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 cancer

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Abstract. Understanding the relationship between the coexistence of inflammatory and neoplastic processes in ovarian cancer, particularly those involving chemokines and their receptors, may help to elucidate the involvement of the studied parameters in tumor pathogenesis and could lead to improved clinical applications. Therefore, the present study aimed to analyze the levels of C-X-C motif chemokine ligand 8 (CXCL8), and its receptors C-X-C chemokine receptor (CXCR)1 and CXCR2, in the serum and peritoneal fluid of women with ovarian cancer, and to evaluate the association between the expression of these parameters in tumor tissue and patient characteristics, particularly the degree of histological differentiation. The study group included women with ovarian cancer diagnosed with serous cystadenocarcinoma International Federation of Gynecology and Obstetrics stage IIIc and a control group, which consisted of women who were diagnosed with a benign lesion (serous cystadenoma). The transcript levels of *CXCL8*, *CXCR1* and *CXCR2* were evaluated using reverse transcription-quantitative PCR (RT-qPCR). The quantitative analysis was carried out using the LightCycler® 480 System and GoTaq® 1-Step RT-qPCR System, according to the manufacturers' instructions. The concentration of CXCL8 in serum and peritoneal fluid was determined using a Human Interleukin-8 ELISA kit, and the concentrations of CXCR1 and CXCR2 were determined using the CLOUD-CLONE ELISA kit. Local and systemic disturbances in immune and inflammatory responses involving the CXCL8 chemokine and its receptors indicated the involvement of these studied parameters in the pathogenesis of ovarian cancer. Immunoregulation of the CXCL8-CXCR1 system may influence the course of the inflammatory process accompanying ovarian cancer development, which may result in the identification of novel clinical applications; however, further studies are required.

Introduction

Ovarian cancer is ranked as one of the most dangerous gynecological cancers, which is associated with a high rate of mortality (1). According to the World Health Organization, ~313,959 cases of ovarian cancer are diagnosed annually and >207,252 deaths are recorded, making ovarian cancer the seventh most common type of cancer and the fifth most common cause of cancer-associated death worldwide (2-5). Notably, >70% of all ovarian cancer cases are diagnosed at a late clinical stage (stage III or IV) according to the International Federation of Gynecology and Obstetrics (FIGO) classification (1,6,7). The reason for the late diagnosis of ovarian cancer is the asymptomatic course of the disease in the early stages, the non-specificity of clinical symptoms and the lack of parameters useful for early diagnosis. The incidence of ovarian cancer is increasing, particularly in developed countries; therefore, there is a need for further research to better understand the mechanisms involved in the pathogenesis of this disease (5,8,9).

An important role in the formation and development of ovarian cancer is attributed to disturbances in the immune system, particularly involving interactions between immune cells and tumor cells. Interactions occurring in the tumor microenvironment include both direct interactions and indirect actions involving soluble mediators, including chemokines and their receptors. Numerous studies have

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reported the involvement of chemokines and their receptors in several physiological and pathological processes, including tumorigenesis and chronic inflammation (10-13). In the body, inflammatory mediators aim to eliminate pathogenic agents and restore homeostasis; however, excessive inflammation can lead to overproduction of cytokines and chemokines, which result in the formation of a network of interrelationships that can directly affect tumor development. Through these networks, the signaling process is disrupted, surrounding cells are stimulated, new blood vessels are formed and, consequently, tumors can grow (14-16). Furthermore, it has been shown that in the course of a number of malignancies, cancer cells have the ability to secrete the chemokine C-X-C motif chemokine ligand 8 (CXCL8, also Interleukin 8-IL-8) in an autocrine or paracrine manner (13). Chemokines can also be produced by tumor-infiltrating leukocytes and tumor-associated fibroblasts. Cancer cells are also capable of taking control of host cell signaling and regulatory mechanisms responsible for the synthesis of various growth factors using chemokine and receptor pathways (17).

The CXCL8 chemokine is regulated through two specific receptors, C-X-C chemokine receptor (CXCR)1 and CXCR2, and has an important role in the pathological mechanism of ovarian cancer. Notably, via its autocrine action on cells, CXCL8 can affect ovarian cancer cell proliferation, invasion and angiogenesis (18). CXCL8 is a chemokine that belongs to the CXC family. Its function is to activate neutrophil granulocytes and recruit granulocytes to sites of inflammation (19). CXCL8 is secreted by a large group of cells, including circulating monocytes in the blood, macrophages present in the alveoli, fibroblasts, endothelial cells and epithelial cells. CXCL8 synthesis occurs under the influence of several factors, including: tumor necrosis factor-α, interleukin (IL)-1, IL-6, and stressors of environmental and chemical origin, such as hypoxia and reactive oxygen species (20,21).

CXCL8-mediated signaling depends on the extracellular binding of the chemokine to CXCR1 or CXCR2, which are both coupled to G protein (20). In addition, CXCR1 has a high specificity to the chemokine CXCL8 compared with CXCR2, which can bind to other ILs (22). CXCR1 and CXCR2 are found on the surface of a variety of cells, both normal and cancerous (23). The interaction between CXCL8 and CXCR1 or CXCR2 has a significant function in the development of the inflammatory process and thus affects different stages of carcinogenesis, leading to the promotion, progression and metastasis of cancer, including ovarian cancer (11-13,24). Notably, the CXCL8-CXCR1/2 signaling axis may serve an important role in tumorigenesis and the formation of secondary tumor foci by controlling the proliferation and self-renewal of cancer stem cells (20). Furthermore, the CXCL8-CXCR1 signaling pathway has been reported to primarily enhance cancer cell proliferation, whereas the CXCL8-CXCR2 pathway can affect angiogenesis (19).

Disturbances in the immune system serve an important role in the formation and development of ovarian cancer, particularly those involving chemokines and their receptors. Understanding the relationship between the coexistence of inflammatory and neoplastic processes may help to elucidate the involvement of the studied parameters in tumor pathogenesis and could lead to improved clinical applications. Therefore, the aim of the present study was to analyze the levels of CXCL8, and its receptors CXCR1 and CXCR2, in the serum and peritoneal fluid of women with ovarian cancer. In addition, the association between the expression levels of CXCL8, CXCR1 and CXCR2, and the degree of histological differentiation, was assessed in ovarian cancer.

Materials and methods

Patients. The present study included 32 patients aged 28-89 years (mean age, 61.3415.55 years), who were hospitalized at the Gynecology and Obstetrics Department with Pregnancy Pathology and Gynecology Oncology Subdivisions, Provincial Specialist Hospital Blessed Virgin Mary (Częstochowa, Poland) and were diagnosed with ovarian serous cystadenocarcinoma III C according to FIGO. In 12 patients, the neoplasm was of G1 histological differentiation grade, in 10 patients it was of G2 histological differentiation grade, and in the remaining 10 patients it was of G3 histological differentiation grade. Patients and control individuals were recruited between May 2019 and February 2022.

The clinical staging classification of ovarian cancer was established based on the FIGO guidelines. The degree of histological differentiation of cancer was graded according to the following criteria: G1, highly differentiated; G2, moderately differentiated; G3, poorly differentiated. The diagnosis was based on clinical symptoms, results of gynecological and histopathological examinations, laboratory tests and exclusion of the coexistence of other diseases of reproductive organs. The women that qualified to the studied group were clinically diagnosed with ovarian tumors confirmed with a histopathological examination. None of the examined women were administered pharmacological treatments in the previous 3 months.

Serum, peritoneal fluid and tumor tissue were examined for all of the recruited patients. In the study group, blood was taken from women after establishing the clinical diagnosis and before surgery. Blood was taken in the morning from the cubital vein and was added to tube containing a clot activator, in order to obtain serum; 30 min after blood collection, it was centrifuged at 1,500 x g for 15 min at room temperature the serum was obtained and maintained at -80˚C until further use. Tumor tissue intended for molecular examination was collected during the planned surgery and frozen at -80˚C until analyses were performed. Peritoneal fluid was collected during laparoscopy for bacteriological examination, was centrifuged at 1,500 x g for 10 min at 4˚C, and the obtained supernatant was partitioned and frozen at -80˚C until the remaining determinations were made.

The control group consisted of 15 women aged between 22 and 77 years (mean age, 52.08±18.00 years) who were diagnosed with a benign lesion (serous cystadenoma). Serum samples were used as control.

The present study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Ethics Committee of Medical University of Silesia in Katowice (Sosnowiec, Poland; protocol code KNW/0022/KB1/49/19). All patients agreed to participate in the present study and provided written informed consent.

Table I. Serum concentrations of CXCL8 and its receptors in women with ovarian cancer and the control group.

Characteristic	Ovarian cancer group (n=32)	Control group (n=15)	P-value
Mean age ± SD, years	61.34±15.55	52.08±18.00	
Median serum CXCL8 concentration (Q1-Q3), pg/ml	48.25 (26.26-77.48)	14.96 (11.03-16.83)	<0.01
Median serum CXCR1 concentration (Q1-Q3), ng/ml	1.19 (0.94-2.26)	1.51 (1.34-1.65)	NS
Median serum CXCR2 concentration (Q1-Q3), ng/ml	1.23 (1.17-1.32)	3.27 (1.19-4.04)	0.001

Serum concentrations are presented as median and interquartile range.

ELISA analyses. The concentration of CXCL8 in the serum and peritoneal fluid was determined by sandwich ELISA using the Human Interleukin-8 ELISA kit (cat. no. RD194558200R; BioVendor LM), according to the manufacturer's instructions. The sensitivity of the assay was 0.5 pg/ml. The concentration of CXCR1 and CXCR2 was determined by sandwich ELISA using the CLOUD-CLONE ELISA kit (cat. nos. SEA019Hu and SEC006Hu; Cloud-Clone Corp.), according to the manufacturer's instructions. The sensitivity of the assay for CXCR1 was 0.054 ng/ml, whereas for CXCR2 it was 0.057 ng/ml.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from tissue samples using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Quantitative analysis of RNA was performed using a nanospectrophotometer (MaestroNano MN-913; MaestroGen, Inc.). Qualitative analysis was performed by electrophoresis on a 1% agarose gel. The mRNA expression levels of *CXCL8, CXCR1* and *CXCR2* were evaluated using RT-qPCR. The quantitative analysis was carried out using LightCycler® 480 System (Roche Diagnostics) and GoTaq® 1-Step RT-qPCR System (Promega GmbH), according to the manufacturers' instructions, under the following conditions: RT at 37°C for 15 min; RT inactivation/Hot-start activation at 95°C for 10 min; followed by 40 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 30 sec, elongation at 72°C for 30 sec. Amplification was performed using KiCqStart SYBR Green oligonucleotide primers (Sigma-Aldrich; Merck KGaA), as follows: *CXCL8*, forward (F) 5'-TGTAAACAT GACTTCCAAGC-3', reverse (R) 5'-AAAACTGCACCT TCACAC-3'; *CXCR1*, F 5'-TTAAGTCACTCTGATCTCTGA C-3', R 5'-TGGTTTGATCTAACTGAAGC-3'; *CXCR2*, F 5'GTGATAGCTGAGAATATGCAG-3', R 5'-ACTTAAATC CTGACTGGGTC-3'; and *β-actin*, F 5'-GACGACATGGAG AAAATCTG-3' and R 5'-ATGATCTGGGTCATCTTCTC-3'. Melting curve analysis and 2% agarose gel electrophoresis were used to confirm the specificity of amplification and the absence of primer dimers. RT-qPCR data were analysed by the $2^{-\Delta\Delta Cq}$ method using β-actin as an internal control (25).

Statistical analysis. The obtained results were statistically analysed using Statistica 13.3 software (StatSoft Polska Sp. z o.o.). The normality of the distribution of the studied variables was assessed using the Shapiro-Wilk test. The significance of differences between two groups was determined with Mann-Whitney U-test. The significance of differences between more than two groups was determined by Kruskal-Wallis test, followed by Dunn's post-hoc analysis. P<0.05 was considered to indicate a statistically significant difference. Correlation was assessed using Pearson correlation for logarithmic data (r).

Results

Concentration of CXCL8, and its receptors CXCR1 and CXCR2, in serum. The levels of CXCL8, and its receptors CXCR1 and CXCR2, in the serum of women with serous ovarian cystadenocarcinoma were evaluated according to histological differentiation stage. It was revealed that the concentration of CXCL8 was significantly increased between G1, G2 and G3 grades (P<0.0001). Analysis of the concentration of the examined parameter in successive grades of histological differentiation in comparison with the control group showed a statistically significant difference only in the G2 and G3 grades (P<0.0001) (Fig. 1A). Further analysis included evaluation of serum CXCR1 levels according to histological differentiation stage. There was a statistically significant difference in these concentrations between grades G1 and G3 (P<0.001), and between G2 and G3 (P<0.05). Furthermore, when compared with the control group, a statistically significant difference was shown only in grade G3 (P<0.001) (Fig. 1B). Regarding serum CXCR2 levels, there were no statistically significant differences between the different degrees of histological differentiation, nor between G1-G3 grades and the control group (Fig. 1C). The obtained results are also shown in Table I.

Concentration of CXCL8, and its receptors CXCR1 and CXCR2, in peritoneal fluid. The concentrations of CXCL8, and its receptors CXCR1 and CXCR2, in the peritoneal fluid of women with ovarian cancer were evaluated according to histological differentiation stage. CXCL8 levels in the peritoneal fluid were only significantly different between grades G2 and G3 (P<0.05; Fig. 1D). Notably, there were no statistically significant differences between the concentration of CXCR1 in the peritoneal fluid between patients with different degrees
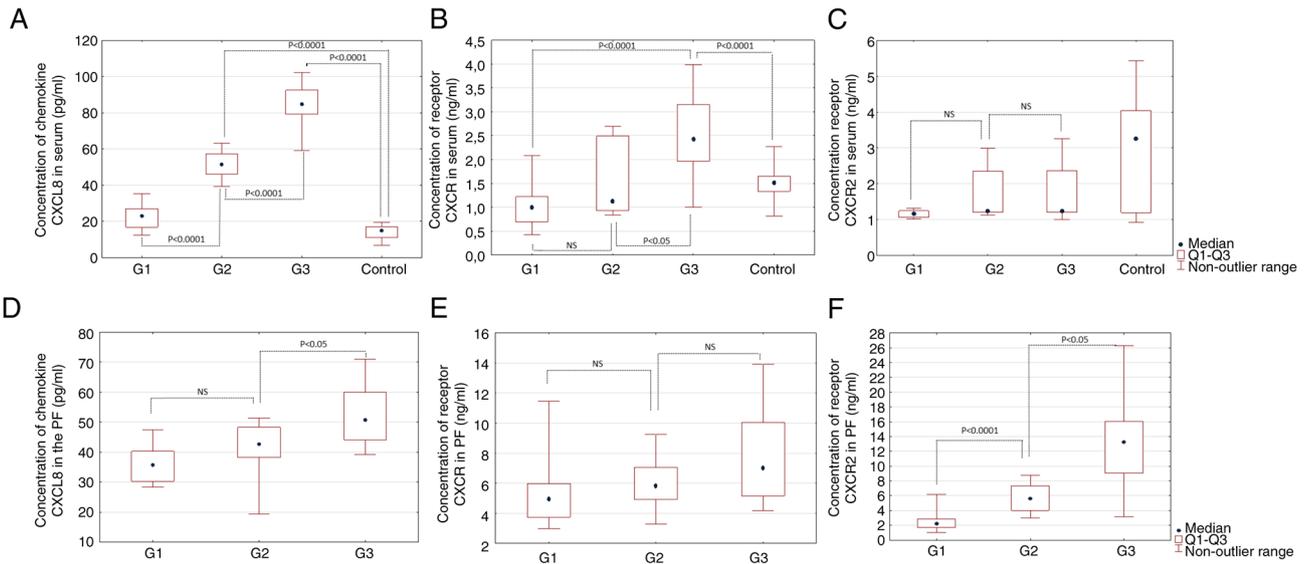
Figure 1. Concentration of (A) CXCL8, (B) CXCR1 and (C) CXCR2 in the serum of control individuals and women with ovarian cancer with different degrees of differentiation. Concentration of (D) CXCL8, (E) CXCR1 and (F) CXCR2 in the PF in women with ovarian cancer with different degrees of differentiation. CXCL8, C-X-C motif chemokine ligand 8; CXCR, C-X-C chemokine receptor; PF, peritoneal fluid.
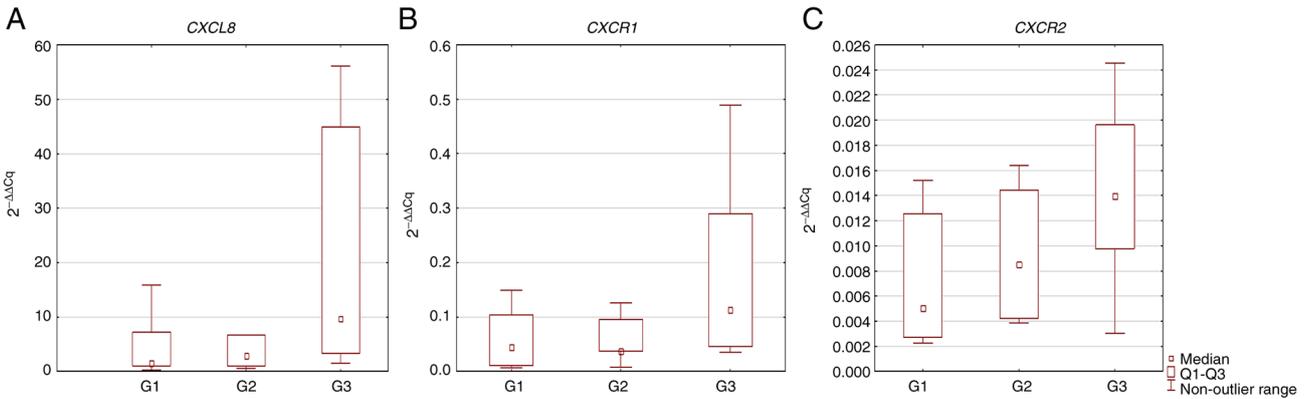
Figure 2. mRNA expression levels of (A) *CXCL8*, (B) *CXCR1* and (C) *CXCR2* in the tumor tissue of patients with ovarian cancer with different degrees of differentiation. CXCL8, C-X-C motif chemokine ligand 8; CXCR, C-X-C chemokine receptor.

of histological differentiation of ovarian cancer (Fig. 1E). By contrast, analysis of CXCR2 concentration in the peritoneal fluid revealed a statistically significant difference between G1 and G2 grades (P<0.001), and between G2 and G3 grades (P<0.05) (Fig. 1F).

mRNA expression levels of CXCL8, and its receptors CXCR1 and CXCR2, in tumor tissue. The highest mRNA expression levels of *CXCL8*, and its receptors *CXCR1* and *CXCR2*, were detected in the tumor tissues obtained from patients with G3 grade ovarian cancer. However, no statistically significant differences were found (P>0.05; Fig. 2).

Positive statistically significant correlations were detected between serum and peritoneal fluid levels of CXCL8, CXCR1 and CXCR2 in women with ovarian cancer (Fig. 3). There were no statistically significant correlations detected between the serum and tissue levels, and between the peritoneal fluid and tissue levels, with regard to CXLC8, CXCR1 and CXCR2 (data not shown).

Discussion

Previous studies have predicted that mortality from ovarian cancer will continue to increase until 2040 (3,26); therefore, research into the biology of ovarian cancer continues, with the aim of understanding the mechanisms involved in its pathogenesis, which may prove useful in developing new diagnostic and therapeutic regimens. Previous studies have reported that the process of ovarian tumorigenesis is accompanied by chronic inflammation (27,28). In this process, an important role is attributed to the CXCL8 chemokine system, along with its receptors CXCR1 and CXCR2, which serve an important role in tumor formation and development by affecting the different stages of carcinogenesis, consequently leading to ovarian cancer progression (10-13).

The aim of the present study was to analyze the expression of the *CXCL8* chemokine, and its receptors *CXCR1* and *CXCR2*, in tumor tissue, and to evaluate the levels of these parameters in serum and peritoneal fluid from women
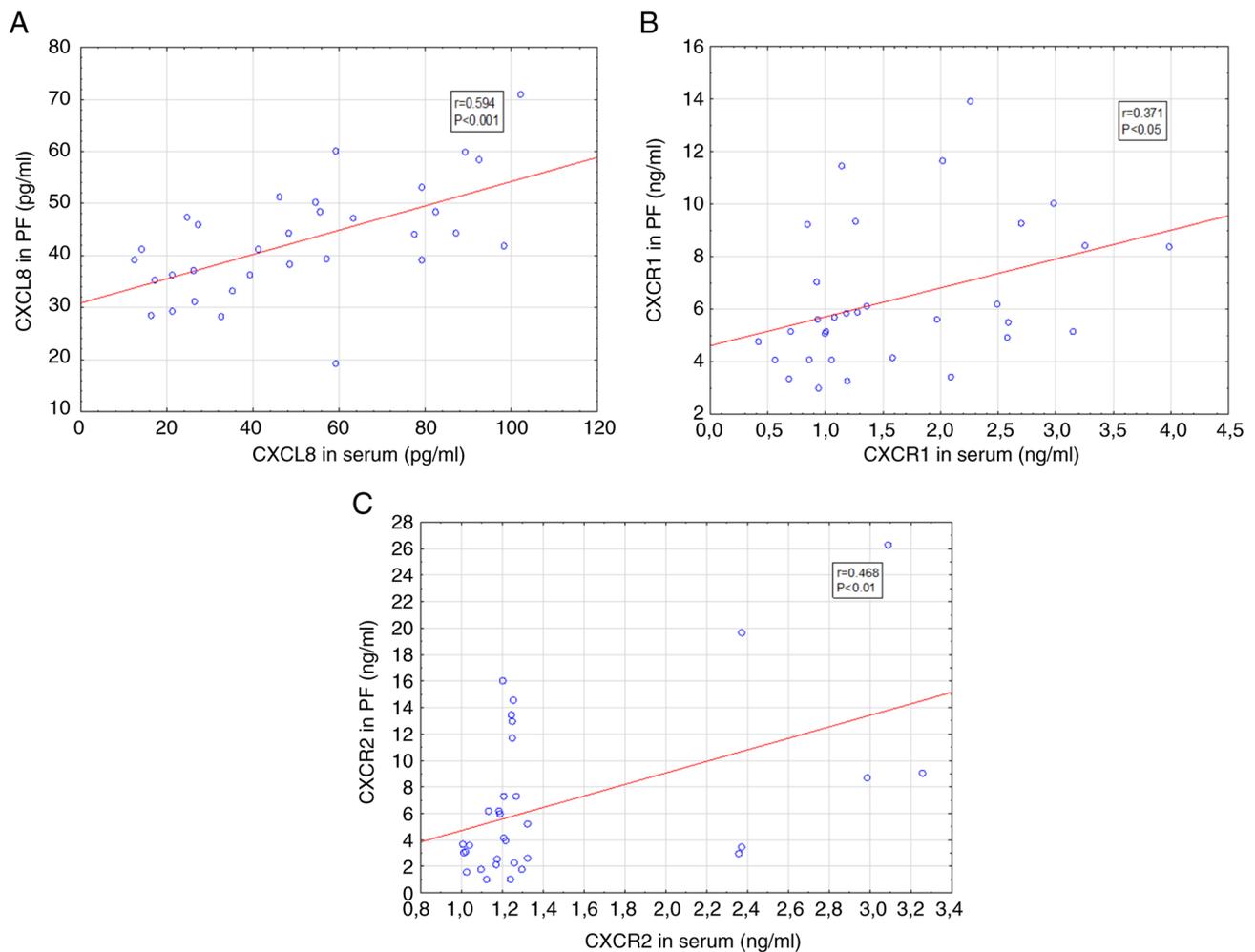
Figure 3. (A) Correlation analysis showing the relationship between CXCL8 in serum and PF. (B) -Correlation analysis showing the relationship between CXCR1 in serum and PF. (C) Correlation analysis showing the relationship between CXCR2 in serum and PF. CXCL8, C-X-C motif chemokine ligand 8; CXCR, C-X-C chemokine receptor; PF, peritoneal fluid.

diagnosed with ovarian cancer, taking into account the histological differentiation of ovarian cancer. The analysis of CXCL8 revealed that the levels were significantly higher in the serum of patients diagnosed with ovarian cancer compared with those in the control group ($P<0.01$), which may indicate the involvement of the studied cytokine in the pathogenesis of ovarian cancer. Moreover, statistical significance was demonstrated between the G1, G2 and G3 grades ($P<0.0001$). These findings indicated that a relationship may exist between CXCL8 secretion and the degree of histological differentiation of ovarian cancer. However, when analyzing CXCL8 concentration in the peritoneal fluid, a statistically significant difference was only found between differentiation grades G2 and G3 ($P<0.05$). A similar tendency was observed regarding *CXCL8* mRNA expression in tumor tissue, where the highest expression levels were detected in the G3 grade; however, no statistically significant differences were noted between the studied groups.

In the pathological mechanism of ovarian cancer development, an important role has been attributed to the proinflammatory chemokine CXCL8, which has chemotactic effects on cells expressing CXCR1 and CXCR2. Moreover, CXCL8 interacting with the tumor microenvironment can

positively influence tumor growth, stimulate new blood vessel formation and promote the formation of secondary tumor foci (29). Browne *et al* (30) detected significantly elevated levels of CXCL8 in each histological subtype of ovarian cancer, and demonstrated the existence of a relationship between CXCL8 chemokine levels and the clinical stage of ovarian cancer. Analogous results were obtained by Crispim *et al* (31); significantly elevated serum levels of CXCL8 were detected in women diagnosed with ovarian malignancy and benign tumors compared with those detected in a group of women without reproductive system conditions. In addition, the authors revealed that the prognosis of patients with ovarian cancer was worse when higher levels of the chemokine persisted during the course of the disease. Furthermore, Zhang *et al* (32) analyzed chemokine levels in the course of ovarian cancer and observed that the stage of ovarian cancer was correlated with the levels of CXCL8. In addition, significantly higher serum levels of CXCL8 were detected in women with stage III and IV ovarian cancer compared with those in women with stage I and II ovarian cancer, according to the FIGO classification.

An important protumor role has been attributed to tumor-associated cells, mainly fibroblasts, neutrophils and macrophages, which can contribute to tumor growth and

invasiveness, and can promote the formation of secondary cancer foci by secreting pro-inflammatory cytokines. Yang *et al* (33) evaluated the relationship between IL-8 and neutrophils during cancer development. Their study showed that the expression of numerous chemokines, particularly IL-8, was significantly higher in ovarian cancer with stronger neutrophil infiltration compared with that in ovarian cancer with little infiltration of these cells. Additionally, the study revealed that higher levels of IL-8 were correlated with an increase in tumor-associated neutrophils; therefore, IL-8 may be involved in attracting neutrophils toward the tumor microenvironment. Thongchot *et al* (34) demonstrated that tumor-associated fibroblasts have an important role in the pathological mechanism of ovarian cancer by mobilizing ovarian cancer cells to form metastases. Moreover, these cells could secrete CXCL8 more intensively compared with physiological fibroblasts. This previous study also revealed that increased serum CXCL8 levels were correlated with disease progression and negatively affected patient prognosis, and increased CXCL8 levels in the peritoneal fluid were correlated with ovarian cancer progression. Furthermore, over-secreted CXCL8 has been reported to act as a chemotactic factor for ovarian cancer cells, facilitating the formation of metastasis (34). Alfaro *et al* (35) reported that CXCL8 was also capable of attracting other cells that express its specific receptors CXCR1 and CXCR2 on their surface. In addition, Ha *et al* (20), showed that stimulated cells, for example those stimulated by various cytokines, were able to produce and secrete CXCL8 10 to 100 times more than under normal conditions, in which CXCL8 was mostly undetectable.

In the present study, CXCR1 levels were further evaluated in the serum and peritoneal fluid. The existence of a statistically significant difference in serum concentrations was demonstrated between grades G1 and G3 (P<0.001), and between grades G2 and G3 (P<0.05), which may indicate the involvement of CXCR1 in autocrine and paracrine signaling associated with CXCL8 in tumor development. Further tests, including assessment of patients with different ovarian cancer stages according to the FIGO classification, may provide more information on the association between serum concentration of CXCR1 and the condition of the patient. In addition, the mRNA expression levels of *CXCR1* in the tumor tissue were highest at G3 grade; however, this difference was not statistically significant.

Browne *et al* (30) observed a correlation between the expression levels of IL-8Ra and cancer stage. By analyzing the levels of IL-8Rb, no significant association was identified in the levels of this IL-8 receptor between the study group and the control group. Their study also revealed that there was a significant association between the levels of IL-8 and IL-8R and the type of cancer. The concentration of these compounds was significantly higher in serous carcinoma, as opposed to the other histological types of ovarian cancer. Furthermore, increased expression levels of IL-8, IL-8Ra and IL-8Rb were detected in benign serous ovarian tumors and benign mucinous tumors. Comparing the levels of IL-8 and its receptors in benign and malignant ovarian tumors showed significantly decreased levels during benign tumor development.

The present study also analyzed the levels of CXCR2; no statistically significant differences were detected in the serum levels of CXCR2 between the histological grades. Similarly,

differences at mRNA level were also not statistically significant. However, in the peritoneal fluid, a statistically significant difference in CXCR2 levels was identified between the G1 and G2 grades (P<0.001), and between the G2 and G3 grades (P<0.05).

Notably, Henriques *et al* (36) evaluated the role of CXCR2 in the pathogenesis of ovarian cancer. The results of this study revealed that CXCR2 was upregulated in patients diagnosed with ovarian cancer. Furthermore, CXCL8 and CXCL2 chemokines regulated by CXCR2, which is located on ovarian cancer cells, showed autocrine activity. Elevated levels of both chemokines in the course of ovarian cancer have been associated with the occurrence of tumor progression, formation of secondary cancer foci and chemoresistance to the applied treatment (13,37,38). In addition, Taki *et al* (39) indicated a significant role of CXCR2 in ovarian cancer progression, as determined using mouse models. The authors demonstrated that CXCR2 not only affected CXCL8, but also interacted with CXCL1 and CXCL2, resulting in the observed chemotaxis of myeloid-derived suppressor cells. These cells in turn may promote tumor metastasis by inducing epithelial-mesenchymal transition, consequently leading to disease progression.

The role of CXCL8-CXCR1 and CXCL8-CXCR2 signaling axes is one of several mechanisms involved in the regulation of the immune system during the antitumor response (40). According to Liu *et al* (19), this pathway may have an important role not only in the pathogenesis of ovarian cancer, but also in the formation of numerous other types of cancer, including breast, prostate, lung, colorectal and gastric cancer, and melanoma. Researchers have suggested that in breast cancer, CXCL8 can directly affect tumor formation; CXCL8 synthesized by cancer cells may initiate the process of neovascularization by stimulating vascular endothelial growth factor. The newly formed blood vessels can thus initiate the process of breast cancer development, and may also supply nutrients to distant metastases (19). Liubomirski *et al* (41) evaluated the role of the inflammatory process and the involvement of inflammatory mediators, including the chemokine CXCL8, in the pathological mechanism of breast cancer development. This previous study showed that CXCL8 directly interacted with cancer cells in triple-negative breast cancer (TNBC), resulting in increased invasiveness and aggressiveness. Using a mouse model of TNBC, it was demonstrated that CXCL8 regulated by CXCR2, and C-C motif chemokine ligand 2 regulated by receptor for chemokine CCL2, interacted with tumor-associated neutrophils and macrophages, affecting their migration to the tumor site where they promoted disease course.

In conclusion, local and systemic disturbances of immune and inflammatory responses involving the CXCL8 chemokine and its receptors indicate the involvement of these studied parameters in the pathogenesis of ovarian cancer. Moreover, immunoregulation of the CXCL8-CXCR1 system may influence the course of the inflammatory process accompanying ovarian cancer development and may have a clinical application; however, further studies are required.

Funding

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AMP was involved in conceptualization, supervision of immunology research, investigation and writing (original draft preparation, review and editing). JMG was involved in conceptualization, supervision of molecular research, investigation, and writing, reviewing and editing. MSK and SS designed and performed immunological research. CKR designed and performed molecular research. MSK, SS, CKR, DW, PKD and AS performed the experiments, and participated in data analysis and interpretation. JS, WS and AW were involved in clinical research conceptualization and data interpretation. AMP and JMG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Ethics Committee of Medical University of Silesia in Katowice, Poland (protocol code KNW/0022/KB1/49/19). All patients agreed to participate in the presnt study and provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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