RESEARCH ARTICLE

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Schlafen 11 (SLFN11) and Tumor-Infiltrating Lymphocytes (TILs): Dual Predictive Biomarkers in Ovarian Serous Carcinoma

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Abstract

Objectives: This study aims to investigate the clinicopathological significance and prognostic impact of *SLFN11* expression and tumor-infiltrating lymphocytes (TILs) in high-grade and low-grade ovarian serous carcinoma. **Methods:** A total of 70 patients diagnosed with high-grade and low-grade serous carcinoma were retrospectively analyzed. Clinical data, including age, Karnofsky Performance Status (KPS), CA125 levels, treatment details, and survival outcomes, were collected. Immunohistochemistry was used to assess *SLFN11* expression in tumor cells and TILs. Statistical correlations were performed with chemotherapy response, recurrence, progression-free survival (PFS), and overall survival (OS). **Results:** High *SLFN11* expression was significantly associated with better response to neoadjuvant chemotherapy ($p = 0.003$), higher histopathologic chemotherapy response score ($p = 0.026$), lower recurrence rate ($p = 0.037$), and improved survival outcomes ($p < 0.001$). High *SLFN11* expression had significantly longer PFS (median= 33.05 months) and OS (median= 66.91 months), compared to those with low *SLFN11* expression (median PFS =7.60 and median OS = 31.50, $p<0.001$). Similarly, high TILs count was associated with improved response to neoadjuvant chemotherapy ($p = 0.008$) and higher response score ($p = 0.007$). TILs-high patients had longer median PFS (25.52 months) and median OS (68.24 months) than TILs-low patients (median PFS = 10.30 and median OS = 30.63 months, $p<0.001$). **Conclusion:** Our findings highlight that immunohistochemical expression of *SLFN11* and the density of TILs may serve as predictive biomarkers for chemotherapy response and survival in ovarian serous carcinoma, with potential implications for personalized treatment strategies.

Keywords: Schlafen 11 (*SLFN11*)- Tumor-Infiltrating Lymphocytes (TILs)- Ovarian Serous Carcinoma- Survival

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Introduction

Serous ovarian carcinoma, the most common and aggressive subtype of ovarian cancer, is characterized by rapid progression and poor prognosis, as most patients diagnosed at advanced stages [1]. Despite advances in cytoreductive surgery and platinum-based chemotherapy, relapse rates remain high, underscoring the need for biomarkers to guide personalized therapies [2]. Schlafen11 (*SLFN11*), a DNA/RNA helicase implicated in DNA damage response and replication stress, has emerged as a promising predictive and prognostic biomarker in serous ovarian carcinoma [3].

Functionally, *SLFN11* induces irreversible replication arrest in response to DNA-damaging agents by blocking cell cycle progression at stalled replication forks. This mechanism has positioned *SLFN11* as a determinant of sensitivity to DNA-damaging agents across cancers, including serous ovarian carcinoma [4]. Immunohistochemical assays have standardized *SLFN11* assessment in serous ovarian carcinoma tissues, revealing variable expression patterns [5]. Clinically, high *SLFN11* expression correlates with improved platinum response, as demonstrated by longer progression-free survival and overall survival in high-grade serous carcinoma (HGSC) patients [6].

Tumor-infiltrating lymphocytes (TILs) have been shown to express *SLFN11*, indicating a potential dual function in both immune modulation and the regulation of cancer cell-intrinsic DNA repair mechanisms [7]. TILs

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themselves are prognostic in HGSC, with higher CD8+ infiltrates associated with improved survival [8].

Standardized immunohistochemistry protocols enable reproducible *SLFN11* scoring, with studies validating its clinical utility [9]. Similarly, intraepithelial TILs-high patients demonstrate superior outcomes, with extending periods of progression-free survival and overall survival [10].

Currently, there is limited data on how *SLFN11* expression in tumor cells and TILs correlate with clinicopathological features and treatment response. This study aims to evaluate the immunohistochemical expression of *SLFN11* in both tumor cells and TILs in serous ovarian carcinoma. It investigates the relationship between *SLFN11* expression and clinicopathological variables as well as responses to platinum-based chemotherapy.

Materials and Methods

Study design and patient selection

This retrospective study analyzed 70 specimens of ovarian serous carcinoma (high-grade and low-grade) obtained from the archives of the Pathology Laboratory at Woman Health Hospital, Assiut University, between 2017 and 2024. Cases were selected based on histological confirmation via H&E-stained slides. Inclusion criteria comprised patients over 40 years of age with histologically confirmed ovarian serous carcinoma (stages I-III and all grades), available follow-up data, and treatment with platinum-based drugs, bevacizumab, or poly-adenosine diphosphate ribose polymerase (PARP) inhibitors. Exclusion criteria involved specimens with secondary infection, extensive necrosis, and incomplete medical records.

Clinical and pathological data collection

Clinicopathological data were retrieved from the Pathology and Oncology Departments at Assiut University and South Egypt Cancer Institute, including patient age, Karnofsky Performance Status (KPS) score, CA125 levels (pre- and post-treatment), surgical procedures, tumor laterality, size, outer capsule status, grade, lymphovascular invasion, pathological stage according to American joint committee on cancer TNM 8th edition and FIGO staging, ascitic fluid cytology, treatment type (adjuvant/neoadjuvant chemotherapy),progression-free survival measured from time of starting treatment till progression or death and overall survival measured from time of diagnosis till death or missed follow up. Surgical interventions included primary cytoreductive surgery (total hysterectomy with bilateral salpingo-oophorectomy and debulking), interval debulking after neoadjuvant chemotherapy, or fertility-sparing unilateral salpingo-oophorectomy for early-stage cases. Chemotherapy regimens included platinum-based therapy (paclitaxel/carboplatin) for HGSC and cytoreduction with adjuvant chemotherapy/hormonal therapy for advanced low-grade serous carcinoma (LGSC) cases [1]. Response assessment following neoadjuvant chemotherapy and interval debulking was performed clinically using Response Evaluation Criteria in Solid Tumors (RECIST) criteria V1.1 and histopathologically using the chemotherapy response score [12]. Chemotherapy response score categorizes HGSC response as chemotherapy response score 1 (CRS 1: no/minimal response), chemotherapy response score 2 (CRS 2: partial response), and chemotherapy response score 3 (CRS 3: complete or near-complete response) based on residual tumor and regression features [13].

Immunohistochemical staining and evaluation

Immunohistochemistry staining was performed on formalin-fixed, paraffin-embedded sections using *SLFN11* mouse monoclonal antibodies (Medaysis, USA, 2023) via the avidin-biotin-peroxidase method. Tissue sections underwent antigen retrieval in ethylenediaminetetraacetic acid (EDTA) buffer (pH 9), peroxidase blocking, and overnight incubation with primary antibody (1:50 dilution). Detection employed horseradish peroxidase (HRP)-conjugated secondary antibody and 3,3′-diaminobenzidine (DAB) chromogen, followed by hematoxylin counterstaining.

SLFN11 expression was scored independently by two pathologists using the Immunoreactivity Score, calculated as the product of intensity (0-3) and distribution (0-3) scores. Immunoreactivity Score categories were defined as negative (0), mild (1-2), moderate (3-4), or high (6-9) (Figure 1) [14]. Tumor-infiltrating lymphocytes (TILs) in high-grade serous carcinoma were assessed in the most prominent hotspot per high-power field (400x) and graded as absent (0), low (1-2), moderate (3-19), or high (≥20) (Figure 2) [8].

Statistical analysis

Data were analyzed using SPSS v.28 (IBM), with significance set at $p < 0.05$ (two-tailed). Continuous variables were reported as mean ± SD (parametric) or frequencies (non-parametric). Categorical variables (e.g., demographics, tumor characteristics, treatment) were compared using Fisher’s exact test. Survival outcomes (progression-free survival and overall survival) were analyzed via Kaplan-Meier curves with 95% confidence intervals (95% CI).

Ethical approval

This study was approved by the Institutional Review Board (IRB), Faculty of Medicine, Assiut University. The IRB local approval number is 17101754.

Results

Clinicopathological findings

This study analyzed 70 serous ovarian carcinoma cases, with 64.3% were ≥50 years old. Most patients (74.3%) had a good performance status (KPS ≥70%). Tumors were predominantly bilateral (75.7%), high-grade (87.1%), and advanced stage (65.7% T3, 74.3% FIGO III). Lymphovascular invasion (81.4%) and elevated CA125 (>35 U/mL, 84.3%) were noted. Treatment involved adjuvant chemotherapy (58.6%) or neoadjuvant chemotherapy (41.4%), with 81.4% completing 6 cycles.

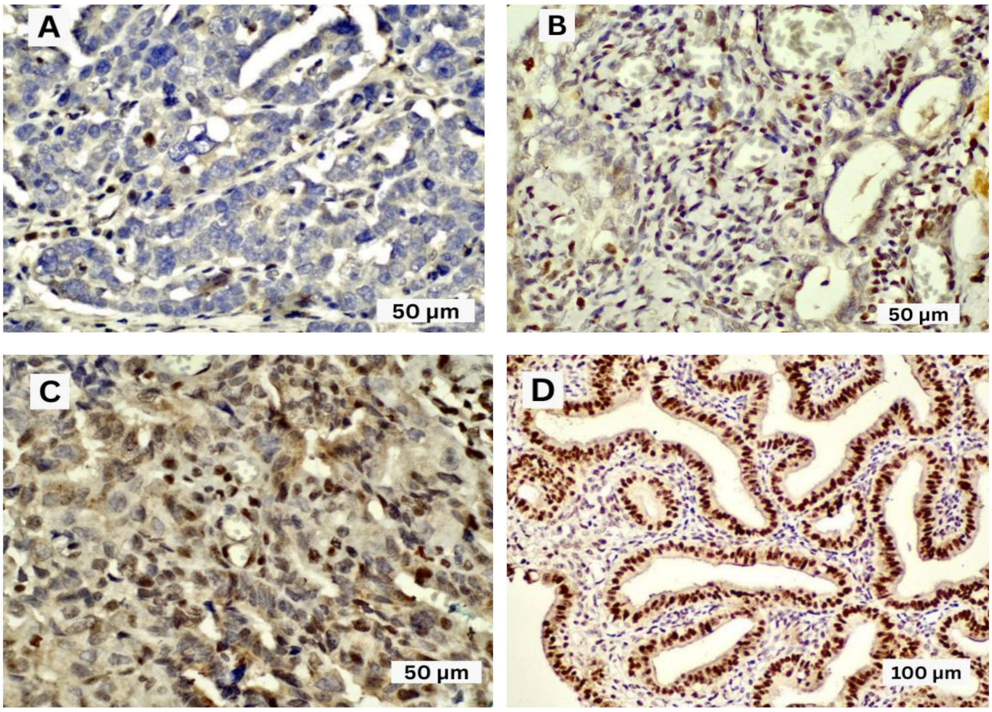

Figure 1. Immunohistochemical staining of *SLFN11* in serous ovarian carcinoma. A: *SLFN11* mild nuclear expression in high-grade serous carcinoma (IHC, x400). B: *SLFN11* moderate nuclear expression in high-grade serous carcinoma (IHC, x400). C: *SLFN11* high nuclear expression in high-grade serous carcinoma (IHC, x400). D: *SLFN11* high nuclear expression in low-grade serous carcinoma (IHC, x200).

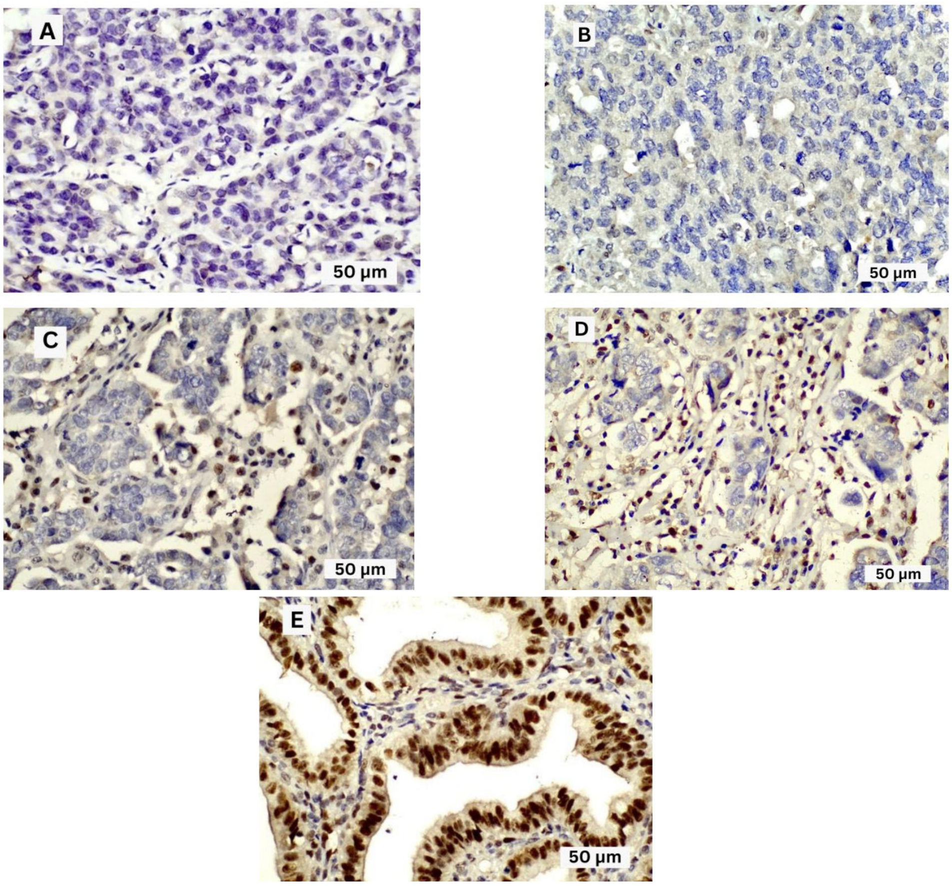

Figure 2. Immunohistochemical Analysis of Tumor-Infiltrating Lymphocytes (TILs) and *SLFN11* Expression in Serous Ovarian Carcinoma. A: High-grade serous carcinoma with absent intra-tumoral TILs, (IHC, x400).B: High-grade serous carcinoma with low intra-tumoral TILs count (IHC, x400).C: High-grade serous carcinoma with moderate intra-tumoral TILs count (IHC, x400).D: High-grade serous carcinoma showing high intra-tumoral TILs count (IHC, x400).E: Low-grade serous carcinoma with absent intra-tumoral TILs (IHC, x400).

Table 1. Demographic Data, Tumor Characteristics, Treatment Modalities, Outcome of Ovarian Serous Carcinoma Cases (n=70), treatment response in NACT cases (n=29), immunoreactivity score (n=70) and TILs count in HGSC patients (n=61).

	N=70	%
Age: (years)		
< 50	25	35.70
≥ 50	45	64.30
Karnofsky performance status (KPS) score (%)		
50 – 60	18	25.70
≥ 70	52	74.30
Tumor size (largest) cm		
< 8.5	34	48.60
8.5	36	51.40
Median (IQR)	8.5 (5.0-15.0)	
Laterality		
Unilateral	17	24.30
Bilateral	53	75.70
Capsule		
Intact	43	61.40
Ruptured	27	38.60
Grade		
Low	9	12.90
High	61	87.10
Lymphovascular invasion (LVI)		
Present	57	81.40
Absent	13	18.60
Lymph nodes		
Positive	27	38.60
Free	14	20.00
Not resected (Nx)	29	41.40
TNM staging		
T		
T1	13	18.60
T2	11	15.70
T3	46	65.70
N		
N0	14	20.00
N1	27	38.60
Nx	29	41.40
FIGO staging		
I	11	15.70
II	7	10.00
III	52	74.30
Cytology:		
Positive	30	42.90
Negative	11	15.70
Not done	29	41.40
CA125 before treatment		
≤ 35	11	15.70
> 35	59	84.30
Median (IQR)	201.0 (48.4-510.0)	
CA125 after treatment:		
≤ 35	59	84.30
> 35	11	15.70
Median (IQR)	13.6 (6.9-28.5)	
Type of treatment		
Adjuvant	41	58.60
Neoadjuvant	29	41.40
Number of chemotherapy cycles:		
< 6 cycles	13	18.60
6 cycles	57	81.40
Outcome		
Alive	53	75.70
Dead	17	24.30
Recurrence		
No	20	28.60
Yes	50	71.40
Response to neoadjuvant chemotherapy in high-grade serous carcinoma cases	N=29	
Clinical response:		
Partial response (PR)	22	75.90
Stable disease (SD)	7	24.10
Histopathological chemotherapy response score (CRS):		
CRS 1 / 2	15	51.70
CRS 3	14	48.30
Immunoreactivity score (IRS)	N=70	
Mild	18	25.70
Moderate	27	38.60
High	25	35.70
Intra-tumoral TILs count in high-grade serous carcinoma	N=61	
Absent	4	6.60
Low	14	23.00
Moderate	34	55.70
High	9	14.80

Note that response to neoadjuvant chemotherapy is only assessed in high-grade serous carcinoma (n=29). Intra-tumoral tumor infiltrating lymphocytes (TILs) were counted in high-grade serous carcinoma cases received adjuvant and NACT (n=61). TNM; Tumor, Node, Metastasis (Union for International Cancer Control (UICC) staging), FIGO; International Federation of Gynecology and Obstetrics, TILs; Tumor-Infiltrating Lymphocytes.

Neoadjuvant cases showed 75.9% response (PR) and 51.7% poor histopathological response (chemotherapy response score 1/2). The mean overall survival of all patients was 36 months (median = 59.03 months), and mean progression-free survival of all patients was 18 months (median = 26.37 months). *SLFN11* expression was high/moderate in 74.3% of cases. *SLFN11* expression in TILs in HGSC cases showed that 55.7% had moderate TILs and 14.8% had high TILs count. Detailed characteristics of the patients are shown in (Table 1).

Table 2. Relationship between *SLFN11* Expression and the Clinicopathological Parameters, Treatment Type, Patient Outcome, Recurrence of the Patients Studied (n= 70) and Response to NACT (n=29).

	SLFN11 expression				P-value
	SLFN11 low (mild)		*SLFN11* High (moderate/high)		
	No.	%	No.	%	
Age: (years)					
< 50	2	11.00	23	44.20	0.064
≥ 50	16	89.00	29	55.70	
Karnofsky performance status (KPS) score (%):					
50 – 60	9	50	9	17.30	0.087
≥ 70	9	50	43	82.60	
Tumor size (cm):					
<8.5	7	38.90	27	52	0.522
8.5	11	61.10	25	48	
Laterality					
Unilateral	4	22.20	13	25	0.976
Bilateral	14	77.80	39	75	
Capsule					
Intact	7	38.90	36	69	0.141
Ruptured	11	61.10	16	31	
Grade					
Low	0	0	9	17.30	0.302
High	18	100	43	82.70	
Lymphovascular invasion (LVI)					
Present	16	89	41	78.80	0.653
Absent	2	11	11	21.00	
Staging T					
T1 / T2	5	27.80	19	36.50	0.897
T3	13	72.20	33	63.50	
Staging N (n=41)					
N0	4	30.80	10	35.70	0.988
N1	9	69.20	18	64.30	
FIGO staging					
I / II	4	22	14	27	0.898
III	14	78	38	73	
Cytology (n=41)					
Positive	11	84.60	19	67.80	0.621
Negative	2	15.40	9	32.20	
CA125 levels before treatment (U/ml)					
≤ 35	0	0	11	21	0.308
> 35	18	100	41	79	
CA125 levels after treatment (U/ml):					
≤ 35	7	38.90	52	100	<0.001*
> 35	11	61.10	0	0	
Type of treatment					
Adjuvant	9	50	32	61.50	0.711
Neoadjuvant	9	50	20	38.50	
Clinical response in NACT patients (n=29)					
Partial response (PR)	2	22.20	20	100	0.003*
Stable disease (SD)	7	77.80	0	0	

*, Statistically significant difference if p <0.05. *SLFN11:* Schlafen 11. T, Tumor (Union for International Cancer Control (UICC) staging); N, Node (Union for International Cancer Control (UICC) staging); FIGO, International Federation of Gynecology and Obstetrics.

Table 2. Continued

	SLFN11 expression				P-value
	SLFN11 low (mild)		*SLFN11* High (moderate/high)		
	No.	%	No.	%	
Histopathological chemotherapy response score (CRS) in NACT patients (n=29)					
CRS 1 / 2	9	100	6	30	0.026*
CRS 3	0	0	14	70	
Patient outcome					
Alive	5	27.70	48	92.30	<0.001*
Dead	13	72.30	4	7.70	
Recurrence:					
No	0	0	20	38.50	0.037*
Yes	18	100	32	61.50	

*, Statistically significant difference if p <0.05. *SLFN11:* Schlafen 11. T, Tumor (Union for International Cancer Control (UICC) staging); N, Node (Union for International Cancer Control (UICC) staging); FIGO, International Federation of Gynecology and Obstetrics.

Relationship between SLFN11 expression in tumor cells and clinicopathological findings and treatment response

Table 2 shows that patients with high *SLFN11* expression exhibited significantly better CA125 normalization post-treatment (100% ≤35 U/mL in high *SLFN11* patients vs. 38.9% in low *SLFN11*, p <0.001). Additionally, clinical response to chemotherapy correlated with *SLFN11* expression (p = 0.003), where all of partial responders had high *SLFN11*, while 77.8% of stable disease cases exhibited low expression. Similarly, the histopathological chemotherapy response score was significantly linked to *SLFN11* (p = 0.026), with all patients showing chemotherapy response score 1 or 2 (poor responders) exhibited low *SLFN11* expression, whereas 70% of patients with chemotherapy response score 3 (good responders) had high *SLFN11*. Mortality was also strongly associated with low *SLFN11* (72.3% of deaths in low expressors vs. 7.7% of deaths in high expressors, p <0.001), and recurrence was more frequent in low-*SLFN11* cases (100% recurrence in low *SLFN11* expression vs. 61.5% in high *SLFN11* expression, p = 0.037).

Borderline associations were noted with age (p = 0.064), where 89% of low-*SLFN11* patients were ≥50 years, and KPS score (p = 0.087), as high *SLFN11* was more common in patients with better performance status (KPS ≥70%, 82.6% in high *SLFN11* patients vs. 50% in low *SLFN11* patients). However, no significant links were found with tumor size, laterality, grade, FIGO staging, or pre-treatment CA125.

Relationship between TILs count and treatment response

Table 3 illustrates that TILs count significantly correlated with treatment response: all patients with high TILs achieved partial response to neoadjuvant chemotherapy, whereas 63.6% of low-TILs cases had stable disease (p =0.008). Similarly, histopathological

Table 3. Relationship between Tumor-Infiltrating Lymphocytes (TILs) Count and Treatment Response (n=29), Patient Outcome and Recurrence of High-Grade Serous Carcinoma Patients (n= 61)

	TILs count Absent/ Low		TILs count Moderate/ High		P-value
Type of treatment (n=61)					
Adjuvant	7	38.90%	25	58.10%	0.457
Neoadjuvant	11	61.10%	18	41.90%	
Clinical response (n=29)					
Partial response	4	36.40%	18	100%	0.008*
Stable disease	7	63.60%	0	0%	
Histopathological chemotherapy response score (CRS) (n=29)					
CRS 1/2	11	100%	4	22.20%	0.007*
CRS 3	0	0%	14	77.80%	
Patient outcome (n=61)					
Alive	5	27.80%	39	90.70%	0.001*
Died	13	72.20%	4	9.30%	
Recurrence (n=61)					
No	2	11.10%	13	30.20%	0.385
Yes	16	88.90%	30	69.80%	

*, Statistically significant if p-value <0.05; TILs, Tumor-Infiltrating Lymphocytes.

Table 4. Progression Free Survival, Overall Survival According to *SLFN11* Expression in the Studied Cases (n=70) and the TILs Count in High-Grade Serous Carcinoma Cases (n=61)

SLFN11 expression (n=70)	Median	95% CI		P-value
		lower	Upper	
Progression-free survival				<0.001*
SLFN11 low (Mild)	7.6	6.29	8.91	
SLFN11 high (Moderate/ High)	33.05	25.6	40.5	
Overall survival				
SLFN11 low (mild)	31.5	21.81	41.19	<0.001*
SLFN11 high (Moderate/ High)	66.91	60.36	73.46	
Intra-tumoral TILs count (n=61)	**Median**	**95% CI**		**P-value**
		lower	Upper	
Progression-free survival				<0.001*
Absent/ Low	10.3	4.85	15.75	
Moderate/ High	25.52	19.7	31.35	
Overall survival				
Absent/ Low	30.63	21.08	40.17	<0.001*
Moderate/ High	68.24	63.15	73.34	

95% CI: 95% Confidence Interval, *, Statistically significant difference if p <0.05, Survival; analysis was done by Kaplan-Meier curve, *SLFN11,* Schlafen 11; TILs, Tumor-Infiltrating Lymphocytes.

response was strongly associated with TILs (p =0.007). All low-TILs cases exhibited poor response (chemotherapy response score 1/2), while 77.8% of high-TILs cases showed chemotherapy response score 3. Survival outcomes were markedly better in high-TILs patients: 90.7% were alive in TILs high patients and 72.2% mortality in low-TILs cases (p =0.001). However, no association was found between TILs count and treatment type (adjuvant/neoadjuvant) or recurrence rates.

Relationship between SLFN11 expression in both tumors cells and TILs count and survival

Table 4 showed survival outcomes regarding *SLFN11* expression in tumor cells and TILs count in HGSC. Survival was markedly influenced by *SLFN11* expression. Patients with high *SLFN11* had significantly longer progression-free survival (median= 33.05 vs. 7.60 months, p <0.001) and overall survival (median= 66.91 vs. 31.50 months, p <0.001). Progression-free survival was significantly longer in high-TILs groups (median= 25.52 vs. 10.30 months, p <0.001), as was the overall survival (median= 68.24 vs. 30.63 months, p <0.001).

Discussion

This study highlights the clinical significance of *SLFN11* expression and TILs in HGSC and LGSC of the ovary. High *SLFN11* expression was significantly associated with improved response to platinum-based chemotherapy and better survival outcomes. Patients with high *SLFN11* exhibited complete CA125 normalization post-treatment. Also, superior clinical response to neoadjuvant chemotherapy and chemotherapy response scores were noted in high *SLFN11* expression. All responding patients achieved partial response not complete response as more than half of the patients had their surgery before starting neoadjuvant treatment and residual was evident and none of patients who were on neoadjuvant chemotherapy received target therapy as no financial covering of target treatment as all our patients had no health insurance covering. Most notably, high *SLFN11* expression predicted significantly longer progression-free survival and overall survival. Higher TILs count further enhanced treatment response and favorable survival outcomes.

Our study revealed characteristic features of serous ovarian carcinoma, including frequent bilaterality (75.7%) and median tumor size of 8.5 cm. The high rate of bilaterality is contributed to the proposed tubal origin of HGSC [15]. LGSC represented 12.9% of cases, consistent with its known rarity [16]. Lymphovascular invasion was prevalent (81.4%), but reported rates vary, due to differences in angioinvasion across tumor grades (HGSC vs LGSC) [17]. 42.9% of patients showed malignant cells in ascitic fluid or peritoneal washings, whereas Purbadi et al. [18] observed a lower rate (28.5%), likely due to a greater proportion of early-stage disease. Notably, 41.4% of patients lacked cytological data -a higher percentage than reported by Bansal et al. (17.6%) [19]- despite recommendations advocating routine cytological assessment, particularly given its diagnostic value in early-stage ovarian and fallopian tube tumors.

Primary debulking surgery was performed in 58.6% of patients, consistent with standard protocols [20], while 81.4% completed six platinum-based chemotherapy cycles - critical for optimal outcomes [21]. Neoadjuvant chemotherapy yielded 75.9% of patients with overall good clinical response rate (partial response) and 24.1% showed stable disease, comparable to Cobb et al. [22] Plett et al. stated that more patients showed complete/partial response (88.2%) and less patients with stable disease (8.2%) [23], with variations attributable to assessment timing or tumor biology. Histopathological assessment showed 48.3% achieving chemotherapy response score 3, exceeding previous reports [24], reflecting methodological differences in tissue sampling/scoring [25].

Recurrence occurred in 71.4% and 75.7% of patients were alive at follow-up - higher than Nogales et al. (30%) [26]. Mean overall survival was 36 months and progression-free survival was 18 months, which aligned with Plett et al. [23], though variations exist due to stage distribution and treatment protocols.

We demonstrated that predominant nuclear *SLFN11* expression in tumor cells, consistent with prior reports (Figure 1) [4, 27], though cytoplasmic localization noted in other studies may reflect tissue processing artifacts or viral-related functions [14, 28]. High *SLFN11* expression significantly correlated with post-treatment CA125 normalization ($p < 0.001$), suggesting enhanced chemosensitivity, which is a novel association reflecting the role of CA125 as a response predictor [29]. Low *SLFN11* expression showed borderline associations with older age (≥50 years, $p = 0.064$) and poorer performance status (KPS, $p = 0.087$). The link with age is consistent with findings in esophageal carcinoma [30], suggesting a potential role for age-related epigenetic silencing, while the association with KPS may reflect additional factors influencing *SLFN11* downregulation. Winkler et al. [2] restricted their study to high performance patients, suggesting that performance status may influence *SLFN11* related tumor biology. Notably, *SLFN11*-high tumors showed superior neoadjuvant chemotherapy response ($p = 0.003$), which aligns with previous reports [6], as well as chemotherapy response score ($p = 0.026$). This strongly supports *SLFN11* as a marker of sensitivity to platinum-based chemotherapy, consistent with its role in DNA damage response [9].

Notably, all LGSC cases and 43 cases of HGSC cases exhibited moderate/high *SLFN11* expression - a novel finding suggesting its preservation in LGSC, which is known to have more indolent behavior and less genomic instability, compared to HGSC [1], akin to its role in chemoresistant ovarian clear cell carcinoma (Figure 1) [6].

We demonstrated *SLFN11*'s strong prognostic value, with high expression associated with significantly longer overall survival (median= 66.91 months, 95% CI: 60.36-73.46 vs. 31.50 months, 95% CI: 21.81-41.19, $p < 0.001$) and progression-free survival (median= 33.05 months, 95% CI: 25.60-40.50 vs. 7.60 months, 95% CI: 6.29-8.91, $p < 0.001$), consistent with prior studies in serous ovarian carcinoma [2,6]. The mechanism likely involves *SLFN11*-mediated impairment of DNA repair, enhancing platinum sensitivity [9]. While most reports support this trend, some studies noted non-significant survival benefits [27,31], possibly due to methodological variability in antibody use, cut-off values, or cohort heterogeneity [9].

This study showed significant association between TILs count and both clinical response to neoadjuvant chemotherapy ($p = 0.008$) and chemotherapy response score ($p = 0.007$). Our analysis included ovarian specimens across all chemotherapy response score categories. These findings are consistent with those of Lee et al. [32], who reported a positive correlation between pre-neoadjuvant chemotherapy TILs and chemotherapy response score. Higher TILs count was also significantly associated with improved overall survival (median= 68.2 months, 95% CI: 63.15-73.34 vs. 30.63 months, 95% CI: 21.08-40.17, $p < 0.001$) and progression-free survival (median= 25.5 months, 95% CI: 19.70-31.35 vs. 10.3 months, 95% CI: 4.85-15.75, $p < 0.001$), supporting their prognostic value and potential utility as markers of chemosensitivity and immunotherapy response. Our overall survival findings align with those of Wang et al. and Goode et al. [8, 33] who demonstrated survival benefits with specific T-cell subsets. Winkler et al. [2] reported associations between progression-free survival and $CD3^+$ TILs, with a trend toward improved outcomes with $CD8^+$ cells, while Machuca-Aguado et al. [10] observed improved overall survival with total TILs count. In contrast, Yaniz-Galende et al. [34] found no overall survival benefit from intraepithelial $CD8^+$ cells alone, although a broader immune infiltrate correlated with improved overall and progression-free survival. Conversely, Lee et al. [32] reported worse post-neoadjuvant chemotherapy outcomes in the presence of high TILs, potentially due to elevated forkhead box P3+ regulatory T lymphocytes ($FoxP3^+$ Tregs). These discrepancies may reflect differences in TILs distribution, lack of standardized assessment protocols, and heterogeneity in treatment approaches.

TILs showed no association with treatment modality (adjuvant vs neoadjuvant chemotherapy), consistent with Yaniz-Galende et al. [34], who noted that intra-tumoral $CD8^+$ TILs count remained unchanged post-neoadjuvant chemotherapy. However, discrepancies with reports of post-neoadjuvant chemotherapy TILs increase, highlighting the impact of evaluation techniques (e.g., H&E vs. immunohistochemistry) and immune subset heterogeneity [35].

We had several strengths in our study, including long-term follow-up that enabled robust survival analysis, inclusion of both HGSC and LGSC subtypes for comparison, and standardized methodologies using RECIST criteria with dual-pathologist *SLFN11* scoring. The findings needed to be interpreted considering certain limitations including a smaller sample size, a single-center design that potentially limits generalizability, possible interobserver variability in pathological assessments, and reliance on archived specimens which could have introduced tissue-quality bias. While strict exclusion criteria strengthened internal validity, the selected patient population may have reduced broader applicability of these results to clinical practice.

Our study addresses an important knowledge gap by demonstrating that *SLFN11* expression and TILs count predict outcomes in both subtypes of serous ovarian carcinoma. There is a critical need to establish standardized protocols for the interpretation of *SLFN11* immunohistochemical expression and TILs quantification in ovarian neoplasms. Moreover, additional studies are warranted to validate the role of *SLFN11* as a predictive biomarker for chemotherapy response in LGSC. Integration of these biomarkers into clinical practice may enhance prognostication and enable more individualized treatment approaches, supporting the development of targeted therapies and improved management of this challenging disease.

In conclusions, this study reinforces the significant predictive and prognostic value of *SLFN11* expression in serous ovarian carcinoma. High nuclear *SLFN11* expression strongly correlated with better response to platinum-based chemotherapy (both clinically and histopathologically) and significantly improved progression-free survival and overall survival. Also, *SLFN11* was expressed in LGSC, suggesting a potential biologic role in this histological subtype with limited molecular data. Higher counts of intra-tumoral TILs were associated with better survival outcomes. These findings support the potential use of *SLFN11* immunohistochemistry and TILs assessment as biomarkers to determine treatment responsiveness and to modulate treatment plans for ovarian serous carcinoma patients.

Author Contribution Statement

Sherry R. Michael: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Dalia M. Badary: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Hisham Ahmed Abou-Taleb: Writing – review & editing, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Doaa A Gamal: Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Rabab Mohamed Mumdouh: Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Youssab A. F. Nazeer: Writing – original draft, Visualization, Software, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. Mahmoud I. Nassar: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Ethical approval

This study was approved ethically by the Institutional Review Board (IRB), Faculty of Medicine, Assiut University. The IRB local approval number is 17101754.

Data Availability: Data is available upon reasonable request.

The current study is not registered in any registration data set.

Conflict of interest

The authors declare no conflict of interest.

Glossary

ATR: Ataxia Telangiectasia and Rad3-related protein
CI: Confidence interval
CR: Complete response
CRS: Chemotherapy response score
DAB: 3,3’-Diaminobenzidine
DNA: Deoxyribonucleic acid
EDTA: Ethylenediaminetetraacetic acid
FIGO: International Federation of Gynecology and Obstetrics
FoxP3+ Tregs: Forkhead box P3+ regulatory T cells
H&E: Hematoxylin and eosin
HGSC: High-grade serous carcinoma
HRP: Horseradish peroxidase
KPS: Karnofsky performance status
LGSC: Low-grade serous carcinoma
OS: Overall survival
PARP: Poly (adenosine diphosphate ribose) polymerase
PFS: Progression free survival
PR: Partial response
RECIST: Response Evaluation Criteria in Solid Tumors
RNA: Ribonucleic acid
SLFN11: Schlafen Family Member 11
SPSS: Statistical Package for the Social Sciences
TILs: Tumor-infiltrating lymphocytes
TNM: Tumor, Node, Metastasis (Staging System)
UICC: Union for International Cancer Control

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