In the final part of the thesis, the author explores predictive modeling as a tool for individualized care. She contributed to an external validation of the ENDORISK model, which uses clinical, histological, and molecular data to predict lymph node metastases and disease-specific survival. The model performed with high accuracy in low-risk patients, highlighting its value in preoperative planning. Another original study showed that incorporating immunohistochemical markers into existing diagnostic models significantly improved risk stratification, further supporting the integration of molecular pathology into daily practice.

MUDr. Petra Bretová, Ph.D., is either the first or corresponding author in a large proportion of the included publications. Her contribution consistently includes the formulation of research hypotheses, design of methodology, clinical data collection, statistical analysis, and manuscript preparation. In multicenter settings, she has

assumed responsibility for coordinating data acquisition, ensuring methodological rigor, and participating in critical manuscript revisions.

In conclusion, this habilitation thesis presents a comprehensive, methodologically rigorous, and clinically relevant body of work that significantly advances individualized management in endometrial cancer. The results are well-validated, thoroughly discussed, and directly applicable to clinical practice. The author's contributions meet all the criteria for independent scientific work and academic promotion.

9 List of Abbreviations

Abbreviation	Full Term
AI	Artificial Intelligence
AUC	Area Under the Curve
BMI	Body Mass Index
CA125	Cancer Antigen 125
CI	Confidence Interval
CT	Computed Tomography
DFS	Disease-Free Survival
DNA	Deoxyribonucleic Acid
DSS	Disease-Specific Survival
EC	Endometrial Cancer
EIN	Endometrial Intraepithelial Neoplasia
ENITEC	European Network of Individualized Treatment in Endometrial Cancer
ER	Estrogen Receptor
ESGO	European Society of Gynaecological Oncology
ESP	European Society of Pathology
ESTRO	European Society for Radiotherapy and Oncology
FIGO	International Federation of Gynecology and Obstetrics
HE4	Human Epididymis Protein 4
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hazard Ratio
ICG	Indocyanine Green
IETA	International Endometrial Tumor Analysis
IF	Impact Factor (according to Journal Citation Reports)
IHC	Immunohistochemistry
ITC	Isolated Tumor Cells
LNM	Lymph Node Metastasis
L1CAM	L1 Cell Adhesion Molecule
LVM	Low-volume Metastasis
LVSI	Lymphovascular Space Invasion
MIC	Micrometastasis
MIS	Minimally Invasive Surgery
MLH1	MutL Homolog 1
MMR	Mismatch Repair
MMRd	Mismatch Repair deficiency
MSI	Microsatellite Instability

Abbreviation	Full Term
MSH2	MutS Homolog 2
MSH6	MutS Homolog 6
NGS	Next-Generation Sequencing
NSMP	No Specific Molecular Profile
OS	Overall Survival
p53mut	p53-Mutation
PARK7	Parkinson's disease-associated protein 7
PMS2	Postmeiotic Segregation Increased 2
POLE	DNA Polymerase Epsilon
POLEmut	DNA Polymerase Epsilon mutated phenotype
PR	Progesterone Receptor
Q1/Q2/Q4	Quartile rankings for journal impact factor (e.g., Q1 = top 25%)
SENECA	Staging ENdomEtrial CAncer based on molecular classification
SLN	Sentinel Lymph Node
SNB	Sentinel Node Biopsy
Tc99	Technetium-99m
TCGA	The Cancer Genome Atlas

10 References

1. Krejčí D, Mužík J, Šnábl I et al. Portál epidemiologie novotvarů v ČR. Masarykova univerzita, Brno. 2024 [online]. Available from: https://www.svod.cz.

2. Creasman W, Odicino F, Maisonneuve P, et al. Carcinoma of the Corpus Uteri. *Int J Gynecol Obstet*. 2006;95(S1). doi:10.1016/S0020-7292(06)60031-3

3. Concin N, Matias-Guiu X, Vergote I, 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

4. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol*. 1983;15(1):10-17. doi:10.1016/0090-8258(83)90111-7

5. Concin N, Planchamp F, Abu-Rustum NR, et al. European Society of Gynaecological Oncology quality indicators for the surgical treatment of endometrial carcinoma. *Int J Gynecol Cancer*. 2021;31(12):ijgc-2021-003178. doi:10.1136/ijgc-2021-003178

6. Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of Endometrial Cancer Risk With Postmenopausal Bleeding in Women: A Systematic Review and Meta-analysis. *JAMA Intern Med*. 2018;178(9):1210. doi:10.1001/jamainternmed.2018.2820

7. Lee NK, Cheung MK, Shin JY, et al. Prognostic Factors for Uterine Cancer in Reproductive-Aged Women: *Obstet Gynecol*. 2007;109(3):655-662. doi:10.1097/01.AOG.0000255980.88205.15

8. Leone FPG, Timmerman D, Bourne T, et al. Terms, definitions and measurements to describe the sonographic features of the endometrium and intrauterine lesions: a consensus opinion from the International Endometrial Tumor Analysis (IETA) group. *Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol*. 2010;35(1):103-112. doi:10.1002/uog.7487

9. Van Den Bosch T, Verbakel JY, Valentin L, et al. Typical ultrasound features of various endometrial pathologies described using International Endometrial Tumor Analysis (IETA) terminology in women with abnormal uterine bleeding. *Ultrasound Obstet Gynecol*. 2021;57(1):164-172. doi:10.1002/uog.22109

10. Smith-Bindman R, Weiss E, Feldstein V. How thick is too thick? When endometrial thickness should prompt biopsy in postmenopausal women without vaginal bleeding. *Ultrasound Obstet Gynecol*. 2004;24(5):558-565. doi:10.1002/uog.1704

11. Heremans R, Van Den Bosch T, Valentin L, et al. Ultrasound features of endometrial pathology in women without abnormal uterine bleeding: results from the International Endometrial Tumor Analysis study (IETA3). *Ultrasound Obstet Gynecol*. 2022;60(2):243-255. doi:10.1002/uog.24910

12. Gemer O, Segev Y, Helpman L, et al. Is there a survival advantage in diagnosing endometrial cancer in asymptomatic postmenopausal patients? An Israeli Gynecology

Oncology Group study. *Am J Obstet Gynecol*. 2018;219(2):181.e1-181.e6. doi:10.1016/j.ajog.2018.05.013

13. Barak F, Kalichman L, Gdalevich M, et al. The influence of early diagnosis of endometrioid endometrial cancer on disease stage and survival. *Arch Gynecol Obstet*. 2013;288(6):1361-1364. doi:10.1007/s00404-013-2898-5

14. Seebacher V, Schmid M, Polterauer S, et al. The presence of postmenopausal bleeding as prognostic parameter in patients with endometrial cancer: a retrospective multi-center study. *BMC Cancer*. 2009;9(1):460. doi:10.1186/1471-2407-9-460

15. The Cancer Genome Atlas Research Network, Levine DA. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67-73. doi:10.1038/nature12113

16. Stelloo E, Nout RA, Osse EM, 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):4215-4224. doi:10.1158/1078-0432.CCR-15-2878

17. Talhouk A, McConechy MK, Leung S, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer*. 2017;123(5):802-813. doi:10.1002/cncr.30496

18. Dundr P, Cibula D, Doležel M, et al. Molecular testing in endometrial carcinoma - joint recommendation of Czech Oncological Society, Oncogynecological Section of the Czech Gynecological and Obstetrical Society, Society of Radiation Oncology, Biology and Physics, and the Society of Czech Pathologists. *Ceska Gynekol*. 2021;86(4):264-272. doi:10.48095/cccg2021264

19. León-Castillo A, Britton H, McConechy MK, et al. Interpretation of somatic *POLE* mutations in endometrial carcinoma. *J Pathol*. 2020;250(3):323-335. doi:10.1002/path.5372

20. Church DN, Stelloo E, Nout RA, et al. Prognostic Significance of POLE Proofreading Mutations in Endometrial Cancer. *JNCI J Natl Cancer Inst*. 2015;107(1). doi:10.1093/jnci/dju402

21. Dong D, Lei H, Liu D, et al. POLE and Mismatch Repair Status, Checkpoint Proteins and Tumor-Infiltrating Lymphocytes in Combination, and Tumor Differentiation: Identify Endometrial Cancers for Immunotherapy. *Front Oncol*. 2021;11:640018. doi:10.3389/fonc.2021.640018

22. Ryan NAJ, Glaire MA, Blake D, Cabrera-Dandy M, Evans DG, Crosbie EJ. The proportion of endometrial cancers associated with Lynch syndrome: a systematic review of the literature and meta-analysis. *Genet Med*. 2019;21(10):2167-2180. doi:10.1038/s41436-019-0536-8

23. León-Castillo A, Gilvazquez E, Nout R, et al. Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas. *J Pathol*. 2020;250(3):312-322. doi:10.1002/path.5373

24. Zhang Y, Zhao D, Gong C, et al. Prognostic role of hormone receptors in endometrial cancer: a systematic review and meta-analysis. *World J Surg Oncol*. 2015;13(1):208. doi:10.1186/s12957-015-0619-1

25. Smith D, Stewart CJR, Clarke EM, et al. ER and PR expression and survival after endometrial cancer. *Gynecol Oncol*. 2018;148(2):258-266. doi:10.1016/j.ygyno.2017.11.027

26. Trovik J, Wik E, Werner HMJ, 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):3431-3441. doi:10.1016/j.ejca.2013.06.016

27. Van Der Putten LJM, Visser NCM, Van De Vijver K, et al. Added Value of Estrogen Receptor, ProgesteroneReceptor, and L1 Cell Adhesion Molecule Expression to Histology-Based Endometrial Carcinoma Recurrence Prediction Models. *Int J Gynecol Cancer*. 2018;28(3):514-523. doi:10.1097/IGC.0000000000001187

28. Ethier JL, Desautels DN, Amir E, MacKay H. Is hormonal therapy effective in advanced endometrial cancer? A systematic review and meta-analysis. *Gynecol Oncol*. 2017;147(1):158-166. doi:10.1016/j.ygyno.2017.07.002

29. Van Weelden WJ, Massuger LFAG, ENITEC, Pijnenborg JMA, Romano A. Anti-estrogen Treatment in Endometrial Cancer: A Systematic Review. *Front Oncol*. 2019;9:359. doi:10.3389/fonc.2019.00359

30. Wang C, Tran DA, Fu MZ, Chen W, Fu SW, Li X. Estrogen Receptor, Progesterone Receptor, and HER2 Receptor Markers in Endometrial Cancer. *J Cancer*. 2020;11(7):1693-1701. doi:10.7150/jca.41943

31. Van Weelden WJ, Reijnen C, Küsters-Vandevelde HVN, et al. The cutoff for estrogen and progesterone receptor expression in endometrial cancer revisited: a European Network for Individualized Treatment of Endometrial Cancer collaboration study. *Hum Pathol*. 2021;109:80-91. doi:10.1016/j.humpath.2020.12.003

32. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer. *Int J Gynecol Cancer*. 2016;26(1):2-30. doi:10.1097/IGC.0000000000000609

33. Yuan Y, Liu X, Cai Y, Li W. Lapatinib and lapatinib plus trastuzumab therapy versus trastuzumab therapy for HER2 positive breast cancer patients: an updated systematic review and meta-analysis. *Syst Rev*. 2022;11(1):264. doi:10.1186/s13643-022-02134-9

34. Łapińska-Szumczyk S, Supernat A, Majewska H, et al. HER2-Positive Endometrial Cancer Subtype Carries Poor Prognosis. *Clin Transl Sci*. 2014;7(6):482-488. doi:10.1111/cts.12207

35. Morrison C, Zanagnolo V, Ramirez N, et al. HER-2 Is an Independent Prognostic Factor in Endometrial Cancer: Association With Outcome in a Large Cohort of Surgically Staged Patients. *J Clin Oncol*. 2006;24(15):2376-2385. doi:10.1200/JCO.2005.03.4827

36. Fader AN, Roque DM, Siegel E, et al. Randomized Phase II Trial of Carboplatin-Paclitaxel Versus Carboplatin-Paclitaxel-Trastuzumab in Uterine Serous Carcinomas That Overexpress Human Epidermal Growth Factor Receptor 2/neu. *J Clin Oncol*. 2018;36(20):2044-2051. doi:10.1200/JCO.2017.76.5966

37. Brümmendorf T, Kenwrick S, Rathjen FG. Neural cell recognition molecule L1: from cell biology to human hereditary brain malformations. *Curr Opin Neurobiol*. 1998;8(1):87-97. doi:10.1016/S0959-4388(98)80012-3

38. Van Der Putten LJ, Visser NC, Van De Vijver K, et al. L1CAM expression in endometrial carcinomas: an ENITEC collaboration study. *Br J Cancer*. 2016;115(6):716-724. doi:10.1038/bjc.2016.235

39. Geels YP, Pijnenborg JMA, Gordon BBM, et al. L1CAM Expression is Related to Non-Endometrioid Histology, and Prognostic for Poor Outcome in Endometrioid Endometrial Carcinoma. *Pathol Oncol Res*. 2016;22(4):863-868. doi:10.1007/s12253-016-0047-8

40. Bosse T, Nout RA, Stelloo E, et al. L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: Pooled PORTEC trial results. *Eur J Cancer*. 2014;50(15):2602-2610. doi:10.1016/j.ejca.2014.07.014

41. Kommoss FK, Karnezis AN, Kommoss F, et al. L1CAM further stratifies endometrial carcinoma patients with no specific molecular risk profile. *Br J Cancer*. 2018;119(4):480-486. doi:10.1038/s41416-018-0187-6

42. Karnezis AN, Leung S, Magrill J, et al. Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification. *J Pathol Clin Res*. 2017;3(4):279-293. doi:10.1002/cjp2.82

43. Doberstein K, Harter PN, Haberkorn U, et al. Antibody therapy to human L1CAM in a transgenic mouse model blocks local tumor growth but induces EMT. *Int J Cancer*. 2015;136(5). doi:10.1002/ijc.29222

44. Schäfer H, Dieckmann C, Korniienko O, et al. Combined treatment of L1CAM antibodies and cytostatic drugs improve the therapeutic response of pancreatic and ovarian carcinoma. *Cancer Lett*. 2012;319(1):66-82. doi:10.1016/j.canlet.2011.12.035

45. Concin N, Matias-Guiu X, Cibula D, et al. ESGO–ESTRO–ESP guidelines for the management of patients with endometrial carcinoma: update 2025. *Lancet Oncol*. 2025;26(8):e423-e435. doi:10.1016/S1470-2045(25)00167-6

46. Lee JY, Kim JH, Seo JW, et al. Detecting Asymptomatic Recurrence in Early-Stage Endometrial Cancer. *Int J Gynecol Cancer*. 2016;26(8):1434-1439. doi:10.1097/IGC.0000000000000797

47. Brennan DJ, Hackethal A, Mann KP, et al. Serum HE4 detects recurrent endometrial cancer in patients undergoing routine clinical surveillance. *BMC Cancer*. 2015;15(1):33. doi:10.1186/s12885-015-1028-0

48. Kim CH, Khoury-Collado F, Barber EL, et al. Sentinel lymph node mapping with pathologic ultrastaging: A valuable tool for assessing nodal metastasis in low-grade endometrial cancer with superficial myoinvasion. *Gynecol Oncol*. 2013;131(3):714-719. doi:10.1016/j.ygyno.2013.09.027

49. Buda A, Di Martino G, Restaino S, et al. The impact on survival of two different staging strategies in apparent early stage endometrial cancer comparing sentinel lymph nodes mapping algorithm and selective lymphadenectomy: An Italian retrospective analysis of two reference centers. *Gynecol Oncol*. 2017;147(3):528-534. doi:10.1016/j.ygyno.2017.09.033

50. Panici PB, Basile S, Maneschi F, et al. Systematic Pelvic Lymphadenectomy vs No Lymphadenectomy in Early-Stage Endometrial Carcinoma: Randomized Clinical Trial. *JNCI J Natl Cancer Inst*. 2008;100(23):1707-1716. doi:10.1093/jnci/djn397

51. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *The Lancet*. 2009;373(9658):125-136. doi:10.1016/S0140-6736(08)61766-3

52. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the management of endometrial cancer. Cochrane Gynaecological, Neuro-oncology and Orphan Cancer Group, ed. *Cochrane Database Syst Rev*. 2017;2017(10). doi:10.1002/14651858.CD007585.pub4

53. Leitao MM, Zhou QC, Gomez-Hidalgo NR, et al. Patient-reported outcomes after surgery for endometrial carcinoma: Prevalence of lower-extremity lymphedema after sentinel lymph node mapping versus lymphadenectomy. *Gynecol Oncol*. 2020;156(1):147-153. doi:10.1016/j.ygyno.2019.11.003

54. Frumovitz M, Plante M, Lee PS, et al. Near-infrared fluorescence for detection of sentinel lymph nodes in women with cervical and uterine cancers (FILM): a randomised, phase 3, multicentre, non-inferiority trial. *Lancet Oncol*. 2018;19(10):1394-1403. doi:10.1016/S1470-2045(18)30448-0

55. Papadia A, Zapardiel I, Bussi B, et al. Sentinel lymph node mapping in patients with stage I endometrial carcinoma: a focus on bilateral mapping identification by comparing radiotracer Tc99m with blue dye versus indocyanine green fluorescent dye. *J Cancer Res Clin Oncol*. 2017;143(3):475-480. doi:10.1007/s00432-016-2297-y

56. Rossi EC, Kowalski LD, Scalici J, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre,

prospective, cohort study. *Lancet Oncol*. 2017;18(3):384-392. doi:10.1016/S1470-2045(17)30068-2

57. Eriksson AG, Beavis A, Soslow RA, et al. A Comparison of the Detection of Sentinel Lymph Nodes Using Indocyanine Green and Near-Infrared Fluorescence Imaging Versus Blue Dye During Robotic Surgery in Uterine Cancer. *Int J Gynecol Cancer*. 2017;27(4):743-747. doi:10.1097/IGC.0000000000000959

58. Martinelli F, Ditto A, Bogani G, et al. Sentinel lymph node mapping in endometrial cancer: performance of hysteroscopic injection of tracers. *Int J Gynecol Cancer*. 2020;30(3):332-338. doi:10.1136/ijgc-2019-000930

59. Ballester M, Dubernard G, Lécuru F, 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(5):469-476. doi:10.1016/S1470-2045(11)70070-5

60. Holloway RW, Gupta S, Stavitzski NM, et al. Sentinel lymph node mapping with staging lymphadenectomy for patients with endometrial cancer increases the detection of metastasis. *Gynecol Oncol*. 2016;141(2):206-210. doi:10.1016/j.ygyno.2016.02.018

61. Ignatov A, Lebius C, Ignatov T, et al. Lymph node micrometastases and outcome of endometrial cancer. *Gynecol Oncol*. 2019;154(3):475-479. doi:10.1016/j.ygyno.2019.07.018

62. Iasonos A, Schrag D, Raj GV, Panageas KS. How To Build and Interpret a Nomogram for Cancer Prognosis. *J Clin Oncol*. 2008;26(8):1364-1370. doi:10.1200/JCO.2007.12.9791

63. Wang Z, Zhang S, Ma Y, Li W, Tian J, Liu T. A nomogram prediction model for lymph node metastasis in endometrial cancer patients. *BMC Cancer*. 2021;21(1):748. doi:10.1186/s12885-021-08466-4

64. Kourou K, Exarchos TP, Exarchos KP, Karamouzis MV, Fotiadis DI. Machine learning applications in cancer prognosis and prediction. *Comput Struct Biotechnol J*. 2015;13:8-17. doi:10.1016/j.csbj.2014.11.005

65. Zagidullin B, Pasanen A, Loukovaara M, Bützow R, Tang J. Interpretable prognostic modeling of endometrial cancer. *Sci Rep*. 2022;12(1):21543. doi:10.1038/s41598-022-26134-w

66. Lucas PJF, Van Der Gaag LC, Abu-Hanna A. Bayesian networks in biomedicine and health-care. *Artif Intell Med*. 2004;30(3):201-214. doi:10.1016/j.artmed.2003.11.001

67. Reijnen C, Gogou E, Visser NCM, et al. Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: A development and validation study. Brenton JD, ed. *PLOS Med*. 2020;17(5):e1003111. doi:10.1371/journal.pmed.1003111