Histological patterns and intra-tumor heterogeneity as prognostication tools in high grade serous ovarian cancers

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HIGHLIGHTS

- In HGSOCs the definition of morphological architectural patterns and intratumor heterogeneity are useful in prognosis.
- HGSOCs with SET features had longer overall and progression free survival
- The Shannon diversity index (SDI) is a proposed method to measure intratumor heterogeneity.
- In HGSOCs higher intratumor heterogeneity by SDI was a negative independent prognostic factor in patients treated with NACT.

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ABSTRACT

Objective. High grade serous ovarian carcinoma (HGSOC) is the most common type of malignant ovarian neoplasm and the main cause of ovarian cancer related deaths worldwide. Although novel biomarkers such as homologous recombination deficiency testing have been implemented into the clinical decision-making algorithm since diagnosis, morphological classification and immunohistochemistry analysis are essential for diagnostic purpose. This study aims at identifying histologic and clinical features that can be predictive of patients' prognosis.

Methods. Morphological and architectural characterization including SET (Solid-Endometroid-Transitional)/Classic features was carried out in a cohort of 234 patients analyzing 695 slides. From each slide tumor infiltrating lymphocyte (TILs), the presence of necrosis, the number of mitoses, the presence of psammoma bodies, giant cells and atypical mitoses were recorded. Morphological heterogeneity was quantified by the Shannon's diversity index (SDI) considering the percentage of each architectural pattern per patient's slide.

Results. The frequency of architectural patterns and morphological variables varied with respect of the surgical strategy (primary debulking surgery vs interval surgery after neoadjuvant chemotherapy). HGSOCs exhibiting SET features had a longer overall as well as progression free survival. Among SET features, pseudo-endometrioid and transitional like patterns had the best outcome, while it was heterogenous for solid pattern, that had better outcome for BRCA 1 negative and less heterogeneous tumors. In patients submitted to neoadjuvant chemotherapy a higher intratumor heterogeneity as defined by SDI was a negative independent prognostic factor.

Conclusions. A comprehensive histological examination considering architectural patterns and their heterogeneity can help in prognostication of HGSOCs.

1. Introduction

High grade serous ovarian carcinoma (HGSOC) is the most common type of ovarian neoplasm [1]. In the majority of women, the disease is diagnosed in advanced stages (FIGO III-IV). The standard treatment

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includes primary debulking surgery (PDS) followed by a platinum-based chemotherapy, or the use of neoadjuvant chemotherapy (NACT) and interval debulking surgery in cases where up-front cytoreduction is not feasible or patient's performance status is poor [2–4]. Patients usually respond well to the first line treatment, but soon after they relapse and most of them die. The mortality rate hasn't changed during the past decades and more than 50% of women still die within five years from diagnosis [5]. Clinical parameters such as optimal cytoreduction, patient's age and tumor stage are consolidated prognostic factors in HGSOC [6,7], but at the molecular level only homologous recombination deficiency (HRD) testing has been implemented into clinical practice [8,9]. Notwithstanding, recently HRD-positive cases were suggested to be classified into epigenetic and non-epigenetic, as epigenetic HRD patients resulted to have a poor prognosis, similar to non HRD patients [10]. Several efforts have also been made at identifying prognostic signatures or discrete molecular classes [11–16], but they have not yet been implemented in clinical routine since they are technically complex and quite expensive. For these reasons, there's also been a parallel interest in the histomorphological analysis for stratifying HGSOC patients and providing prognostic information for patient management. The classification of ovarian cancer is now highly reproducible using modern diagnostic criteria supplemented where necessary by immunohistochemistry [17]. Soslow et al. [15,18] described a sub-group of HGSOC, termed 'SET', which included solid, pseudo-endometrioid and transitional-like patterns that, if compared with tumor having classic features, namely classic and micropapillary, was characterized by higher mitotic index, higher number of TILs, higher necrosis. Furthermore, SET group was more frequently associated with BRCA1 inactivation [15]. Similarly, another study identified specific morphologic features that can be predictive of BRCA1 mutational status in HGSOC [19], but the effective role of tumor morphology in predicting response to chemotherapy or patients' survival is still unclear. Bromley et al. [20] characterized the morphological patterns of 70 patients diagnosed with advanced HGSOC treated with NACT or PDS correlating them with response to chemotherapy, but without any significant association.

Here we describe the morphological heterogeneity of HGSOC, analyzing tumor architectural patterns and SET/Classic groups in a retrospective cohort of patients submitted to NACT followed by surgery or PDS. Our aim was at identifying histologic and clinical features that can be predictive of patients' prognosis.

2. Patients and methods

2.1. Cases selection

The H&E tissue slides and the respective paraffin-embedded blocks, were collected at the Centro di Riferimento Oncologico of Aviano (CRO). All patients gave informed consent before enrollment in the study that was conducted in accordance with the Declaration of Helsinki. The study was approved by the institutional review board (protocol number 1213, 24/01/2017). Inclusion criteria were: women who had i) stage IIIC or IV HGSOC, ii) partial or complete clinical information available and iii) available H&E slides. Clinical data were obtained from the hospital information system or medical records. Accordingly, the following variables were gathered: patients' age, residual tumor after surgery, FIGO stage, surgical strategy (PDS/NACT), presence of positive lymph nodes, primary platinum response, follow-up data for overall survival (OS) and progression-free survival (PFS).

The cohort included 301 patients diagnosed with HGSOC who underwent surgery at CRO of Aviano between December 1998 and April 2017. An initial histological revision was performed (V.C.) to select slides with adequate tumor purity. All available tumor's slides were then reviewed by two pathologists (R.B. and G.S.). Samples with ambiguous HGSOC morphology were discarded or revaluated by immunohistochemistry (IHC) using the biomarkers panel modified from Kobel et al. [21], as listed in the Supplementary Table 1. Tumors presenting ambiguous IHC features were discarded and a detailed histomorphological analysis was performed on all the selected H&E slides (mean number of slides per case: 3). In order to account for intra-patient heterogeneity, tumor lesions were taken from different anatomical sites: ovaries, peritoneal implants and lymph nodes.

2.2. Histopathological review

The following histo-morphological features were recorded: (1) pattern of tumor infiltrating lymphocyte (TILs), namely if TILs were present in both tumor epithelium and intratumoral stroma (IES pattern) or just in the intratumoral stroma (IS pattern); (2) number of TILs (x1 HPF) (mean value obtained analyzing 10 HPFs for each slide); (3); presence of necrosis; (4) number of mitoses (x10 HPF); (5) presence of psammoma bodies; (6) presence of giant cells and atypical mitoses and (7) tumor growth patterns.

All features were assessed in each anatomical location except TILs pattern which was not assessed in lymph nodes specimens. TILs were evaluated in selected areas after scanning all slide at 20× magnification; an average estimation of TILs number was then determined after viewing the selected areas at 1× HPF. Necrosis was assessed using a four-level score from 0 (absence) to 3 (1- up to 10% of the total slide, 2-moderate- 11% - 44% of the total slide; 3–45-60% or more of the total slide; comedo-like and geographic necrosis were grouped together) while giant cells and atypical mitoses were both classified in "prominent" or "not prominent". Tumor architectures were evaluated at 20× magnification and classified in seven patterns according with Soslow and colleagues [15]. The patterns were: pseudo-endometrioid (PSE), infiltrative micropapillae (INF MP), micropapillary (MP), papillary (PA), papillary infiltrative (PA INF), solid (SD) and transitional-like (TR). Representative images of HGSOC architectural patterns are depicted in Fig. 1. In each slide, the pattern percentages were visually estimated and quantified in increments of 5% and, in tumors having multiple architectures, the most prominent pattern was recorded. Tumor samples were classified as "SET" when ≥40% of the tumor displayed one or more of the following patterns: solid, pseudo-endometrioid and transitional-like. This cut-off was chosen as mean of the values used by Soslow (25%) and Ritterhouse (50%) rounded up to the nearest multiple of five [15,18]. The diversity of tumor patterns within each single slide was measured using the Shannon diversity index (SDI) [22]: $(H) = -\sum \text{pi ln pi}$, where pi was the percentage of one specific tumor pattern.

2.3. Correlation BRCA1 expression with morphological and clinical features

BRCA1 immunohistochemistry was performed in 311 samples from 156 patients. To set-up the method, samples submitted to BRCA1/BRCA2 genomic testing were used as positive and negative controls. Immunostaining BRCA1 procedures and detailed results on BRCA1 immunohistochemistry results are reported in detail in the Supplementary file.

2.4. Statistical analysis

Statistical analyses were carried out using R software (version 3.6.0) and GraphPad Prism 8 (La Jolla, CA, USA). For continuous variables, comparisons between groups were performed using parametric or non-parametric test according to variables' distribution. Categorical variables were compared by Pearson $\chi 2$ test.

Overall survival (OS) was defined as the time from HGSOC diagnosis to death or end of follow-up information, whichever came first. Progression free survival (PFS) was defined as the time from the starting point of first line chemotherapy to the progression of the disease or death whichever comes first. Primary platinum response was classified in "never progressed" for patients without progression after first line

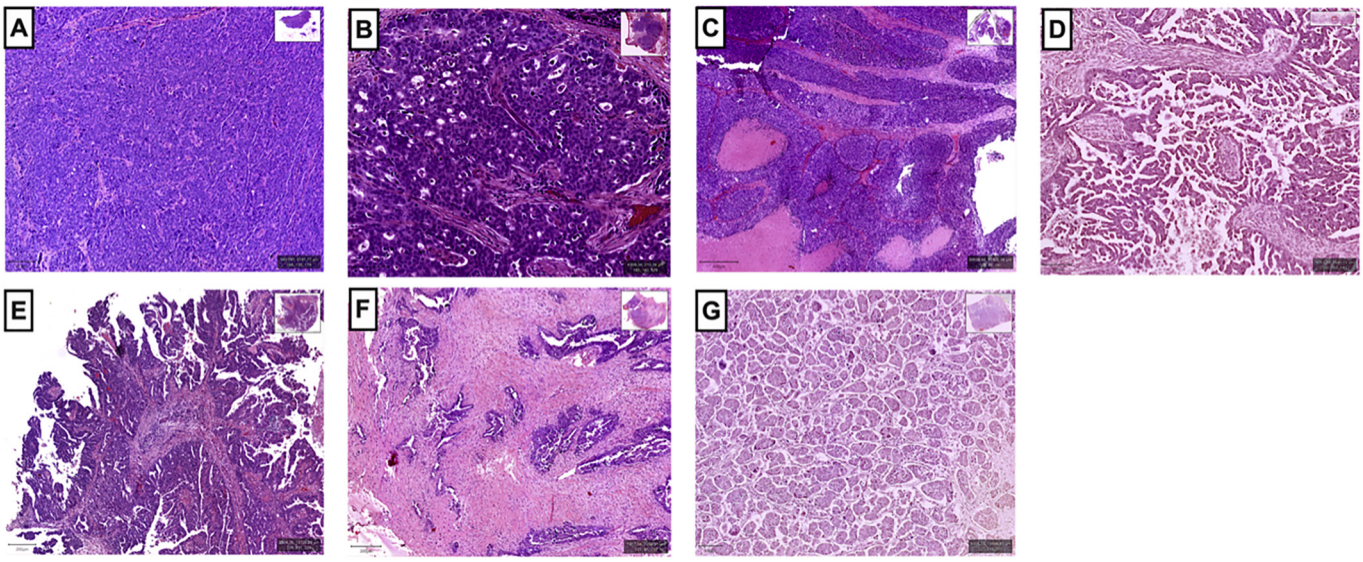

Fig. 1. Representative images of HGSOC growth patterns. Solid (A); Pseudo-endometrioid (B); Transitional-like (C); Micropapillary (D); Papillary (E); Papillary infiltrative (F); Infiltrative micropapillae (G). Micropapillary compressed pattern was excluded as it was not detected in our series.

chemotherapy, "platinum sensitive" for patients with PFI > 6 months and "platinum resistant" for patients with PFI < 6 months.

Clinical and histological parameters were dichotomized with respect to their median value in "low" or "high" status. Variables influencing OS e PFS were analyzed by log-rank test or univariate Cox regression and then used in a multivariate regression model to evaluate the covariates joint effect. The proportional hazard assumption was checked by Shoenfeld's residuals method. *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Clinicopathologic features of the study cohort

From the initial 301 HGSOCs cases, 234 were confirmed to be HGSOC while 67 were discarded for ambiguous morphology or ambiguous biomarkers' expression. The clinicopathologic characteristics of the cohort of women, stratified according to the surgical strategy into PDS and NACT patients, are summarized in the Supplementary Table 3. Briefly, median age at diagnosis was 61 years (range 31–82 years). All patients had advanced-stage disease: 73% had FIGO stage III and 27% stage IV. The primary treatment in 70% women was PDS while in 30% it was NACT prior to interval surgery. Of the 69 patients submitted to NACT treatment 43 resulted to have chemotherapy response score (CRS) 1, 24 had CRS 2 and 3 CRS 3. During follow-up, 149 patients (82%) experienced recurrence while for 68 women data were not available mainly because of patient's referral to local hospital for medical therapy after surgery. Optimal cytoreduction was achieved in 68 patients (34%) while for 34 this information was not available. After first line chemotherapy, 32 women (18%) never progressed, 78 (44%) were sensitive to platinum treatment and 67 (38%) were resistant.

Comparing PDS and NACT groups, no differences were detected with respect to age, FIGO stage and residual tumor after surgery, but a significant higher percentage of recurrences ($p = 0.0003$) and resistance to platinum therapy (53% vs 31%) were recorded in NACT patients. For the 147 patients with assessable lymph node metastasis, higher rates of positive lymph-nodes were recorded in PDS patients in comparison to NACT ones ($p = 0.005$).

In the whole cohort, the median PFS and OS were 12 months and 32 months, respectively. In patients who underwent PDS, the median overall survival (42 months) was higher when compared to those submitted to a NACT regimen (25 months), but the two groups had similar median progression free survival of 12 months. The results of univariate Cox regression analysis (Supplementary Table 4) pointed out that FIGO stage ($p < 0.0001$), surgical strategy ($p < 0.0001$) and residual tumor after surgery ($p = 0.003$) were significant prognostic factors for overall survival. For PFS, optimal cytoreduction was the only favorable prognostic factor ($p = 0.002$).

3.2. HGSOC architectural patterns

Histological analysis was carried out in 695 tissue slides derived from 234 patients. Since infiltrative patterns, namely infiltrative micropapillae and papillary infiltrative, had similar behavior they were merged in the group "infiltrative" (INF). Therefore, the total number of HGSOC architectures analyzed was six.

Overall, 52% of the analyzed slides were primary tumors from the two ovaries, 35% peritoneal implants and 13% lymph nodes. High morphological heterogeneity was detected within each tumor sample since more than one HGSOC growth pattern commonly coexisted (mean number of patterns/slide 2; range 1–5). When considering the predominant architecture, papillary and solid were the most common (42% and 20% respectively), followed by pseudo-endometrioid (16%), infiltrative (15%), transitional-like (4%) and micropapillary (2%). Pattern's frequency differed between PDS versus NACT-treated patients, where a lower rate of pseudo-endometrioid pattern ($p = 0.02$) and absence of micropapillary architecture ($p = 0.06$) were detected, suggesting a possible role of chemotherapy in the modification or selection of specific tumor architectures.

Cytological and histological parameters were then investigated with respect of the six tumor morphologies and the surgical strategy adopted. The results obtained highlighted marked differences between the group of women submitted to PDS and those submitted to interval surgery after NACT. Detailed results for both groups are reported in the Supplementary file and Supplementary Table 5.

3.3. Correlation of the HGSOC architectural patterns with clinical variables

The correlations between HGSOC growth patterns and the clinicopathological features of women submitted to PDS and NACT are shown in Table 1. Considering the predominant tumor architecture in each patient, our results showed that specific HGSOC patterns were

Table 1
Clinical features correlated with HGSOC growth patterns in PDS and NACT group.

Features	Primary debulking surgery (PDS) ($N = 165$), n (%)							Neoadjuvant chemotherapy (NACT) ($N = 69$), n (%)						
	INF (25)	MP (6)	PA (71)	PSE (26)	SD (29)	TR (8)	*P*	INF (10)	MP (0)	PA (35)	PSE (8)	SD (15)	TR (1)	*P*
Age at diagnosis (mean, [range])	66 (48–79)	67 (43–70)	61 (32–82)	61 (34–76)	59 (37–79)	68 (38–77)	0.6	63 (42–70)	/	58 (43–81)	64 (31–71)	59 (42–76)	46 (46–46)	0.5
FIGO stage														
IIIC	17 (81)	2 (67)	42 (67)	19 (76)	15 (60)	8 (100)	0.4	8 (89)	/	22 (67)	7 (87)	10 (83)	1 (100)	0.5
IV	4 (19)	1 (23)	21 (33)	6 (24)	10 (40)	0 (0)		1 (11)	/	11 (33)	1 (13)	2 (17)	0 (0)	
NA	4	3	8	1	4	0		1		2	0	3	0	
Primary platinum response														
Never progressed	3 (18)	1 (33)	8 (15)	9 (39)	5 (24)	5 (63)	0.02*	0 (0)	/	1 (4)	0 (0)	0 (0)	0 (0)	0.8
Resistant	9 (53)	1 (33)	22 (41)	2 (9)	6 (28)	0 (0)		5 (83)	/	13 (50)	2 (40)	6 (46)	1 (100)	
Sensitive	5 (29)	1 (33)	24 (44)	12 (52)	10 (48)	3 (37)		1 (17)	/	12 (46)	3 (60)	7 (54)	0 (0)	
NA	8	3	17	1	8	0		1		9	3	2	1	
Positive lymph-nodes														
No	1 (11)	1 (33)	8 (17)	5 (26)	3 (15)	1 (20)	0.3	0 (0)	/	8 (42)	4 (57)	4 (33)	1 (100)	0.1
NV	16	3	24	7	9	3		5	/	15	1	3	0	
Yes	8 (89)	2 (67)	39 (83)	14 (74)	17 (75)	4 (80)		5 (100)	/	11 (58)	3 (43)	8 (67)	0 (0)	
Residual tumor after surgery	2 (10)	1 (33)	15 (25)	11 (46)	12 (46)	5 (63)	0.02*	1 (11)	/	13 (41)	5 (71)	2 (18)	1 (100)	0.05*
No	17 (90)	2 (67)	45 (75)	13 (54)	14 (54)	3 (37)		8 (89)	/	19 (59)	2 (29)	9 (82)	0 (0)	
Yes	6	3	15	1	3	0		1		3	0	4	0	
NA														

INF = infiltrative; MP = micropapillary; PA = papillary; PSE = pseudo-endometrioid; SD = solid; TR = transitional-like. PDS = primary debulking surgery; NACT = neoadjuvant chemotherapy. NV = not evaluable. NA = data not available. *$P < 0.05$

linked to different responses to platinum therapy ($p = 0.02$) and debulking outcomes ($p = 0.02$), but only in patients submitted to PDS. In PDS group, patients with predominant transitional-like and pseudo-endometrioid tumors had the highest rate of no progression after first line chemotherapy (63% and 39% respectively), while those ones with infiltrative and papillary tumors were the most resistant (53% and 41% respectively). Accordingly, the highest frequencies of platinum sensitivity were recorded in pseudo-endometrioid, papillary and solid patterns (52%, 44% and 48% respectively). In women with predominant transitional-like features no resistance to chemotherapy was observed.

Residual tumor after debulking was higher in women with predominant infiltrative (90%), micropapillary (67%) and papillary (75%) patterns, while the highest rates of optimal cytoreduction were achieved in predominant pseudo-endometrioid, solid and transitional-like patients (46%, 46% and 63% respectively).

In NACT patients no evident associations between HGSOC growth patterns and clinical variables were detected, except for the rates of optimal cytoreduction ($p = 0.05$). High rates of tumor residual were frequently detected in predominant infiltrative (89%), papillary (59%) and solid (82%) tumors while low rates (29%) or absence (0%) of tumor were achieved in women with predominant pseudo-endometrioid and transitional-like patterns.

3.4. Histological features of SET and Classic groups

The histological analysis was carried out by grouping the 695 tumor slides into those exhibiting SET and Classic features. The results highlighted marked differences with respect to tumor histology and cytology, but only in patients submitted to PDS (Supplementary file and Supplementary Table 6).

3.5. Clinical features of SET and Classic groups

Associations between clinicopathological variables and the prevalence of SET or Classic features were investigated in patients submitted to PDS or treated with NACT (Table 2). In PDS group, women with predominant SET tumors were less resistant to platinum agents when compared to Classic ones (18% vs 48% respectively) and more frequently they did not relapse (34% vs 14%) ($p = 0.0006$). Moreover, SET predominant patients presented more often no residual tumor after debulking surgery ($p = 0.007$).

No evident associations between SET/Classic features and clinical variables were detected in NACT treated women.

3.6. Morphological heterogeneity in HGSOC

Morphological heterogeneity and its possible association with patient's outcome were searched between HGSOC patterns and the anatomical sites (ovaries, peritoneal implants and lymph nodes). In PDS group transitional-like, pseudo-endometrioid, papillary and solid architectures were more frequent in ovaries, while infiltrative and micropapillary patterns were typically observed in peritoneal implants ($p < 0.0001$). Lymph nodes were not associated to any morphology, although papillary and solid patterns were more frequent in this site. In NACT patients no significant associations were detected.

Since multiple HGSOC architectures coexist often in the same tumor slide, intratumor heterogeneity was measured by the Shannon diversity index and analyzed with respect of the clinical-histological parameters, sorting patients by the therapeutical strategies, namely NACT and PDS treatments. SDI was similar in patients submitted to PDS and to NACT ($p = 0.4$). The distribution of SDI differed with anatomical sites, showing higher values in ovaries and peritoneal implants when compared to lymph nodes in both PDS and NACT patients ($p = 0.0004$ and $p = 0.01$ respectively). No associations were found between the SDI and the clinical variables.

3.7. Survival analysis

Patients' OS and PFS were analyzed with respect of the architectural patterns, SET/Classic features and SDI (Fig. 2). OS differed significantly in patients treated with NACT and PDS among the six HGSOC patterns ($p = 0.001$ and $p = 0.05$ respectively). In PDS group, patients with predominant pseudo-endometrioid and transitional-like features had the best outcomes with a median survival of 92 and 91 months respectively. Micropapillary, solid and papillary patterns had intermediate outcomes (median survivals were respectively 30, 43 and 45 months), while infiltrative architecture had the poorest prognosis with a median survival of 22 months. Similarly, in NACT-treated patients, the predominant infiltrative and the pseudo-endometrioid patterns exhibited the poorest

Table 2
Clinical features correlated with SET and Classic groups in PDS and NACT patients.

	PDS (N = 165), n (%)			NACT (N = 69), n (%)		
Features	CLASSIC (*N* = 86)	SET (*N* = 79)	*P*	CLASSIC (*N* = 41)	SET (*N* = 28)	*P*
Age at diagnosis (mean, [range])	64 (32–82)	60 (34–79)	0.3	61 (42–81)	59 (31–76)	1
FIGO stage						
IIIC	49 (69)	53 (73)	0.6	28 (74)	20 (80)	0.6
IV	22 (31)	20 (27)		10 (26)	5 (20)	
NA	15	6		3	3	
Primary platinum response						
Never progressed	9 (14)	23 (34)	0.0006*	1 (3)	0 (0)	0.6
Resistant	28 (48)	12 (18)		16 (55)	11 (50)	
Sensitive	23 (39)	32 (48)		12 (41)	11 (50)	
NA	26	12		12	6	
Positive lymph-nodes						
No	8 (16)	11 (20)	0.3	7 (32)	10 (45)	0.09
NV	37	25		18	6	
Yes	41 (84)	43 (80)		15 (68)	12 (55)	
Residual tumor after surgery						
No	14 (21)	32 (43)	0.006*	11 (30)	11 (48)	0.2
Yes	51 (79)	42 (57)		26 (70)	12 (52)	
NA	21	5		4	5	

SET = "Solid, pseudo-Endometrioid, Transitional-like" features. Classic = "Classic" features. PDS = primary debulking surgery; NACT = neoadjuvant chemotherapy. NA = data not available. NV = not evaluable. *$P < 0.05$

and the best outcome respectively (median survival 13.5 vs 50 months), while solid and papillary had an intermediate prognosis (median survivals 25 and 33.5 months). The only patient with transitional-like features in NACT group had a survival of 20 months.

HGSOC growth patterns also influenced patients' PFS, but only in PDS group, where predominant transitional-like tumors tended to relapse significantly later. Furthermore, women with predominant pseudo-endometrioid tumors had longer PFS compared to those with predominant infiltrative and micropapillary tumors ($p = 0.008$ and $p = 0.01$ respectively).

Sorting patients by SET/Classic features, women with SET tumors had significant longer OS versus those with Classic ones in both PDS (median survival 60 vs 42 months; $p = 0.02$) and NACT (median survival 33 vs 23.5 months; $p = 0.05$) groups. Furthermore, SET features conferred longer PFS compared to Classic in patients submitted to PDS (median progression 16 vs 10 months; $p = 0.009$).

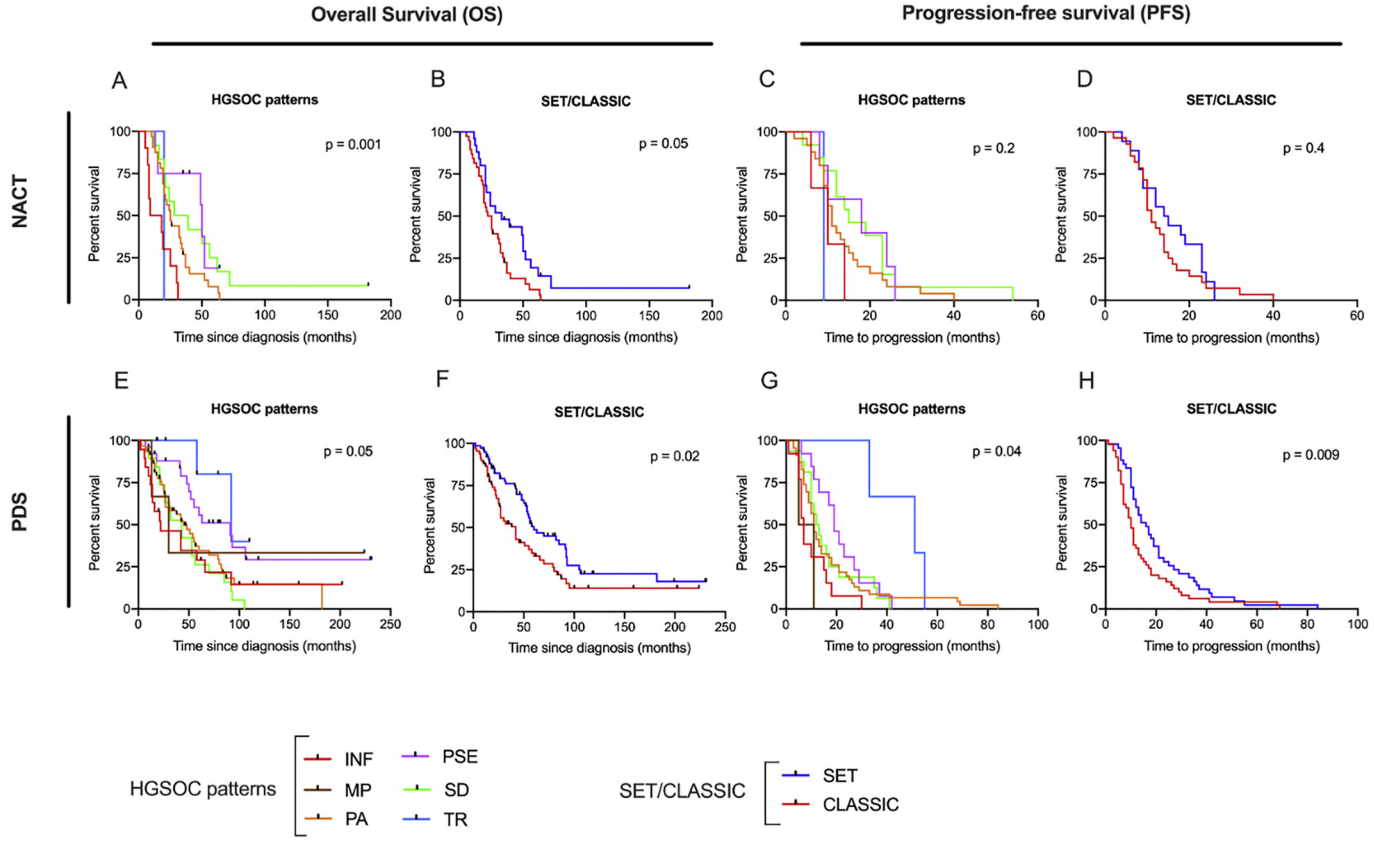

Fig. 2. Association between HGSOC growth patterns, SET/Classic features and survival (overall survival and progression-free survival) in patients treated with NACT (A-D) or submitted to PDS (E-H). PDS = primary debulking surgery; NACT = neoadjuvant chemotherapy. INF = infiltrative; MP = micropapillary; PA = papillary; PSE = pseudo-endometrioid; SD = solid; TR = transitional-like. SET = "Solid, pseudo-Endometrioid, Transitional-like features"; Classic = "Classic features".

Tumor heterogeneity, as defined by Shannon diversity index, did not influence patients' OS in univariate analysis, however, in PDS group, patients with high SDI relapsed earlier compared to those with low one (median progression at 11 vs 13 months respectively; $p = 0.03$) (Supplementary file -Supplementary Fig. 4).

Multivariate Cox regression was run to estimate the joint effects of the HGSOC patterns, SET/Classic features, SDI and clinical variables on patients' survival. According to histological and clinical information, OS analysis was carried out for 131 patients submitted to PDS and 58 patients submitted to NACT. The results showed that FIGO stage and SET/Classic features were independent prognostic factors for patients' OS independently from the therapeutical strategy. SDI and the presence of tumor residuals after primary cytoreduction were associated with patients' outcome only in the NACT-treated patients (Table 3).

In the alternative multivariate model considering HGSOC growth patterns (Table 3), infiltrative pattern resulted an independent predictor of poor OS, irrespective of the surgical strategy. In PDS group, patients with predominant infiltrative tumors had a risk of death about 3 times higher than patients with pseudo-endometrioid tumors (HR = 0.38; $p = 0.01$) and even 5 times higher than those with transitional-like ones (HR = 0.20; $p = 0.04$). In NACT group, the risk of death for predominant infiltrative patients was around 4.5 times higher than for those with pseudo-endometrioid tumors (HR = 0.22; $p = 0.02$) and almost 8 times higher than those having solid ones (HR = 0.13; $p \leq 0.0001$).

For PFS, 88 women in PDS group and 47 in NACT group were included in the analysis. In PDS group SET/Classic features were not associated with tumor progression but only a borderline association for the residual tumor after surgery ($p = 0.05$). In NACT-treated women no variable was prognostic (Table 3).

For PFS with HGSOC architectural patterns (Table 3), in PDS group a risk of recurrence almost 4 time higher for patients with infiltrative tumors than those with transitional-like ones (HR = 0.26; p = 0.05) was recorded. Similarly, in NACT group, the recurrence risk for patients with infiltrative pattern was around 3 times higher than for women with solid tumors (HR = 0.32; $p = 0.04$).

In the multivariate Cox regression, the growth patterns were an independent prognostic factor for OS in both PDS and NACT-treated groups and for PFS only in patients submitted to PDS.

Table 3
Prognostic factors for OS and PFS identified by multivariate Cox regression analysis in PDS and NACT patients.

Overall Survival (OS)						
	PDS ($N = 131$)			NACT ($N = 58$)		
Cox Model 1	HR	95% CI	*P*	HR	95% CI	*P*
Age at diagnosis	1.02	1.00–1.04	0.08	1.01	0.97–1.04	0.7
FIGO stage (IIIC/IV)	2.45	1.52–3.93	0.0002*	1.90	0.97–3.72	0.06
Residual tumor after surgery (Yes/No)	1.35	0.83–2.26	0.2	1.96	1.05–3.65	0.03*
SET/CLASSIC features	1.59	1.02–2.46	0.04*	2.64	1.33–5.24	0.006*
SDI (High/Low)	1.05	0.68–1.63	0.8	1.98	1.08–3.62	0.03*
		PhTest: p = 0.2			*PhTest: p = 0.9*	
Cox Model 2						
Age at diagnosis	1.02	1.00–1.04	0.06	1.01	0.97–1.05	0.7
FIGO stage (IIIC/IV)	2.41	1.46–4.01	0.001*	1.75	0.88–3.50	0.1
Residual tumor after surgery (Yes/No)	1.19	0.71–2.00	0.5	1.88	1.02–3.45	0.04*
HGSOC growth patterns						
Infiltrative*	1			1		
Micropapillary	0.51	0.10–2.52	0.4			
Papillary	0.58	0.29–1.15	0.1	0.45	0.20–1.02	0.06
Pseudo-endometrioid	0.36	0.16–0.80	0.01*	0.22	0.06–0.81	0.02*
Solid	0.56	0.26–1.22	0.1	0.13	0.04–0.39	<0.0001*
Transitional-like	0.20	0.04–0.91	0.04*	2.44	0.26–22.65	0.4
SDI (High/Low)	0.99	0.63–1.55	1	1.84	1.02–3.32	0.04*
		PhTest: p = 0.1			*PhTest: p = 0.9*	
Progression free survival (PFS)						
	PDS ($N = 88$)			NACT ($N = 47$)		
Cox Model 1	HR	95% CI	*P*	HR	95% CI	*P*
Age at diagnosis	1.00	0.99–1.02	0.7	0.98	0.95–1.01	0.2
FIGO stage (IIIC/IV)	1.02	0.63–1.65	0.9	1.84	0.85–3.99	0.1
Residual tumor after surgery (Yes/No)	1.72	1.00–2.97	0.05	1.58	0.83–3.00	0.2
SET/CLASSIC features	1.36	0.86–2.14	0.2	1.31	0.64–2.64	0.5
SDI (High/Low)	1.38	0.88–2.17	0.2	1.21	0.62–2.38	0.6
		PhTest: p = 0.1			*PhTest: p = 0.9*	
Cox Model 2						
Age at diagnosis	1.00	0.99–1.02	0.5	0.98	0.95–1.01	0.4
FIGO stage (IIIC/IV)	1.08	0.65–1.81	0.8	2.04	0.90–4.68	0.09
Residual tumor after surgery (Yes/No)	1.57	0.90–2.75	0.1	1.78	0.91–3.46	0.09
HGSOC growth patterns						
Infiltrative*	1			1		
Micropapillary	1.77	0.38–8.24	0.5			
Papillary	0.56	0.28–1.10	0.09	0.59	1.21–1.61	0.3
Pseudo-endometrioid	0.49	0.21–1.13	0.09	0.40	0.11–1.44	0.2
Solid	0.56	0.25–1.26	0.2	0.32	0.11–0.96	0.04*
Transitional-like	0.26	0.07–0.98	0.05*	2.48	0.26–23.6	0.4
SDI (High/Low)	1.35	0.83–2.18	0.2	1.35	0.68–2.67	0.4
		PhTest: p = 0.07			*PhTest: p = 1*	

HR indicates Hazard Ratio; CI, confidence intervals; PDS, primary debulking surgery; NACT, neoadjuvant chemotherapy; SDI, Shannon Diversity Index; OS, overall survival; PFS, progression-free survival; PhTest, Schoenfeld's residual test. *$P < 0.05$. * Infiltrative pattern is considered as baseline.

4. Discussion

In the present study we investigated the associations between morphological patterns, clinical factors and patients' survival in advanced HGSOC. Architectural patterns as defined by Soslow and colleagues [15] were identified in 695 samples from 234 patients. Our results indicate that a detailed morphological analysis of HGSOC with the definition of characteristic architectural patterns can help in prognostication. Architectural patterns grouped as SET/classic resulted, indeed, to be an independent variable influencing overall survival independently from the surgical strategy (interval surgery after NACT and PDS) conferring a hazard ratio of 1.8 for classic features. This result was also valid sorting cases by surgical strategies. However, the OS curves for each architectural pattern within SET group varied greatly considering the therapeutical strategies. In patients submitted to PDS, women with pseudo-endometrioid and transitional architectures survived significantly longer than those with a solid pattern, in agreement with Winterhoff and colleagues who found improved outcome in stage III/IV patients with endometrioid-like HGSOC [23]. On the contrary, in women treated with NACT those with solid pattern tumors had a better prognosis characterized by longer overall survival. This apparent discrepancy on solid pattern is more likely due to the small sample size of the NACT group rather than an effect of the chemotherapy. Possible therapeutical effects on the architectural patterns include the overall decrement of mitoses and increment of psammoma bodies in NACT, as observed in our series. It is well known, indeed, that chemotherapy has an effect on the morphology of ovarian cancers where pronounced stromal changes, including many free psammoma bodies, have been reported as effects of NACT in responders [24] and a decrement in mitoses was also observed [20]. Naïve SET tumors are characterized by higher mitotic counts representing a possible prerequisite of response to taxanes which are mitosis inhibitors [25]. The better prognosis of SET tumors is supported by the observation that in our cohort 63% of transitional-like tumors submitted to PDS did not progress during the follow-up period and had the highest rate of optimal cytoreduction among HGSOC patterns. Similar results by other authors indicated that ovarian carcinomas containing predominant transitional-like pattern have an excellent response to different chemotherapy regimens, surgical complete response and longer survival [26–28]. Architectural pattern have been also recently characterized by means of biomechanical tools showing a higher softness, therefore higher deformability and aggressiveness, for micropapillary and partially for papillary patterns [29].

Solid HGSOCs are characterized in our cohort by the lowest immunohistochemical expression of BRCA1 (mean H score 15) as surrogate of silencing mutations of the gene. Theoretically, BRCA1 mutated HGSOC should be chemosensitive and have improved survival [30]. That result is clearly visible in solid HGSOC of NACT treated patients, because they represent a sub-group of patients who had at least a partial response to NACT since they were submitted to interval surgery. Notwithstanding, in PDS treated patients, solid HGSOCs seem to be more heterogeneous in terms of outcome as only solid HGSOCs exhibiting higher mitotic counts and lower heterogeneity, as defined by the Shannon index, had a longer survival (see Supplementary Fig. 5 and 6).

The infiltrative pattern resulted in our series an independent predictor of poor OS, independently from the surgical strategy in agreement with Hussein and colleagues [31]. As in PDS patients infiltrative pattern is characterized by lower mitotic counts and the highest BRCA1 expression (mean value 79) this could be a possible indication of non-response to chemotherapy. The mean value of BRCA1 H score found in infiltrative pattern is indicative of BRCA1 proficiency, in agreement with other authors who reported that cut-off value to consider loss of BRCA1 expression was 10% of positive cells or H score cut-offs ranging from 47 to 70 [32]. Although not directly proven in the present study, there is the possibility that infiltrative morphological pattern together with positive BRCA1 immunohistochemistry could be indicative of proficient homolog recombination. In addition, infiltrative pattern was recorded more frequently in the peritoneal sites compared to ovaries, suggesting a higher dissemination potential or a particular trophism for peritoneal tissues.

Most associations of architectural patterns with clinical data were confined to patients treated with PDS in our series. We acknowledge that the sample size of the NACT patients is small for further subdivision in architectural patterns, therefore results obtained in that group should be confirmed. Nonetheless, possible changes to morphological features, including cytological alterations and altered histologic typing in NACT could be ascribed to the effect of chemotherapy [24]. In agreement with Bromley et al. [20], lower rate of tumors with pseudo-endometrioid and transitional features were detected in NACT in comparison to PDS group.

Our study has also been dealing with morphological intratumor heterogeneity, which we have tried to quantify for the first time by the application of the Shannon diversity index. HGSOCs are, as a matter of common knowledge, characterized by a higher level of intratumor heterogeneity, which is one of the main causes of its acquired resistance to chemotherapy [33]. In our study we have classified the different slides from the same patients considering the prevalent architectural pattern. Intratumor heterogeneity was highlighted both intra-slide, recording multiple architectures, and intra-patient among different anatomical sites. Taken this heterogeneity, it is highly misleading to characterize patient's tumor based on the evaluation of only one single slide. The analysis of a proper number of slides from different tumor locations is of paramount importance for the stratification of patients using histomorphological criteria. Heterogeneity as defined by Shannon index influenced negatively both patients' overall survival in NACT group on multivariate analysis (Table 3) and progression free survival in PDS group on univariate analysis (Supplementary file- Supplementary Fig. 4). Those results can have an explanation in the presence of different cells' populations that can respond differently to chemotherapy. As a matter of fact, in naïve SET group, only architectural patterns at low index of diversity (transitional-like and pseudo-endometrioid) had better outcomes. Solid pattern, instead, which has been frequently found in association with other architectures and in particular with papillary (data not shown) had an average overall survival. Nevertheless, solid tumors with lower Shannon diversity index had significant better outcomes compared to the higher ones (see Supplementary Fig. 6).

We acknowledge as limitations of the present study that only platinum-based chemotherapy was included among treatments although in recent years also PARP inhibitors and Bevacizumab have been introduced in clinical practice. Furthermore, our study is monocentric and the selection of patients for NACT was based merely on the clinical evaluation of the surgeons rather than to randomization. However, our results show that a comprehensive histologic examination considering architectural patterns and their heterogeneity can help in prognostication.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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