doi: 10.48095/cccg2024377

Evaluation of cytokine immunostaining in ovarian neoplasms and endometriomas

Hodnocení cytokinového imunobarvení u ovariálních novotvarů a endometriomů

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Summary: **Objective:** The objective of our study was to quantify and compare the immunostaining of IL-2, IL-5, IL-6, IL-8, and TNF-α in endometriomal tissue, non-neoplastic tumors, benign neoplasms, and malignant ovarian neoplasms. **Materials and methods:** The study involved 90 patients: 15 non-neoplastic ovarian lesions, 28 ovarian benign neoplasms, 28 ovarian malignant neoplasms, and 19 ovarian endometriomas were diagnosed. Immunohistochemistry was performed for cytokines IL-2, IL-5, IL-6, IL-8, and TNF-α and their concentrations were compared in these groups. Fisher's exact test was used, requiring a P-value of < 0.05 for significance. **Results:** IL-5 and IL-8 epithelial immunostaining is stronger in endometriomas than in ovarian cancer (P-values of 0.0046 and 0.0149, resp.). The stromal immunostaining of TNF-α, IL-5, IL-6, and IL8 is stronger in endometriomas than in ovarian cancer (P-values of 0.0008, < 0.0001, 0.0003, and 0.0006, resp.). **Conclusions:** Stronger immunostaining of some cytokines in endometriomas compared to ovarian cancer reflects an inflammatory and immune response that could be future targets for new discoveries about the infiltrative behavior of endometriosis.

Key words: endometriosis – ovarian neoplasms – cytokines – immunology

Souhrn: **Cíl:** Cílem naší studie bylo kvantifikovat a porovnat imunobarvení IL-2, IL-5, IL-6, IL-8 a TNFα ve tkáni endometriomu, nenádorových nádorech, benigních novotvarech a maligních novotvarech vaječníků. **Materiál a metody:** Studie se zúčastnilo 90 pacientek; bylo diagnostikováno 15 nenádorových lézí ovaria, 28 ovariálních benigních novotvarů, 28 ovariálních maligních novotvarů a 19 ovariálních endometriomů. Imunohistochemie byla provedena pro cytokiny IL-2, IL-5, IL-6, IL-8 a TNFα a jejich koncentrace byly v těchto skupinách porovnány. Použili jsme Fisherův exaktní test, podle něhož jsou významné hodnoty p < 0,05. **Výsledky:** Imunobarvení epitelu na přítomnost IL-5 a IL-8 je silnější u endometriomů než u karcinomu vaječníků (p hodnoty 0,0046 a 0,0149). Stromální imunobarvení TNFα, IL-5, IL-6 a IL8 je silnější u endometriomů než u rakoviny vaječníků (p hodnoty 0,0008; < 0,0001; 0,0003 a 0,0006). **Závěr:** Silnější imunobarvení některých cytokinů u endometriomů ve srovnání s karcinomem ovaria odráží zánětlivou a imunitní odpověď, která by mohla být budoucím cílem nových objevů o infiltrativním chování endometriózy.

Klíčová slova: endometrióza – ovariální novotvary – cytokiny – imunologie

Introduction

Endometriosis is a benign disease of the female genital system that is characterized by the presence of ectopic endometrial tissue and may present in a diffuse manner with pelvic implants, and in a localized manner as in ovarian endometrioma. It is a chronic disease that is difficult to diagnose. Literature data indicate a prevalence of 5% to 10% in women of childbearing age [1]. Ovarian endometriomas are found in 17–44% of patients diagnosed with endometriosis [2].

Cytokines are the main mediators of the immune system. They have an immunological-regulatory role, which can be pro- or anti-inflammatory. To remain effective, the immune response requires a delicate balance between these actions. Thus, any dysregulation of cytokines becomes an important aspect in the progression of numerous pathological conditions, including endometriosis. It is known that in women with

endometriosis, the peritoneal fluid contains a higher concentration of pro-inflammatory cytokines. However, further studies are needed to elucidate the role of cytokines in the mechanisms of endometriotic implants and their growth [3].

Studies report a link between endometriosis and ovarian cancer. Kok et al. (2015) showed that patients diagnosed with endometriosis are 4-times more likely to have ovarian cancer [4]. In another study, it was shown that 19% of ovarian epithelial tumors were related to endometriosis [5].

In the search for new therapeutic possibilities, studies have been carried out on the role of immunology in the pathophysiology of endometriosis. These have led to the hypothesis of dysregulation in the immune system of women with endometriosis and resulting in failure to contain the growth of endometrial tissue in the pelvic cavity and even stimulation of angiogenesis in this tissue [3].

The objective of this study was to quantify and compare epithelial and stromal immunostaining of IL-2, IL-5, IL-6, IL-8, and TNF-α in endometriomal tissue, non-neoplastic tumors, benign neoplasms, and malignant ovarian neoplasms.

Materials and methods

Patients

Patients were evaluated and treated at the Pelvic Mass Outpatient Clinic of our service with an indication for surgical treatment according to pre-established criteria [6]. The histopathological results of paraffin-embedded tissues and the anatomopathological results were reviewed, and patients with a confirmed diagnosis of non-neoplastic ovarian lesions (N = 15), benign neoplasias, (N = 28), malignant neoplasias (N = 28), and endometriomas (N = 19) were included in the study.

Exclusion criteria were secondary malignant ovarian neoplasms (metastasis), previous treatment, use of immunosuppressive medications, and comorbidity with diseases that lead to immunosuppression and relapse.

The study was approved by the Research Ethics Committee of the institution (protocol 1,408) and informed consent was obtained from all participants.

Anatomopathological study

The anatomopathological evaluation and staging of the cases were performed according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) [7]. The histopathological study was performed by the Surgical Pathology Service on paraffin-embedded sections.

Immunohistochemistry study

Immunohistochemistry was performed for cytokines IL-2, IL-5, IL-6, IL-8, and TNF-α using standard techniques as described briefly below.

Slices (4 μm) of the paraffin-embedded tissues from the selected cases were adhered to silanized slides (ATPS – Silane, Sigma® A3648), and then processed using the Novolink™ Polymer Detection System (Leica Biosystems Inc.). The slides were kept in an oven at a temperature of 56 °C for a period of 24 hod, and then they were dewaxed in two xylol baths, remaining 5 min in each bath and dehydrated in three baths of absolute alcohol and one bath of 80% alcohol. The slides were then placed in a PBS bath (pH 7.2) for 5 min for hydration.

Heat-mediated antigen retrieval was then performed as follows. The slides were placed in cytology tubes containing a 10 mM citrate buffer solution (pH 6.0) or Tris-EDTA, according to the manufacturer's instructions, and placed in a distilled water bath for 30 minutes at a temperature of 100 °C. The tubes were then removed from the bath and placed on the bench for cooling to an ambient temperature of 22 °C.

The slides were placed on a rack and washed with distilled water. The slides were then wiped dry adjacent to the tissue sections while not allowing the tissue sections to dry out. The slides were placed on an incubation board and endogenous peroxidase was neutralized with a Peroxidase Blocker (3% hydrogen peroxide) applied to each tissue section for 5 min. The slides were then washed 3-times in a PBS buffer for 5 min per wash. The slides were then incubated with a Protein Blocker for 5 min and then washed 3-times in PBS buffer for 5 min per wash. Specific anti-TNF-α, anti-IL2, anti-IL5, anti-IL6, and anti-IL8 antibodies were diluted in Bovine Serum Albumin (BSA, Sigma®) and incubated with the prepared tissue sections in a humid chamber at 4 °C following the manufacturer's specifications. These preparations of bovine albumin (2% BSA) and primary antibody serve to block nonspecific binding and reduce background color.

Positive controls were used for each antibody according to the manufacturer's directions. After incubation with the primary antibodies, the slides were washed twice with PBS buffer for 5 min per wash. The slides were then incubated with a post-primary antibody for 30 min and then washed twice with PBS buffer for 5 min. The slides were then incubated with a Novolink™ Polymer for 30 min and were washed twice with PBS buffer for 5 min per wash. The slides were then developed by adding chromogenic solution (DAB–diaminobenzidine), and incubating for 5 min followed by a wash in running water and counterstaining with Harris' hematoxylin. Finally, the slides were immersed in three baths of absolute alcohol for 10 s each to remove excess water, one bath of phenylated xylol, and three baths of xylol for 5 min each. Coverslips were added over the slides with Entellan® (Merck Millipore) mounting medium for further analysis.

Two observers evaluated the slides, observing immunostaining in the epithelium and stroma. The immunostaining intensity was assessed subjectively using a scale of 0–3:

- 0 (no staining);
- 1 (weak staining);
- 2 (moderate staining);
- 3 (strong staining) (Fig. 1).

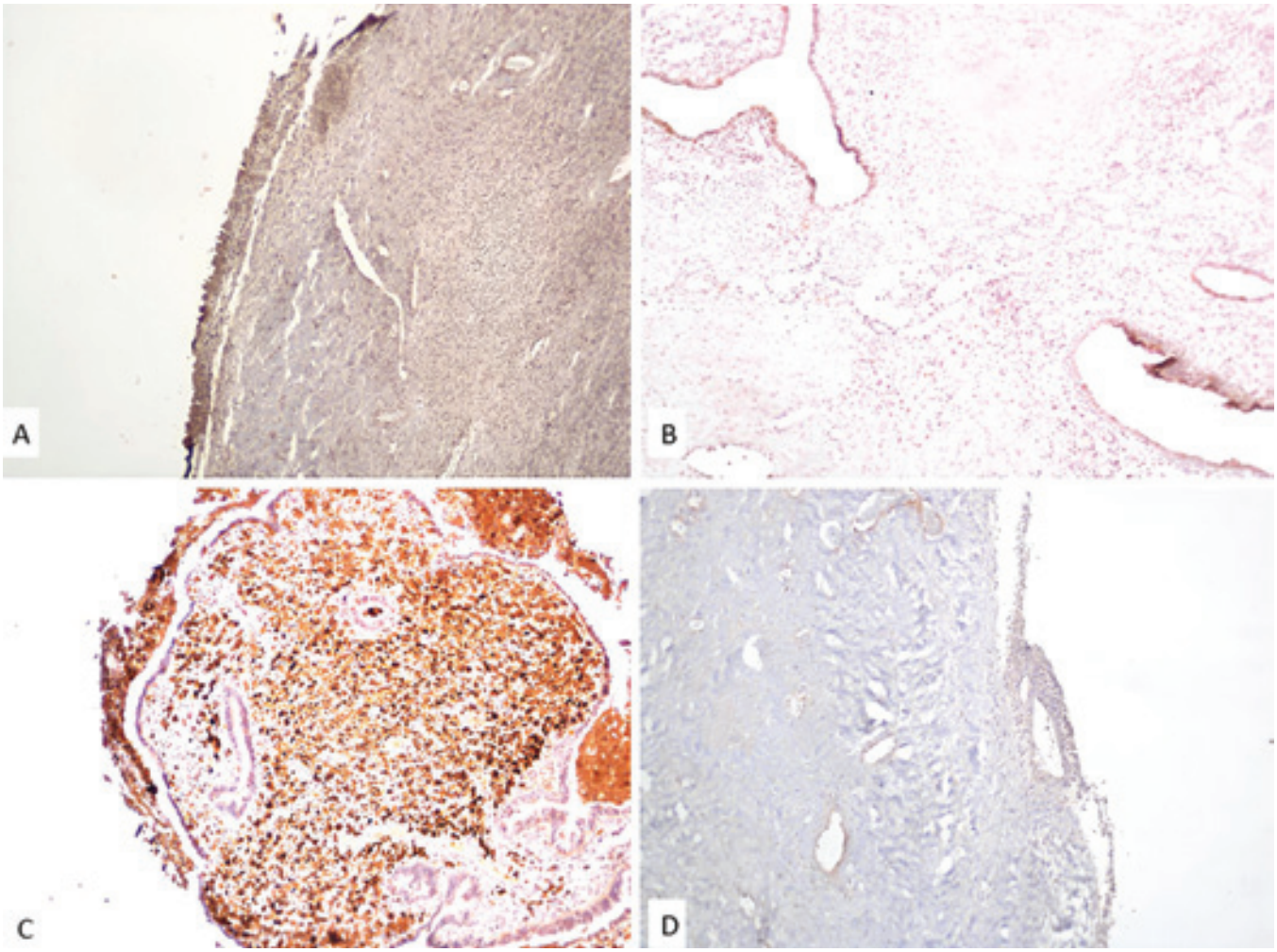

Fig. 1. Histological section of endometriomas, showing immunohistochemical staining of polyclonal anti-TNF-α (A, 100×); polyclonal anti-IL-6 (B, 100×); polyclonal anti-IL-8 (C, 100×); polyclonal anti-IL-5 (D, 100×).
Obr. 1. Histologický řez endometriomy, ukazující imunohistochemické barvení polyklonální anti-TNF-α (A, 100×); polyklonální anti-IL-6 (B, 100×); polyklonální anti-IL-8 (C, 100×); polyklonální anti-IL-5 (D, 100×).

Statistical analysis

The data were analyzed using the GraphPad Prism 6 software. Fisher's exact test was used to assess immunohistochemistry staining intensity between the tissue types. Statistical significance was considered when the P-value was ≤ 0.05. The Kappa test was performed to verify agreement among the observers. Discordant cases were reviewed together and results were obtained by consensus. Agreement between two observers was performed through kappa: $\kappa < 0.4$: weak agreement; $0.4 \leq \kappa < 0.8$: moderate agreement; $0.8 \leq \kappa < 1.0$: strong agreement; $\kappa = 1.0$: perfect agreement. The cut-off value used was immunostaining 1. Above immunostaining 1 (immunostaining 2 and 3), we consider strong immunostaining. Below or equal to 1 (immunostaining 0 and 1), we consider weak immunostaining. Sensitivity, specificity, and accuracy in endometriomas and ovarian cancer diagnosis were calculated.

Results

The evaluated groups were: non-neoplastic ovarian lesions (N = 15), benign neoplasias, (N = 28), malignant neoplasias (N = 28), and endometriomas/endometrial cysts (N = 19).

The median age of the study participants was 46 years (35–82 years) in the non-neoplastic group, 43 years (20–61 years) in the endometrioma group, 48 years (18–69 years) in the benign neoplasm group, and 51 years (25–73 years) in the group of malignant neoplasms.

The staging of malignant neoplasms according to FIGO were: IA (nine patients; 32.14%), IB (two patients; 7.14%), IC2 (two patients; 7.14%), IIA (one patient; 3.57%), IIB (one patient; 3.57%), IIIB (one patient; 3.57%), IIIC (ten patients; 35.71%), and IVB (two patients; 7.14%). The histological grading of the malignant neoplasms was: grade 1 (11 patients; 39.28%), grade 2 (11 patients; 39.28%), and grade 3 (six patients; 21.42%).

Tab. 1. Differences in epithelial immunostaining of cytokines in malignant ovarian neoplasms, benign ovarian neoplasms, endometriomas and non-neoplastic lesions of the ovary.
Tab. 1. Rozdíly v epiteliálním imunobarvení cytokinů u maligních ovariálních novotvarů, benigních ovariálních novotvarů, endometriomů a nenádorových lézí ovaria.

		Immunostaining 2/3	P-value
TNF-α	malignant neoplasms x	25 (89.3%)	**0.01**
	benign neoplasms	16 (57.1%)	
	endometriomas x	16 (84.2%)	0.0632
	malignant neoplasms	16 (57.1%)	
	endometriomas x	16 (84.2%)	**< 0.0001**
	benign neoplasms	2 (7.1%)	
	endometriomas x	16 (84.2%)	0.238
	non-neoplastic lesions	15 (100%)	
IL-2	malignant neoplasms x	2 (7.1%)	**0.0001**
	benign neoplasms	26 (92.9%)	
	endometriomas x	8 (42.1%)	**0.0002**
	malignant neoplasms	26 (92.9%)	
	endometriomas x	8 (42.1%)	**0.0009**
	benign neoplasms	25 (89.3%)	
	endometriomas x	8 (42.1%)	0.2714
	non-neoplastic lesions	3 (20%)	
IL-5	malignant neoplasms x	22 (78.6%)	**0.02**
	benign neoplasms	13 (46.4%)	
	endometriomas x	17 (89.5%)	**0.0046**
	malignant neoplasms	13 (46.4%)	
	endometriomas x	17 (89.5%)	1.0
	benign neoplasms	24 (85.7%)	
	endometriomas x	17 (89.5%)	**0.0035**
	non-neoplastic lesions	6 (40%)	
IL-6	malignant neoplasms x	20 (71.4%)	1.0
	benign neoplasms	20 (71.4%)	
	endometriomas x	15 (78.9%)	0.7365
	malignant neoplasms	20 (71.4%)	
	endometriomas x	15 (78.9%)	1.0
	benign neoplasms	22 (78.6%)	
	endometriomas x	15 (78.9%)	0.113
	non-neoplastic lesions	15 (100%)	
IL-8	malignant neoplasms x	18 (64.3%)	1.0
	benign neoplasms	17 (60.7%)	
	endometriomas x	18 (94.7%)	**0.0149**
	malignant neoplasms	17 (60.7%)	
	endometriomas x	18 (94.7%)	0.064
	benign neoplasms	20 (71.4%)	
	endometriomas x	18 (94.7%)	**1.0**
	non-neoplastic lesions	15 (100%)	

Comparison between immunostaining 0 and 1 vs. 2 and 3 of each cytokine, in the epithelial environment. Fisher's exact test, with a significance level lower than 0.05 are highlighted.

The histological types of ovarian malignancies were: borderline mucinous tumors (nine patients; 32.14%), serous papillary cystadenocarcinomas (eight patients; 28.57%), mucinous cystadenocarcinomas (two patients; 7.14%), serous cystadenocarcinomas (two patients; 7.14%), and one patient each (3.57%) for adenocarcinoma, papillary anaplastic adenocarcinoma, endometrioid adenocarcinoma, grade III poorly differentiated adenocarcinoma, clear cell carcinoma, borderline serous tumor, and atypical borderline proliferative endometrioid tumor.

The histological types of benign ovarian neoplasms were: serous cystadenomas (16 patients; 57.14%), mucinous cystadenomas (nine patients; 32.14%), mucinous cystadenomas with Brenner's tumor (two patients; 7.14%), and serous papillary cystadenoma with Brenner's tumor (one patient; 3.57%).

The histological types of non-neoplastic ovarian lesions were: simple cysts (five patients; 33.33%), theca-lutein cysts (four patients; 26.66%), follicular cysts (three patients; 20%), inclusion cyst (one patient; 6.66%), hemorrhagic cyst (one patient; 6.66%), and corpus luteum hematoma (one patient; 6.66%).

Immunohistochemical study

Agreement between observers from the Kappa test ranged from strong to perfect. Tab. 1 compares the cytokine epithelial immunostaining in malignant and benign ovarian neoplasms, endometriomas, and non-neoplastic lesions. There was stronger TNF-α immunostaining in benign neoplasms compared to malignant neoplasms ($P = 0.01$), and in endometriomas compared to benign neoplasms ($P < 0.0001$). With IL-2, there was stronger immunostaining in malignant neoplasms compared to benign neoplasms ($P = 0.0001$), malignant neoplasms compared to endometriomas ($P = 0.0002$), and in benign neoplasms in relation to endometriomas ($P = 0.0009$). With IL-5, there was stronger

immunostaining in benign neoplasms compared to malignant neoplasms (P = 0.02), in endometriomas compared to malignant neoplasms (P = 0.0046), and in endometriomas compared to non-neoplastic lesions (P = 0.0035). With IL-8, there was stronger immunostaining in endometriomas than in malignant neoplasms (P = 0.0149).

Tab. 2 compares cytokine stromal staining in malignant and benign ovarian neoplasms, endometriomas, and non-neoplastic lesions. For TNF-α, there was stronger immunostaining in benign neoplasms in relation to malignant ovarian neoplasms (P = 0.001), stronger immunostaining in endometriomas compared to malignant neoplasms (P = 0.0008), and stronger immunostaining in endometriomas compared to benign neoplasms (P < 0.0001). With IL-2, there was stronger immunostaining in malignant neoplasms than in benign ovarian neoplasms (P = 0.004), and stronger immunostaining in benign neoplasms compared to endometriomas (P = 0.0434). For IL-5, there was stronger immunostaining in benign neoplasms compared to malignant ovarian neoplasms (P = 0.0003), stronger immunostaining in endometriomas than in malignant neoplasms (P < 0.0001), stronger immunostaining in endometriomas than in benign ovarian neoplasms (P = 0.009), and stronger immunostaining in endometriomas than in non-neoplastic lesions (P = 0.0135). With IL-6, there was stronger immunostaining in endometriomas than in malignant ovarian neoplasms (P = 0.0003), and stronger immunostaining in endometriomas than in benign neoplasms (P < 0.0001). For IL-8, there was stronger immunostaining in malignant than in benign neoplasms (P = 0.004), and stronger immunostaining in endometriomas than in malignant ovarian neoplasms (P = 0.0006).

Sensitivity, specificity, and accuracy in endometriomas and ovarian cancer diagnosis were calculated. The epithelial immunostaining of IL-5 and IL-8 is stronger

Tab. 2. Differences in stromal immunostaining of cytokines in malignant ovarian neoplasms, and benign neoplasms, endometriomas and non-neoplastic lesions of the ovary.

Tab. 2. Rozdíly ve stromálním imunobarvení cytokinů u maligních ovariálních novotvarů a benigních novotvarů, endometriomů a nenádorových lézí ovaria.

		Immunostaining 2/3	P-value
TNF-α	malignant neoplasms x	22 (78.6%)	**0.001**
	benign neoplasms	9 (32.1%)	
	endometriomas x	16 (84.2%)	**0.0008**
	malignant neoplasms	9 (32.1%)	
	endometriomas x	16 (84.2%)	**< 0.0001**
	benign neoplasms	0 (0%)	
	endometriomas x	16 (84.2%)	0.238
	non-neoplastic lesions	15 (100%)	
IL-2	malignant neoplasms x	0 (0%)	**0.004**
	benign neoplasms	8 (28.6%)	
	endometriomas x	7 (36.8%)	0.7507
	malignant neoplasms	8 (28.6%)	
	endometriomas x	7 (36.8%)	**0.0434**
	benign neoplasms	19 (67.9%)	
	endometriomas x	7 (36.8%)	0.4513
	non-neoplastic lesions	3 (20%)	
IL-5	malignant neoplasms x	19 (67.9%)	**0.0003**
	benign neoplasms	5 (17.6%)	
	endometriomas x	15 (78.9%)	**< 0.0001**
	malignant neoplasms	5 (17.6%)	
	endometriomas x	15 (78.9%)	**0.009**
	benign neoplasms	11 (39.3%)	
	endometriomas x	15 (78.9%)	**0.0135**
	non-neoplastic lesions	5 (33.3%)	
IL-6	malignant neoplasms x	11 (39.3%)	0.57
	benign neoplasms	8 (28.6%)	
	endometriomas x	16 (84.2%)	**0.0003**
	malignant neoplasms	8 (28.6%)	
	endometriomas x	16 (84.2%)	**< 0.0001**
	benign neoplasms	5 (17.6%)	
	endometriomas x	16 (84.2%)	0.238
	non-neoplastic lesions	15 (100%)	
IL-8	malignant neoplasms x	5 (17.6%)	**0.04**
	benign neoplasms	13 (46.4%)	
	endometriomas x	18 (94.7%)	***0.0006***
	malignant neoplasms	13 (46.4%)	
	endometriomas x	18 (94.7%)	**0.2154**
	benign neoplasms	22 (78.6%)	
	endometriomas x	18 (94.7%)	**1.0**
	non-neoplastic lesions	15 (100%)	

Comparison between immunostaining 0 and 1 vs. 2 and 3 of each cytokine, in the stromal environment. Fisher's exact test, with a significance level lower than 0.05 are highlighted.

in endometriomas than in ovarian cancer. For epithelial IL-5, sensitivity, specificity, and accuracy were 89.5%, 53.6%, and 68.1%, resp. For epithelial IL-8, sensitivity, specificity, and accuracy were 94.7%, 39.3%, and 61.7%, resp.

The stromal immunostaining of TNF-α, IL-5, IL-6, and IL-8 is stronger in endometriomas than in ovarian cancer. For stromal TNF-α, sensitivity was 84.2%, specificity was 67.9%, and accuracy was 74.5%. For stromal IL-5, sensitivity, specificity, and accuracy were 78.9%, 82.1%, and 80.8%, resp. For stromal IL-6, sensitivity was 84.2%, specificity was 71.4%, and accuracy was 76.6%. For stromal IL-8, sensitivity, specificity, and accuracy were 94.7%, 53.6%, and 70.2%, resp.

Discussion

Association between endometriosis and ovarian malignant tumors have been reported [8–10]. The pathophysiology of endometriosis is not well established. There are multiple and complex endocrine-immunological interactions involved in this process, and the immune response participates in this context. The study of the role of the immune response in this disease may lead to the discovery of new therapeutic targets [11,12].

Endometriosis is a disease with a prolonged inflammatory response. The presence of ectopic tissues in the peritoneal cavity, without a clear mechanism, reaches a state of equilibrium with a high level of pro-inflammatory cytokines and immune cells [13]. It is well established that women with endometriosis have immune dysfunction in the form of intensified local and systemic inflammation [3,14]. Studies have shown that inflammation is a risk factor for ovarian cancer, and the inflammatory response is involved in almost all stages of tumor development [15,16]. In tumors, histological analysis has shown varying degrees of infiltration of immune cells, suggesting the recruitment of these cells in response to neoplastic proliferation [17], leading to chronic inflammation and tumor development and progression [18].Thus, local inflammation and immune dysregulation characterize both endometriosis and cancer. Like cancer, endometriosis resembles a chronic wound that does not heal [19]. The lymphatic vessels invaded in endometriosis can serve as a channel for the infiltration of immune cells, which further increases the inflammatory state in the endometriotic microenvironment [13].

Inflammatory behavior of the peritoneal fluid in patients with endometriosis is complex, and some studies have demonstrated the role of cytokines in this environment [12,20,21]. One study used mass cytometry to assess immune cell compartments in endometriosis, demonstrating the dynamic spectrum of cell signatures across disease stages [20]. Modifications to this immune microenvironment can account for the mechanisms of recruitment and functional regulation of Tregs in endometriotic lesions [21]. In our study, we observed and measured epithelial and stromal immunostaining of IL-2, IL-5, IL-6, IL-8, and TNF-α in endometriomal tissue, non-neoplastic tumors, benign neoplasms, and malignant neoplasms of the ovary. Our results show endometriotic tissue, both epithelial and stromal, is rich in immunological cytokines.

Immune disorders have been suggested to contribute to the development and progression of endometriosis, creating a microenvironment that supports the survival and implantation of endometriotic cells [22–24]. In our study, there was stronger epithelial and stromal TNF-α immunostaining in benign neoplasms compared to malignant neoplasms, and in endometriomas compared to benign neoplasms. For stromal immunostaining, we observed stronger immunostaining in endometriomas compared to ovarian cancer. It is suggested that the inflammatory response in endometriosis increases due to cytokines such as TNF-α [25,26]. The presence of TNF-α polymorphisms increases the risk of endometriosis and its development [27,28], as suggested by elevated levels of TNF-α in peritoneal fluid and the positive regulation of TNF-α in peritoneal macrophages and peripheral blood monocytes [29,30].

We observed stronger stromal immunostaining of IL-6 in endometriomas when compared to malignant and benign ovarian neoplasms. Similarly, Tsudo et al. (2000) detected a significant increase in IL-6 expression in endometriotic tissue derived from cell stroma [31]. A study demonstrated a significant production of IL-1β, IL-6, and TNF-α levels in endometriotic tissue and endometrium, with significant differences between tissue types, indicating a deviating cytokine pattern in both endometriotic tissue and endometrium from women with endometriosis compared with that from healthy controls [32].

In the present study, IL-8 immunostaining was strong in endometriomas, both in epithelial and stromal components, and in agreement with two studies that analyzed the immunostaining of this cytokine in ectopic endometrial tissues. These researchers suggested that IL-8 and its receptor system would be involved in the pathogenesis of endometriosis [33,34]. A stronger stromal immunostaining of IL-8 was observed in malignant neoplasms compared to benign neoplasms. Increased expression of IL-8 is associated with increased cell proliferation, angiogenesis, and metastases during cancer progression, with a worse prognosis noted in prostate and ovarian cancer [35].

The association of a panel of cytokines (IL-1α, IL-1β, and IL-6) in endometrial fluid aspiration was able to predict stage 3–4 endometriosis [36]. Peritoneal fluid is also an interesting environment for the study of endometriosis. One study evaluated some cytokines in the serum and peritoneal fluid of women with endometriosis. IL-8 and TGF-β levels were higher in patients with endometriosis

compared to the control group [37]. In our study, there was stronger IL-8 immunostaining in malignant than in benign neoplasms, and stronger immunostaining in endometriomas than in ovarian cancer.

IL-2 modulates the expression of receptors for other cytokines and transcription factors, promoting or inhibiting cytokine cascades that correlate with each T helper cell differentiation state [38]. Velasco et al. (2007) demonstrated that clinical treatment with IL-2 in rats significantly reduced the size of endometriosis implants. Thus, this interleukin could become an effective alternative for the non-hormonal treatment of endometriosis [39]. In our study with IL-2, there was stronger epithelial and stromal immunostaining in malignant neoplasms compared to benign neoplasms, and in benign neoplasms in relation to endometriomas. Only epithelial staining was stronger in malignant neoplasms compared to endometriomas.

There are few conclusions in the literature on the role of IL-5 in the pathophysiology of ovarian neoplasms. In an immunohistochemical evaluation of endometriosis tissues, an increase in eosinophil peroxidase (a marker of eosinophil degranulation) and IL-5 expression was observed during the evaluation of eutopic endometrial tissue [40]. In our study, there was stronger epithelial and stromal immunostaining in benign neoplasms compared to malignant neoplasms, in endometriomas compared to malignant neoplasms, and in endometriomas compared to non-neoplastic lesions. Only in tumor stroma, there was stronger immunostaining in endometriomas than in benign ovarian neoplasms.

It is well established that the study of biomarkers can contribute to the management of ovarian cancer, distinguishing benign and malignant pelvic masses and monitoring the response to treatment [41]. Similarly, the histopathological associations between endometriosis and epithelial ovarian cancer may have prognostic value and help to clarify some pathogenic mechanisms of endometriosis that are not yet clearly understood. The presence and type of cellular infiltrate in endometriotic tissue and its location can be considered in a study of prognostic factors.

The main limitation of the study was heterogeneity of the histological types evaluated, the presence of a certain type of cellular infiltrate in endometriotic tissue, and its location can be considered in a study of prognostic factors. Another limitation was the inclusion of borderline tumors in the group of malignant neoplasms. On the other hand, this is the first study in the literature that evaluates an immunohistochemical panel of cytokines both in the stroma and in the epithelium of ovarian tumors and endometriomas. Our findings demonstrate significantly different levels of cytokine expression in the two tissue microenvironments, the epithelium and stroma. This work may also be the basis for further studies on the inflammatory response and immunology of endometriomas, and their relationship with carcinogenesis.

Conclusion

Epithelial immunostaining of IL-5 and IL-8 is stronger in endometriomas than in ovarian cancer. The stromal immunostaining of TNF-α, IL-5, IL-6, and IL8 is stronger in endometriomas than in ovarian cancer. The stronger immunostaining of some cytokines in endometriomas compared to ovarian cancer reflects inflammatory and immune responses that could be future targets for new discoveries about the infiltrative behavior of endometriosis.

References

1. Mao AJ, Anastasi JK. Diagnosis and management of endometriosis: the role of the advanced practice nurse in primary care. J Am Acad Nurse Pract 2010; 22(2): 109–116. doi: 10.1111/j.1745-7599.2009.00475.x.

2. Ruderman R, Pavone ME. Ovarian cancer in endometriosis: an update on the clinical and molecular aspects. Minerva Ginecol 2017; 69(3): 286–294. doi: 10.23736/S0026-4784.17.04042-4.

3. Ahn SH, Monsanto SP, Miller C et al. Pathophysiology and immune dysfunction in endometriosis. Biomed Res Int 2015; 2015: 795976. doi: 10.1155/2015/795976.

4. Kok VC, Tsai HJ, Su CF et al. The risks for ovarian, endometrial, breast, colorectal, and other cancers in women with newly diagnosed endometriosis or adenomyosis: a population-based study. Int J Gynecol Cancer 2015; 25(6): 968–976. doi: 10.1097/IGC.0000000000000454.

5. Kumar S, Munkarah A, Arabi H et al. Prognostic analysis of ovarian cancer associated with endometriosis. Am J Obstet Gynecol 2011; 204(1): 63.e1–63.e7. doi: 10.1016/j.ajog.2010.08.017.

6. Murta EF, Nomelini RS. Early diagnosis and predictors of malignancy in the evaluation of adnexal mass. Curr Opin Obstet Gynecol 2006; 18(1): 14–19. doi: 10.1097/01.gco.0000192967.67567.e9.

7. Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynecol Obstet 2014; 290(5): 839–842. doi: 10.1007/s00404-014-3364-8.

8. Jiang X, Hitchcock A, Bryan EJ et al. Microsatellite analysis of endometriosis reveals loss of heterozygosity at candidate ovarian tumor suppressor gene loci. Cancer Res 1996; 56(15): 3534–3539.

9. Li J, Liu R, Tang S et al. Impact of endometriosis on risk of ovarian, endometrial and cervical cancers: a meta-analysis. Arch Gynecol Obstet 2019; 299(1): 35–46. doi: 10.1007/s00404-018-4968-1.

10. Králíčková M, Laganà AS, Ghezzi F et al. Endometriosis and risk of ovarian cancer: what do we know? Arch Gynecol Obstet 2020; 301(1): 1–10. doi: 10.1007/s00404-019-05358-8.

11. Crispim PC, Jammal MP, Murta EF et al. Endometriosis: what is the influence of immune cells? Immunol Invest 2021; 50(4): 372–388. doi: 10.1080/08820139.2020.1764577.

12. Zou G, Wang J, Xu X et al. Cell subtypes and immune dysfunction in peritoneal fluid of endometriosis revealed by single-cell RNA-sequencing. Cell Biosci 2021; 11(1): 98. doi: 10.1186/s13578-021-00613-5.

13. Li WN, Hsiao KY, Wang CA et al. Extracellular vesicle-associated VEGF-C promotes lymphangiogenesis and immune cells infiltration in endometriosis. Proc Natl Acad Sci U S A 2020; 13; 117(41): 25859–25868. doi: 10.1073/pnas.1920037117.

14. Kyama CM, Debrock S, Mwenda JM et al. Potential involvement of the immune system in the development of endometriosis. Reprod Biol Endocrinol 2003; 1: 123. doi: 10.1186/1477-7827-1-123.

15. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5): 646–674. doi: 10.1016/j.cell.2011.02.013.

16. Wang X, Wang E, Kavanagh JJ et al. Ovarian cancer, the coagulation pathway, and in-

flammation. J Transl Med 2005; 3: 25. doi: 10.1186/1479-5876-3-25.
17. Restifo NP, Wunderlich JR. Molecular biology of cancer: cancer immunology. In: DeVita VT, Hellman S, Rosenberg SA (eds). Cancer: principles and practice of oncology. Philadelphia: Lippincott Williams & Wilkins 2005: 139.
18. Plewka D, Kowalczyk AE, Jakubiec-Bartnik B et al. Immunohistochemical visualization of proinflammatory cytokines and enzymes in ovarian tumors. Folia Histochem Cytobiol 2014; 52(2): 124–137. doi: 10.5603/FHC.2014.0015.
19. Qu H, Li L, Wang TL et al. Epithelial cells in endometriosis and adenomyosis upregulate STING expression. Reprod Sci 2020; 27(6): 1276–1284. doi: 10.1007/s43032-019-00127-z.
20. Guo M, Bafligil C, Tapmeier T et al. Mass cytometry analysis reveals a distinct immune environment in peritoneal fluid in endometriosis: a characterisation study. BMC Med 2020; 18(1): 3. doi: 10.1186/s12916-019-1470-y.
21. Wang XQ, Zhou WJ, Luo XZ et al. Synergistic effect of regulatory T cells and proinflammatory cytokines in angiogenesis in the endometriotic milieu. Hum Reprod 2017; 32(6): 1304–1317. doi: 10.1093/humrep/dex067.
22. D'Hooghe TM, Debrock S, Hill JA et al. Endometriosis and subfertility: is the relationship resolved? Semin Reprod Med 2003; 21(2): 243–254. doi: 10.1055/s-2003-41330.
23. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril 2001; 75(1): 1–10. doi: 10.1016/s0015-0282 (00)01630-7.
24. Tamaresis JS, Irwin JC, Goldfien GA et al. Molecular classification of endometriosis and disease stage using high-dimensional genomic data. Endocrinology 2014; 155(12): 4986–4999. doi: 10.1210/en.2014-1490.
25. Montgomery GW, Nyholt DR, Zhao ZZ et al. The search for genes contributing to endometriosis risk. Hum Reprod Update 2008; 14(5): 447–457. doi: 10.1093/humupd/dmn016.
26. Govindan S, Ahmad SN, Vedicherla B et al. Association of progesterone receptor gene polymorphism (PROGINS) with endometriosis, uterine fibroids and breast cancer. Cancer Biomarkers 2007; 3(2): 73–78. doi: 10.3233/cbm-2007-3201.
27. Babaabasi B, Ahani A, Sadeghi F et al. The association between TNF-alpha gene polymorphisms and endometriosis in an Iranian population. Int J Fert Steril 2019; 13(1): 6–11. doi: 10.22074/ijfs.2019.5542.
28. Saliminejad K, Memariani T, Ardekani AM et al. Association study of the TNF-α-1031T/C and VEGF +450G/C polymorphisms with susceptibility to endometriosis. Gynecol Endocrinol 2013; 29(11): 974–977. doi: 10.3109/09513590.2013.824956.
29. Keenan JA, Chen TT, Chadwell NL et al. IL-1 beta, TNF-alpha, and IL-2 in peritoneal fluid and macrophage-conditioned media of women with endometriosis. Am J Reprod Immunol 1995; 34(6): 381–385. doi: 10.1111/j.1600-0897.1995.tb00968.x.
30. Braun DP, Gebel H, House R et al. Spontaneous and induced synthesis of cytokines by peripheral blood monocytes in patients with endometriosis. Fertil Steril 1996; 65(6): 1125–1129.
31. Tsudo T, Harada T, Iwabe T et al. Altered gene expression and secretion of interleukin-6 in stromal cells derived from endometriotic tissues. Fertil Steril 2000; 73(2): 205–211. doi: 10.1016/s0015-0282(99)00496-3.
32. Bergqvist A, Bruse C, Carlberg M et al. Interleukin 1beta, interleukin-6, and tumor necrosis factor-alpha in endometriotic tissue and in endometrium. Fertil Steril 2001; 75(3): 489–495. doi: 10.1016/s0015-0282(00)01752-0.
33. Ulukus M, Ulukus EC, Seval Y et al. Expression of interleukin-8 receptors in endometriosis. Human Reprod 2005; 20(3): 794–801. doi: 10.1093/humrep/deh675.
34. Ulukus M, Ulukus EC, Tavmergen Goker EN et al. Expression of interleukin-8 and monocyte chemotactic protein 1 in women with endometriosis. Fertil Steril 2009; 91(3): 687–693. doi: 10.1016/j.fertnstert.2007.12.067.
35. Gatla HR, Singha B, Persaud V et al. Evaluating cytoplasmic and nuclear levels of inflammatory cytokines in cancer cells by western blotting. Methods Mol Biol 2014; 1172: 271–283. doi: 10.1007/978-1-4939-0928-5_25.
36. Llarena NC, Richards EG, Priyadarshini A et al. Characterizing the endometrial fluid cytokine profile in women with endometriosis. J Assist Reprod Genet 2020; 37(12): 2999–3006. doi: 10.1007/s10815-020-01989-y.
37. Pizzo A, Salmeri FM, Ardita FV et al. Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. Gynecol Obstet Invest 2002; 54(2): 82–87. doi: 10.1159/000067717.
38. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr Opin Immunol 2011; 23(5): 598–604. doi: 10.1016/j.coi.2011.08.003.
39. Velasco I, Quereda F, Bermejo R et al. Intraperitoneal recombinant interleukin-2 activates leukocytes in rat endometriosis. J Reprod Immunol 2007; 74(1–2): 124–132. doi: 10.1016/j.jri.2006.12.001.
40. Blumenthal RD, Samoszuk M, Taylor AP et al. Degranulating eosinophils in human endometriosis. Am J Pathol 2000; 156(5): 1581–1588. doi: 10.1016/S0002-9440(10)65030-4.
41. Giampaolino P, Foreste V, Della Corte L et al. Role of biomarkers for early detection of ovarian cancer recurrence. Gland Surg 2020; 9(4): 1102–1111. doi: 10.21037/gs-20-544.

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Submitted/Doručeno: 28. 4. 2024
Accepted/Přijato: 30. 4. 2024

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Publication ethics: The Editorial Board declares that the manuscript met the ICMJE uniform requirements for biomedical papers.

Publikační etika: Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

Conflict of interests: The authors declare they have no potential conflicts of interest concerning the drugs, products or services used in the study.

Konflikt zájmů: Autoři deklarují, že v souvislosti s předmětem studie/práce nemají žádný konflikt zájmů.

Dedication: The authors wish to acknowledge the funding received from CNPq (National Council for Scientific and Technological Development - Brazil, process No.: 01115/2018-4 and research grant for Rosekeila Simões Nomelini process No.: 303086/2022-0), FAPEMIG (Minas Gerais Research Funding Foundation, process No.: APQ-00888-21 and RED-00011-14), FUNEPU (Uberaba's Teaching and Research Foundation, process No.: 02 2011), CAPES (Brazilian Federal Agency fot the Support and Evaluation of Graduate Education, process No.: 32012012001P5), and AREMG Medical Residency Support Association, process No.: TCPRM 2019).

Dedikace: Autoři chtějí ocenit finanční prostředky získané od CNPq (Národní rada pro vědecký a technologický rozvoj – Brazílie, proces č.: 01115/2018-4 a výzkumný grant pro Rosekeila Simões Nomelini proces č.: 303086/2022-0), FAPEMIG (Minas Gerais Research Funding Foundation, číslo procesu: APQ-00888-21 a RED-00011-14), FUNEPU (Uberaba's Teaching and Research Foundation, proces č.: 02 2011), CAPES (Brazilská federální agentura pro podporu a hodnocení Graduate Education, proces č.: 32012012001P5), a AREMG Medical Residency Support Association, proces č.: TCPRM 2019).