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ORIGINAL ARTICLE

The Clinicopathological and Prognostic Values of Chemotherapy Response Score in Tubo-Ovarian High-Grade Serous Carcinoma

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Abstract

Purpose Tubo-ovarian high-grade serous carcinoma is one of the aggressive ovarian tumors. Neoadjuvant chemotherapy has been used in managing the affected patients. The chemotherapy response score (CRS) system has been introduced to evaluate the patient's response to neoadjuvant chemotherapy and their prognosis. The current study aimed to investigate the prognostic and clinicopathological significance of the CRS system in patients with tubo-ovarian high-grade serous carcinoma.

Methods A total of 39 patients with tubo-ovarian high-grade serous carcinoma were included in the current study. We retrospectively investigated the progression-free survival (PFS), overall survival (OS), and their clinicopathological and surgical parameters. We also studied the Ki67 protein expression of included patients using immunohistochemistry (IHC). Besides, we investigated the significance of Ki67 expression in tubo-ovarian high-grade serous carcinoma development using the GSE73064 and GSE126308 datasets.

Results Ki67 expression was not alerted in ascites, metastatic, and primary tubo-ovarian high-grade serous carcinoma. Also, Ki67 expression was not changed in the early tubo-ovarian high-grade serous carcinoma compared to late tubo-ovarian high-grade serous carcinoma. We showed that Ki67 protein expression was elevated in CRS1 patients with tubo-ovarian high-grade serous carcinoma compared to CRS3 patients. The bleeding amount and operation time were substantially lower in CRS3 patients compared to CRS1 patients, and there was a strong positive association between CRS3 and optimal resection. Furthermore, CRS3 patients had substantially improved PFS compared to CRS1 patients.

Conclusion This study has highlighted the valuable role of the CRS system in determining the PFS and some clinicopathological features of tubo-ovarian high-grade serous carcinoma patients.

Keywords Ovarian neoplasms · Neoadjuvant therapy · Tubo-ovarian high-grade serous carcinoma · Chemotherapy · Prognosis

Introduction

In 2022, it is estimated that ovarian cancer affects 19,880 individuals and is responsible for the death of 12,810 affected patients [1]. Based on the dualistic model of epithelial ovarian cancers, low-grade and high-grade serous carcinomas have different clinical manifestations [2]. High-grade serous carcinoma is a highly aggressive tumor and is considered the most common malignancy originating from ovaries [3]. Considering the prevalence and the aggressive nature of tubo-ovarian high-grade serous carcinoma, it is a considerable burden on the health care system.

Randomized clinical trials have shown that neoadjuvant chemotherapy is associated with non-inferior overall survival (OS) of advanced ovarian cancers [4, 5]. Besides, it

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has been reported that neoadjuvant chemotherapy improves patients' quality of life with advanced ovarian cancers [5]. Given the significance of neoadjuvant chemotherapy for advanced ovarian cancer patients, Böhm et al. have developed a scoring system, i.e., chemotherapy response score (CRS), for histological investigation of tubo-ovarian high-grade serous carcinoma regression. Based on the omental studies, this scoring system stratifies affected patients into three groups, i.e., complete/near-complete response to neoadjuvant chemotherapy (CRS3), partial response to neoadjuvant chemotherapy (CRS2), and no/minimal response to neoadjuvant chemotherapy (CRS1) [6]. In 2015, the International Collaboration on Cancer Reporting (ICCR) recommended applying this three-tier scoring system following its promising inter-observer reproducibility and its correlation with clinical features; however, it suggested further studies [7]. Recently, several studies have shown the prognostic values of the CRS scoring system for high-grade serous tubo-ovarian carcinoma patients treated with neoadjuvant chemotherapy [8, 9].

Sustained aberrant cell proliferation is one of the characteristics of malignant cells proposed by Hanahan and Weinberg [10]. Based on preclinical findings, Ki67 knockout suppresses tumorogenesis, and Ki67 increased expression promotes tumor development [11]. Ki67 is widely used in the pathological examination of tumor tissues, and it is considered a proliferation maker following its strong association with tumor proliferation [12]. Ki67 expression level has been upregulated in ovarian carcinomas compared to benign or borderline tumors of epithelial tumors; also, increased expression of Ki67 has been associated with increased tumor invasion, poor prognosis, and poor chemotherapy response [13]. However, there is no sufficient evidence of the possible association between Ki67 expression level and the CRS scoring tool in high-grade serous tubo-ovarian carcinoma patients treated with neoadjuvant chemotherapy.

In the current study, we investigated the possible association between CRS and the clinicopathological features of high-grade serous tubo-ovarian carcinoma patients treated with neoadjuvant chemotherapy; besides, we studied its prognostic values in terms of determining the OS and progression-free survival (PFS) of affected patients. Furthermore, we investigated the association between the Ki67 protein expression and this scoring system and shed light on the significance of Ki67 in ovarian cancer. Indeed, the current study is the first study that investigates the association of Ki67 protein expression, a well-established maker of proliferation implicated in malignant invasion, with the CRS scoring tool. Furthermore, this study investigates the prognostic and clinicopathological significance of the CRS scoring tool for tubo-ovarian high-grade serous carcinoma patients and highlights the significance of Ki67 in the different phases of tubo-ovarian high-grade serous carcinoma development. Given the simple nature of this scoring system, the results of the present study can be applied to predicting the outcome of affected patients in clinical settings.

Patients and Methods

Study Participants

The current study was conducted after approval from the Ethics Committee of Tabriz University of Medical Sciences. We searched the pathology database of Alzahra Hospital, a university hospital of Tabriz University of Medical Sciences, to identify patients diagnosed with tubo-ovarian high-grade serous carcinoma between March 21, 2011, and March 20, 2020. We only included the cases where immunohistochemistry (IHC) studies confirm the high-grade serous carcinomas. Patients with the histologically, clinically, and radiologically confirmed International Federation of Gynecology and Obstetrics (FIGO) stage III_c and IV tubo-ovarian high-grade serous carcinomas were included in this study. Thirty-six patients had omental metastasis, and a considerable number of patients had an invasion of the liver/diaphragm (ten patients), bowel (ten patients), and lung/pleura (seven patients). Three patients had a tumoral invasion of the spleen, and one patient had a malignant invasion of the urinary bladder. Following supportive care for their metastasis, like plural and ascitic tap, they were consulted with an oncologist for neoadjuvant chemotherapy. The included patients underwent platinum-based neoadjuvant chemotherapy and debulking surgery. The radiological responses (CT-scan findings) following neoadjuvant chemotherapy were the resolution of adhesions, ascites, pleural effusion, and tumor and omental shrinkage. The patients were selected for surgery following the resolution of pleural effusion, ascites, tumor, and omentum shrinkage; all patients had appropriate operative candidates. The surgical procedures were performed by experienced gynecologic oncologists; the debulking surgery included midline laparotomy, hysterectomy, salpingo-oophorectomy, omentectomy, and lymphadenectomy of the pelvis and paraaortic regions. In the case of gross involvement due to visceral metastasis, a consult with oncosurgens was made for the management. This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.398). Written informed consent was obtained from the patients.

Pathology Review and IHC

The slides of ovarian tumor tissues, fallopian tumor tissues, lymph nodes, peritoneum, and omentum were retrieved

from the Alzahra Hospital pathology department; these slides were formalin-fixed and stained with hematoxylin and eosin (H&E) based on standard procedures. An experienced gynecologic pathologist reviewed the available slides and calculated the score for each slide based on the CRS system [6]. In brief, CRS1 indicates no or minimal tumor regression, CRS2 indicates partial chemotherapy response, and CRS3 indicates complete tumor regression without residual malignant cells. For IHC staining, the paraffin-embedded blocks were cut into 4 µm slices using a microtome. Anti-Ki67 rabbit monoclonal antibody (clone SP6) was used to detect the nuclear Ki67 expression in post-neoadjuvant chemotherapy tissues. The cells in the late G1, S, G2, and M phases are positive for Ki67, and cells in the G0 phase are not immunostained. The proliferation index, which is obtained from the nuclear expression of Ki67, is calculated based on the IHC results; therefore, the proliferation index indicates the mitotic status of cells. In line with the previous study, PFS determined the time between chemotherapy initiation and documented relapse or death; the time between tubo-ovarian high-grade serous carcinoma diagnosis and the time of the affected patient's death was defined as OS [14].

In Silico Studies

In silico studies were conducted on the mRNA expression of malignant tissues to study the significance of Ki67 mRNA expression in different phases of tubo-ovarian high-grade serous carcinoma leveraging the GSE73064 and GSE126308 datasets from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The TCGA dataset for ovarian cancer was accessed using the UCSC Cancer Browser (https://xenabrowser.net/) to investigate the significance of Ki67 mRNA expression for determining the OS and PFS of affected patients. Aside from providing valuable insights into the tumorigenesis of tubo-ovarian high-grade serous carcinoma, these in silico results might pave the way for mRNA-based prognostic tools for the affected patients.

Ki67 Expression Based on GEO Datasets

We accessed the GSE73064 and GSE126308 datasets using the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). We leveraged the GSE73064 dataset to investigate the Ki67 expression pattern in the primary tumors, ascites, and metastases of high-grade serous ovarian cancer patients. The GSE73064 applied the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array to study the mRNA expression of primary tumors, ascites, and metastases of high-grade serous ovarian cancers; the tissues were included from five patients with high-grade serous ovarian cancer. We also used the GSE126308 dataset to study whether Ki67 expression is different in tissues of high-grade serous ovarian cancer patients with early progression and late progression. The GSE126308 dataset applied the GPL17586 [HTA-2_0] Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version] to sequence the mRNA expression levels. We used R (version 4.1.3) to normalize the expression values of each dataset, depict box plots, assign each probe to the related gene symbol, and perform statistical analyses.

The Prognostic Significance of Ki67

We accessed the TCGA-ovarian cancer dataset to investigate the prognostic value of Ki67 in patients with high-grade ovarian cancer. We investigated the significance of Ki67 expression levels in determining the OS and PFS of affected patients.

Statistical Analyses

We used R (version 4.1.3) and GraphPad Prism (version 8.0.2) to analyze the data. The Kaplan–Meier method and log-rank test were used to analyze the PFS and OS data; 95% confidence intervals (CIs) were also calculated for the obtained hazard ratios. We used the Student T test and chi-square test to investigate the difference between quantitative and categorical variables. P values less than 0.05 were considered statistically significant.

Results

The Clinicopathological Features of Included Patients

We gained access to the clinicopathological findings of 39 patients histopathologically diagnosed with tubo-ovarian high-grade serous carcinoma. The included patient's median age in our retrospective study is 55 years old, and all recruited patients received carboplatin with paclitaxel as neoadjuvant chemotherapy. Most of the patient's CRS is CRS1, and the patient's median Ki67 score is 5%. Table. 1 demonstrates the clinicopathological features of the included patients in this study.

The Clinicopathological Significance of Ki67

To demonstrate the clinicopathological significance of Ki67 expression in tubo-ovarian high-grade serous carcinoma, we studied its expression level in primary tubo-ovarian high-grade serous carcinoma, its metastasis, and ascites. Following normalizing the expression values, our results have shown that the expression level of Ki67 is not altered in primary, metastasis, and ascites of tubo-ovarian high-grade

Table 1 The clinicopathological features of included patients

No. of patients (%)	
Age (years old)	
Median	55
Range	44–76
Histological type	
HGSC	39 (100%)
FIGO stage	
IIIC	15 (38.46%)
IV	24 (61.54%)
NAC cycle	
Three	30 (76.92%)
Four	9 (23.08%)
Regimen of NAC	
Carboplatin + Paclitaxel	39 (100%)
CRS score	
CRS1	29 (74.36%)
CRS2	0
CRS3	10 (25.64%)
Family history of breast or ovarian cancer	
Positive	15 (38.46%)
Negative	19 (48.72%)
Unknown	5 (12.82%)
Affected ovary	
Bilateral	21 (53.85%)
Unilateral	18 (46.15%)
OCP history	
Positive	12 (30.77%)
Negative	19 (48.72%)
Unknown	8 (20.51%)
Peritoneal seeding	
Positive	36 (92.31%)
Negative	3 (7.69%)
Ascites	
Positive	31 (79.49%)
Negative	8 (20.51%)
Ki67 (%)	
Median	5%
Range	0–60%
Surgery	
Optimal	21 (53.85%)
Sub-optimal	18 (46.15%)
Operation time (min)	
Median	215
Range	150–310
Bleeding in surgery (cc)	
Median	400
Range	100–1000

FIGO International federation of gynecology and obstetrics *NAC* Neoadjuvant chemotherapy *CRS* Chemotherapy response score and *OCP* Oral contraceptive pill

serous carcinoma (Fig. 1A, B). Also, we investigate whether the expression level of Ki67 remains unchanged in the early and advanced progression of tubo-ovarian high-grade serous carcinoma or not. After normalizing the expression values, our results have indicated that the expression level of Ki67 is not altered in the early progression of tubo-ovarian high-grade serous carcinoma compared to the advanced one (Fig. 1C, D).

The Prognostic Significance of Ki67

After demonstrating the role of Ki67 in various phases of tubo-ovarian high-grade serous carcinoma, we aimed to study its prognostic value in high-grade ovarian tumors. For this aim, we accessed the TCGA-ovarian cancer dataset. Although increased expression of Ki67 is associated with the inferior OS of affected patients, this is not statistically significant (P value = 0.4789) (Fig. 2A). Our results have demonstrated that Ki67 does not have prognostic value in terms of determining the PFS of affected patients (Fig. 2B).

The Prognostic Significance of CRS in Determining the OS and PFS of Affected Patients

We investigated the prognostic significance of CRS in determining the OS and PFS of included patients with tubo-ovarian high-grade serous carcinoma. Our results have shown that the OS of patients with CRS3 is not significantly different from the OS of patients with CRS1 (P value = 0.8889) (Fig. 3A). However, our results have indicated that the PFS of patients with CRS3 is significantly better than the PFS of patients with CRS1 (HR = 0.4522, 95% CI = 0.2192—0.9332, and P value = 0.0318) (Fig. 3B).

The Association Between CRS and Clinicopathological Factors

We investigated the possible association between CRS and clinicopathological factors in the included patients. Our results have demonstrated that tubo-ovarian high-grade serous carcinoma patients with CRS3 have significantly low Ki67 expression levels compared with tubo-ovarian high-grade serous carcinoma patients with CRS1 (P value = 0.0006) (Table 2). Nevertheless, there were no significant associations with the age, gestation, OCP history, number of the affected ovary, and family history of breast/ovarian cancer of the included patients (Table 2).

The Association Between CRS and Surgical Parameters

Besides, we studied the possible association between CRS and the surgical factors in the included patients. Our results

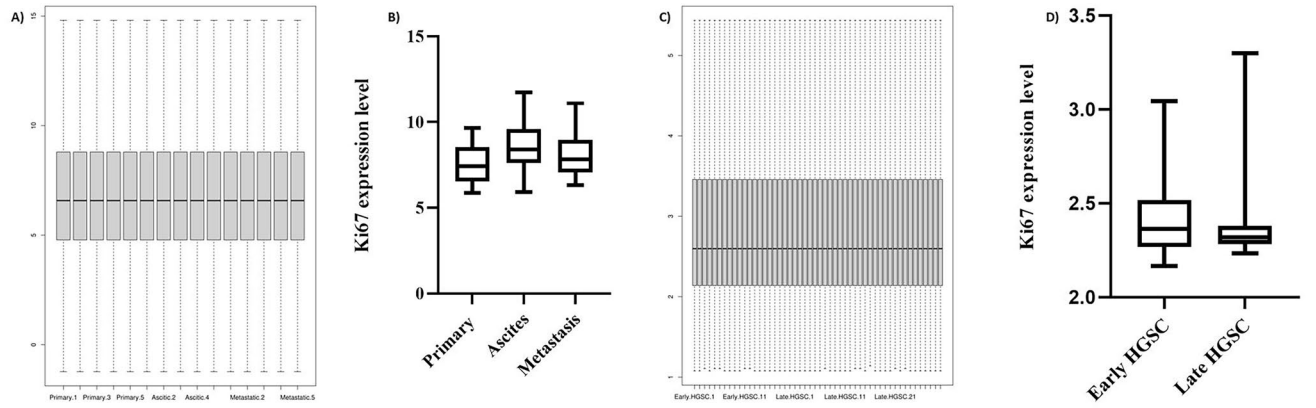

Fig. 1 The clinicopathological significance of Ki67. **A** Normalizing the expression values of the GSE73064 dataset. **B** There is no difference between the expression level of Ki67 in the primary, ascites, and metastasis of high-grade serous ovarian carcinoma tissues. **C** Normalizing the expression values of the GSE126308 dataset. **D** There is no significant difference between the expression level of Ki67 in the early and late progressed high-grade serous ovarian carcinomas

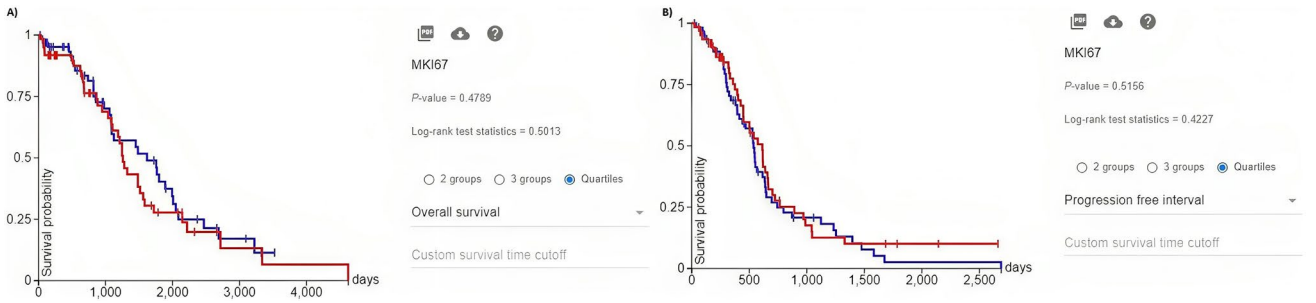

Fig. 2 The prognostic significance of Ki67 in high-grade ovarian cancers using the TCGA-ovarian cancer dataset. **A** Although increased expression of Ki67 is associated with the inferior OS of affected patients, this trend is not statistically significant. **B** There is no prognostic value for Ki67 expression level in determining the PFS of affected patients

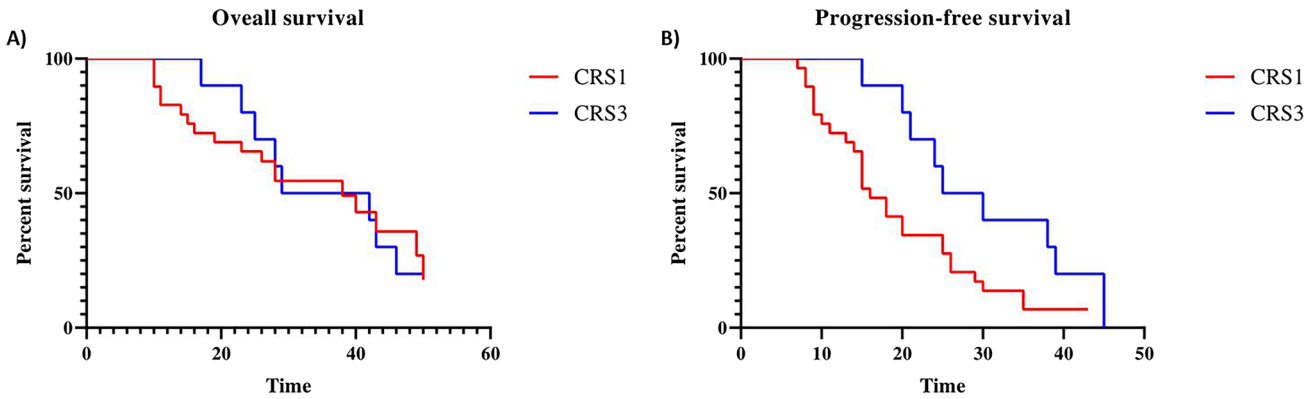

Fig. 3 The prognostic value of CRS in determining the OS and PFS of included patients. **A** The OS of patients with CRS3 is similar to the OS of patients with CRS1. **B** The PFS of patients with CRS3 is significantly better than the PFS of patients with CRS1

have demonstrated that patients with CRS1 have increased time of operation and increased bleeding amount in operation compared to patients with CRS3 (P value < 0.0001, and P value $= 0.0002$, respectively) (Table 3). Also, our results have shown a strong statically significant association between CRS3 with optimal resection (OR = 14.73, 95%

Table 2 The association between CRS scoring with clinicopathological factors

		CRS 3	CRS 1	*P* value
Age #		54.5 (47.5–59)	56 (49–67.5)	0.4696
Gestation #		5 (1–7)	5 (3–8)	0.4324
History of OCP	Positive	4	8	0.7039
	Negative	5	14	
Affected ovary	Bilateral	3	18	0.1406
	Unilateral	7	11	
Family history of Breast/ ovarian cancer	Positive	7	8	> 0.9999
	Negative	10	9	
Ki67		0% (0–5%)	13% (2.5–30%)	0.0006

OCP Oral contraceptive pill, and *CRS* Chemotherapy response score; # the data are presented as mean and range between Q25 and Q75

Table 3 The association between CRS scoring with surgical parameters

		CRS 3	CRS 1	*P* value
Operation time (min) #		180 (170–200)	210 (200–260)	*< 0.0001*
Bleeding in operation (cc) #		275 (200–300)	400 (350–475)	*0.0002*
Resection status	Optimal	9	11	*0.0084*
	Sub-optimal	1	18	
Ascites	Positive	6	25	0.1672
	Negative	4	4	
Peritoneal seeding	Positive	8	28	0.1559
	Negative	2	1	

CRS Chemotherapy response score # the data are presented as mean and range between Q25 and Q75

CI = 2.104—168.8, *P* value = 0.0084) (Table 3). However, no statistically significant association was identified between CRS and peritoneal seeding and ascites (Table 3).

Discussion

Tubo-ovarian high-grade serous carcinoma is among the common and aggressive ovarian tumors. It is believed that most cases of tubo-ovarian high-grade serous carcinoma develop from serous tubal intraepithelial carcinoma, a precursor lesion in the distal fallopian tube [15]. This cancer is characterized by a high mitotic rate, chromosome instability, and nuclear atypia [16]. Given its burden, there is a need to assess the prognosis of affected patients following neoadjuvant chemotherapy and resection. In the current study, we investigated the prognostic and clinicopathological significance of the CRS system in patients with tubo-ovarian high-grade serous carcinoma following neoadjuvant chemotherapy. Besides leveraging *in silico* data, we investigated the relationship between CRS and Ki67 expression in these patients.

Singh et al. have investigated the prognostic values of omental and adnexal CRS systems for patients with tubo-ovarian high-grade serous carcinoma. Their results have shown that omental CRS has prognostic value in determining the PFS of affected patients; however, adnexal CRS is not associated with the PFS and OS of affected patients. Besides, similar CRS scoring of adnexal and omental tissues has been recorded in 54.5% of patients [17]. Besides, Ditzel et al. have shown that tubo-ovarian high-grade serous carcinoma patients with CRS1/2 have substantially inferior PFS compared to patients with CRS3 [14]. Coghlan et al. have demonstrated that CRS has prognostic value in determining the OS and PFS of tubo-ovarian high-grade serous carcinoma [18]. Recently, Santoro et al. have shown that the CRS system has significant prognostic value in determining the OS and PFS of high-grade serous ovarian carcinoma patients [19]. In the current study, our results have demonstrated that CRS3 patients have significantly improved PFS compared to CRS1 patients (HR = 0.4522, 95% CI = 0.2192—0.9332, and *P* value = 0.0318); however, CRS has not demonstrated valuable prognostic value for determining the OS of patients.

In terms of studying clinicopathological factors, it has been shown that the CRS system is significantly associated with necrosis, inflammation, and residual tumor in omental deposits; however, no significant association has been identified between CRS with fibrosis and macrophages in

omental deposits of high-grade serous ovarian carcinoma patients [20]. In patients with high-grade serous ovarian carcinoma, there are significant negative correlations between omental CRS with the average per cell TP53 mutational load and TP53 variant allele frequency, which is associated with poor prognosis [21]. Our study has shown that the bleeding amount and the required time for operation are significantly decreased in CRS3 patients compared with CRS1 patients. Also, our study has shown a significant positive association between optimal surgery and CRS3 in high-grade serous ovarian carcinoma patients (OR = 14.73, 95% CI = 2.104—168.8, *P* value = 0.0084). Therefore, besides having prognostic significance, the CRS system has clinicopathological value as well.

Dysregulated proliferation is one of the hallmarks of malignant tumors, and proliferation status can predict chemotherapy response [22]. As a nucleus-residing protein, Ki67 is expressed in the active phase of the cell cycle, i.e., G1, S, G2, and mitosis [23]. In line with this, Ki67 is significantly enriched for regulation of the mitotic cell cycle, mitotic nuclear division, chromosome segregation, and chromosome organization. Ki67 knockdown can substantially inhibit tumoral proliferation and clonogenicity and stimulate apoptosis in malignant cells [24, 25]. Ki67 can be used as a potential predictive biomarker for affected patients. Kaya et al. have reported that the decreased expression of Ki67 is associated with favorable relapse-free survival of unresectable ovarian cancer patients undergoing neoadjuvant chemotherapy [26]. Also, increased expression of Ki67 has been associated with poor overall survival of patients with epithelial ovarian cancer [27]. Our *in silico* results have demonstrated that Ki67 expression is not altered in the early progression of tubo-ovarian high-grade serous carcinoma compared to the advanced one. Also, we have shown that the Ki67 expression level of primary tubo-ovarian high-grade serous carcinoma is similar to the Ki67 expression level of ascites and metastatic tubo-ovarian high-grade serous carcinoma. These results indicate the consistently stable expression of Ki67 in various phases of tubo-ovarian high-grade serous carcinoma pathogeneses. Since the CRS score reflects the malignancy response to chemotherapeutic cell toxicity and it can be inversely associated with the proliferation status of malignant cells, we studied the potential association between Ki67 protein expression and CRS scoring. Our results have indicated that tubo-ovarian high-grade serous carcinoma patients with CRS3 have lower Ki67 expression than CRS1 patients. These results highlight the role of Ki67 in various phases of tubo-ovarian high-grade serous carcinoma development and the chemotherapy response of affected patients.

The current study has some strengths. First, we investigated the association between the CRS system and clinicopathological and surgical parameters. Second, we studied the interplay between Ki67 protein expression and the CRS system. Third, we applied deep *in silico* studies to investigate the significance of Ki67 in the various phases of tubo-ovarian high-grade serous carcinoma development. However, the current study has some limitations, as well. First, this study is retrospective in nature. Second, the number of registered cases was limited. Third, we did not perform Ki67 IHC staining in the pre-neoadjuvant chemotherapy tissues. Overall, this study has provided new insights into the significance of the CRS system in determining the prognosis and clinicopathological factors in patients with tubo-ovarian high-grade serous carcinoma. From the clinical point of view, the present study has shown that CRS3 patients have favorable PFS compared to CRS1 patients; also, CRS3 status is positively associated with optimal resection in the affected patients. Besides, the bleeding amount and the operation time are considerably lower in CRS3 patients than in CRS1 ones.

Conclusion

The results of the current study have indicated that the CRS system has prognostic value in determining the PFS of tubo-ovarian high-grade serous carcinoma patients. Also, CRS3 patients with tubo-ovarian high-grade serous carcinoma have a substantially lower amount of bleeding in operation and lower operation time compared to CRS1 patients. Our results have shown a strong positive association between CRS3 and optimal resection in tubo-ovarian high-grade serous carcinoma patients. Given the demonstrated significance of Ki67 in cell proliferation and its role in various phases of tubo-ovarian high-grade serous carcinoma development, CRS1 patients with tubo-ovarian high-grade serous carcinoma display increased Ki67 expression compared to CRS3 patients. Collectively, the current study has shed light on the significance of the CRS system in determining the prognosis and clinicopathological features of tubo-ovarian high-grade serous carcinoma patients.

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Author Contributions RD was involved in the investigation, methodology, data collection and analysis, writing—original draft, and writing—review and editing. AM contributed to the conceptualization, methodology, writing—original draft, and writing—review and editing. MSM assisted in the conceptualization and writing—review and editing. MJS was involved in the conceptualization, methodology, and writing—review and editing. VR contributed to the conceptualization, methodology, and writing—review and editing. MV assisted in the conceptualization, methodology, and writing—review and editing. ADT was involvd in the conceptualization, data analysis, and

writing—review and editing. PMG contributed to the conceptualization, methodology, funding acquisition, supervision, writing—original draft, and writing—review and editing.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.398).

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