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Original contribution

Prognostic significance of Ki-67 levels and hormone receptor expression in low-grade serous ovarian carcinoma: an investigation of the Tumor Bank Ovarian Cancer Network☆

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Summary Low-grade serous ovarian carcinoma (LGSOC) has recently come up as a distinct rare entity of epithelial ovarian cancer. Predictive and prognostic markers are not well studied yet. Because Ki-67 and hormone receptors (HR) have been established as relevant cancer biomarkers in several malignant tumors, we evaluated Ki-67 and HR expression rates by immunohistochemistry in 68 patients with LGSOC. We used a standardized cutoff finder algorithm to analyze prognostic significance for overall survival (OS) and progression-free survival (PFS). Cox regression showed a significant continuous decrease in OS for higher proliferation rates with an HR of 1.07% (95% confidence interval, 1.01%-3.67%; $P = .048$) but not in PFS ($P = .86$). Cutoff finder analysis revealed the best possible cutoff for OS at 6.28% ($P = .04$) and for PFS at 1.85% proliferative activity ($P = .04$). Estrogen receptors (ERs) were expressed in most LGSOC patients (n = 61; 89.7%), progesterone receptor (PR) in about half of patients (n = 33; 48.5%). For both ER/PR, a statistically significant cutoff for PFS could be determined, which was at 75% of positive tumor cells for ER ($P = .02$) and at 15% of positive tumor cells for PR ($P = .03$). For OS, HR expression showed a tendency toward better OS for HR-positive tumors but did not turn out statistically significant. Our results show that

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Ki-67 is a valuable prognostic marker in the subgroup of LGSOC. We could also show that most LGSOCs express HRs but that this expression is associated with a better PFS, a finding valuable in times of antihormonal therapy in LGSOC.

1. Introduction

Epithelial ovarian cancer (EOC) is a deadly disease with estimated 14 080 cancer deaths in 2017 in the United States and about 150 000 worldwide [1].

Among all the epithelial tumors, serous tumors represent the largest subgroup. Serous tumors used to be graded by a 3-tiered grading system, for example, according to Silverberg [2]. However, recent morphologic, immunohistochemical, and molecular studies showed that low- (LGSOC) and high-grade (HGSOC) serous ovarian carcinomas vary significantly and are rather 2 different entities indeed [3]. LGSOC is characterized by mutations in BRAF, KRAS, and ERBB2 [4-6] and few point mutations. It is probably derived from borderline tumors and develops in a multistep fashion. In contrast, HGSOC shows frequent p53 and BRCA mutations and high genetic instability and is probably derived from tubal epithelium [4]. In this definition, LGSOC is a rare entity accounting about 5% of all serous ovarian carcinomas [7] and is not well studied yet. It is characterized by younger age, better clinical outcome, and lower response to conventional chemotherapy [8,9] compared with HGSOC [10]. LGSOC in early stages shows a much better clinical outcome compared with HGSOC, but comparing advanced stages, histology loses significance as an independent prognosticator [11]. Because, on the one hand, diagnosis of LGSOC is mostly made in advanced stages and, on the other hand, chemoresistance does not allow chemotherapy to be effective, there is a grave need for individualized treatments such as hormonal therapy or targeted agents to avoid ineffective treatments or overtreatment. Although there are some studies on hormone receptor (HR) expression in LGSOC already published as the one by Buttarelli et al [12], very few data are available to describe estrogen receptor (ER) and progesterone receptor (PR) expression in LGSOC as prognostic or predictive biomarkers.

Ki-67 was discovered in the 1980s of the past century as a marker in the "nuclear matrix" during cell division [13]. Little was known about the nature and biochemical characteristics of the antigen. The role of Ki-67 in cell division is still not entirely clear, but it seems to prevent chromosomes from sticking together by binding with one end to the chromosome and repelling other chromosomes with the positively charged other end [14]. Because Ki-67 is expressed only during the active G1, S, and G2 phases of the cell cycle and because it can be easily detected by immunohistochemistry [15], its prognostic and predictive role has been analyzed for different cancer types. Although in routine pathological diagnostics estimating the percentage of Ki-67–positive cells still remains common practice, in large studies, methods have been refined over manual counting large amounts of cells to automated readers [16]. Another issue is the distribution of Ki-67. Because most tumors are heterogenous in proliferation rate, the reliability of tissue microarrays (TMAs) [17] versus examination of hotspots has been discussed.

Probably the most extensively studied tumor category for Ki-67 proliferation rate is breast cancer. Studies on Ki-67 proliferation rate in association with HR expression [18], adjuvant [19] or neoadjuvant therapy [20], or metastatic disease have been conducted. Because Yerushalmi et al [21] studied crystallization, Ki-67 is a valid prognostic marker, but the usefulness depends on the analysis of consistent, reproducible, and valid scores in appropriate cohorts. For EOC, few studies have evaluated Ki-67 proliferation rate, and the differentiation between histotypes seems to be a frequent problem [22-25].

In this study, we have determined the proliferation rate of Ki-67 systematically with digital image analysis in a cohort of 68 patients with LGSOC and evaluated the prognostic significance for overall survival (OS) and progression-free survival (PFS). Furthermore, we analyzed the percentage of HR expression in LGSOC and its prognostic significance.

2. Materials and methods

2.1. Study population and histopathologic examination

Within our tumor bank ovarian cancer project (http://www.toc-network.de), we identified 68 patients with LGSOC, with available paraffin specimens diagnosed between January 2000 and November 2015. Surgical procedure and determination of residual tumor mass were performed as previously described [26,27]. For patient characteristics, refer to Table 1.

Histopathologic analysis was made at the Institute of Pathology of the Charité University Hospital, and for study purposes, histologic type has been reconfirmed by an experienced, board-approved pathologist, applying World Health Organization criteria [28].

2.2. Immunohistochemical staining

For Ki-67 analysis, slides were stained and a representative spot was chosen. ER-α and PR were determined on TMAs,

Table 1 Patients' characteristics

Parameter	
Total patients, n (%)	68 (100)
Age at diagnosis (y), median (range)	55.0 (20-81)
Preoperative serum CA-125 (U/mL)	
Median	130
Range	13-14 315
Follow-up (mo)	
Median	54
Range	1-183
FIGO stage (2014), n (%)	
Ia	4 (5.9)
Ic	1 (1.5)
IIa	2 (2.9)
IIb	1 (1.5)
IIIa	2 (2.9)
IIIb	13 (19.1)
IIIc	38 (55.9)
IVb	7 (10.3)
Lymph node involvement, n (%)	
N0	14 (20.6)
N1	31 (45.6)
Nx	23 (33.8)
Ascites, n (%)	
None	36 (52.9)
<500 mL	22 (32.4)
>500 mL	10 (14.7)
Other malignancies, n (%)	7 (11.8)
Breast cancer	2 (2.9)
Cervical cancer	4 (5.9)
Endometrial cancer	1 (1.5)
Family history of ovarian and/or breast cancer, n (%)	5 (7.4)
Hormonal status	
Premenopausal	30 (44.1)
Postmenopausal	38 (55.9)
Residual tumor mass after surgery, n (%)	15 (22.1)
Peritoneal carcinomatosis, n (%)	57 (83.8)
Platinum based first-line chemotherapy, n (%)	61 (89.7)
Response to chemotherapy, n (%)	
Responders	38 (62.3)
Nonresponders	10 (16.4)
Not applicable	13 (21.3)
Chemotherapy lines	
Median	1
Range	0-9
Neoadjuvant chemotherapy, n (%)	11 (16.2)

which were prepared as described previously [29] with 2 spots for each tumor. For Ki-67 analysis, immunohistochemical staining was performed using a mouse monoclonal antibody (Mib-1 clone, "M7240" by Dako, 5301 Stevens Creek Blvd. Santa Clara, CA 95051 United States dilution 1:100). 3,3′-Diaminobenzidine peroxide substrate (DAB^+) of the "ultraView Universal DAB detection kit" (Ventana Medical Systems, Tucson, AZ) was used as a chromogen. A rabbit monoclonal primary antibody against ER (clone SP1 by Ventana) and a mouse monoclonal antibody against PR (clon1E2 by Ventana) were used for HR evaluation. Staining procedure was performed using a Ventana Benchmark XT Autostainer.

2.3. Ki-67 Quantification

We used the Ki-67 Quantifier, a computer program that facilitates Ki-67 analysis by mainly 3 steps: first, using for cell detection method an image analysis framework. Second, the distinction between tumor and nontumor (eg, stroma or lymphocytes) cells is determined by an algorithm based on cell distances. To determine positive versus negative stained cells, the image is separated into 2 intensity classes. Finally, a manual intervention to separate tumor and nontumor areas is possible to enhance accuracy even further. Thus, the computer program is fast, robust, and specific and was validated in a large cohort of >1000 patients with breast cancer by our group [16].

2.4. Immunohistochemical evaluation of ER and PR on the TMA slides

ER and PR statuses were determined visually by an experienced pathologist on scanned sections, supported by the TMA Evaluator 2.2 software (VMScope, Berlin, Germany). Cases with at least 1% positive tumor cells were considered positive. Hormonal expression rates were then summarized in 10% steps, with the aim to find an optimal cutoff for different risk-related groups.

2.5. Digital image analysis–assisted evaluation of Ki-67

Slides were explored with a conventional light microscope and a microscope camera by an experienced pathologist. A representative field for Ki-67 evaluation was selected; in case of heterogenous staining, a hotspot (= area with high Ki-67 expression) was chosen. A digital photograph was taken, and the picture was uploaded to the CognitionMaster platform (VMScope) with integrated Ki-67 Quantifier Software for Ki-67 evaluation. The digital image analysis classified the detected cells into nontumor, as well as Ki-67–negative and Ki-67–positive tumor cells. A manual correction of tumor and nontumor areas was performed if necessary (Fig. 1).

2.6. Statistical analyses

For Ki-67 analysis, we used the Charité online cutoff finder (http://molpath.charite.de/cutoff/) to determine optimal cutoff for OS and PFS [30]. All data are presented as frequency and rate for categorical variables or median and range for continuous variables. Comparisons between groups were performed using the Fisher exact test, χ^2 test, Kendall τb, or Mann-Whitney U test where appropriate. Estimates of median survival and survival rates were calculated using the Kaplan-Meier method. Log-rank tests were used for

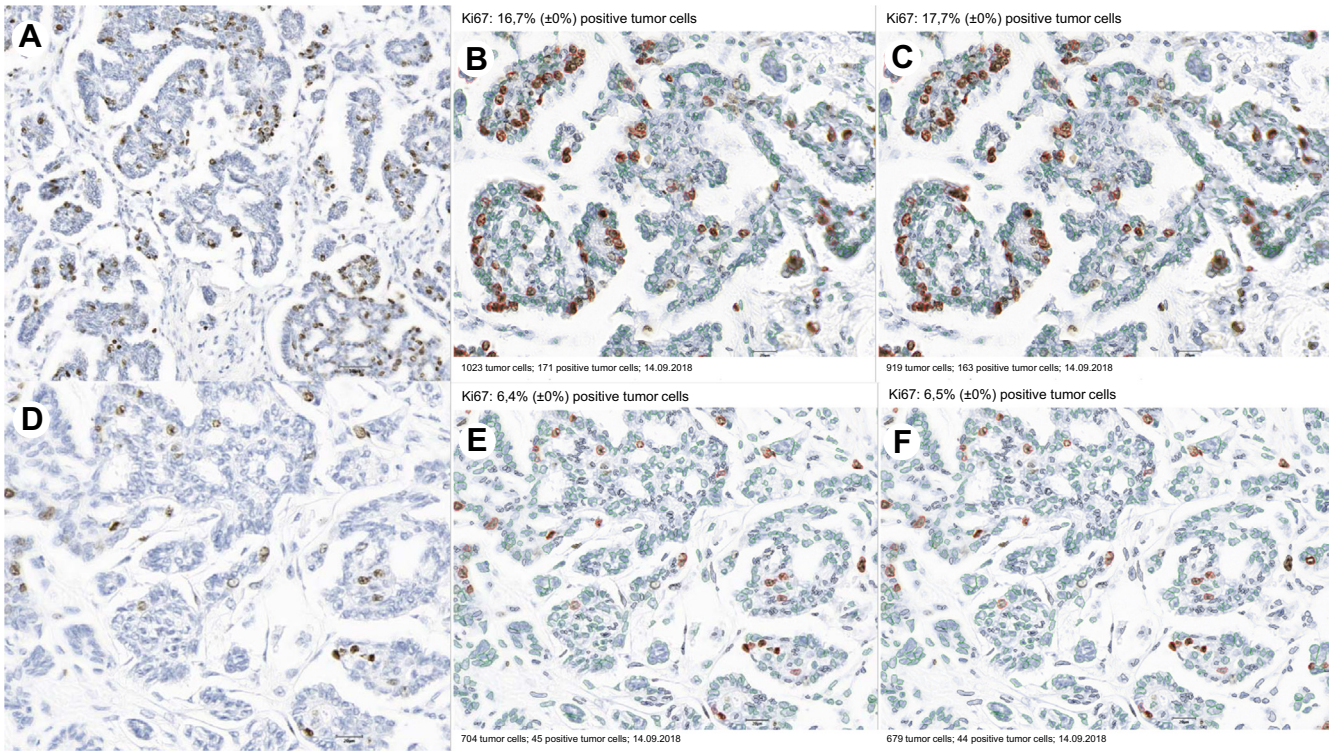

Fig. 1 A: Low-grade serous ovarian carcinoma immunohistochemistry for Ki-67. B: Initial Ki-67 evaluation by digital image analysis. C: Ki-67 evaluation after manual tumor/stroma separation by an experienced pathologist. D: Low-grade serous ovarian carcinoma immunohistochemistry for Ki-67. E: Initial Ki-67 evaluation by digital image analysis. F: Ki-67 evaluation after manual tumor/stroma separation by an experienced pathologist.

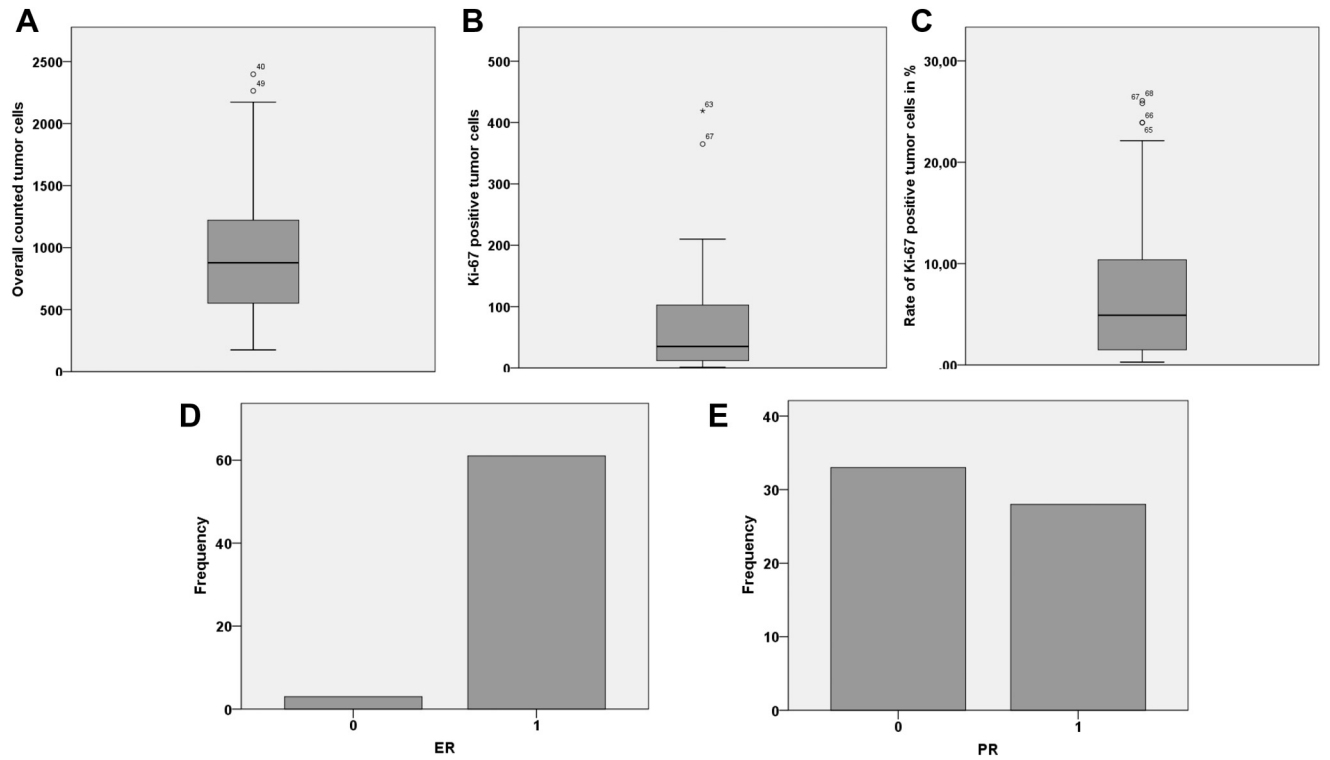

Fig. 2 Distribution of Ki-67 proliferation rate. A: Number of overall counted tumor cells. B: Number of Ki-67 positive tumor cells. C: Percentage of Ki-67 positive tumor cells. D: 0 = ER negative vs. 1 = ER positive tumor cells. E: 0 = PR negative vs. 1 = PR positive tumor cells.

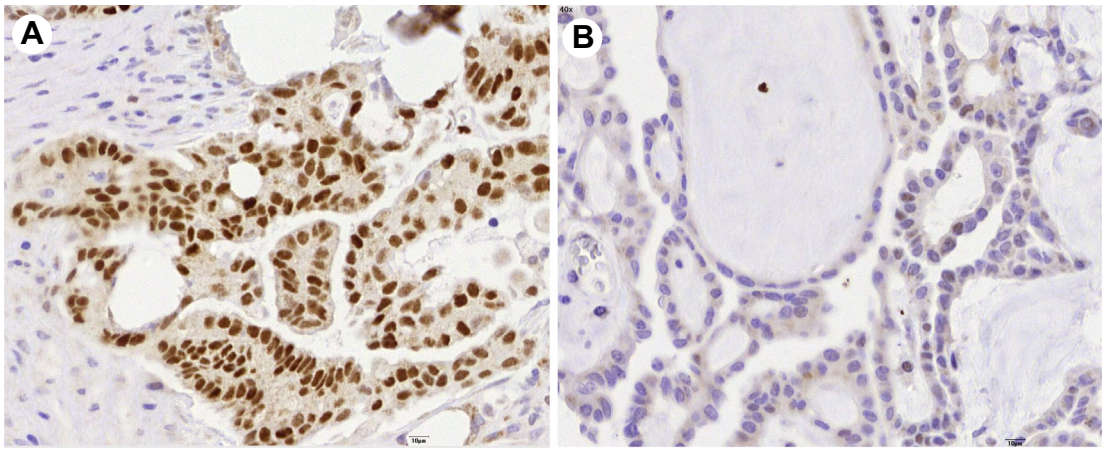

Fig. 3 Hormone receptor expression in low-grade serous ovarian carcinoma A: Low-grade serous ovarian carcinoma with strong positivity for estrogen receptor in the majority of tumor cells. B: Low-grade serous ovarian carcinoma with few, only moderately positive cells for estrogen receptor.

univariate statistical comparisons. Unadjusted and adjusted hazard ratios and 95% confidence interval (CI) were estimated using the Cox proportional hazards model. All data were analyzed using IBM SPSS Statistics 23 (SPSS, an IBM Company, Chicago, IL), and $P < .05$ was considered statistically significant.

3. Results

3.1. Ki-67 index

Overall, the mean (SD) number of 925 (487) tumor cells (range, 175-2398) was evaluated; in the mean (SD), we found

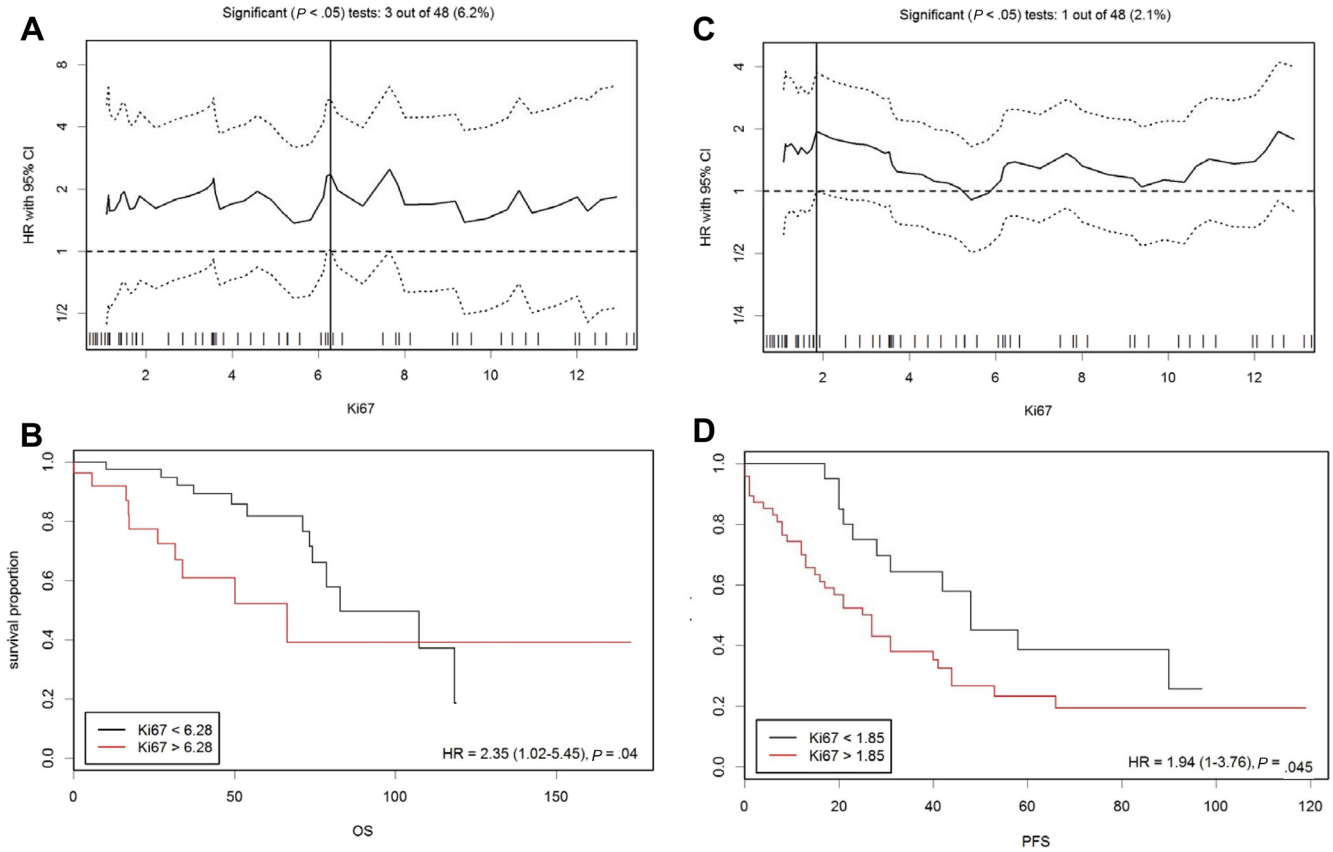

Fig. 4 Systematic cut off determination for Ki-67 as a prognostic marker in low-grade serous ovarian carcinoma. A: Hazard ratio (HR) for overall survival according to cutoff. B: Cutoff Finder Kaplan–Meier analysis at optimal cutoff for overall survival of 6.28% proliferative activity with Ki-67. C: Hazard ratio (HR) for progression free survival according to cutoff. D: Cutoff Finder Kaplan–Meier analysis at optimal cutoff for progression free survival of 1.85% proliferative activity with Ki-67.

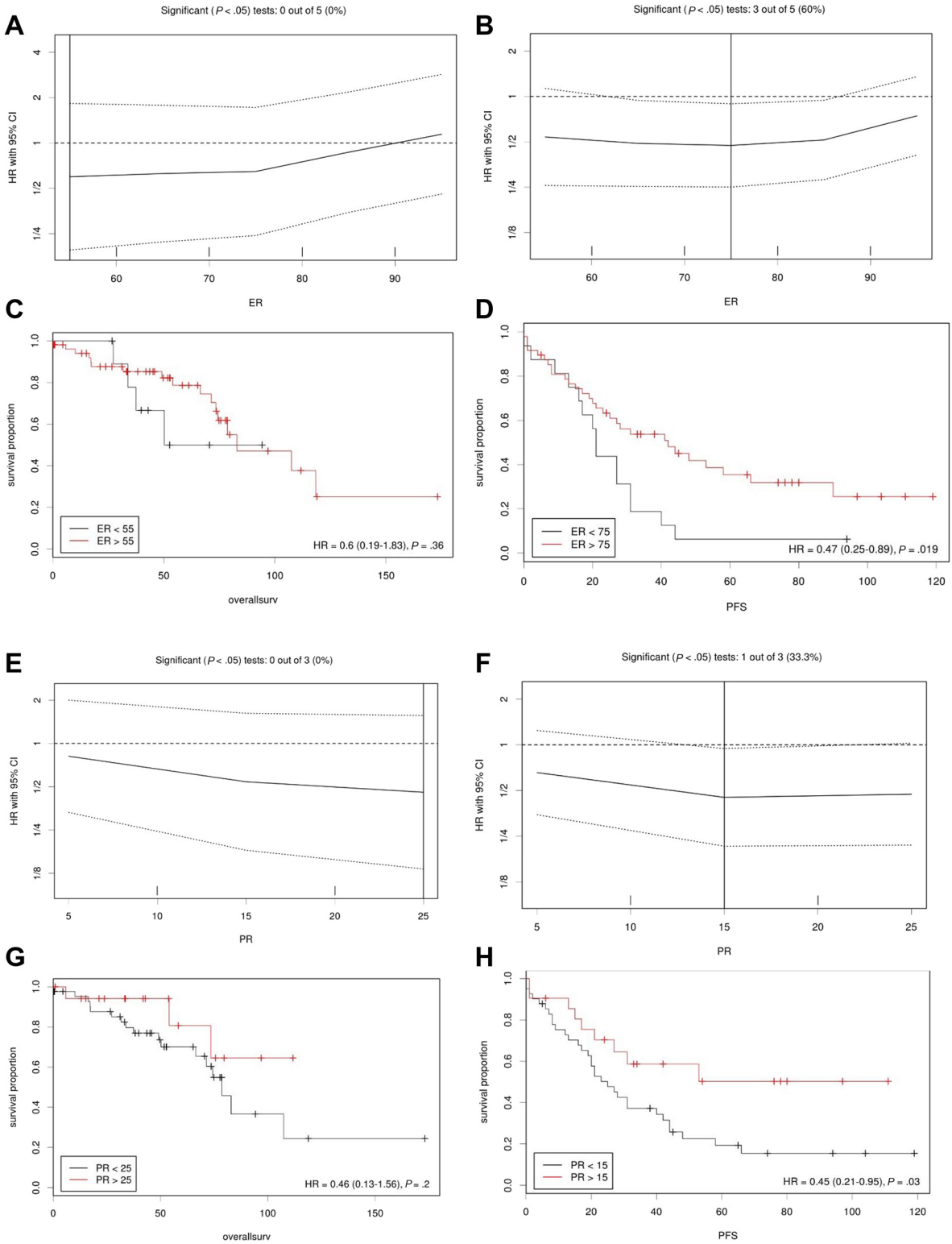

Fig. 5 Systematic cut off determination for ER and PR as a prognostic marker in low-grade serous ovarian carcinoma. A: Hazard ratio (HR) for ER in overall survival according to cutoff. B: Hazard ratio (HR) for ER in progression free survival according to cutoff. C: Cutoff Finder Kaplan–Meier analysis for ER expression in overall survival. D: Cutoff Finder Kaplan–Meier analysis for ER expression in progression free survival. E: Hazard ratio (HR) for PR in overall survival according to cutoff. F: Hazard ratio (HR) for PR in progression free survival according to cutoff. G: Cutoff Finder Kaplan–Meier analysis for PR expression in overall survival. H: Cutoff Finder Kaplan–Meier analysis for PR expression in progression free survival.

Table 2 Comparison of tumors with low and high Ki-67 expression

	Ki-67			
	<6.28% (n = 41)	≥6.28% (n = 27)	Total (n = 68)	*P*
Age (y), median (range)	55 (20-76)	56 (20-81)	55.5 (20-81)	.910
FIGO				.503
IA-IIIB	12 (29.3)	10 (37)	22 (32.4)	
IIIC-IV	29 (70.7)	17 (63)	46 (67.6)	
Residual tumor				.069
0 mm	35 (85.4)	18 (66.7)	53 (77.9)	
>0 mm	6 (14.6)	9 (33.3)	15 (22.1)	
Ascites				.520
No	23 (56.1)	13 (48.1)	36 (52.9)	
Yes	18 (43.9)	14 (51.9)	32 (47.1)	
ER%, mean (SD)	80 (31)	82 (28)	81 (30)	.814
PR%, mean (SD)	17 (29)	21 (31)	19 (30)	.766
CA-125 (presurgery), mean (SD)	1000 (2737)	360 (483)	771 (2223)	.235
Follow-up median (mo)	71	45	54	.071
Death by disease	13 (31.7)	10 (37)	23 (38.3)	
OS median (mo)	83	66	83	.040
5-y OS rate (%)	82	52	72	
Recurrent disease	24 (58.5)	30 (37)	34 (50)	
PFS median (mo)	42	44	42	.762
5-y PFS rate (%)	34	46	37	

68 (81) cells positive for Ki-67 (range, 1-419), resulting in a mean (SD) proliferative rate of 6.9% (6.1%; range, 0.28%-26.07%; Fig. 2A-C).

3.2. HR expression

HRs were expressed in tumor cell nuclei (Fig. 3). Most of the tumors were positive for ER (61 positive; 89.7%), and only 3 tumors were entirely negative (4.4%). More than half of the tumors showed strong nuclear staining for ER in more than 80% of tumor cells (39; 57.4%). PR was expressed in about half of tumors (33; 48.5%), whereas the other half was negative (28; 41.2%; Fig. 2D and E).

3.3. Survival analysis

3.3.1. Ki-67

Cox regression showed a significant continuous decrease in OS for higher proliferation rate with an HR per percent of 1.07 (95% CI, 1.01-3.67; $P = .048$) but not for PFS (HR per percent, 0.99; 95% CI, 0.93-1.06; $P = .86$). Because no established cutoff for the proliferative activity with Ki-67 exists in LGSOC, we used the online tool cutoff finder to determine the cutoff for OS and PFS [28]. For PFS, the cutoff was slightly lower (at 1.85% proliferative activity, $P = .04$) than for OS where the optimal cutoff was at 6.28% proliferative activity ($P = .04$; Fig. 4).

Median OS in the group of Ki-67 > 6.28% was 66 months (range, 23-109 months) as opposed to 83 months (range, 49-117 months) for lower proliferation rate of the tumors. Median time for PFS at the optimal cutoff of >1.85% Ki-67 proliferation rate was 27 months (range, 19-35 months), whereas in the group of patients with lower proliferation rate, median time to progression was 90 months (range, 41-139 months).

3.3.2. Estrogen receptor/progesterone receptor

To determine prognostic significance of HR expression in LGSOC, we again used the cutoff finder online tool [30]. For both ER and PR, a statistical significant cutoff for PFS could be determined, which was at 75% positive tumor cells for ER ($P = .02$) and at 15% positive tumor cells for PR ($P = .03$). For OS, a tendency toward better survival for HR-positive tumor could be seen, but a statistical significant cutoff could not be determined (ER best possible cutoff with 55% positive tumor cells [$P = .36$] and PR best possible cutoff with 25% positive tumor cells [$P = .2$]; Fig. 5).

3.4. Comparison of tumors with low and high Ki-67 expression in univariate and multivariate analyses

A detailed comparison of low and high Ki-67 tumors is shown in Table 2, where clinical variables were analyzed according to our 6.28% Ki-67 cutoff.

We already described the significant continuous decrease in OS for higher proliferation rates above, but applying the 6.28% Ki-67 cutoff in univariate analysis, we could identify Ki-67 also to be a significant factor for a poorer OS (HR, 2.35; 95% CI, 1.02-5.45; $P = .046$). Residual disease (1 to >10 mm) was identified as a variable with the strongest difference in Ki-67 low/high groups but turned out not to be a

Table 3 Cox regression analysis of patients with primary LGSOC for age, Ki-67, FIGO stage, residual disease, and ascites

	PFS				OS			
	P	HR	95% CI		*P*	HR	95% CI	
			Lower	Upper			Lower	Upper
Age	.325	0.990	0.971	1.010	.488	1.010	0.982	1.038
Ki-67 >6.28/<6.28	.783	1.112	0.521	2.373	.007 [a]	3.766	1.441	9.842
FIGO IIIC-IV *vs* IA-IIIB	.038 [a]	2.784	1.060	7.314	.039 [a]	3.971	1.070	14.731
Residual tumor >0	.537	1.312	0.554	3.105	.334	1.619	0.609	4.299
Ascites >500 mL	.640	0.844	0.414	1.719	.892	1.066	0.421	2.700

[a] Statistically significant.

significant factor for OS, neither in univariate analysis (HR, 2.35; 95% CI, 0.89-6.22; $P = .089$) nor in multivariate analysis (HR, 2.47; 95% CI, 0.98-6.22; $P = .055$). Ki-67 adjusted to residual disease in contrast remained an independent parameter and to be a significant factor for OS (HR, 2.60; 95% CI, 1.09-6.20, $P = .031$).

In multivariate analysis, when adjusting to all variables as shown in Table 3, only International Federation of Gynecology and Obstetrics (FIGO) stage (HR, 6.91; 95% CI, 1.07-14.73; $P = .039$) and Ki-67 (HR, 3.77; 95% CI, 1.44-9.84; $P = .007$) remained significant independent risk factors for poorer OS.

HRs were not included in the multivariate analysis because of the small number of cases. Anyway, we could not demonstrate any correlation between Ki-67 and ER/PR neither with a linear approach (Ki-67–ER: Pearson correlation coefficient [PCC] = −0.058, $P = .0644$; Ki-67–PR, PCC = 0.077, $P = .533$;) nor using the cutoff 6.28%, where positive/negative HR tumors were distributed equally (PCC = 0.042, $P = .736$). While analyzing our 4 complete HR-negative tumors (ER 0% and PR 0%), we found the corresponding median Ki-67 at 4.1% (range, 0.28%-23.89%). In comparison to complete HR-positive tumors (ER 100% and PR 80%-100%, n = 8), the median Ki-67 was found at 6.0% (range, 0.97%-15.61%).

As far as the tumor marker cancer antigen (CA) 125 is concerned, we observed an inverse correlation using the 6.28% Ki-67 cutoff (see Table 2). However, looking at tumors with very low Ki-67 expression (<1%), the median CA-125 was 120 U/mL (range, 13-289 U/mL; n = 9), whereas tumors with high Ki-67 expression (>15%) showed also a higher median CA-125 with 270 U/mL (range, 116-2206 U/mL; n = 8).

4. Discussion

Our study demonstrates for the very first time that Ki-67 index measured by digital image analysis as a continuous marker is an independent prognostic factor for LGSOC, and it remained so in multivariate analysis. Furthermore, we investigated HR expression and found 95.6% of LGSOC to express at least some HRs. Percentage of HR expression proved to be a positive prognostic marker for PFS, whereas it only showed a tendency for OS.

To our knowledge, this is the first time that such a large cohort of LGSOC was systematically analyzed for proliferative activity by Ki-67 and HR expression.

Our study is in line with previous studies about proliferation rate by Ki-67 analysis in serous ovarian carcinoma. Because the concept of distinguishing between LGSOC and HGSOC in 2 distinct entities is quite recent, we tried to analyze data from this point of view. In 2008, Köbel et al [22] analyzed Ki-67 expression among other biomarkers in a large cohort of 500 ovarian cancers and could demonstrate a positive prognostic effect for the whole cohort. When looking at the 12 included LGSOCs, a prognostic significance could not be detected. Also, Kritpracha et al [23] analyzed, similar to Köbel et al [22], proliferative activity in different histotypes together and described a cutoff of 7.6% Ki-67 proliferative activity, which is rather close to our determined optimal cutoff of 6.28% for OS. Mishra and Crasta [31] analyzed LGSOC and HGSOC separately in 2010 and found the median proliferative activity of LGSOC to be around 20%, which is much higher than our median proliferation rate by 7%. They described furthermore a significant difference between LGSOC und HGSOC where median Ki-67 expression was 42.1%. Also, Chen et al [24] in 2016 analyzed 318 women with HGSOC Ki-67 proliferation indices in a range from 3% to 95% with a median of 40%. Low Ki-67 expression was significantly associated with decreased PFS (22% versus 34% for 5-year PFS, $P < .001$) as well as decreased OS (31% versus 55%, $P < .001$), and low Ki-67 was also significantly associated with platinum resistance.

In 2017, Mahadevappa et al [25] analyzed 40 patients with EOC (75% were HGSOC) and underlined the robust and cost-effective advantages of Ki-67 as a good approach to get an impression on biology of the carcinoma. Interestingly, they also pointed out the gap between the proliferation rate of LGSOC in comparison to HGSOC; for example, CA-125 levels did not have a significant correlation with Ki-67 in their study, as it was not the case in our study either.

Other studies have focused on Ki-67 analysis in other tumor categories, and certainly, some general considerations should be adopted also to LGSOC. As Denkert et al [32] stated

with regard to breast cancer, Ki-67 values rise rather gradually and have not a tendency to jump or concentrate at a clear cutoff. We could confirm these findings in our study, and statistical significance for Ki-67 as a continuous marker in Cox regression analysis could be demonstrated, but when using the cutoff finder tool, a few solitary cutoffs could be tracked down. In our opinion, this could be helpful in daily clinical use.

As for HR expression in LGSOC, our results of their frequent expression are congruent with previous studies. Probably the largest study regarding this question is the one by Sieh et al [33], who analyzed a big cohort of LGSOCs among other histotypes. HR expression was observed in most cases (PR, 57%; ER, 87%). With 104 LGSOCs, their cohort was much bigger, but when reducing the group to primary LGSOCs only resulting in 64 patients, it is quite comparable to our cohort. Sieh et al did not analyze prognostic significance in PFS, but they reported, similar to us, a prognostic significance for OS in HR-positive tumors.

Further studies regarding HR expression in LGSOC are the ones by Escobar et al [34] and Buttarelli et al [12]. Although Escobar et al tested various antibodies in a cohort of 27 LGSOC patients and found the majority to be HR positive, Buttarelli et al analyzed immunohistochemical expression of progesterone and different isotypes of estrogen in 25 primary LGSOCs. Buttarelli et al also found most LGSOCs to be HR positive. Neither Escobar et al nor Buttarelli et al analyzed prognostic significance.

Gershenson et al [9] reported in their retrospective analysis a significant benefit in PFS for the group of patients with LGSOC who underwent primary surgery and systematic chemotherapy followed by an antihormonal maintenance therapy. HR expression or Ki-67 was not reported.

In our analysis, none of the patients received a maintenance therapy so we were not able to correlate our findings with a similar approach. Nevertheless, further trials should translate our cutoffs to predictive values. Our data were already published in American Society of Clinical Oncology 2017 under abstract number 5562 [35]. We included 80 patients with LGSOC and stated that no differences in clinical outcome were seen in patients with different ER and PR statuses. Under revision of the data, we decided to exclude 12 patients with missing ER or PR data and noticed a tendency toward better survival for HR-positive tumor. A statistical significant cutoff could not be determined. The results concerning Ki-67 did not change during revision of the data.

Further limitations of our findings are questions of potential changes at several times of recurrences and regarding the biological function and interaction of Ki-67 and HRs in LGSOCs. However, our study—as a biomarker investigation—was not designed to address those questions. The high percentage of FIGO III/IV stages may bias our results. This is, though with a total number of 68 patients in our study, not avoidable, and FIGO stage remained an independent prognostic factor in multivariate analysis. We decided not to include patients with recurrent disease of LGSOC to avoid possible differences on Ki-67 percentages in primary sites and in relapses.

In conclusion, our result underlines the value of Ki-67 as a prognostic marker in LGSOC. Furthermore, we found most LGSOCs to be positive for HR expression, which is associated with a better PFS, a finding valuable in times of controversial discussion about antihormonal therapy in LGSOC.

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