Gynecologic Oncology 182 (2024) 148–155

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Reviving peritoneal cytology: Exploring its role in endometrial cancer molecular classification

Anna-Sophie Villiger [a], Selma Zurbriggen [b], Sara Imboden [a], Wiebke Solass [c], Lucine Christe [c], Flurina A.M. Saner [a], Andrea Gmür [a], Tilman T. Rau [d], Michael D. Mueller [a], Franziska Siegenthaler [a,*]

[a] Department of Obstetrics and Gynecology, Bern University Hospital and University of Bern, Bern, Switzerland
[b] University of Bern, Bern, Switzerland
[c] Institute of Tissue Medicine and Pathology, University of Bern, Bern, Switzerland
[d] Institute of Pathology, Universitätsklinikum Düsseldorf, Düsseldorf, Germany

HIGHLIGHTS

- Patients with p53abn endometrial cancer showed the highest rate of positive peritoneal cytology.
- Positive peritoneal cytology is associated with worse oncological outcome in NSMP and p53abn endometrial cancer patients.
- In women with p53abn endometrial cancer, positive peritoneal cytology is an independent predictor of recurrence and death.

ARTICLE INFO

Article history:
Received 2 November 2023
Received in revised form 26 December 2023
Accepted 6 January 2024
Available online xxxx

Keywords:
Endometrial cancer
Molecular classification
Peritoneal cytology
Overall survival
Recurrence rate
Surgical treatment

ABSTRACT

Objective. The prognostic significance of positive peritoneal cytology in endometrial cancer has long been debated. In 2009, the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) removed cytology as a staging criterion from the endometrial cancer staging system. However, there is still evidence that positive peritoneal cytology may decrease survival among patients with endometrial cancer. The aim of this study was to determine the prognostic significance of positive peritoneal cytology among the different molecular subgroups.

Methods. This study included patients with endometrial cancer who underwent primary surgical treatment between 2004 and 2015 at the Bern University Hospital, Switzerland, with molecular classification of the primary tumor and peritoneal cytology performed.

Results. A total, 250 patients with endometrial cancer were enrolled. Peritoneal cytology was assessed in 206 patients, of whom 24% were positive: 25% of the *POLE*mut, 16% of the MMRd, 41% of the p53abn, and 24% of the NSMP cases. The mean follow-up was 128.7 months. Presence of positive peritoneal cytology was associated with significantly decreased mean recurrence-free and overall survival in patients with p53abn ($p = .003$ and $p = .001$) and NSMP ($p = .020$ and $p = .049$) endometrial cancer. In multivariable Cox regression analysis, positive peritoneal cytology remained an independent predictor of recurrence ($p = .033$) and death ($p = .008$) in p53abn endometrial cancer patients.

Conclusion. Positive peritoneal cytology is associated with worse oncologic outcomes in NSMP and p53abn endometrial cancer and remains an independent predictor of recurrence and death in patients with p53abn endometrial cancer.

1. Introduction

Endometrial carcinoma is one of the most common malignant gynecological cancers with a worldwide incidence of 382′000 cases annually [1]. Especially in high-income countries, its incidence is increasing due to the greater prevalence of its risk factors, including obesity, metabolic syndrome, and age [2]. In general, endometrial cancer has a favorable prognosis, with an overall 5-year survival rate of 80% [3]. Despite its commonly beneficial outcome, about 18% of endometrial cancer patients experience recurrence, and in these patients, treatment options are limited, and mortality remains high [4,5]. Due to the good prognosis of this disease, it is not only of great importance to identify patients with

* Corresponding author at: Department of Obstetrics and Gynecology, Bern University Hospital and University of Bern, Friedbühlstrasse 19, 3010 Bern, Switzerland.
E-mail address: Franziska.siegenthaler@insel.ch (F. Siegenthaler).

https://doi.org/10.1016/j.ygyno.2024.01.006

aggressive tumor biology and poor outcomes despite optimal treatment but also patients who may not benefit from adjuvant treatment and are currently over-treated.

Positive peritoneal cytology, defined as the presence of malignant cells in the peritoneal washing collected during staging surgery, is suspicious for microscopic peritoneal metastasis and spreading outside the uterine cavity. Peritoneal cytology is highly predictive of survival in multiple gynecological malignancies [6]. In early-stage endometrial cancer, the prognostic importance of positive peritoneal cytology has long been debated [7]. In 2009, the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) removed cytology as a staging criterion from the endometrial cancer staging system and positive cytology has to be reported separately without changing the stage [8]. Nonetheless, multiple studies have shown an association of positive peritoneal cytology with decreased survival in patients with endometrial cancer [9–12]. Furthermore, treatment of patients with positive cytology with adjuvant chemotherapy was associated with increased survival [9].

The understanding of endometrial cancer at the molecular level has seen an incredible evolution over the past decade. In 2013, The Cancer Genome Atlas (TCGA) collaborative endometrial project determined four molecular subgroups [13]. This was further developed into a simplified molecular classifier called the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), which identifies four molecular subtypes: polymerase epsilon ultramutated (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53abn), and non-specific molecular profile (NSMP) [14]. Since then, the prognostic significance of the molecular classification of endometrial cancer has been demonstrated by various study groups [15–19]. The molecular subgroups were integrated in the 5th edition of the WHO Classification of Female Genital Tumors, finding their definitive place in endometrial cancer diagnosis [20]. Furthermore, in 2021, the European Society of Gynecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP) published updated guidelines for the determination of risk groups in endometrial cancer, integrating molecular classification into risk classification and recommendations for adjuvant treatment [21].

While the prognostic independence of the molecular classification from several histopathological factors appears demonstrated [16,22–24], data on the association of peritoneal cytology with the new molecular subgroups is missing. The aim of this study is to evaluate the impact of positive peritoneal cytology on oncological outcomes in each molecular subgroup.

2. Material and methods

2.1. Patient population

This retrospective cohort study includes patients with endometrial cancer who underwent primary surgical treatment between 2004 and 2015 at the Bern University Hospital, Switzerland. As patients were selected without reference to tumor type, the cohort represents a population-based cohort of all patients with endometrial cancer treated at the Bern University Hospital, Switzerland during the study period. This patient population consists of the Swiss part of the KimBer (Swedish and Swiss endometrial cancer cohort), of which the analysis, as well as its oncological outcomes, has been published previously [17,19,24,25]. Surgical treatment included hysterectomy, bilateral salpingo-oophorectomy, and lymph node staging if indicated. Pelvic irrigation for peritoneal cytology was conducted at the beginning of the surgery using 200 ml of lactated Ringer's solution. All pathology slides were reviewed by reference pathologists as previously described [17]. Follow-up data on recurrence and survival were available through standardized databases and follow-up controls. Ethical approval was obtained from the local ethics committees in Bern (reference number: 2018–00479). All patients provided written informed consent for using their tissue and clinical data for research. Molecular analysis was conducted according to the WHO Classification of Tumors, 5th Edition [20]. Immunohistochemistry for p53 and MMR proteins was performed on a tissue microarray as published previously [17,19,25]. Tumors were analyzed for mutations of *POLE* gene (NM.006231) exons 9–14 by Sanger sequencing. This patient population consists of the Swiss part of the KimBer (Swedish and Swiss endometrial cancer cohort), of which the analysis, as well as its oncological outcomes, has been published previously [17,19,24,25].

2.2. Oncological outcomes

Patients received follow-up examination according to international guidelines [21,26]. Recurrence-free survival was defined as the time from primary staging surgery to the first recurrence or death of any cause. Overall survival was defined as the time from primary staging surgery to death of any cause. Patients who were alive were censored at the date of their last follow-up. Recurrences were classified into locoregional, abdominal, and distant recurrences according to the first site of recurrence. Locoregional recurrences included vaginal and pelvic; abdominal recurrences refer to peritoneal carcinomatosis, omental metastasis, and para-aortic lymph node involvement; distant recurrences entail lung, liver, bone, and brain metastases as well as lymph node involvement other than pelvic and para-aortic.

2.3. Statistical data analysis

Statistical calculations were performed using the Statistical Package for Social Sciences (IBM SPSS Statistic version 28.0.1.1). Categorical variables were reported as frequencies and percentages, while continuous variables were reported as means and standard deviations. Patients, tumor, and treatment characteristics were analyzed using chi-square statistics and Fisher's exact test in case of categorical and analysis of variance (ANOVA) for continuous variables. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. Cox regression analyses was conducted to assess the relationship between the risk of recurrence and death with peritoneal cytology and other prognostic factors. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. General characteristics of the study cohort

The whole study cohort included 250 patients with endometrial cancer, whose tumors were classified as *POLE*mut in 4.0%, MMRd in 32.0%, p53abn in 16.4%, and NSMP in 47.6%. The mean age was 66 years, and the mean BMI was 30.5 kg/m^2. The majority of the patients had FIGO stage I disease (65.6%) and low-grade tumors (77.6%) with endometrioid histology (85.2%). Table 1 shows a detailed description of the main clinicopathological characteristics.

3.2. Association of peritoneal cytology with clinicopathological characteristics and oncological outcome

Peritoneal cytology was assessed in 206 patients, of which 157 (76.2%) patients showed negative and 49 (23.8%) patients showed positive peritoneal cytology. In 44 patients, data on peritoneal cytology was missing. Positive peritoneal cytology was significantly associated with negative prognostic factors such as advanced tumor stage (FIGO stage III and IV, $p < .001$), high-grade tumors ($p = .025$), larger tumor size ($p < .001$), myometrial invasion ($p = .027$) and lymph node metastasis ($p < .001$). There was no association of peritoneal cytology with histological subtype ($p = .466$) and preoperative hysteroscopy ($p = .469$). More details are provided in Table 2.

Table 1
Patients demographics and histological baseline characteristics of the whole study cohort.

Whole study cohort (n = 250)	
Mean age, years ± SD	65.9 ± 10.9
Mean BMI, kg/m^2 ± SD	30.5 ± 7.8
Menopausal status, n (%)	
premenopausal	11 (4.4)
postmenopausal	227 (90.8)
perimenopausal	12 (4.8)
Surgical approach, n (%)	
laparoscopic	212 (84.8)
robotic	2 (0.8)
laparotomy	22 (8.8)
conversion to laparotomy	14 (5.6)
Surgical lymph node staging performed, n (%)	204 (81.6)
FIGO stage, n (%)	
I	164 (65.6)
II	25 (10.0)
III	44 (17.6)
IV	17 (6.8)
Tumor size, mm ± SD	34.1 ± 21.5
Grading, n (%)	
G1	90 (36.0)
G2	104 (41.6)
G3	56 (22.4)
Histological subtype, n (%)	
endometrioid	213 (85.2)
serous	10 (4.0)
clear cell	5 (2.0)
carcinosarcoma	2 (0.8)
mixed	19 (7.6)
neuroendocrine	1 (0.4)
Lymphovascular space invasion, n (%)	57 (22.8)
Blood vessel invasion, n (%)	51 (20.4)
Lymph node metastasis, n (%)	43 (17.2)
Molecular classification, n (%)	
*POLE*mut	10 (4.0)
MMRd	80 (32.0)
p53abn	41 (16.4)
NSMP	119 (47.6)
Adjuvant treatment, n (%)	
none	86 (34.4)
vaginal brachytherapy	81 (32.4)
chemotherapy	3 (1.2)
chemoradiation	72 (28.8)
vaginal brachytherapy and external beam radiation	7 (2.8)
hormonal therapy	1 (0.4)

Abbreviations: N = number, SD = standard deviation, BMI = Body mass index, FIGO = Federation International de Gynecologie et Obstetrique, POLEmut = polymerase epsilon ultramutated, MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile,

The mean follow-up was 128.7 (95% CI 123.1–134.3) months for the whole study cohort. 55 (22.0%) patients suffered at least one recurrence, and 100 (40.0%) patients died during follow-up. Location of recurrence was locoregional in 15, abdominal in 16, distant in 21, progression in two, and unknown in one patient. Positive peritoneal cytology was significantly associated with non-locoregional recurrences (88.2% vs. 59.3%, $p = .040$). Patients with positive peritoneal cytology showed a significantly worse mean recurrence-free survival (88.0 months, 95% CI 65.3–110.6) compared to patients with negative peritoneal cytology (143.3 months, 95% CI 129.0–157.7, log-rank $p < .001$). Mean overall survival was significantly longer in patients with negative (152.2 months, 95% CI 139.0–165.5) compared to patients with positive (99.5 months, 95% CI 77.3–121.6) peritoneal cytology (log-rank $p < .001$). Positive peritoneal cytology was significantly associated with recurrence (HR 2.6, 95% CI 1.4–4.8, $p = .002$) and death (HR 2.2, 95% CI 1.4–3.5, $p < .001$) in univariable Cox regression analysis. In multivariable Cox regression analysis including stage, lymphovascular space invasion, and grading, positive peritoneal cytology was an independent predictor of death (HR 1.8, 95% CI 1.1–2.8, $p = .020$) but not for recurrence (HR 1.8, 95% CI 0.9–3.4, $p = .069$) in the overall study cohort.

Table 2
Association of peritoneal cytology with clinicopathological characteristics.

	Negative peritoneal cytology N = 157	Positive peritoneal cytology N = 49	p-value[a]
Age at diagnosis, years ± SD	65.4 ± 10.7	64.5 ± 11.7	0.595
BMI, kg/m2 ± SD	30.1 ± 7.7	29.9 ± 5.8	0.875
Preoperative hysteroscopy, n (%)	80 (51.0)	24 (49.0)	0.469
FIGO stage, n (%)			
I	116 (73.9)	19 (38.8)	
II	19 (12.1)	3 (6.1)	
III	19 (12.1)	17 (34.7)	
IV	3 (1.9)	10 (20.4)	**<0.001**
High-grade tumors, n (%)	28 (17.8)	16 (32.7)	**0.025**
Endometrioid histology, n (%)	134 (85.4)	41 (83.7)	0.466
Tumorsize, mm ± SD	30.3 ± 17.2	44.7 ± 26.3	**<0.001**
Myometrial invasion >50%, n (%)	65 (41.4)	31 (63.3)	**0.027**
Lymph node involvement, n (%)	19 (12.1)	19 (38.8)	**<0.001**
Molecular subgroup, n (%)			
*POLE*mut	6 (3.8)	2 (4.1)	
MMRd	57 (36.3)	11 (22.4)	
p53abn	19 (12.1)	13 (26.5)	
NSMP	75 (47.8)	23 (46.9)	0.066
Adjuvant treatment, n (%)	102 (65.0)	40 (81.6)	**0.019**

Abbreviations: N = number, SD = standard deviation, BMI = Body mass index, POLEmut = polymerase epsilon ultramutated, MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile, BMI = Body mass index, LVSI = lymphovascular space invasion.

[a] p values reflect χ^2 statistics or Fisher's exact test for categorical and ANOVA for continuous variables. A statistically significant p-value lower than 0.05 was marked bold in the table.

3.3. Prognostic value of peritoneal cytology among the different molecular subgroups

3.3.1. POLEmut

Ten patients presented with *POLE*mut endometrial cancer and information on peritoneal cytology was available in eight of these patients. Of these, 80% had FIGO stage I disease, and 70% low-grade tumors. The histological subtype was endometrioid in 80% and clear cell in 20% of the patients. One patient presented with lymph node metastasis, and 25% showed positive peritoneal cytology. During mean follow-up of 131.9 months (95% CI 112.1–151.7), no patient developed recurrence, and one patient died. Due to the low number of events, no further calculations on oncological outcome were performed in the *POLE*mut subgroup.

3.3.2. MMRd

In this subgroup, 63.7% of the patients presented with stage I disease, 78.8% with low-grade tumors, and 86.3% with endometrioid histology. 20% of all women with MMRd endometrial cancer had lymph node metastasis and 16.2% positive peritoneal cytology. Among these patients, positive peritoneal cytology was associated with advanced tumor stage ($p < .001$), lymph node metastasis ($p = .036$), and larger tumor size ($p < .001$). There was no association of peritoneal cytology with tumor grading ($p = .696$) or histological subtype ($p = .489$). 21 (26.3%) patients suffered a recurrence, and 37 (46.3%) patients died during mean follow-up of 132.6 months (95% CI 120.4–144.8). There was no significant difference in mean recurrence-free survival between patients with MMRd endometrial cancer and positive (72.6 months, 95% CI 38.0–107.1) compared to negative (127.4 months, 95% CI 103.0–151.8) peritoneal cytology (log-rank, $p = .212$, Fig. 1a). Furthermore, mean overall survival did not differ significantly in MMRd

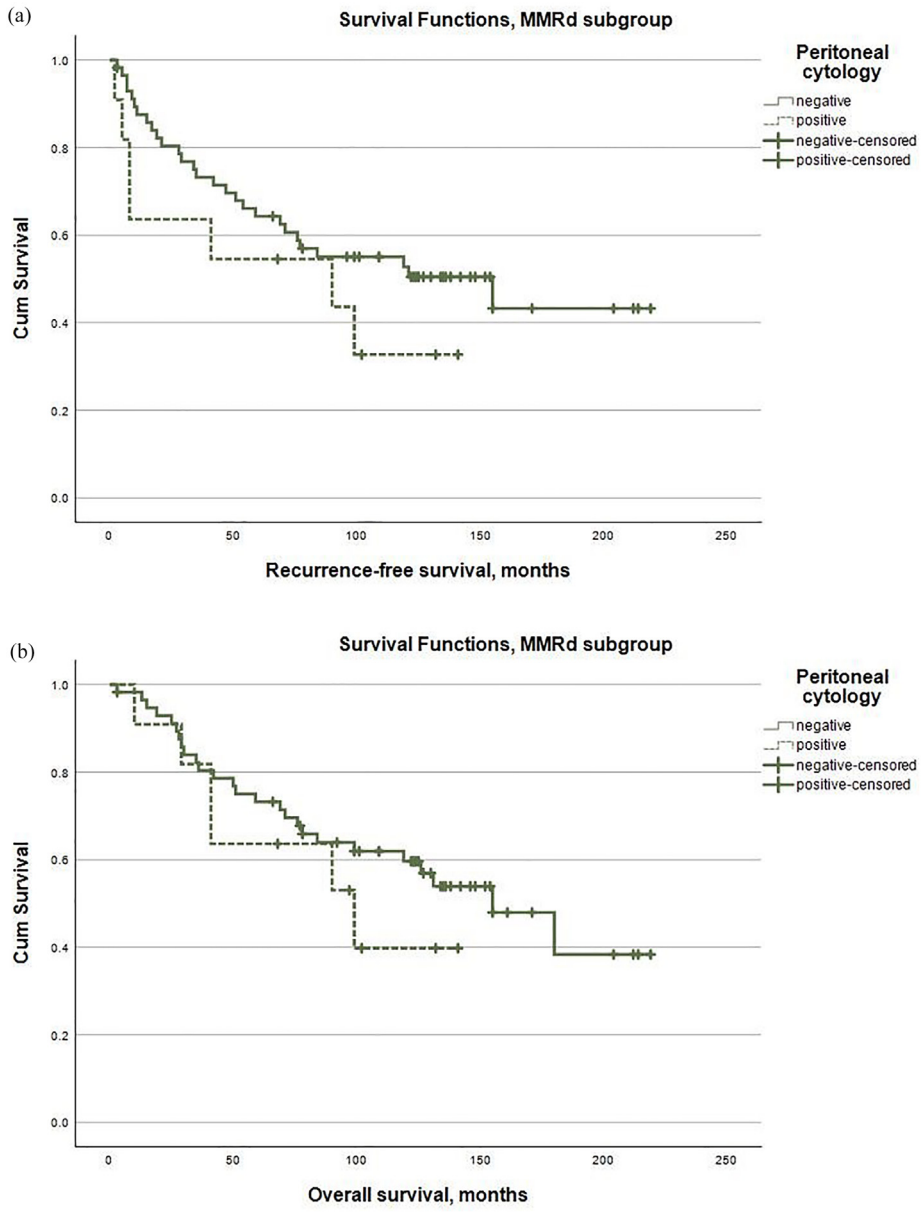

Fig. 1. Kaplan-Meier survival curves according to peritoneal cytology in the MMRd subgroup for (a) recurrence-free survival (log-rank, p = .212) and (b) overall survival (log-rank, p = .378).

patients with positive peritoneal cytology (89.8 months, 95% CI 60.2–119.3) compared to MMRd patients with negative (137.9 months, 95% CI 115.3–160.5) peritoneal cytology, (log-rank, $p = .378$, Fig. 1b).

3.3.3. p53abn

The p53abn tumors presented with the most advanced tumor stages (34.2%), high-grade tumors (41.5%), non-endometrioid histologies (24.4%), lymph node metastasis (22.0%), and the highest rate of positive peritoneal cytology (40.6%) compared to the other molecular subgroups. Among patients with p53abn endometrial cancer, positive peritoneal cytology was associated with advanced tumor stage ($p = .004$), lymph node metastasis ($p = .017$), and larger tumor size (p = .004). There was no association of peritoneal cytology with tumor grading ($p = .149$) or histological subtype ($p = .427$) in p53abn tumors. In this subgroup 69% of the patients with positive peritoneal cytology presented with advanced tumor stage. The mean follow-up was 122.4 months (95% CI 109.2–135.7) in this subgroup. Nine patients suffered at least one recurrence, and 16 patients died. Mean recurrence-free survival was significantly lower in patients with positive (52.6 months, 95% CI 17.3–87.9) compared to patients with negative (134.7 months, 95% CI 11.2–159.1) peritoneal cytology (log-rank, $p = .003$, Fig. 2a). The same correlations were seen for overall survival with a significant difference between patients with positive (57.2 months, 95% CI 22.9–91.6) and negative (177.7 months, 95% CI 143.8–211.5) peritoneal cytology (log-rank, $p = .001$, Fig. 2b). In multivariable Cox regression analysis including tumor stage, lymphovascular space invasion, and grading, positive peritoneal cytology remained an independent predictor of recurrence (HR 6.4, 95% CI 1.2–35.5, $p = .033$) and death (HR 6.2, 95% CI 1.6–23.8, $p = .008$) in patients with p53abn tumors.

3.3.4. NSMP

NSMP endometrial cancer patients form the largest subgroup. 68.9% of all NSMP tumors were stage I at primary diagnosis, with the highest proportion of low-grade (84.0%) and endometrioid (88.2%) histologies among all molecular subgroups. 14.3% presented with lymph node metastasis, and 23.5% had positive peritoneal cytology. In this subgroup,

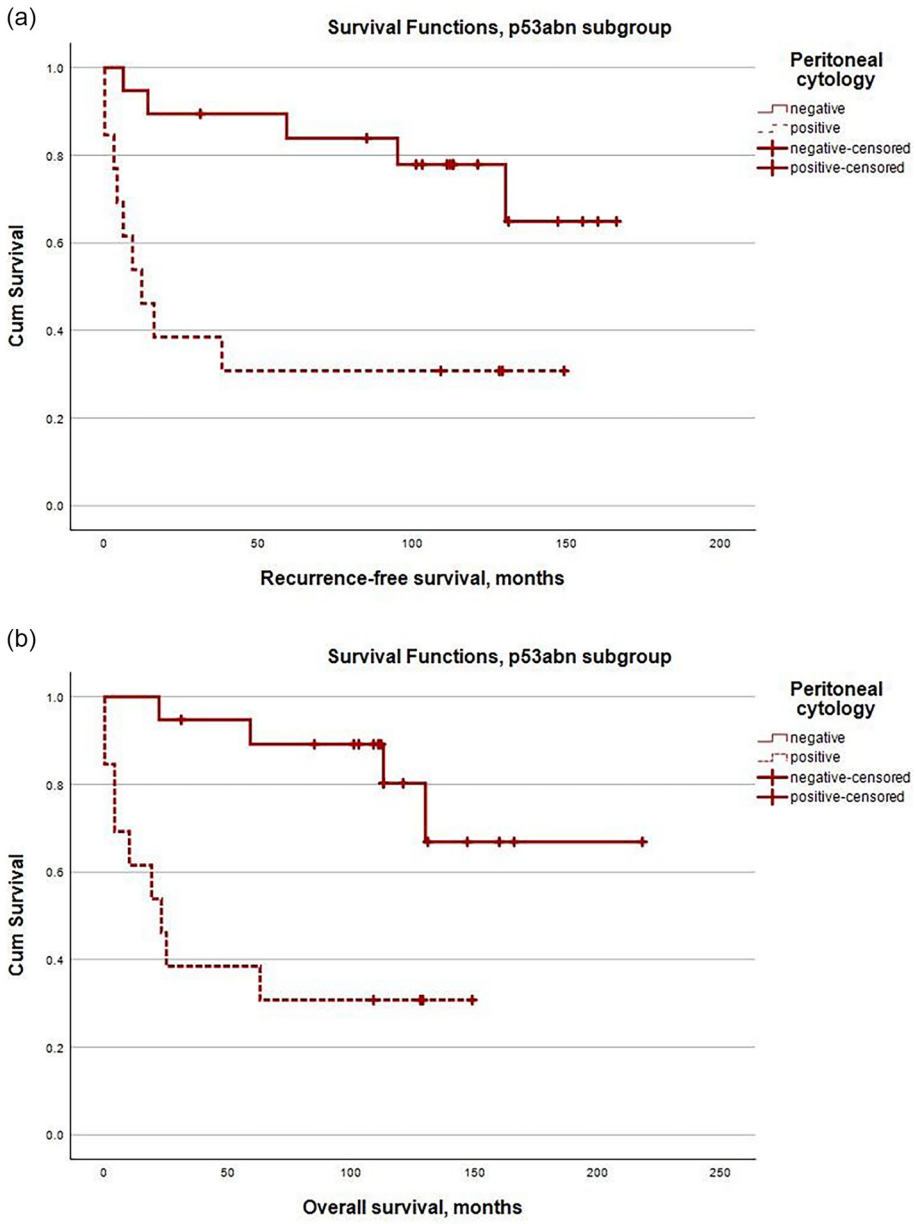

Fig. 2. Kaplan-Meier survival curves according to peritoneal cytology in the p53abn subgroup for (a) recurrence-free survival (log-rank, $p = .003$) and (b) overall survival (log-rank, $p = .001$).

positive peritoneal cytology was associated with advanced tumor stage ($p = .006$). There was no association of peritoneal cytology with tumor size ($p = .118$), lymph node metastasis ($p = .110$), tumor grading ($p = .177$), or histological subtype ($p = .497$) in this subgroup. During a mean follow-up of 127.7 months (95% CI 120.7–134.7), 25 patients suffered a recurrence and 46 patients died. Mean recurrence-free survival was significantly worse in patients with positive (95.7 months, 95% CI 64.7–126.7) compared to patients with negative (133.7 months, 95% CI 117.8–149.5) peritoneal cytology (log-rank, $p = .020$, Fig. 3a). Similar results showed up for overall survival with a mean of 110.3 months (95% CI 80.4–140.2) in patients with positive peritoneal cytology and 147.6 months (95% CI 131.2–164.1) in patients with negative peritoneal cytology (log-rank, $p = .049$, Fig. 3b). In a multivariable analysis including tumor stage, lymphovascular space invasion, and grading, positive peritoneal cytology was no longer an independent predictor of recurrence (HR 1.2, 95% CI 0.4–3.4, $p = .714$) or death (HR 1.4, 95% CI 0.7–2.8, $p = .415$) among patients with NSMP endometrial cancer.

4. Discussion

During the past years, the management of endometrial cancer has become more and more personalized, mainly as a result of the introduction of the new molecular classification [27], which is considered one of the most important prognostic markers both in the ESGO/ESTRO/ESP risk stratification [21] and the new FIGO 2023 staging system [28]. While some traditional histopathological factors such as lymphovascular space invasion and histological subtype were able to keep their place in the new classification systems, peritoneal cytology was removed from FIGO as a staging criterion in 2009[8] and currently has no impact on adjuvant treatment recommendations for women with endometrial cancer. However, evidence showed a diverse biological nature of endometrial carcinomas depending on the molecular classification [19,29,30]. In our study, we investigated if peritoneal cytology impacts patient prognosis among the molecular subgroups of endometrial cancer.

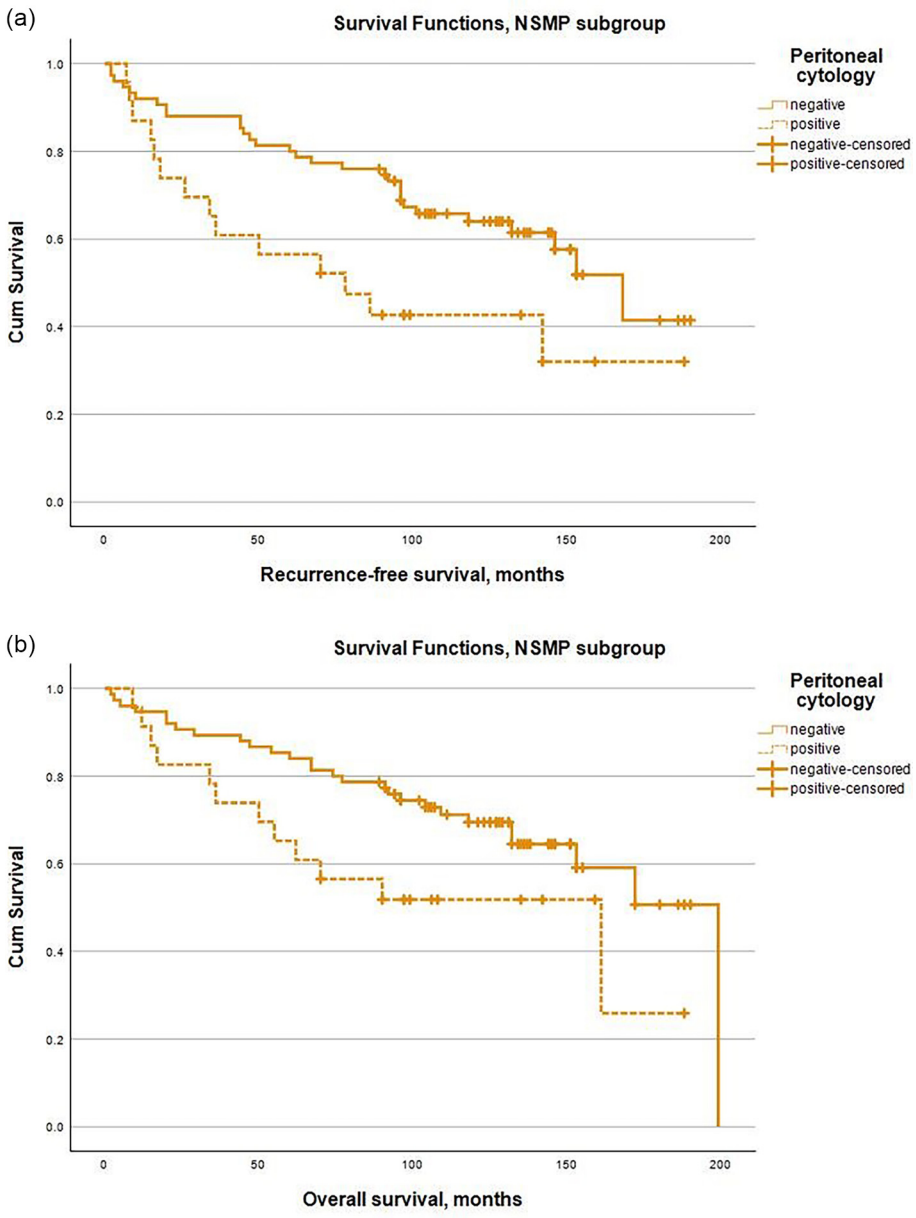

Fig. 3. Kaplan-Meier survival curves according to peritoneal cytology in the NSMP subgroup for (a) recurrence-free survival (log-rank, $p = .020$) and (b) overall survival (log-rank, $p = .049$).

In our study population of patients with endometrial cancer, 24% showed positive peritoneal cytology, which is rather high compared to current literature, revealing rates between 5 and 22% [31,32]. This might be explained by the fact that our study cohort also included advanced tumor stages and generally corresponds to a high-risk population of a tertiary center. As previously described, positive peritoneal cytology significantly correlated with negative prognostic factors such as advanced tumor stage, high-grade tumors, larger tumor size, myometrial invasion, and lymph node metastasis in our cohort [33]. But contrary to current literature [34,35], peritoneal cytology did not correlate with histological subtype or preoperative hysteroscopy in this study. In our study population of patients with endometrial cancer, positive peritoneal cytology was associated with a worse oncological outcome, including recurrence-free and overall survival, consistent with previous studies [9–12]. Furthermore, our result showed that patients with positive peritoneal cytology suffered more non-locoregional recurrences. This association was known in the literature before [12,36] and might be interpreted in the context of the persistence of microscopic disease in the abdominal cavity after primary surgical treatment.

Of our study patients, 4% were classified as POLEmut, 32% as MMRd, 16% as p53abn, and 48% as NSMP endometrial cancer, corresponding to the common distribution found in the literature [14]. Positive peritoneal cytology most frequently occurred in p53abn endometrial cancer patients, followed by the *POLE*mut, NSMP, and MMRd subgroups. To date, we do not only know that the molecular classification in endometrial cancer is prognostic [15] and predictive [30,37] but there is also evidence for different intrinsic tumor biology among the molecular subgroups [19]. MMRd endometrial cancers show a unique metastasis and recurrence pattern involving retroperitoneal lymph nodes [19,29], and there is evidence that adjuvant radiotherapy improves survival in MMRd tumors [30]. Consistent with these findings, the positive peritoneal cytology rate was lowest in the MMRd subgroup and did not correlate with the oncological outcome in our study. This further underlines

the importance of the lymphatic dissemination pathway in these tumors. On the other hand, p53abn tumors showed the highest rate of positive peritoneal cytology in our study. Only in this subgroup positive peritoneal cytology was an independent predictor of recurrence and death. As the p53abn endometrial cancers share genomic characteristics such as *TP53* mutations with ovarian serous carcinomas, a comparison is obvious. This is further supported by the fact that patients with p53abn endometrial cancer have a higher risk of intra-abdominal spread and experience most frequently abdominal recurrences [19,38]. Also in NSMP endometrial cancer, positive peritoneal cytology was associated with worse oncological outcomes. However, this association was not significant in multivariable Cox regression analysis.

4.1. Clinical relevance of our findings

The optimization of surgical and adjuvant therapy in endometrial cancer based on personalized risk stratification has advanced extremely during the last decade [13,39]. An assessment of independent prognostic factors is needed to obtain an ideally tailored management. Current evidence on the prognostic impact of peritoneal cytology in endometrial cancer is controversial [9–12,40] and to date, peritoneal cytology is not decisive for treatment decisions. Our study demonstrates that positive peritoneal cytology is an independent risk factor for recurrence and death in p53abn endometrial cancer. These tumors have a different biological behavior with a predominant abdominal dissemination [19,38]. We should therefore consider reintroducing peritoneal cytology for staging purposes in these tumors, analogous to ovarian cancer. Furthermore, we should incorporate peritoneal cytology for adjuvant treatment decisions in p53abn tumors since the evidence showed increased survival in patients with positive peritoneal cytology treated with adjuvant chemotherapy [9]. Particularly in patients with stage IA p53abn tumors, adjuvant chemotherapy should be discussed in case of positive peritoneal cytology. On the other hand, our results showed no impact of peritoneal cytology on prognosis in MMRd endometrial cancer. As these tumors are already known to disseminate predominantly by the lymphatic pathway [19,29], to our opinion peritoneal cytology might be omitted in these patients. If peritoneal cytology has a prognostic or predictive value in NSMP endometrial cancer independently of stage has to be further assessed. As these tumors form the biggest subgroup with a generally intermediate prognosis, new prognostic markers are welcome to further guide treatment decision for these patients.

4.2. Strengths and weaknesses

To our best knowledge, this is the first study to investigate the prognostic significance of positive peritoneal cytology in correlation with the molecular classification in endometrial cancer. The major strengths of this study include its large cohort size and the length of follow-up. The most important limitations are the retrospective study design and the missing information on peritoneal cytology in one-fifth of the patients. Furthermore, the numbers in this study were too small to identify statistically significant differences in all the molecular subgroups, especially in patients with *POLE*mut tumors.

5. Conclusion

In conclusion, molecular subgroups showed different rates of positive peritoneal cytology, with p53abn tumors associated with the highest and MMRd with the lowest rates. Furthermore, peritoneal cytology was associated with recurrence-free and overall survival among patients with NSMP and p53abn endometrial cancer. In patients with p53abn endometrial cancer, positive peritoneal cytology remained an independent predictor of recurrence and death in multivariable analysis, including stage, lymphovascular space invasion, and grading.

CRediT authorship contribution statement

Anna-Sophie Villiger: Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Selma Zurbriggen:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **Sara Imboden:** Conceptualization, Methodology, Validation. **Wiebke Solass:** Methodology, Validation. **Lucine Christe:** Data curation, Validation. **Flurina A.M. Saner:** Data curation, Methodology. **Andrea Gmür:** Formal analysis, Investigation. **Tilman T. Rau:** Data curation, Investigation, Resources, Validation. **Michael D. Mueller:** Conceptualization, Methodology, Validation. **Franziska Siegenthaler:** Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

All authors declare no conflict of interest.

Acknowledgements

The authors thank patients participating in this study and their families. We would also like to thank Madeleine Köchli for her extremely valuable medical documentation.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global Cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. (2021), https://doi.org/10.3322/caac.21660.
[2] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2021, CA Cancer J. Clin. 71 (1) (2021) 7–33, https://doi.org/10.3322/caac.21654.
[3] N. Colombo, C. Creutzberg, F. Amant, et al., ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up, Ann. Oncol. 27 (1) (2016) 16–41, https://doi.org/10.1093/annonc/mdv484.
[4] L. Ouldamer, S. Bendifallah, G. Body, et al., Incidence, patterns and prognosis of first distant recurrence after surgically treated early stage endometrial cancer: results from the multicentre FRANCOGYN study group, Eur. J. Surg. Oncol. 45 (4) (2019) 672–678, https://doi.org/10.1016/j.ejso.2019.01.011.
[5] F. Legge, S. Restaino, L. Leone, et al., Clinical outcome of recurrent endometrial cancer: analysis of post-relapse survival by pattern of recurrence and secondary treatment, Int. J. Gynecol. Cancer 30 (2) (2020) 193–200, https://doi.org/10.1136/ijgc-2019-000822.
[6] R.E. Zuna, A. Behrens, Peritoneal washing cytology in gynecologic cancers: long-term follow-up of 355 patients, J. Natl. Cancer Inst. 88 (14) (1996) 980–987, https://doi.org/10.1093/jnci/88.14.980.
[7] G.P. Sutton, The significance of positive peritoneal cytology in endometrial cancer, Oncology 4 (6) (1990) 21–32.
[8] S. Pecorelli, Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium, Int. J. Gynecol. Obstet. 105 (2) (2009) 103–104.
[9] B.L.L. Seagle, A.L. Alexander, T. Lantsman, S. Shahabi, Prognosis and treatment of positive peritoneal cytology in early endometrial cancer: matched cohort analyses from the National Cancer Database, Am. J. Obstet. Gynecol. 218 (3) (2018) 329.e1–329.e15, https://doi.org/10.1016/j.ajog.2017.11.601.
[10] B. Lee, D.H. Suh, K. Kim, J.H. No, Y.B. Kim, Influence of positive peritoneal cytology on prognostic factors and survival in early-stage endometrial cancer: a systematic review and meta-analysis, Jpn. J. Clin. Oncol. 46 (8) (2016) 711–717, https://doi.org/10.1093/jjco/hyw063.
[11] K. Matsuo, A. Yabuno, M.S. Hom, et al., Significance of abnormal peritoneal cytology on survival of women with stage I–II endometrioid endometrial cancer, Gynecol. Oncol. 149 (2) (2018) 301–309, https://doi.org/10.1016/j.ygyno.2018.02.012.
[12] F. Siegenthaler, S. Johann, S. Imboden, et al., Prospective multicenter trial assessing the impact of positive peritoneal cytology conversion on oncological outcome in endometrial cancer patients undergoing minimally invasive surgery with the use of an intrauterine manipulator, Ann. Surg. Oncol. (2022), https://doi.org/10.1245/s10434-022-12356-9.
[13] C. Kandoth, M.D. McLellan, F. Vandin, et al., Mutational landscape and significance across 12 major cancer types, Nature. 502 (7471) (2013) 333–339, https://doi.org/10.1038/nature12634.
[14] A. Talhouk, M.K. McConechy, S. Leung, et al., A clinically applicable molecular-based classification for endometrial cancers, Br. J. Cancer 113 (2) (2015) 299–310, https://doi.org/10.1038/bjc.2015.190.
[15] S. Kommoss, M.K. McConechy, F. Kommoss, et al., Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series, Ann. Oncol. 29 (5) (2018) 1180–1188, https://doi.org/10.1093/annonc/mdy058.
[16] L. Vermij, V. Smit, R. Nout, T. Bosse, Incorporation of molecular characteristics into endometrial cancer management, Histopathology. 76 (1) (2020) 52–63, https://doi.org/10.1111/his.14015.

[17] S. Imboden, D. Nastic, M. Ghaderi, et al., Implementation of the 2021 molecular ESGO/ESTRO/ESP risk groups in endometrial cancer, Gynecol. Oncol. 162 (2) (2021) 394–400, https://doi.org/10.1016/j.ygyno.2021.05.026.
[18] J. McAlpine, A. Leon-Castillo, T. Bosse, The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses, J. Pathol. 244 (5) (2018) 538–549, https://doi.org/10.1002/path.5034.
[19] F. Siegenthaler, K. Lindemann, E. Epstein, et al., Time to first recurrence, pattern of recurrence, and survival after recurrence in endometrial cancer according to the molecular classification, Gynecol. Oncol. (2022), https://doi.org/10.1016/j.ygyno.2022.02.024.
[20] WHO Classification of Tumours Editorial Board, Female genital tumours: WHO classification of tumours, Female Genital Tumours: Medicine Series, 5th edition, 2020.
[21] N. Concin, X. Matias-Guiu, I. Vergote, et al., ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma, Int. J. Gynecol. Cancer 31 (1) (2021) 12–39, https://doi.org/10.1136/ijgc-2020-002230.
[22] A. Talhouk, M.K. McConechy, S. Leung, et al., Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer, Cancer. 123 (5) (2017) 802–813, https://doi.org/10.1002/cncr.30496.
[23] E. Stelloo, R.A. Nout, E.M. Osse, 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), https://doi.org/10.1158/1078-0432.CCR-15-2878.
[24] F. Siegenthaler, E. Epstein, C. Büchi, et al., Prognostic value of lymphovascular space invasion according to the molecular subgroups in endometrial cancer, Int J Gynecol Cancer (2023), Advance on. https://doi.org/10.1136/ijgc-2023-004606.
[25] T.T. Rau, E. Bettschen, C. Büchi, et al., Prognostic impact of tumor budding in endometrial carcinoma within distinct molecular subgroups, Mod. Pathol. 34 (1) (2021) 222–232, https://doi.org/10.1038/s41379-020-0626-9.
[26] G. Emons, E. Steiner, D. Vordermark, et al., Interdisciplinary diagnosis, therapy and follow-up of patients with endometrial cancer. Guideline (S3-level, AWMF registry number 032/034-OL, April 2018) - part 2 with recommendations on the therapy and follow-up of endometrial cancer, palliative care, ps, Geburtshilfe Frauenheilkd. 78 (11) (2018) 1089–1109, https://doi.org/10.1055/a-0715-2964.
[27] C.G.A.R. Network, C. Kandoth, N. Schultz, et al., Integrated genomic characterization of endometrial carcinoma, Nature. 497 (7447) (2013) 67–73, https://doi.org/10.1038/nature12113.
[28] J.S. Berek, X. Matias-Guiu, C. Creutzberg, et al., FIGO staging of endometrial cancer: 2023, Int. J. Gynecol. Obstet. (2023), https://doi.org/10.1002/ijgo.14923.
[29] S.R. Kim, A. Tone, R.H. Kim, et al., Understanding the clinical implication of mismatch repair deficiency in endometrioid endometrial cancer through a prospective study, Gynecol. Oncol. 161 (1) (2021) 221–227, https://doi.org/10.1016/j.ygyno.2021.01.002.
[30] C. Reijnen, H.V.N. Küsters-Vandevelde, C.F. Prinsen, et al., Mismatch repair deficiency as a predictive marker for response to adjuvant radiotherapy in endometrial cancer, Gynecol. Oncol. (2019), https://doi.org/10.1016/j.ygyno.2019.03.097.
[31] G. Garg, F. Gao, J.D. Wright, A.R. Hagemann, D.G. Mutch, M.A. Powell, Positive peritoneal cytology is an independent risk-factor in early stage endometrial cancer, Gynecol. Oncol. 128 (1) (2013) 77–82, https://doi.org/10.1016/j.ygyno.2012.09.026.
[32] M. Kanno, M. Yunokawa, M. Nakabayashi, et al., Prognosis and adjuvant chemotherapy for patients with positive peritoneal cytology in stage IA endometrial cancer, Sci. Rep. (2022), https://doi.org/10.1038/s41598-021-03975-5.
[33] K. Matsuo, S. Matsuzaki, D.J. Nusbaum, et al., Association between adjuvant therapy and survival in stage II–III endometrial Cancer: influence of malignant peritoneal cytology, Ann. Surg. Oncol. (2021), https://doi.org/10.1245/s10434-021-09900-4.
[34] S. Maricic, A. Mandic, T. Vasiljević, B. Gutic, N. Stevanovic, T. Maksimovic, Association of peritoneal cytology with other prognostic factors in endometrial cancer, J Cytol. (2022), https://doi.org/10.4103/joc.joc_53_22.
[35] I. Takač, B. Žegura, Office hysteroscopy and the risk of microscopic extrauterine spread in endometrial cancer, Gynecol. Oncol. 107 (1) (2007) 94–98, https://doi.org/10.1016/j.ygyno.2007.05.031.
[36] P. Padilla-Iserte, V. Lago, C. Tauste, et al., Impact of uterine manipulator on oncological outcome in endometrial cancer surgery, Am. J. Obstet. Gynecol. (2021), https://doi.org/10.1016/j.ajog.2020.07.025.
[37] A. Leon-Castillo, S.M. De Boer, M.E. Powell, 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. 38 (29) (2020) 3388–3397, https://doi.org/10.1200/JCO.20.00549.
[38] W.T. Creasman, M.F. Kohler, F. Odicino, P. Maisonneuve, P. Boyle, Prognosis of papillary serous, clear cell, and grade 3 stage I carcinoma of the endometrium, Gynecol. Oncol. (2004), https://doi.org/10.1016/j.ygyno.2004.08.019.
[39] A. Papadia, S. Imboden, F. Siegenthaler, et al., Laparoscopic Indocyanine green sentinel lymph node mapping in endometrial Cancer, Ann. Surg. Oncol. 23 (7) (2016) 2206–2211, https://doi.org/10.1245/s10434-016-5090-x.
[40] P.M. Tebeu, Y. Popowski, H.M. Verkooijen, et al., Positive peritoneal cytology in early-stage endometrial cancer does not influence prognosis, Br. J. Cancer (2004) https://doi.org/10.1038/sj.bjc.6602035.