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Clinical factors and biomarker profiles associated with patient outcome in endometrioid ovarian carcinoma - Emphasis on tumor grade

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HIGHLIGHTS

- Disease stage and grade are independent clinical prognostic factors in endometrioid ovarian carcinoma.
- 3-tier grading system is supported by distinct survival and gradual change of markers between grades.
- Markers of favorable outcome were PR, ER, nuclear β-catenin and vimentin positivity.
- Abnormal expression of p53, overexpression of p16 and L1CAM positivity were associated with aggressive disease.

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ABSTRACT

Objective. The role of clinicopathological factors and molecular markers in prognostic classification of endometrioid ovarian carcinoma (EnOC) is not established. Tumor grade is used in risk assessment, but the role of current 3-tier grading system has been challenged.

Methods. Clinicopathological factors and 12 immunohistochemical biomarkers (PR, ER, β-catenin, vimentin, ARID1A, HNF1-β, p53, p16, MIB-1, E-cadherin, c-erb-B2 and L1CAM) were analyzed as regards patient outcome in 215 contemporarily classified EnOCs.

Results. Of clinical parameters, grade and stage appeared as strong independent prognostic factors both for disease-free and disease-specific overall survival. Grades 1–3 distinguished clearly from each other in the survival analysis, whereas stages I-II and stages III-IV clustered with each other. PR, ER, nuclear β-catenin and vimentin positivity were associated with favorable overall outcome and clinical parameters, whereas abnormal expression of p53, overexpression of p16 and L1CAM positivity were associated with aggressive disease characteristics and poor survival. The frequency of good-prognosis markers PR and β-catenin gradually decreased and poor-prognosis markers p53, p16 and L1CAM gradually increased from grade 1–3. However, vimentin and ER were expressed at similar frequencies across different grades and presented with independent prognostic significance.

Conclusions. We found histological grade and disease stage, but not residual tumor, to be independent clinical prognostic factors in EnOC. A set of good-prognosis markers (PR, ER, β-catenin and vimentin) and poor-prognosis markers (p53, p16 and L1CAM) were identified. Our findings support continuation of the use of the 3-tier grading system for EnOC and provide clinically feasible IHC biomarkers for prognostic profiling.

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1. Introduction

Ovarian carcinomas are divided by morphology into five main types: high-grade serous, endometrioid, clear cell, mucinous, and low-grade serous carcinomas, which differ in clinical behavior and prognosis [1–3]. Endometrioid type is the second most common ovarian malignancy accounting for 10–15% of ovarian carcinomas. Typically the prognosis of endometrioid ovarian carcinoma (EnOC) is good. However, in a significant proportion this is not the case reflecting heterogeneity of the molecular background of the disease [4,5]. Classification of ovarian cancers into clinically meaningful subgroups is needed to better understand the pathogenesis, estimate prognosis and tailor treatment in the era of targeted therapy.

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EnOC is found in association with endometriosis in about 40% of cases [2]. The most common genetic changes in EnOC are somatic mutations of *ARID1A*, *CTNNB1*, *PIK3CA* and *PTEN* (2,4–7). The same molecular changes have also been found in adjacent endometriosis [2,6,8]. This and the often seen morphological continuum, suggest that endometriosis is a precursor of at least a subset of EnOC. However, the histogenesis of high-grade EnOC is not well established. It may be found in association with well/moderately differentiated areas and harbor molecular alterations typical to endometrioid neoplasias, suggesting evolution from low- to high-grade carcinoma. On the other hand, high grade endometrioid carcinomas can possess characteristics similar to high grade serous ovarian carcinoma (particularly *TP53* mutations), which can make the differential diagnosis challenging.

As EnOC shares morphologic and molecular features with endometrioid endometrial carcinoma, TCGA-based classification of endometrial cancer has been evaluated in EnOC. However, the frequencies of POLE-mutated (3–10% of cases) and MMR (mismatch repair) deficient (8–19%) cases have been lower than in the endometrial counterpart, and the NSMP (non-specific molecular profile) group with variable patient outcome accounts for the majority of the cases [4,9–11]. Individual molecular markers, *e.g.* estrogen and progesterone receptor, p53, p16, L1CAM and β-catenin [9,12–19] have been studied in EnOC but none of them have had an established clinical role. Recently, a whole exome sequencing (WES) study of 112 EnOCs suggested PRISTINE algorithm using mutations of *TP53* and *CTNNB1* as determinants of distinct patient outcome [7].

Disease stage, size of residual tumor in surgery and patient age are acknowledged prognostic factors in epithelial ovarian cancer overall [20–22]. However, these conclusions were reached in studies that included all histological types of ovarian carcinoma and their validity has not been conclusively demonstrated as concerns less common subtypes including EnOC.

Degree of differentiation is not used in histopathological reporting of serous (both high- and low-grade) and clear cell carcinomas, but EnOC is graded according to the 3-tiered (G1-G3) grading system by WHO classification [23]. It is not known whether the grades are different entities by molecular background or represent different evolutionary stages of the same disease. Also the prognostic impact of grade (1 through 3) has remained disputed. In endometrioid endometrial carcinoma, it has been suggested that grades 1 and 2 could be combined as one entity [24]. In EnOC Assem et al. [25] reported similarity between grades 2 and 3, whereas based on early-stage EnOCs Leskelä et al. suggested maintaining the current 3-tier grade classification [26]. Krämer et al. found no survival difference between grades in early-stage EnOC, whereas including all stages the grades 1–3 were prognostically distinct [10].

Our aim was to investigate clinicopathological factors and a larger set of immunohistochemical biomarkers as regards their ability to discern prognostically distinct subclasses of EnOC. The associations between clinical characteristics and biomarkers as well as mutual correlations between biomarkers were analyzed. The biomarker profiles in different tumor grades were analyzed separately to reveal similarities or differences in their molecular background.

2. Materials and methods

2.1. Patients

Initially the study consisted of 249 patients treated for EnOC at the Department of Obstetrics and Gynecology of the Helsinki University Central Hospital between January 1, 1989 and December 31, 2013. This is a tertiary hospital where the treatment of ovarian cancer is centralized. Consecutive patients treated for endometrioid ovarian carcinoma were searched according to pathological records. The study was approved by the Ethics Committee of the Helsinki University Hospital and by the National Supervisory Authority of Welfare and Health. The clinical information of the patients was obtained from the hospital records, and additional survival information was collected from the Population Register Center. This material has been described in detail in our previous work on the role of L1CAM in EnOC [17].

All the cases of the original dataset were reviewed by a gynecological pathologist. All the endometrioid ovarian carcinoma samples were pure samples. Mixed carcinomas were excluded. To reassure exclusion of serous carcinomas, additional immunohistochemistry was performed using tissue microarrays to detect WT1 expression. All WT1-positive cases were re-reviewed by 1 fellow pathologist (J. S.-M.) and 2 gynecological pathologists (A.P., R.B.). The re-evaluation was based on morphological criteria set by WHO Classification 2014. Confirmatory endometrioid features (CEFs – squamous metaplasia, endometriotic/adenofibromatous background, borderline component) [27,28] were searched for especially in the absence of typical low-grade endometrioid component or nuclear features. Expression of other immunohistochemical markers, such as p53, p16, vimentin, ER, PR, ARID1A and beta-catenin were also taken into account in problematic cases. 34 ovarian carcinomas were excluded, majority of which were WT1+/p53abnormal high-grade ovarian carcinomas probably representing endometrioid-like high-grade serous carcinomas. Eventually 215 endometrioid carcinomas remained in the cohort.

The tumors were staged according to the year 2009 FIGO staging system, and graded according to WHO grading system [23]. Response to therapy was evaluated after the initial 6–8 cycles of chemotherapy on the ground of gynecological examination, vaginal ultrasonography, CA125 measurement, and/or computed tomography scan, or second look laparotomy (in the late 1980s and in the beginning of the 1990s). Patients who did not receive chemotherapy were evaluated 5–6 months after the primary surgery. Disease-specific overall survival (DSS) was estimated from the date of diagnosis (primary surgery) to death from ovarian carcinoma. Disease-free survival (DFS) was calculated for patients who had complete response following the primary treatment. Of the 215 patients, 36 had residual disease at completion of primary treatment and 176 had complete response. In three patients the data of the primary response was not available. Sixty-seven patients died from ovarian cancer and 26 patients died from other causes. For four patients the cause of death was unknown and they were recorded as censored in the survival analyses.

2.2. Tissue microarray construction

Histologic slides were reviewed by a gynecological pathologist who marked representative areas of each tumor on 1–2 slides. Four 0.8-mm cores were drawn from the corresponding area of the paraffin blocks and were inserted in the recipient TMA block with a manual tissue microarrayer (Beecher MTA-1, Beecher Instruments) operated by an experienced laboratory technician.

2.3. Immunohistochemistry

The following monoclonal antibodies were used for immunohistochemistry (IHC): ERa (SP1, Roche/Ventana), PR (16, Novocastra), p53 (DO-7, Dako), p16 (E6H4, CINtec Histology), MIB-1 (MIB-1, Dako), Her2 (4B5, Roche/Ventana), E-cadherin (HECD-1, Invitrogen), L1CAM (SIG-3911, Covance, clone 14.10), ARID1A (HPA005456, Sigma-Aldrich), beta-catenin (CAT—5H10, Zymed), HNF1b (CLO374, Atlas Antibodies), vimentin (V9, Dako) and WT1 (6F—H2, Cell Marque).

Scoring was performed by a pathologist blinded to clinical data (A.P., J.S.-M.). A second investigator (R.B.) examined problematic cases and a consensus was reached.

Stainings were scored only on carcinoma cells; stromal cells and inflammatory cells served as internal control, when applicable. Samples with scarce carcinoma cells or completely negative staining of the internal control were discarded.

ER and PR: The cut-off for ER/PR positivity *i.e.*, >10% of the tumor nuclei staining with any intensity, was adopted based on breast cancer studies and studies on endometrial carcinoma [29,30].

p53: Aberrant p53 staining (p53 abn) was defined as strong and diffuse nuclear staining, completely negative ("null") staining or cytoplasmic staining in carcinoma cells. Weak and heterogeneous staining was classified as wild type expression [31].

p16: positive result was defined as strong and diffuse (>50% of cells) nuclear and cytoplasmic staining.

MIB-1: the proportion of carcinoma cells showing nuclear staining of any intensity was scored as for breast cancer: negative (<5%), low (5–14%), moderate (15–29%) and strong (≥30%). In dichotomous comparisons, strong positivity was compared to samples with MIB-1 < 30%.

c-erb-B2: membranous staining was classified positive or negative.

Positive: tumor displays complete, intense circumferential membranous staining in >10% of tumor cells (IHC 3+, strong positive) or weak to moderate complete membrane staining observed in >10% of invasive tumor cells (IHC 2+).

Negative: incomplete faint membrane staining and within >10% of invasive tumor cells (IHC 1+) or no staining observed or incomplete faint/barely perceptible membrane staining within ≤10% of invasive tumor cells (IHC 0).

E-cadherin: loss of E-cadherin was defined as diffuse or clonal lack of membranous staining; weakened or positive staining were classified normal.

L1CAM: expression was scored as described before [17], with ≥10% of membranous staining considered positive.

ARID1A: staining was classified negative when tumoral cells presented diffuse or clonal type loss of nuclear expression. As indicated by a previous mutational study, heterogeneous "checkerboard" pattern of staining and diffuse nuclear staining were considered positive [32].

β-catenin: membranous staining was considered normal; abnormal staining was defined as focal or diffuse nuclear positivity.

HNF1-β: any quantity of nuclear staining of at least moderate intensity was scored as positive.

Vimentin: any quantity of cytoplasmic staining was considered positive.

WT1: any unequivocal nuclear staining >5% of tumor cell nuclei was considered positive.

2.4. Statistical analysis

Categorical variables were analyzed with Pearson $\chi 2$ test and Fisher's exact test. The disease-specific overall survival and disease-free survival was evaluated using the Kaplan-Meier method and the log rank test was used to compare differences between groups. For multivariate survival analysis Cox proportional hazards model was used with the following covariates significant in univariate analysis: FIGO stage (I-II *vs.* III-IV), grade (1 *vs.* 2 *vs.* 3), residual tumor (presence *vs.* absence), preoperative serum CA125 level (normal <35 kU/l *vs.* abnormal ≥35 kU/l), age (<59 years *vs.* ≥59 years (median)), PR expression (≥10% *vs.* <10%), ER expression (≥10% *vs.* <10%), β-catenin expression (nuclear *vs.* other), vimentin expression (positive *vs.* negative), p53 expression (abnormal *vs.* normal), p16 expression (overexpression *vs.* other) and L1CAM expression (positive *vs.* negative). Statistical significance was set at $p < 0.05$. Data was analyzed using IBM SPSS version 25 software.

3. Results

3.1. The association of clinicopathological factors with survival

The distribution of different clinicopathological variables is depicted in Table 1. Higher disease stage, higher grade, residual tumor at primary surgery, higher patient age and higher preoperative CA125 value were associated with shorter disease-specific overall survival (DSS) (Table 1, Fig. 1). For disease-free survival (DFS) significant association with shorter survival was found for higher disease stage, higher grade and residual tumor at primary surgery (Table 1).

Interestingly, stages I-II and stages III-IV clustered with each other (Fig. 1A). There was no survival difference between stage I and stage II ($p = 0.73$), or between stage III-IV ($p = 0.33$). However, grades 1–3 distinguished clearly from each other in the survival analysis (Fig. 1B): grade 1 *vs.* grade 2 ($p = 0.005$) and grade 2 *vs.* grade 3 ($p < 0.001$).

When only low-stage tumors (stage I-II) were analyzed, tumor grade was still a significant prognostic factor both for DSS ($p < 0.0001$) and DFS ($p < 0.0001$) (S1). The poorest outcome was for grade 3 cases; whereas, there was no significant difference between grade 1 and grade 2 cases ($p = 0.43$ for DSS and $p = 0.69$ for DFS).

In multivariate analysis for DSS, independent prognostic factors were stage and grade. In multivariate survival analysis for DFS, the same factors, stage and grade, presented with independent prognostic value (S2).

3.2. Expression of the markers and their association with survival

The frequencies of expression of the specific markers are depicted in Table 2. PR and ER positivity, nuclear β-catenin and vimentin positivity were associated with favorable outcome; whereas, abnormal expression of p53, overexpression of p16 and L1CAM positivity were associated with poor DSS (Table 2; Figs. 2 and 3). The same markers, except ER and vimentin, were also associated with DFS (Table 2). Examples of IHC stainings in a case with favorable outcome are seen in S3 and in a case with poor outcome in S4.

When biomarkers were included in multivariate analysis for DSS, vimentin and estrogen receptor expression were independent prognostic factors in addition to stage and grade. In multivariate survival analysis for DFS, only clinical factors, stage and grade, remained as independent prognostic factors (S5).

Due to rarity of EnOC the time period of the tumor samples was relatively long. To address the possibility of a sample age-related distortion of the findings, we repeated the DSS survival analyses of the good- and poor- prognosis markers by stratification according to sample age into older and newer cohort by the median of the operation date (January 10, 2006). The associations with patient outcome were similar and statistically significant for PR ($p = 0.001$, $p = 0.005$), ER ($p = 0.002$, $p = 0.002$), β-catenin ($p = 0.02$, $p = 0.008$), p16 ($p = 0.03$, $p = 0.02$) and L1CAM ($p = 0.02$, $p < 0.0001$) both for the older and the newer cohort (respectively) as well as for the newer cohort of vimentin ($p = 0.003$) and older cohort of p53 ($p < 0.0001$). For the newer cohort of p53 there was a similar tendency for the abnormal p53 group to have worse survival, but the *p*-value was not significant ($p = 0.23$) due to smaller sample size, shorter follow-up period and better prognosis of the disease in both p53 abnormal and normal groups in the later period. For vimentin, there was no association with survival in the older cohort possibly due to degradation of tissue antigens as the proportion of cases with positive expression of vimentin was lower in the older cohort (53%) as compared with the newer cohort (71%). However, the association of vimentin with better DSS was even stronger in the newer cohort ($p = 0.003$) than in the whole cohort ($p = 0.02$), which confirms the finding of vimentin being a good-prognosis marker.

3.3. Association with response after primary therapy

Of clinical factors, lower stage and presence of no residual tumor were associated with complete response after primary treatment (surgery and adjuvant chemotherapy) ($p < 0.0001$ and $p < 0.0001$, respectively).

Of biomarkers, PR and ER positivity, as well as nuclear β-catenin were associated with complete response after primary treatment ($p < 0.0001$, $p < 0.0001$ and $p = 0.008$, respectively). Weaker association was found for vimentin positivity, wild type expression of p53 and L1CAM negativity ($p = 0.01$, $p = 0.02$ and $p = 0.01$, respectively).

Table 1
Clinicopathological characteristics and their association with disease-specific overall survival (DSS) and disease-free survival (DFS) in patients with endometrioid ovarian carcinoma. HR, hazard ratio.

Clinicopathological factor	Number of cases	Association with DSS (p-value)	DSS HR (95%CI)	Association with DFS (p-value)	DFS HR (95%CI)
Age at diagnosis					
<59 years (median)	112 (52%)	**0.016**	**1.81**	NS	1.71
≥59 years (median)	103 (48%)		(1.11–2.97)		(0.93–3.15)
Histological grade					
1	114 (53%)	**<0.0001**	**2.53**	**<0.0001**	**2.68**
2	72 (34%)		(1.84–3.48)		(1.80–3.99)
3	29 (13%)				
Disease stage					
I-II	153 (72%)	**<0.0001**	**12.26**	**<0.0001**	**7.59**
III-IV	60 (28%)		(7.11–21.17)		(4.08–14.11)
Tumor size					
<5 cm	26 (12%)	NS	1.16	NS	0.90
5–10 cm	66 (31%)		(0.81–1.65)		(0.59–1.35)
>10 cm	121 (57%)				
Ascites					
No	81 (38%)	NS	0.70	NS	0.88
Yes	130 (62%)		(0.42–1.19)		(0.47–1.63)
Residual tumor					
No	170 (81%)	**<0.0001**	**6.36**	**<0.0001**	**3.91**
Yes	40 (19%)		(3.83–10.55)		(1.86–8.20)
CA125					
Normal	43 (21%)	**0.018**	**2.51**	NS	2.46
Elevated	166 (79%)		(1.14–5.49)		(0.97–6.27)

Statistically significant p-values (<0.05) and HRs are bolded.

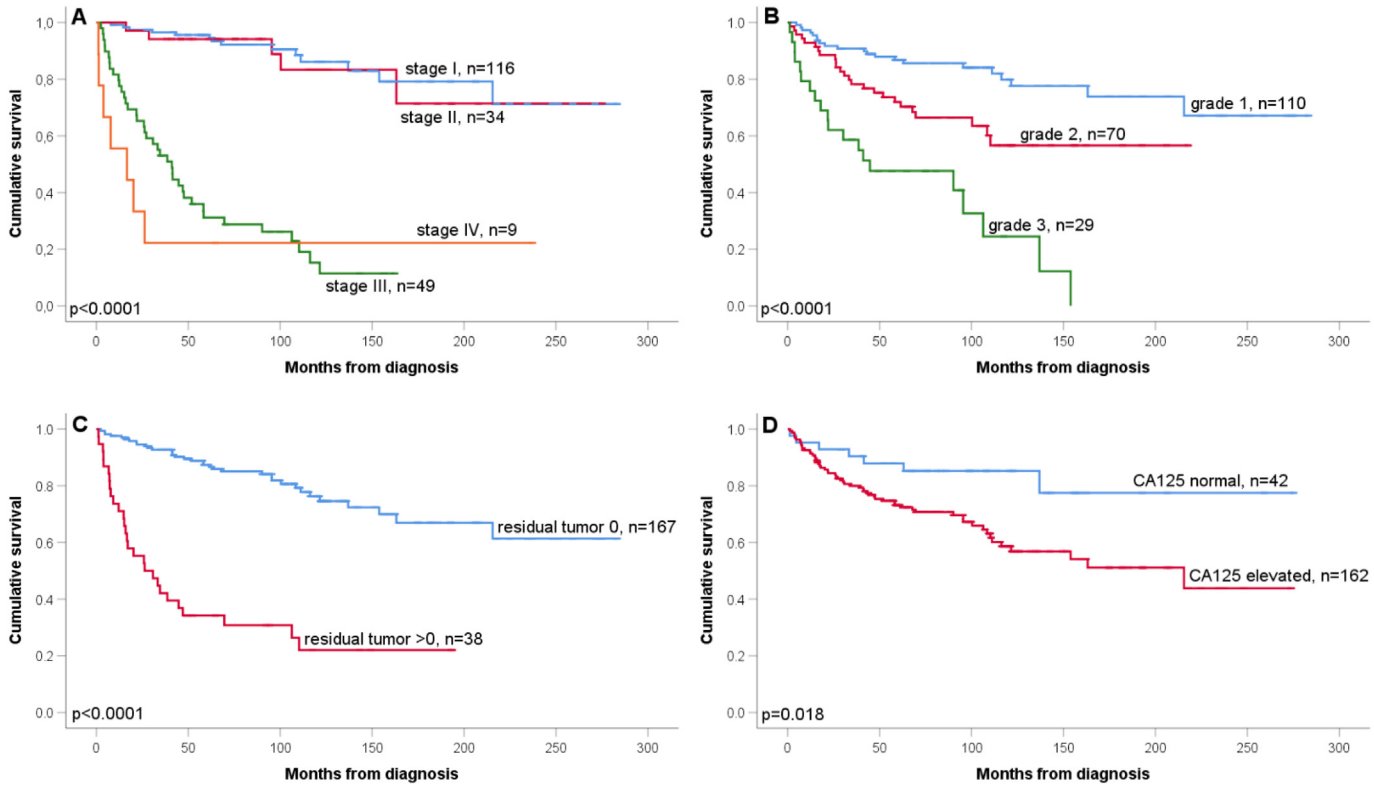

Fig. 1. Disease-specific overall survival in endometrioid ovarian carcinoma patients according to A) stage, B) grade, C) residual tumor and D) CA125 status.

3.4. Association of markers with clinicopathological factors

Positive expression of PR was strongly associated with lower histological grade and disease stage, absence of residual tumor at surgery and lower age (S6). Positive expression of ER was associated with absence of residual tumor at surgery, lower disease stage and absence of ascites.

Nuclear expression of β-catenin was associated with favorable clinical factors: lower histological grade and disease stage, as well as lower age and normal serum level of CA125. Positive expression of HNF1-β

Table 2
Expression of the biomarkers and their association with disease-specific overall survival (DSS) and disease-free survival (DFS) in patients with endometrioid ovarian carcinoma. HR, hazard ratio; NS, not significant.

Marker	No of cases	Association with DSS (p-value)	DSS HR (95%CI)	Association with DFS (*p*-value)	DFS HR (95%CI)
PR positivity	138/202 (68%)	**<0.0001**	**0.33** (0.20–0.56)	**0.02**	**0.48** (0.25–0.91)
ER positivity	174/205 (85%)	**<0.0001**	**0.28** (0.16–0.49)	NS	0.47 (0.20–1.12)
nuclear β-catenin	56/205 (27%)	**<0.0001**	**0.25** (0.11–0.57)	**0.04**	**0.44** (0.19–0.99)
vimentin positivity	129/207 (62%)	**0.02**	**0.56** (0.34–0.91)	NS	0.69 (0.37–1.29)
ARID1A loss	34/205 (17%)	NS	1.01 (0.50–2.05)	NS	0.45 (0.14–1.47)
HNF1-β positivity	86/207 (42%)	NS	0.96 (0.58–1.58)	NS	0.92 (0.49–1.73)
E-cadherin loss	16/205 (8%)	NS	1.59 (0.58–4.40)	NS	1.08 0.38–3.04)
c-erb-B2 positivity	7/201 (4%)	NS	1.63 (0.51–5.21)	NS	1.73 (0.41–7.21)
MIB-1 ≥ 30%	75/201 (37%)	NS	1.11 (0.86–1.44)	NS	1.06 (0.77–1.47)
abnormal p53	65/207 (31%)	**<0.0001**	**2.36** (1.44–3.86)	**0.007**	**2.30** (1.24–4.26)
p16 overexpression	48/205 (23%)	**0.002**	**2.20** (1.32–3.68)	**<0.0001**	**3.34** (1.79–6.23)
L1CAM positivity	25/201 (12%)	**<0.0001**	**3.05** (1.69–5.50)	**0.01**	**2.70** (1.18–6.16)

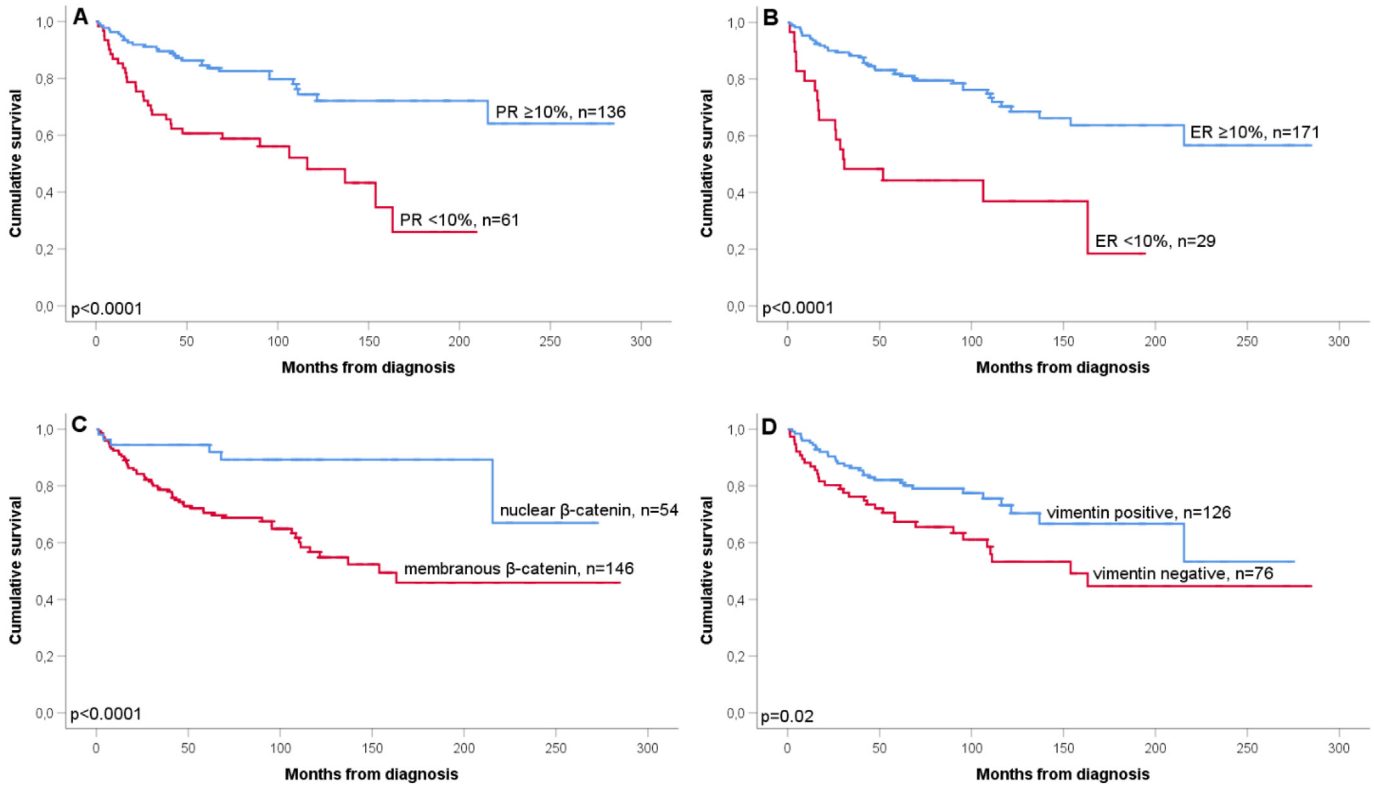

Fig. 2. Disease-specific overall survival in endometrioid ovarian carcinoma patients according to A) PR, B) ER, C) β-catenin and D) vimentin status.

was also associated with lower grade and stage. However, vimentin positivity and ARID1A loss were only associated with lower age.

Associations with aggressive disease characteristics were found for abnormal p53 as well as overexpression of p16 and L1CAM positivity. All of those were associated with higher histological grade and disease stage, presence of residual tumor at surgery and higher age. Abnormal p53 was also associated with the presence of ascites.

C-erb-B2 positivity was only associated with higher patient age. MIB-1 and E-cadherin were not associated with any of the clinical parameters.

3.5. Associations between different markers

Markers associated with good prognosis, positive PR and ER, β-catenin and vimentin, were positively correlated with each other. On the other hand, markers associated with aggressive disease characteristics (abnormal p53, overexpression of p16 and L1CAM) positively correlated with each other and negatively correlated with PR, ER, β-catenin, ARID1A and vimentin (S7). Abnormal p53, overexpression of p16 and L1CAM positivity were associated with higher proliferation index, whereas β-catenin correlated with lower proliferation index. HNF1-β was negatively correlated with ER, vimentin and E-cadherin.

3.6. Histological grade – biomarker profiles and patient outcome

Half of the cases (53%) were grade 1, one third (33%) grade 2 and 14% of grade 3. The frequency of expression of good-prognosis markers PR and β-catenin as well as HNF1-β decreased from grade 1 through 3, and the frequency of poor-prognosis markers p53, p16 and L1CAM increased from grade 1 through 3 (Table 3). There was no significant change in the frequency of expression of ER, vimentin, ARID1A, E-cadherin, c-erb-B2 and MIB-1. However, the expression of poor-

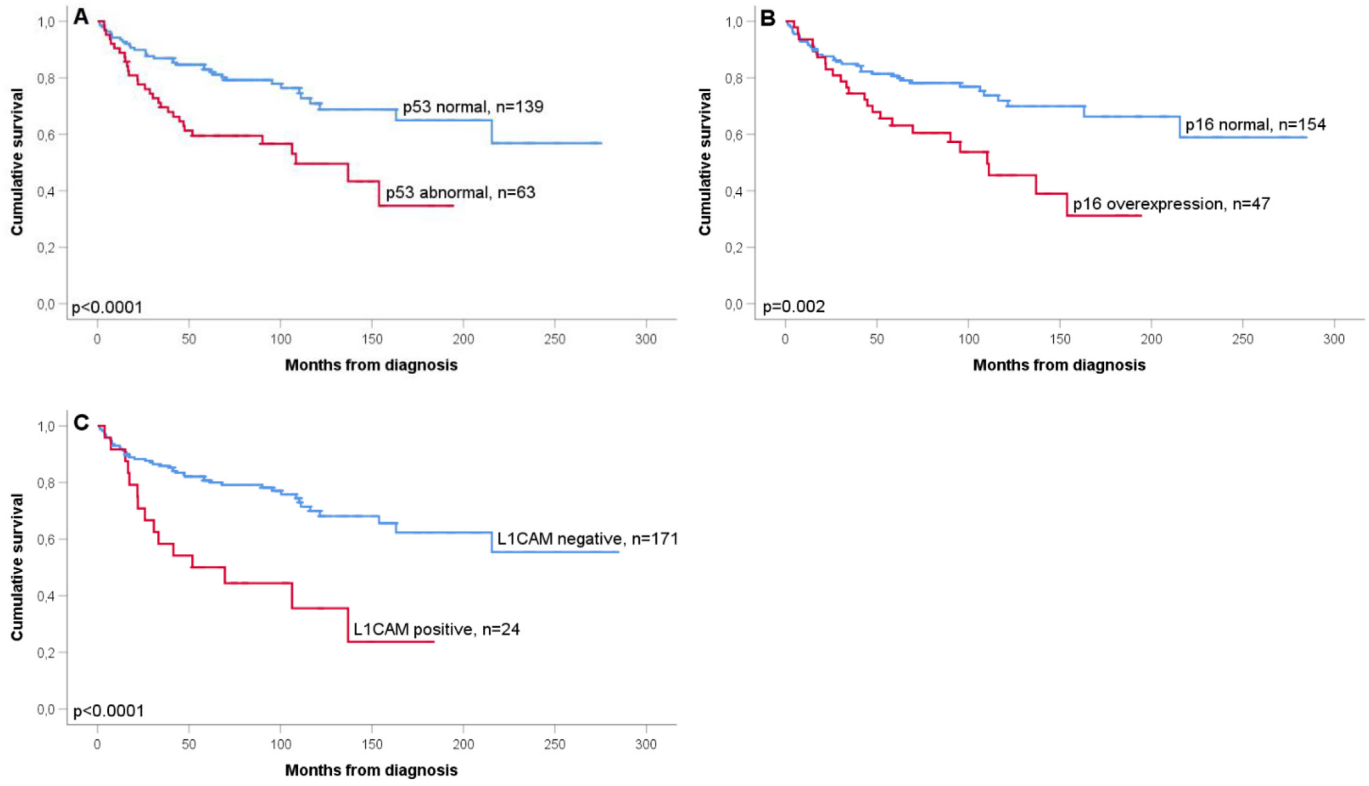

Fig. 3. Disease-specific overall survival in endometrioid ovarian carcinoma patients according to A) p53, B) p16, and C) L1CAM status.

prognosis markers was not limited to grade 3 as for example 10% of grade 1 tumors expressed p53 or p16. Also, good-prognosis markers PR, ER, β-catenin and vimentin were found in a significant proportions of grade 3 carcinomas (36%, 82%, 15% and 64%, respectively).

Higher histological grade was associated with worse DSS (Fig. 1B; S8A) as well as worse DFS (S8B) ($p < 0.0001$ for both). As some authors have suggested the use of WT1+/p53abn as more definitive criteria of high grade serous histology, we performed a confirmatory analysis on the role of grade as prognostic factor by excluding all WT1+/p53abn cases from the analysis (one G1 tumor, eleven G2 tumors and ten G3 tumors). Still the findings remained similar: grade was strongly associated with DSS (S8C) as well as DFS (S8D) ($p < 0.0001$ for both).

4. Discussion

In the present study we evaluated clinicopathological factors and a set of 12 immunohistochemically determined polypeptide markers in 215 contemporarily classified EnOCs treated at one institution. Grade and stage appeared as strong independent prognostic factors both for DFS as well as DSS. Grades from 1, 2 and 3 were clearly separated from one another as regards outcome, whereas stages I-II and stages III-IV clustered together and clearly separated from each other. Residual tumor had prognostic significance only in univariate, but not in multivariate analysis. These findings are to some extent different from ovarian cancer overall (which depicts mostly high grade serous carcinoma), where the size of residual tumor at primary surgery has an independent prognostic role in addition to stage [20,21]. In a recent multicenter study of 511 endometrioid ovarian carcinomas [10] residual tumor was found to be of independent prognostic significance; however, similar to our results stage was the strongest prognosticator. Also according to their findings grade was an independent factor for survival and the grades 1 to 3 separated from each other in the whole cohort. However, in low-stage disease they did not find any prognostic significance for grade [10], whereas in our cohort grade was a significant prognostic factor in low-stage disease as well, due to poor prognosis in grade 3 disease.

We identified a set of markers that identify patients with good outcome and another set of markers, which clustered with poor prognosis in EnOC. The set of markers with good outcome included positivity of hormone receptors PR and ER, nuclear expression of β-catenin and vimentin positivity. In contrast, a set of markers - aberrant p53, expression of p16 and L1CAM - were associated with poor outcome. The associations of these two classes of markers with outcome were detected in response to primary therapy as well as in DFS and DSS. These findings are in line with previous studies on individual markers showing prognostic significance in EnOC: ER, PR and nuclear β-catenin expression have been associated with better survival [12–14,19,33], and p53, p16 and L1CAM with shorter survival [9,10,15–17]. To our knowledge, this is the first study to report prognostic significance of vimentin, and independent prognostic value for ER and vimentin in EnOC. The recent WES based study of 112 EnOCs suggested a prognostic algorithm where the poorest outcome is found in *TP53* mutated cases and the best outcome in *CTNNB1* mutated cases [7]. Our results correspond well with these findings. Interestingly, the good prognosis of *CTNNB1* mutated cases is in contrast to findings in endometrioid endometrial cancer, where β-catenin activation has been associated with aggressive subset of low-grade and low-stage disease [34,35].

We found that the markers of good prognosis (PR, ER, β-catenin) were associated overall with positive clinicopathological characteristics such as younger age, lower histological grade and surgical stage as well as smaller residual tumor size in primary surgery. Interestingly, vimentin was correlated only with younger age, but not with other indicators of indolent disease. On the other hand, the markers of poor prognosis (p53, p16 and L1CAM) were associated with characteristics of aggressive disease such as higher grade and stage, larger residual tumor volume as well as higher age. The strongest predictors of residual tumor at primary surgery were negative expression of ER and aberrant expression of p53. In addition, in the present study we were able to

Table 3
Expression of the biomarkers in grade 1, grade 2 and grade 3 endometrioid ovarian carcinomas.

Marker	Grade 1	Grade 2	Grade 3	
	Expression (%)	Expression (%)	Expression (%)	Association of grade with different markers (p-value)
PR positivity	83/108 (77%)	45/66 (68%)	10/28 (36%)	**<0.0001**
ER positivity	96/107 (90%)	55/70 (79%)	23/28 (82%)	0.12
nuclear β-catenin	39/109 (36%)	13/69 (19%)	4/27 (15%)	**0.01**
vimentin positivity	69/109 (63%)	42/70 (60%)	18/28 (64%)	0.88
ARID1A loss	21/108 (19%)	11/69 (16%)	2/28 (7%)	0.29
HNF1-β positivity	60/109 (55%)	24/70 (34%)	2/28 (7%)	**<0.0001**
E-cadherin loss	9/108 (8%)	5/69 (7%)	2/28 (7%)	0.96
c-erb-B2 positivity	2/104 (2%)	4/70 (6%)	1/27 (4%)	0.41
MIB-1 ≥ 30%	33/106 (31%)	30/69 (44%)	12/26 (46%)	0.16
abnormal p53	11/108 (10%)	31/71 (44%)	23/28 (82%)	**<0.0001**
p16 overexpression	11/109 (10%)	21/68 (31%)	16/28 (57%)	**<0.0001**
L1CAM positivity	3/104 (3%)	13/70 (19%)	9/27 (33%)	**<0.0001**

analyze the reciprocal associations of the markers. Two sets of markers clustered together: the markers of good prognosis (PR, ER, β-catenin, vimentin) correlated positively with each other and negatively with markers of poor prognosis (p53, p16 and L1CAM), and *vice versa*.

Other markers included in our study (ARID1A, HNF1-β, MIB-1, E-cadherin and c-erb-B2) did not cluster into the two described sets of prognostic markers. Negative staining of ARID1A protein, which suggests *ARID1A* loss regarded as an early change in the pathogenesis of EnOC [6], was detected in 17% of the endometrioid carcinomas, which is comparable to previous publications [15,26]. Expression of ARID1A correlated with younger age at diagnosis, positive expression of PR, wild type 53 and normal p16 expression, but not with other clinical factors or disease outcome.

C-erb-B2 is known to play a role in a subset of mucinous ovarian carcinoma [2]. In our series of endometrioid carcinomas, the prevalence of c-erbB2 hyperexpression was low and significant clinical or prognostic correlations were not seen. Proliferation index MIB-1 was positively correlated with the markers of poor outcome (p53, p16, L1CAM) and negatively correlated with β-catenin. However, interestingly, MIB-1 itself did not have any prognostic significance nor clinical associations.

Ovarian endometrioid carcinoma is typically considered to be an early-stage low-grade disease with positive hormone receptors and mutations of *ARID1A*, *CTNNB1*, *PIK3CA, PTEN* and *KRAS*, whereas in higher grades the proportion of cases with aberrant p53 increases [2,4,5,7,10,13,14,25]. Our findings of clinical outcome and marker profiles were not dichotomous. There was a gradual decrease in the frequency of good-prognosis markers PR and β-catenin as well as HNF1-β from grade 1 through 3, and gradual increase in the frequency of poor-prognosis markers p53, p16 and L1CAM. Expression of good-prognosis markers was also seen in grade 3 tumors and expression of poor-prognosis markers in grade 1 tumors. Furthermore, the level of expression of ER and vimentin was similar irrespective of the grade. These findings give support to the currently used 3-tier grading system for EnOC.

The morphology based differential diagnostics of high-grade serous and endometrioid carcinomas is challenging. WT1-protein is considered as marker for serous differentiation and *TP53* mutation is known to be a fundamental early step in the development of high-grade serous carcinoma. Hence, WT1-positivity-p53-abnormality has been suggested to indicate high-grade serous nature of the tumor [36]. However, some WT1+/p53abn tumors fit better into endometrioid category by ancillary tests such as mutation/aberrant expression ARID1A, β-catenin or PIK3CA or morphological confirmatory endometrioid features [25,27,37]. Based on these properties we had included 22 cases presenting with WT1+/p53abn IHC pattern in our cohort. A confirmatory survival analysis was performed by excluding these 22 cases with WT1+/p53abn IHC pattern: grade was still a strong prognostic factor for DFS and DSS, and the grades clearly separated from each other.

Altogether 31% of the cases in our cohort presented with aberrant p53 expression, which is similar to some of the previous studies (24%–29%; [7,9,15]), but higher than in some studies, (10–13%; [10,25,26]). The difference is not fully explained by the use of WT1+/p53abn IHC pattern as definitive exclusion criteria [10,25,26], because higher frequency of *TP53* mutations (26%) was also seen in the WES study using this criteria [7]. Inactivation of p53 is more frequently seen in high-grade form of EnOC, but still 10% of grade 1 tumors in our cohort presented with aberrant p53 expression. The role of p53 inactivation in EnOC is controversial: does TP53 mutation represent a late event in tumor progression or is it an early event, like in high-grade serous carcinoma, taking place in a cell-type committed to endometrioid differentiation. In the study of 166 early-stage endometrioid ovarian carcinomas 32% of the p53 abnormal cases presented with nuclear β-catenin positivity suggesting that *CTNNB1* mutation could have been an initial event in these tumors [11]. In the WES study of 112 EnOCs *TP53* and *CTNNB1* mutations were nearly mutually exclusive, but other mutations typical of EnOC (*PTEN*, *ARID1A*, *KRAS*, *PIK3CA*) were found in the *TP53* mutated cases [7].

TP53 mutation, early or late, may ultimately lead to genomic instability and a phenotype that is difficult to discern from high-grade serous carcinoma. Tumors with abnormal p53 may also carry homologous recombination deficiency (HRD), which renders them susceptible to PARP inhibitor therapy [38]. The prevalence of HRD in contemporarily classified EnOC is not well established. A WES study of 26 endometrioid ovarian carcinomas identified HRD-mutational signature in 19% of cases as compared to 59% in high grade serous carcinomas. The HRD-positivity seemed more common in high grade tumors, but was found in low grade endometrioid carcinoma cases as well [5]. Similarly, we found p53 abnormality not only in grade 3 but also in grade 1 and 2 tumors.

In conclusion, tumor grade and disease stage were identified as independent clinical prognostic factors associated with patient outcome in this large cohort of contemporarily classified EnOCs. In addition to these classical prognostic factors, biomarker clusters were found: a set of good-prognosis markers (PR, ER, β-catenin and vimentin) and poor-prognosis markers (p53, p16 and L1CAM). The frequency of good-prognosis markers PR and β-catenin gradually decreased and poor-prognosis markers p53, p16 and L1CAM gradually increased from grade 1–3. The level of expression of vimentin and ER was similar across different grades, and vimentin and ER proved to be independent prognostic factors for disease-specific survival. Grades from 1 to 3 separated clearly from each other in the survival analysis. Our findings support the current practice of 3–tier grading system for EnOC, and provide clinically feasible IHC biomarkers for prognostic profiling.

Conflict of interest statement

All authors declare no conflict of interest related to this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2021.10.078.

References

[1] WHO, Classification of Female Genital Tumours, 5th ed. International Agency for Research on Cancer, 2020.
[2] J. Prat, E. D'Angelo, I. Espinosa, Ovarian carcinomas: at least five different diseases with distinct histological features and molecular genetics, Hum. Pathol. 80 (2018) 11–27.
[3] R.J. Kurman, I. Shih, The dualistic model of ovarian carcinogenesis: revisited, revised, and expanded, Am. J. Pathol. 186 (4) (2016) 733–747.
[4] P. Cybulska, A.D.C. Paula, J. Tseng, M.M. Leitao Jr., A. Bashashati, D.G. Huntsman, et al., Molecular profiling and molecular classification of endometrioid ovarian carcinomas, Gynecol. Oncol. 154 (3) (2019) 516–523.
[5] W.E. Pierson, P.N. Peters, M.T. Chang, L.M. Chen, D.A. Quigley, A. Ashworth, et al., An integrated molecular profile of endometrioid ovarian cancer, Gynecol. Oncol. 157 (1) (2020) 55–61.
[6] K.C. Wiegand, S.P. Shah, O.M. Al-Agha, Y. Zhao, K. Tse, T. Zeng, et al., ARID1A mutations in endometriosis-associated ovarian carcinomas, N. Engl. J. Med. 363 (16) (2010) 1532–1543.
[7] R.L. Hollis, J.P. Thomson, B. Stanley, M. Churchman, A.M. Meynert, T. Rye, et al., Molecular stratification of endometrioid ovarian carcinoma predicts clinical outcome, Nat. Commun. 11 (1) (2020) 4995 (-020-18819-5).
[8] T. Matsumoto, M. Yamazaki, H. Takahashi, S. Kajita, E. Suzuki, T. Tsuruta, et al., Distinct β-catenin and PIK3CA mutation profiles in endometriosis-associated ovarian endometrioid and clear cell carcinomas, Am. J. Clin. Pathol. 144 (3) (2015) 452–463.
[9] C. Parra-Herran, J. Lerner-Ellis, B. Xu, S. Khalouei, D. Bassiouny, M. Cesari, et al., Molecular-based classification algorithm for endometrial carcinoma categorizes ovarian endometrioid carcinoma into prognostically significant groups, Mod. Pathol. 30 (12) (2017) 1748–1759.
[10] P. Krämer, A. Talhouk, M.A. Brett, D.S. Chiu, E.S. Cairns, D.A. Scheunhage, et al., Endometrial cancer molecular risk stratification is equally prognostic for endometrioid ovarian carcinoma, Clin. Cancer Res. 26 (20) (2020) 5400–5410.
[11] S. Leskela, I. Romero, J.M. Rosa-Rosa, T. Caniego-Casas, E. Cristobal, B. Pérez-Mies, et al., Molecular heterogeneity of endometrioid ovarian carcinoma: an analysis of 166 cases using the endometrial cancer subrogate molecular classification, Am. J. Surg. Pathol. 44 (7) (2020) 982–990.
[12] R.L. Hollis, B. Stanley, Y. Iida, J. Thomson, M. Churchman, T. Rye, et al., Hormone receptor expression patterns define clinically meaningful subgroups of endometrioid ovarian carcinoma, Gynecol. Oncol. 155 (2) (2019) 318–323.
[13] W. Sieh, M. Köbel, T.A. Longacre, D.D. Bowtell, A. de Fazio, M.T. Goodman, et al., Hormone-receptor expression and ovarian cancer survival: an ovarian tumor tissue analysis consortium study, Lancet Oncol. 14 (9) (2013) 853–862.
[14] P. Rambau, L.E. Kelemen, H. Steed, M.L. Quan, P. Ghatage, M. Köbel, Association of hormone receptor expression with survival in ovarian endometrioid carcinoma: biological validation and clinical implications, Int. J. Mol. Sci. 18 (3) (2017) 515.
[15] M. Heckl, E. Schmoeckel, L. Hertlein, M. Rottmann, U. Jeschke, D. Mayr, The ARID1A, p53 and ß-catenin statuses are strong prognosticators in clear cell and endometrioid carcinoma of the ovary and the endometrium, PLoS One 13 (2) (2018), e0192881.
[16] P.F. Rambau, R.A. Vierkant, M.P. Intermaggio, L.E. Kelemen, M.T. Goodman, E. Herpel, et al., Association of p16 expression with prognosis varies across ovarian carcinoma histotypes: an ovarian tumor tissue analysis consortium study, J. Pathol. Clin. Res. 4 (4) (2018) 250–261.
[17] P. Soovares, A. Pasanen, R. Bützow, H. Lassus, L1CAM expression associates with poor outcome in endometrioid, but not in clear cell ovarian carcinoma, Gynecol. Oncol. 146 (3) (2017) 615–622.
[18] D.G. Rosen, Z. Zhang, B. Chang, X. Wang, E. Lin, J. Liu, Low membranous expression of beta-catenin and high mitotic count predict poor prognosis in endometrioid carcinoma of the ovary, Mod. Pathol. 23 (1) (2010) 113–122.
[19] L. Wang, P.F. Rambau, L.E. Kelemen, M.S. Anglesio, S. Leung, A. Talhouk, et al., Nuclear β-catenin and CDX2 expression in ovarian endometrioid carcinoma identify patients with favourable outcome, Histopathology 74 (3) (2019) 452–462.
[20] S. Tingulstad, F.E. Skjeldestad, T.B. Halvorsen, B. Hagen, Survival and prognostic factors in patients with ovarian cancer, Obstet. Gynecol. 101 (5 Pt 1) (2003) 885–891.
[21] A. du Bois, A. Reuss, E. Pujade-Lauraine, P. Harter, I. Ray-Coquard, J. Pfisterer, Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux pour les etudes des cancers de l'Ovaire (GINECO), Cancer 115 (6) (2009) 1234–1244.
[22] L.C. Chang, C.F. Huang, M.S. Lai, L.J. Shen, F.L. Wu, W.F. Cheng, Prognostic factors in epithelial ovarian cancer: a population-based study, PLoS One 13 (3) (2018), e0194993.
[23] R.J. Kurman, M.L. Carcangiu, R.H. Young, C.S. Herrington, WHO Classification of Tumours of Female Reproductive Organs, International Agency for Research on Cancer, 2014.
[24] R.A. Soslow, C. Tornos, K.J. Park, A. Malpica, X. Matias-Guiu, E. Oliva, 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. 38 (Suppl. 1) (2019) S64–S74 (Iss 1 Suppl 1).
[25] H. Assem, P.F. Rambau, S. Lee, T. Ogilvie, A. Sienko, L.E. Kelemen, et al., High-grade endometrioid carcinoma of the ovary: a clinicopathologic study of 30 cases, Am. J. Surg. Pathol. 42 (4) (2018) 534–544.
[26] S. Leskela, I. Romero, E. Cristobal, B. Pérez-Mies, J.M. Rosa-Rosa, A. Gutierrez-Pecharroman, et al., The frequency and prognostic significance of the histologic type in early-stage ovarian carcinoma: a reclassification study by the Spanish Group for Ovarian Cancer Research (GEICO), Am. J. Surg. Pathol. 44 (2) (2020) 149–161.
[27] D. Lim, R. Murali, M.P. Murray, E. Veras, K.J. Park, R.A. Soslow, Morphological and immunohistochemical reevaluation of tumors initially diagnosed as ovarian Endometrioid carcinoma with emphasis on high-grade tumors, Am. J. Surg. Pathol. 40 (3) (2016) 302–312.
[28] W.G. McCluggage, Endometriosis-related pathology: a discussion of selected uncommon benign, premalignant and malignant lesions, Histopathology 76 (1) (2020) 76–92.
[29] V. Jongen, J. Briët, R. de Jong, K. ten Hoor, M. Boezen, A. van der Zee, 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. 112 (3) (2009) 537–542.
[30] 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.
[31] H. Lassus, A. Leminen, J. Lundin, P. Lehtovirta, R. Butzow, Distinct subtypes of serous ovarian carcinoma identified by p53 determination, Gynecol. Oncol. 91 (3) (2003) 504–512.
[32] B. Guan, T.L. Mao, P.K. Panuganti, E. Kuhn, R.J. Kurman, D. Maeda, et al., Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma, Am. J. Surg. Pathol. 35 (5) (2011) 625–632.
[33] R.E. Zyla, E. Olkhov-Mitsel, Y. Amemiya, D. Bassiouny, A. Seth, B. Djordjevic, et al., CTNNB1 mutations and aberrant β-catenin expression in ovarian endometrioid carcinoma: correlation with patient outcome, Am. J. Surg. Pathol. 45 (1) (2021) 68–76.
[34] Y. Liu, L. Patel, G.B. Mills, K.H. Lu, A.K. Sood, L. Ding, et al., Clinical significance of CTNNB1 mutation and Wnt pathway activation in endometrioid endometrial carcinoma, J. Natl. Cancer Inst. 106 (9) (2014) dju245.
[35] K.C. Kurnit, G.N. Kim, B.M. Fellman, D.L. Urbauer, G.B. Mills, W. Zhang, et al., CTNNB1 (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence, Mod. Pathol. 30 (7) (2017) 1032–1041.
[36] M. Köbel, K. Rahimi, P.F. Rambau, C. Naugler, C. Le Page, L. Meunier, et al., An immunohistochemical algorithm for ovarian carcinoma typing, Int. J. Gynecol. Pathol. 35 (5) (2016) 430–441.
[37] M. Köbel, B.M. Ronnett, N. Singh, R.A. Soslow, C.B. Gilks, W.G. McCluggage, Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility, Int. J. Gynecol. Pathol. 38 Suppl 1 (Iss 1 Suppl 1) (2019) S123–S131.
[38] B. Pothuri, R. O'Cearbhaill, R. Eskander, D. Armstrong, Frontline PARP inhibitor maintenance therapy in ovarian cancer: a society of gynecologic oncology practice statement, Gynecol. Oncol. 159 (1) (2020) 8–12.