Submitted: 13 February, 2023 Accepted: 09 June, 2023 Published: 15 February, 2024 DOI:10.22514/ejgo.2024.003

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Epithelial IL-8 immunostaining associated with overall survival in ovarian cancer

Marilha B. M. Neves[1], Letícia M. M. C. Scofoni[1], Agrimaldo Martins-Filho[1], Millena P. Jammal[1], Renata M. Etchebehere[2], Eddie F. C. Murta[1], Rosekeila S. Nomelini[1,3,*]

[1]Department of Gynecology and Obstetrics, Federal University of Triângulo Mineiro, 38025-440 Uberaba, MG, Brazil
[2]Surgical Pathology Service, Federal University of Triângulo Mineiro, 38025-440 Uberaba, MG, Brazil
[3]Graduate Program in Gynecology and Obstetrics of the Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), 14049-900 Ribeirão Preto, SP, Brazil

***Correspondence**
rosekeila.nomelini@uftm.edu.br
(Rosekeila S. Nomelini)

Abstract

Cytokines have major role in regulating the biological processes such as cell growth and activation, chemotaxis, inflammation, immunity and tissue repair. The cytokines involvement in oncogenesis shows their role in signaling between inflammatory cells and invasive neoplastic tissue. This study assessed the association of epithelial and stromal immunostaining of panel of cytokines with overall survival and disease-free survival in malignant ovarian neoplasia women. The study included 28 ovarian cancer patients. The patients' age, staging, differentiation grade, histological type of tumors, treatment type and survivals (disease-free survival and overall survival) were evaluated. Immunohistochemical analysis was made to assess the epithelial and stromal immunostaining of interleukins (IL-2, IL-5, IL-6, IL-8, IL-10 and tumor necrosis factor alpha (TNF-α). Cut-off values for the immunostaining cytokines were calculated through Receiver operating characteristic (ROC) curves. Kaplan-Meier curves were designed to evaluate the survival followed by Cox regression. ROC curves relating death to epithelial IL-8 staining and stromal TNF-alpha staining exhibited a cut-off value more than staining 1 for both cytokines. There was no statistical significance found in the evaluation of other cytokines and disease-free survival. Overall survival was shorter in patients with epithelial IL-8 staining 2/3 (p = 0.049). No statistical significance was found regarding TNF-alpha stromal. Multivariate analysis revealed that epithelial IL-8 staining 2/3 was an independent variable linked to lower overall survival ((Odds ratio (OR) = 18.515. 95% confidence interval (CI): (1.160–295.549)). Therefore, epithelial IL-8 immunostaining predicts overall survival of ovarian malignancy patients. This cytokine can be the target for discovering other types of management systems for epithelial ovarian cancer and borderline ovarian tumor.

Keywords

Ovarian neoplasms; Cytokines; Interleukin-8; Survival; Immunology

1. Introduction

The new cases of ovarian malignancy totaled 313,959 with ~207,252 deaths in 2020, being 13th leading tumor-related deaths among women [1]. This neoplasm is the 2nd common gynecological cancer. The ovarian cancer incidence was 6650 new cases in Brazil which was 3.0% of women cancer new cases in 2020 [2]. There were 4123 deaths from ovarian cancer in Brazil in the year 2019 [3].

Tumor stroma has been studied for improving prognosis in various cancers. Fibroblasts are highly differentiated and heterogeneous stromal cell type that promote cancer invasion, vasculogenesis and angiogenesis. Tumor stroma enhances cancer cells resistance toward some therapies, limiting the access of therapeutic agents to target tissues. Moreover, chemotherapy-induced DNA damage in tumor microenvironment (TME) stimulates the secretions of stromal factors which promote survival, proliferation, invasion and metastasis of cancer cells. The intratumoral stroma contains specific biomarkers for predicting clinical chemotherapeutic response and preventing the toxicity and tumor progression. Thus, new treatment approaches combine the anticancer and anti-stromal therapies [4, 5].

The studies demonstrate cytokines involvement in tumor progression [6–8]. The cytokine IL-6 has a role as inflammatory marker. This glycoprotein stimulates the production of other inflammatory cytokines which favor angiogenesis. Higher serum IL-6 levels are associated with worse prognosis and shorter overall survival [9, 10]. A study evaluated the serum IL-6 and IL-8 wherein their high levels were associated with worse prognosis in malignant ovarian neoplasms [6].

Paclitaxel and carboplatin chemotherapy resistant tumors expressed higher levels of IL-6 and IL-8 (and Programmed Death-Ligand 1 (B7-H4), Indoleamine 2,3-dioxygenase-1

(IDO1), T cell immunoglobulin and mucin domain 3 (Tim3)) and were studied for improving the immunotherapy in high-grade serous ovarian carcinoma women [11]. Another study showed IL-8 inhibitor having anti-tumor effect for developing new cancer treatments [12]. In clear cell renal carcinomas, the expressions of Cluster of Differentiation 44 (CD44) and TNF-α as evaluated by immunohistochemistry were correlated with higher stage of primary tumor, distant metastasis and poor prognosis. TNF-α may thus be linked with progression of this type of cancer, and sunitinib resistance [13].

Malignant ovarian neoplasms have complex TME, which causes changes in cytokine production as exhibited in peritoneal fluid [14–18], and tumor tissue [18]. Furthermore, studies have revealed systemic changes in cytokine production in ovarian cancer women [17–20]. The immune response study in these compartments can assist in better understanding of immune response against ovarian cancerous tumor and tumor escape mechanisms. The inflammatory and immune response studies may discover prognostic markers and targeted therapies for ovarian cancer. Study of panel of tumor epithelial and peritumoral stromal cytokines may define prognostic and survival markers for ovarian cancer.

This study was aimed to demonstrate the stromal and epithelial immunostaining of panel of cytokines (interleukins, IL-2, IL-5, IL-6, IL-8, IL-10 and TNF-α) in epithelial ovarian cancer and borderline ovarian tumors. Moreover, it was assessed whether immunostaining had an association with overall survival and disease-free survival of these women.

2. Materials and methods

2.1 Patient selection and review of the anatomopathological results

A cross-sectional study was carried out at the Laboratory of Applied Sciences for Women (LaCam) of Department of Gynecology and Obstetrics at Federal University of Triângulo Mineiro (UFTM). The study included 28 patients undergone ovarian tumor surgery and had confirmed postoperative diagnosis of epithelial ovarian cancer or borderline ovarian tumor. Women having any factor interfering with cytokines production were excluded from the study (previous treatment of ovarian cancer, immunosuppressive drugs usage, and ovarian torsion observed during surgery).

The patients' age, staging, differentiation grade, histological type of tumors, treatment type and survival were evaluated. Staging was recorded as per the International Federation of Gynecology and Obstetrics (FIGO) criteria. Type I ovarian tumors included borderline serous tumors, low-grade serous carcinomas, mucinous tumors, endometrioid tumors and clear cell tumors. Type II tumors were the high-grade serous carcinomas, mixed malignant mesodermal tumors (carcinosarcomas), and undifferentiated tumors [21, 22].

The disease-free survival (DFS) and overall survival (OS) were studied. DFS was considered from first surgery date (when diagnosis was made) till the first recurrence date, in months. OS was taken from first surgery date (when diagnosis was made) till the date of death.

The anatomopathological results were reviewed by an experienced pathologist in Gynecological Oncology from Surgical Pathology Service of Federal University of Triângulo Mineiro. Pathologist reviewed paraffin results, selected the best slides representing epithelium and stroma, and submitted for immunohistochemistry experiments.

2.2 Immunohistochemical study

Immunohistochemical experiments were conducted as per the polymer technique. The following primary antibodies were employed: IL-2 antibody (H-133, sc-7896, Lot H0811, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), IL 5 antibody (H-85, sc-7887, Lot D0708, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), NLC-L-IL-6 antibody (Lot L155309, Leica Biosystems, Washington, DC, USA), IL-8 RB (E-2) antibody (sc-7304, Lot F1510, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), IL-10 antibody (Lot 11042807, cat No. 250713, Abbiotec, Escondido, CA, USA), and TNF (be 274) antibody (sc-130220, Lot B0509, Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

The slides representing epithelium and stroma were evaluated. Immunostaining was classified as follows: 0 (no staining), 1 (weak), 2 (moderate) and 3 (strong) (Fig. 1). The staining intensity was monitored by 3 observers with strong interobserver agreement.

2.3 Statistical analysis

The software employed for statistical analysis were GraphPad InStat (GraphPad Software, version 3.00, Domatics, Boston, MA, USA), and IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). Fisher's exact test was utilized to evaluate the association of epithelial and stromal immunostaining of panel of cytokines (IL-2, IL-5, IL-6, IL-8, IL-10 and TNF-α) with DFS and OS of malignant ovarian neoplasia women. Cut-off values for immunostaining cytokines were calculated *via* the ROC curves. Kaplan-Meier curves were designed to assess survival followed by the Cox regression. The considered significance level was <0.05.

In immunohistochemical study, the interobserver agreement was checked using kappa: $\kappa < 0.4$: poor agreement; $0.4 \leq \kappa < 0.8$: moderate agreement; $0.8 \leq \kappa < 1.0$: strong agreement; and $\kappa = 1.0$: perfect agreement.

3. Results

The study included 28 patients. The median age was 51 years (25–73 years). The median was 2 deliveries (0–8) in relation to parity.

The histological types were as follows: 10 serous cystadenocarcinomas (35.7%), 10 borderline ovarian tumors (35.7%), 2 endometrioid adenocarcinomas (7.1%), 2 mucinous cystadenocarcinomas (7.1%), 3 adenocarcinomas (10.7%), and 1 clear cell carcinoma (3.6%).

Tumor stages were categorized as follows: 9 (32.1%) stage IA, 2 (7.1%) IB, 2 (7.1%) IC2, 1 (3.6%) IIA, 1 (3.6%) IIIA2, 1 (3.6%) IIIB, 10 (35.7%) IIIC and 2 (7.1%) IVB.

Regarding the classification into type I and type II tumors, 15 (53.6%) were classified as type I, and 13 (46.4%) as type II.

In immunohistochemical readings, there was strong agree-

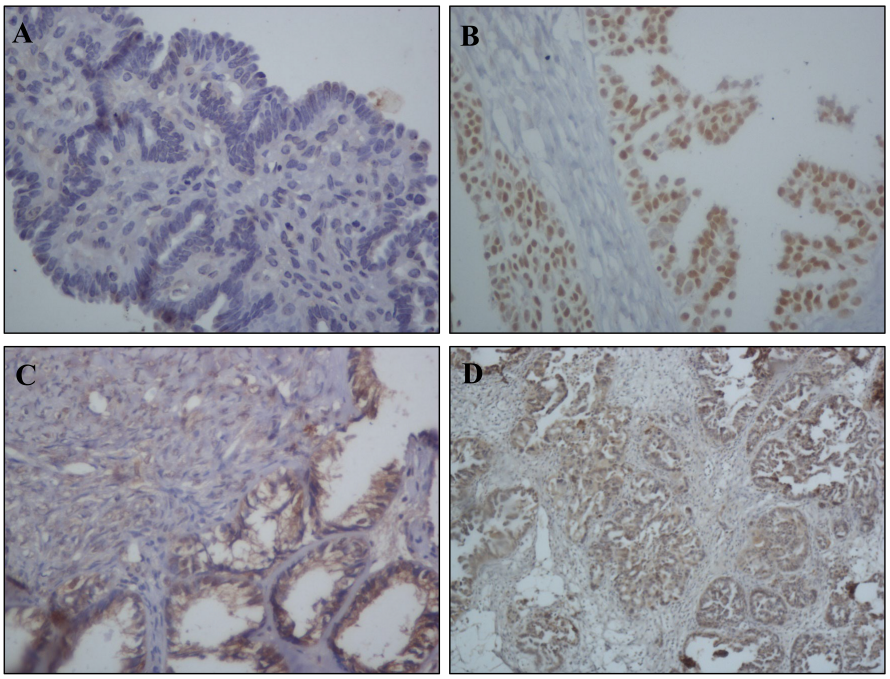

FIGURE 1. Immunostaining in epithelial ovarian cancer. (A) Serous cystadenocarcinoma (400×): epithelium (anti-IL-2; staining 2) and stroma (anti-IL-2; staining 1). (B) Serous cystadenocarcinoma (400×): epithelium (anti-IL-6; staining 3) and stroma (anti-IL-6; staining 0). (C) Endometrioid cystadenocarcinoma (400×): epithelium (anti-IL-8; staining 3) and stroma (anti-IL-8; staining 2). (D) Serous cystadenocarcinoma (100×): epithelium (anti-IL-10; staining 3) and stroma (anti-IL-10; staining 2).

ment. Cohen's kappa coefficient was 0.881. Interobserver agreement was 94.05% (336 slides were evaluated, and there was disagreement in 20 of them; these slides were reassessed, and the final result was established by consensus).

ROC curves were drawn to verify whether the epithelial and stromal immunostaining of each cytokine had significant association with death and recurrence. ROC curves relating death to epithelial IL-8 staining (sensitivity = 88.9%, specificity = 52.6%, area under curve (AUC) = 0.74, p = 0.007) and stromal TNF-α staining (sensitivity = 55.6%, specificity = 78.9%, AUC = 0.78, p = 0.023) determined cut-off value more than staining 1 for the both cytokines (Fig. 2). No association with death was found in relation to IL-2, IL-5, IL-6 and IL-10. Moreover, no association was obtained in relation to recurrence.

Kaplan-Meier curves were constructed to explore whether epithelial IL-8 and stromal TNF-α were associated with overall survival. OS was shorter in patients of epithelial IL-8 staining 2/3 (p = 0.049), however no statistical significance was assessed for stromal TNF-α.

For multivariate analyses, variable ages (>50 years versus ≤50 years), type (type II versus type I), differentiation grade (poorly differentiated versus well and moderately differentiated), staging (stages III-IV versus stages I–II), and epithelial IL-8 immunostaining (2/3 staining versus 0/1 staining) were considered. Borderline tumors were considered well differentiated tumors for the analysis. Multivariate analysis revealed that epithelial IL-8 staining 2/3 was an independent variable related to lower overall survival ((OR = 18.515. 95% CI: (1.160–295.549), p = 0.037) (Table 1, Fig. 3).

Table 2 provided the distribution of IL-8 epithelial immunostaining readings according to histological subtypes and staging of ovarian cancer.

4. Discussion

The study of inflammatory and immunological response in ovarian tumors is essential for discovering new prognostic factors and survivals. Immune system is critical in ovarian tumor evolution and in patients' prognosis. Immunological responses can facilitate their utility as accurate prognostic factors and help in better decisions regarding therapeutic approach [6]. Cytokines may act as potential prognostic and diagnostic biomarkers for some cancer types [6, 23].

A study evaluated the panel of cytokines in peritoneal fluid. Cytokines were known to be found in peritoneal fluid of ovarian tumors women. Study revealed the association of high IL-6 levels in peritoneal fluid with following hemogram parameters: neutrophil-lymphocyte ratio >3.18, and platelet-lymphocyte ratio >219.23. Moreover, IL-6 elevated levels in this environment were related to stage IIIC, serum cancer antigen (CA)-125 levels >35 U/mL, and shorter DFS. Higher IL-8 levels in peritoneal fluid were associated with platelet-lymphocyte ratio >219.23, and serum CA-125 levels >35 U/mL. Multivariate survival analysis showed that IL-6 levels in peritoneal fluid had role as an independent variable related to OS [15]. Our study found that the stronger stromal IL-8 immunostaining was linked to shorter OS.

A cytokine IL-8 was produced by several cells in response to inflammation and inflammatory processes associated with malignancy. Zuccari *et al.* [24] (2011) exhibited the correlation of IL-8 expression in breast stroma with local metastasis and/or

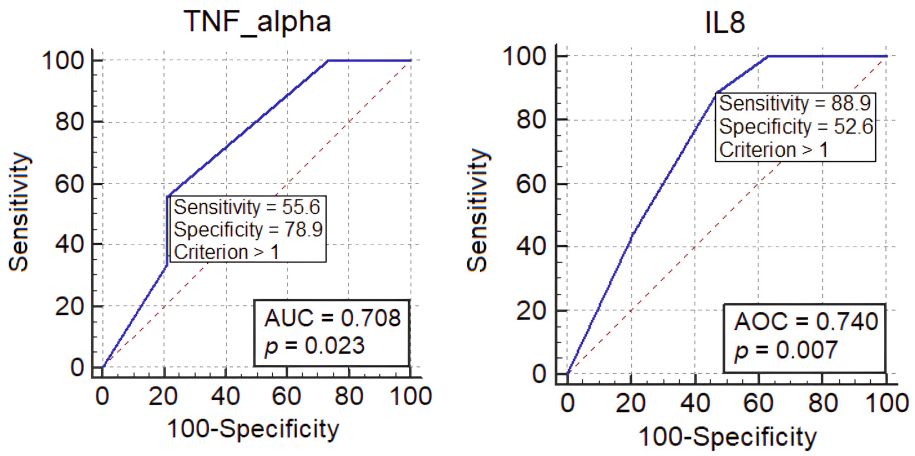

FIGURE 2. ROC curves: epithelial IL-8 staining, stromal TNF-alpha staining and death. TNF: tumor necrosis factor; IL: interleukins; AUC: area under curve.

TABLE 1. Univariate analysis and multivariate analysis of the variables age, type, histological grade, staging, surgery type, chemotherapy and epithelial IL-8 staining considering ovarian cancer and overall survival.

Variable	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*p*-value	RR (95% CI)	*p*-value
>50 years *vs.* ≤50 years	1.173 (0.5526 to 2.489)	1.000	1.392 (0.221–8.746)	0.724
Type II *vs.* type I	1.810 (0.8560 to 3.825)	0.227	11.726 (0.963–142.761)	0.054
Poorly *vs.* well/moderately differentiated tumors	4.222 (0.9411 to 18.943)	0.063	5.803 (0.687–49.044)	0.106
Stages III–IV *vs.* stages I–II	2.111 (1.0650 to 4.187)	0.103	0.820 (0.073–9.194)	0.872
Chemotherapy (Yes *vs.* no)	1.535 (0.9812 to 2.403)	0.195	0.607 (0.029–12.703)	0.748
Unsatisfactory debulking *vs.* complete surgery/satisfactory debulking	2.111 (0.3515 to 12.680)	0.574	1.278 (0.115–14.175)	0.842
Epithelial IL-8 (stainings 2/3 *vs.* stainings 0/1)	1.877 (1.1070 to 3.180)	0.049	18.515 (1.160–295.549)	0.039

IL: interleukin; RR: risk ratio; CI: confidence interval.

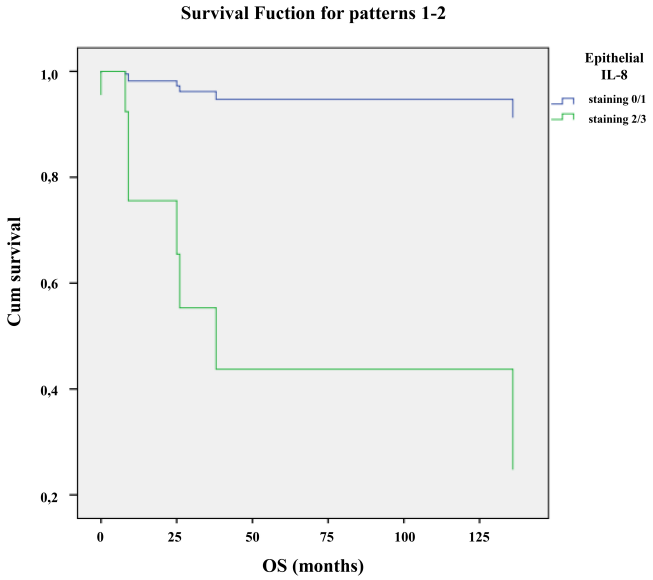

FIGURE 3. Cox regression: epithelial IL-8 staining 2/3 is an independent factor for lower overall survival. IL: interleukin; OS: overall survival.

TABLE 2. Distribution of IL-8 epithelial immunostaining readings according to histological subtypes and staging of ovarian cancer.

Patient number	Histology	Stage	IL-8 epithelial immunostaining
1	Serous cystadenocarcinoma	IIIC	3
2	Borderline ovarian tumor	IIIC	2
3	Borderline ovarian tumor	IIIA2	3
4	Endometrioid adenocarcinoma	IA	3
5	Borderline ovarian tumor	IC2	0
6	Adenocarcinoma	IIIC	1
7	Serous cystadenocarcinoma	IVB	2
8	Endometrioid adenocarcinoma	IIIC	3
9	Borderline ovarian tumor	IA	2
10	Mucinous cystadenocarcinomas	IVB	0
11	Serous cystadenocarcinoma	IC2	2
12	Serous cystadenocarcinoma	IIIC	0
13	Adenocarcinoma	IIIC	0
14	Borderline ovarian tumor	IA	1
15	Adenocarcinoma	IIIC	3
16	Serous cystadenocarcinoma	IIIC	3
17	Borderline ovarian tumor	IA	0
18	Serous cystadenocarcinoma	IIA	2
19	Borderline ovarian tumor	IB	2
20	Serous cystadenocarcinoma	IB	2
21	Serous cystadenocarcinoma	IIIC	3
22	Borderline ovarian tumor	IA	2
23	Clear cell carcinoma	IA	2
24	Serous cystadenocarcinoma	IIIB	1
25	Serous cystadenocarcinoma	IIIC	0
26	Mucinous cystadenocarcinomas	IA	0
27	Borderline ovarian tumor	IA	1
28	Borderline ovarian tumor	IA	3

IL: interleukin.

recurrence. Women treated through adjuvant chemotherapy and radiotherapy depicted lower IL-8 expressions in those with local recurrence [24]. A study revealed that IL-8 levels and the expressions of its receptors (CXCR1 and CXCR2) were correlated to histological grade, staging and lymph node metastasis in ovarian malignancy. Moreover, *in vitro*, the IL-8 promoted ovarian cancer cell proliferation in part *via* Wnt/β-catenin-mediated epithelial-mesenchymal transition [25]. This data expressed the complexity of IL-8 role in tumor environment and reinforced it as a potential prognostic marker.

IL-8 enhanced the proliferation of ovarian cancer cells grown on 3D spheroids [26]. IL-8 secreted by cancer-associated fibroblasts activated the normal ovarian fibroblasts and stimulated the ovarian cancer cells growth in animals [27]. A recent study evaluated the factors linked with complications and delays in adjuvant chemotherapy of ovarian malignancy patients treated through primary cytoreductive surgery. Patients with postoperative adversities had higher serum IL-6 and IL-8 levels than those without complications [28].

Regarding TNF-α, study by Asschert *et al.* [29] (1999) revealed that this cytokine had stimulating role for IL-6 to enhance autocrine growth in ovarian-carcinoma cell lines. IL-6 role in ovarian tumor cell proliferation raised question of how spontaneous and TNF-induced IL-6 expression in ovarian cancer was regulated. TNF-α did not induce IL-6 expression without the co-stimulatory signal. IFN-γ (Interferon-

γ) was essential for TNF-α to induce IL-6 in THP-1 (is a human leukemia monocytic cell line) monocytic cells. IFN-γ triggered IL-6 gene expression in human monocytes through change in amount and phosphorylation of Sp1, along with IRF-1 (Interferon regulatory factor 1) induction and activation. These factors worked synergistically with p65-NFκB homodimer which was activated by TNF-α [30]. The exact regulatory mechanisms of ovarian cancer needed further studies. In our study, the ROC curve drawn to verify whether stromal TNF-α immunostaining had significant association with death determined cut-off value more than staining 1. However, the Kaplan-Meier curve did not show statistical significance for OS or DFS.

The heterogeneity of histological types of ovarian neoplasms was a limitation of this study. Moreover, the study lacked larger number of patients' sample pool. However, based on these results, other studies might provide new perspectives regarding cytokines function in malignant ovarian neoplasms survivals. This study already demonstrated the IL-8 importance in peritumoral stroma as prognostic marker. The strong stromal IL-8 immunostaining could assist the oncologists in individualizing the patients' treatment and set the target for future ovarian cancer therapies. Cytokines could act as survival marker and guide for the preferred treatment option such as chemotherapeutic choice and time management which might result in better cure and improved patient life quality.

Any other study could not be found in literature that evaluated number of tissue cytokines at the same time in epithelium and peritumoral stromal compartments and their association with survivals in ovarian cancer. In future, tissue immunostaining of cytokines could be employed for prognosis and survival outcomes. Moreover, the immunohistochemical method could be utilized in routine clinical practice and made accessible to oncology services.

5. Conclusions

Epithelial IL-8 immunostaining predicts overall survival in ovarian malignancy patients. This cytokine can be the target of studies for discovering other management systems of epithelial ovarian cancer and borderline ovarian tumor.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

RSN and EFCM—protocol/project development. MBMN, LMMCS and RME—data collection or management. AMF, MPJ and RME—immunohistochemical experiments. AMF, MPJ, RSN and EFCM—data analysis. MBMN, LMMCS, AMF, MPJ and RSN—manuscript writing/editing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Research Ethics Committee of the Federal University of Triângulo Mineiro approved this study (1408/2009). All patients who participated in the study signed informed consent.

ACKNOWLEDGMENT

Not applicable.

FUNDING

The authors wish to acknowledge the funding received from the CNPq (Grant No.: 301115/2018-4), FAPEMIG (Grant No.: RED-00011-14), FUNEPU (Grant No.: 02 2011), and CAPES (Grant No.: 32012012001P5), AREMG (Grant No.: TCPRM 2019).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] The Global Cancer Observatory—World Health Organization. Ovary source: globocan 2020. 2020. Available at: https://gco.iarc.fr/today/data/factsheets/cancers/25-Ovary-fact-sheet.pdf (Accessed: 10 February 2023).

[2] INCA. Estimativa de Câncer no Brasil. 2020. Available at: https://www.inca.gov.br/publicacoes/livros/estimativa-2020-incidencia-de-cancer-no-brasil (Accessed: 10 February 2023).

[3] INCA. MS/INCA/Coordenação de Prevenção e Vigilância/Divisão de Vigilância e Análise de Situação. 2021. Available at: https://www.inca.gov.br/en/node/2244 (Accessed: 10 February 2023).

[4] Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. Nature Reviews Clinical Oncology. 2018; 15: 366–381.

[5] Hale MD, Hayden JD, Grabsch HI. Tumour-microenvironment interactions: role of tumour stroma and proteins produced by cancer-associated fibroblasts in chemotherapy response. Cellular Oncology. 2013; 36: 95–112.

[6] Sanguinete MMM, Oliveira PHD, Martins-Filho A, Micheli DC, Tavares-Murta BM, Murta EFC, *et al*. Serum IL-6 and IL-8 correlate with prognostic factors in ovarian cancer. Immunological Investigations. 2017; 46: 677–688.

[7] Baghdadi M, Endo H, Takano A, Ishikawa K, Kameda Y, Wada H, *et al*. High co-expression of IL-34 and M-CSF correlates with tumor progression and poor survival in lung cancers. Scientific Reports. 2018; 8: 418.

[8] Endo H, Hama N, Baghdadi M, Ishikawa K, Otsuka R, Wada H, *et al*. Interleukin-34 expression in ovarian cancer: a possible correlation with disease progression. International Immunology. 2020; 32: 175–186.

[9] Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, *et al*. Interleukin-6 as a therapeutic target in human ovarian cancer. Clinical Cancer Research. 2011; 17: 6083–6096.

[10] Kim S, Gwak H, Kim HS, Kim B, Dhanasekaran DN, Song YS. Malignant ascites enhances migratory and invasive properties of ovarian cancer cells with membrane bound IL-6R *in vitro*. Oncotarget. 2016; 7: 83148–83159.

[11] Niu N, Shen W, Zhong Y, Bast RC, Jazaeri A, Sood AK, *et al*. Expression of B7–H4 and IDO1 is associated with drug resistance and poor prognosis in high-grade serous ovarian carcinomas. Human Pathology. 2021; 113: 20–27.

[12] Lin C, He H, Liu H, Li R, Chen Y, Qi Y, *et al*. Tumour-associated

macrophages-derived CXCL8 determines immune evasion through autonomous PD-L1 expression in gastric cancer. Gut. 2019; 68: 1764–1773.

[13] Mikami S, Mizuno R, Kosaka T, Saya H, Oya M, Okada Y. Expression of TNF-α and CD44 is implicated in poor prognosis, cancer cell invasion, metastasis and resistance to the sunitinib treatment in clear cell renal cell carcinomas. International Journal of Cancer. 2015; 136: 1504–1514.

[14] de Lima CA, Silva Rodrigues IS, Martins-Filho A, Côbo Micheli D, Martins Tavares-Murta B, Candido Murta EF, *et al*. Cytokines in peritoneal fluid of ovarian neoplasms. Journal of Obstetrics and Gynaecology. 2020; 40: 401–405.

[15] Rodrigues ISS, Martins-Filho A, Micheli DC, Lima CAD, Tavares-Murta BM, Murta EFC, *et al*. IL-6 and IL-8 as prognostic factors in peritoneal fluid of ovarian cancer. Immunological Investigations. 2020; 49: 510–521.

[16] Batchu RB, Gruzdyn OV, Kolli BK, Dachepalli R, Umar PS, Rai SK, *et al*. IL-10 signaling in the tumor microenvironment of ovarian cancer. Advances in Experimental Medicine and Biology. 2021; 1290: 51–65.

[17] Kumar P, Ranmale S, Tongaonkar H, Mania-Pramanik J. Immune profile of blood, tissue and peritoneal fluid: a comparative study in high grade serous epithelial ovarian cancer patients at interval debulking surgery. Vaccines. 2022; 10: 2121.

[18] Smycz-Kubańska M, Stępień S, Gola JM, Kruszniewska-Rajs C, Wendlocha D, Królewska-Daszczyńska P, *et al*. Analysis of CXCL8 and its receptors CXCR1/CXCR2 at the mRNA level in neoplastic tissue, as well as in serum and peritoneal fluid in patients with ovarian cance. Molecular Medicine Reports. 2022; 26: 296.

[19] Micheli DC, Jammal MP, Martins-Filho A, Côrtes JRXDM, Souza CND, Nomelini RS, *et al*. Serum cytokines and CXCR2: potential tumour markers in ovarian neoplasms. Biomarkers. 2020; 25: 474–482.

[20] Kampan NC, Kartikasari AER, Deceneux C, Madondo MT, McNally OM, Flanagan KL, *et al*. Combining TNFR2-expressing tregs and IL-6 as superior diagnostic biomarkers for high-grade serous ovarian cancer masses. Cancers. 2023; 15: 667.

[21] Kurman RJ, Shih I. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. The American Journal of Surgical Pathology. 2010; 34: 433–443.

[22] Kurman RJ, Shih I. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer—shifting the paradigm. Human Pathology. 2011; 42: 918–931.

[23] Spacek J, Vocka M, Netikova I, Skalova H, Dundr P, Konopasek B, *et al*. Immunological examination of peripheral blood in patients with colorectal cancer compared to healthy controls. Immunological Investigations. 2018; 47: 643–653.

[24] Zuccari DAPDC, Leonel C, Castro R, Gelaleti GB, Jardim BV, Moscheta MG, *et al*. An immunohistochemical study of interleukin-8 (IL-8) in breast cancer. Acta Histochemica. 2012; 114: 571–576.

[25] Wen J, Zhao Z, Huang L, Wang L, Miao Y, Wu J. IL-8 promotes cell migration through regulating EMT by activating the Wnt/β-catenin pathway in ovarian cancer. Journal of Cellular and Molecular Medicine. 2020; 24: 1588–1598.

[26] Uddin MM, Gaire B, Vancurova I. Interleukin-8 induces proliferation of ovarian cancer cells in 3D spheroids. Methods in Molecular Biology. 2020; 2108: 117–124.

[27] Ji Z, Tian W, Gao W, Zang R, Wang H, Yang G. Cancer-associated fibroblast-derived interleukin-8 promotes ovarian cancer cell stemness and malignancy through the notch3-mediated signaling. Frontiers in Cell and Developmental Biology. 2021; 9: 684505.

[28] Ade CJ, Dockery L, Walter AC, Benbrook DM, Vesely SK, Hammond ST, *et al*. Association between serum biomarkers with postoperative complications and delay of adjuvant chemotherapy initiation in ovarian cancer patients undergoing primary cytoreductive surgery: a pilot study. Nutrition and Cancer. 2023; 75: 662–669.

[29] Asschert JGW, Vellenga E, Ruiters MHJ, de Vries EGE. Regulation of spontaneous and TNF/IFN-induced IL-6 expression in two human ovarian-carcinoma cell lines. International Journal of Cancer. 1999; 82: 244–249.

[30] Sancéau J, Kaisho T, Hirano T, Wietzerbin J. Triggering of the human interleukin-6 gene by interferon-gamma and tumor necrosis factor-alpha in monocytic cells involves cooperation between interferon regulatory factor-1, NFκB, and Sp1 transcription factors. Journal of Biological Chemistry. 1995; 270: 27920–27931.

How to cite this article: Marilha B. M. Neves, Letícia M. M. C. Scofoni, Agrimaldo Martins-Filho, Millena P. Jammal, Renata M. Etchebehere, Eddie F. C. Murta, *et al*. Epithelial IL-8 immunostaining associated with overall survival in ovarian cancer. European Journal of Gynaecological Oncology. 2024; 45(1): 10-16. doi: 10.22514/ejgo.2024.003.