PRACE ORYGINALNE
ginekologia

COX-2 expression pattern is related to ovarian cancer differentiation and prognosis, but is not consistent with new model of pathogenesis

Ekspresja COX-2 jest związana z różnicowaniem raka jajnika i prognozą ale nie wiąże się z nowym modelem patogenezy

Magdalena Magnowska[1], Mikołaj Zaborowski[1], Paweł Surowiak[2,3], Ewa Nowak-Markwitz[1], Maciej Zabel[2,4], Marek Spaczyński[1]

[1] Department of Gynecology, Obstetrics and Gynecologic Oncology, Division of Gynecologic Oncology, Poznan University of Medical Sciences, Poznan, Poland
[2] Department of Histology and Embryology, Wroclaw Medical University, Wroclaw, Poland
[3] Lower Silesian Oncology Centre, Wroclaw, Poland
[4] Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland

Abstract

Objective: *Numerous studies suggest that cyclooxygenase-2 (COX-2) is overexpressed in cancer. Our objective was to investigate the relationship between COX-2 expression in ovarian carcinoma and clinicopathological factors. An emphasis was put on the association with the new pattern of tumorigenesis that divides tumors into type I – less aggressive, and type II – more aggressive one. The prognostic significance of COX-2 expression was evaluated.*

Methods: *Ovarian cancer tissues were obtained from 65 patients in FIGO III stage (23 with type I and 42 with type II ovarian cancer). COX-2 expression was evaluated by immunohistochemistry. The statistical analysis was performed in order to assess the connection between COX-2 expression and characteristic factors of ovarian cancer patients as well as the new division for type I and type II ovarian cancer.*

Results: *COX-2 expression was detected in 91% of tissue samples. It was markedly elevated in well differentiated tumors (p=0.0041). The platinum - resistant tumors had significantly higher expression of COX-2 (p=0.0337). There was no difference between COX-2 expression in type I and type II ovarian cancer (p=0.6720). The COX-2 staining was not associated to age, CA125 level, the presence of ascites or any special histological type. An increased expression of COX-2 was an unfavorable prognostic factor for overall survival (p=0.0369) and progression-free survival (p=0.0218). Multivariate analysis confirmed that COX-2 overexpression is an independent unfavorable prognostic factor of shorter progression-free survival (p=0.048).*

Conclusions: *COX-2 expression is an unfavorable prognostic factor for progression-free survival and overall survival in ovarian cancer. There is no relationship between COX-2 expression in ovarian cancer tissue and the examined model of ovarian cancer pathogenesis.*

Key words: **ovarian cancer** / **pathogenesis** / **COX-2** / **immunohistochemistry** /

Correspondence to:
Magdalena Magnowska
Division of Gynecologic Oncology, Poznan University of Medical Sciences,
ul.Polna 33, 60-535 Poznan, Poland
phone: +48 618 419 330
fax: +48 616 599 654
e-mail: magna5@op.pl

Otrzymano: **12.10.2013**
Zaakceptowano do druku: **30.01.2014**

Streszczenie

Cel: *Liczne badania pokazują, że cyclooxygenaza 2 ulega nadekspresji w nowotworach złośliwych. Celem tego opracowania była analiza związku pomiędzy ekspresją COX-2 w raku jajnika a czynnikami kliniczno-patologicznymi. Szczególny nacisk położono na zbadanie związku z nowym modelem patogenezy raka jajnika, który dzieli nowotwór na typ I – mniej agresywny i typ II – bardziej agresywny. Oceniono prognostyczne znaczenie ekspresji COX-2.*

Metoda: *Tkankę raka jajnika uzyskano od 65 pacjentek w stopniu FIGO III (23 z typem I i 42 z typem II raka jajnika). Ekspresję COX-2 oceniono przy pomocy immunohistochemii. W analizie statystycznej zbadano związek między ekspresją COX-2 a charakterystycznymi cechami kliniczno-patologicznymi oraz nowy podział raka jajnika na typ I i II.*

Wyniki: *Ekspresję COX-2 wykazano w 91% tkanek raka jajnika i była ona istotnie zwiększona w guzach dobrze zróżnicowanych (p=0,0041). Platynooporne guzy miały istotnie wyzszą ekspresję COX-2 (p=0,0337). Nie znaleziono różnicy pomiędzy ekspresją COX-2 w typie I i II raka jajnika (p=0,6720). Ekspresja COX-2 nie była związana z wiekiem, poziomem CA125, obecnością wodobrzusza czy typem histopatologicznym. Zwiększona ekspresja COX-2 okazała się niekorzystnym czynnikiem rokowniczym dla całkowitego czasu przeżycia (p=0,0369) oraz czasu wolnego od wznowy (p=0,0218). Analiza wieloczynnikowa potwierdziła, że zwiększona ekspresja COX-2 jest niezależnym niekorzystnym czynnikiem prognostycznym czasu wolnego od wznowy (p=0,048).*

Wnioski: *Ekspresja COX-2 jest niekorzystnym czynnikiem prognostycznym dla czasu wolnego od wznowy i całkowitego przeżycia w raku jajnika. Nie ma związku pomiędzy ekspresją COX-2 w tkance raka jajnika a badanym modelem patogenezy raka jajnika.*

Słowa kluczowe: **rak jajnika** / **patogeneza** / **COX-2** / **immunohistochemia** /

Introduction

A proposed model of ovarian cancer tumorigenesis distinguishes two groups of tumors that differ in the mechanisms leading to malignancy. Briefly, low grade serous, endometrioid, mucinous, clear cell ovarian cancer are considered less aggressive and classified as type I. It is hypothesized that they develop from precursors such as borderline tumors, cystadenomas and endometriosis. On the other hand, high-grade serous and undifferentiated ovarian cancer are assigned to type II subgroup with a more aggressive course of disease. Precursors of type II cancers remain unspecified [1,2]. Cyclooxygenases are the enzymes, which take part in the conversion of arachidonic acid to prostaglandins (PGs) and are responsible for inflammatory reactions. There are two COX isoenzymes: *COX-1* gene is constitutively expressed in many types of cells, while *COX-2* gene is mainly induced by inflammatory stimuli and mitogens, such as growth factors and cytokines. *COX-2* expression is very low and almost undetectable in physiologic conditions. However it is overexpressed in inflammed and neoplastic tissues [3].

The role of COX-2 pathway has been underscored in many neoplasms. Prostaglandins have been reported to stimulate an angiogenesis and suppress cytotoxic immune response [4]. The increased COX-2 expression has been detected in many malignant tumors, among others in: colon cancer, gastric, esophageal, pancreatic, bladder, cervix and endometrial cancer [4-8]. The aim of this research is to analyze how cyclooxygenase-2 expression is related to the hypothesis of ovarian cancer pathogenesis and other clinicopathological factors.

Materials and methods

Patients

Immunohistochemical examination was performed retrospectively on tissue samples taken during the first look laparotomy for routine diagnostic purposes. Sixty-five patients operated in 2000-2004 due to ovarian cancer in Department of Obstetrics and Gynecology (Poznań University of Medical Sciences, Poland) were enrolled in this study. The study was approved by the Institutional Review Board and the patients gave their informed consent before enrollment into the study. The cases were not stratified for any known prognostic factors. The patients distribution in each clinicopathological feature in connection with COX-2 expression level is summarized in table 1. In order to investigate the overall survival (OS) and the progression free survival (PFS) rate, a follow-up examination was performed every 2 months. The mean follow-up period was 37.2 months (range 24-74). The progression free survival time was defined as the time between the end of first line chemotherapy and the diagnosis of the recurrence. Type I and II ovarian cancer groups have been designated on the basis of histopathology and grading, as defined by Kurman and Shih [1, 2]. The type I group includes low-grade (G1) serous, low-grade endometrioid, clear cell and mucinous carcinomas, whereas high-grade (G2 and G3) serous, mesodermal and undifferentiated carcinomas belong to the type II group.

Tissue

Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. In each case, hematoxylin and eosin stained preparations were subjected to histopathological evaluation by two pathologists. The stage of the tumors was assessed according to the FIGO criteria [9]. Tumors were graded according to the Silverberg grading system [10].

Immunohistochemistry

Formalin-fixed, paraffin embedded tissue was freshly cut (4 um). The sections were mounted on Superfrost slides (Menzel Gläser, Germany), de-waxed with xylene, and gradually hydrated. Activity of endogenous peroxidase was blocked by 5 min exposure to 3% H_2O_2. All the studied sections were boiled for 15 min at 250W in the Antigen Retrieval Solution (DakoCytomation, Denmark). Then, immunohistochemical reactions were

performed with the use of the following antibody: monoclonal anti-COX-2 (mouse), (Santa Cruz Biotechnology, Santa Cruz, CA), dilution 1:2000 in Antibody Diluent, Background Reducing (DakoCytomation, Denmark) as described previously [5-8]. Tested sections were incubated with antibody for 1h at room temperature. Subsequent incubations involved biotinylated antibodies (15 min, room temperature) and streptavidin-biotinylated peroxidase complex (15 min, room temperature) (LSAB+, HRP, DakoCytomation, Denmark). DAB (DakoCytomation, Denmark) was used as a chromogen (7 min, at room temperature). All the sections were counterstained with Meyer's hematoxylin (DakoCytomation, Denmark). In every case, control reactions, in which specific antibody was substituted by the Primary Mouse Negative Control (DakoCytomation, Denmark), were included.

Control reactions

In our previous study we have described the control reactions performed in order to evaluate specificity of the COX-2 antibody (Cayman Chemical Company, Ann Arbor, MI, USA) [6].

Evaluation of reaction intensity

Intensity of immunohistochemical reactions was estimated independently by two pathologists. When in doubt, a re-evaluation was performed using a double-headed microscope and staining was discussed until a consensus was achieved. In order to evaluate the COX-2 expression, a semi-quantitative scale of ImmunoReactive Score (IRS) was applied. It took into account intensity of the colour reaction that was scored 0 to 4 as well as proportion of positive cells that was scored 0 to 3. The final score represented the product of points given for individual characters and ranged between 0 and 12 [11]. Low and high intensity of COX-2 expression was defined as IRS within ranges 0-4 and 6-12, respectively.

Statistical analysis

For statistical analyses, GraphPad Prism 6 for Windows from GraphPad Software and Microsoft Excel were used. The Mann-Whitney test was used for comparing the intensity of immunoreaction between studied subgroups. To estimate the significance of differences in overall survival time (OS) and progression-free survival time (PFS), Kaplan-Meier statistics (log-rank test) and Cox proportional hazard model were used. If applicable, the relationships between COX expression and clinicopathological features was expressed as Spearman's correlation coefficient. The outcome was recognized as statistically significant when $p<0.05$. All p-values are given for two-sided tests.

Results

The expression of COX-2 was present in 91% of examined tissues, in each histopathologic type (Figure 1). We did not observe the COX-2 expression in 5 cases (2 serous, 2 undifferentiated, 1 mucinous).

Table I presents an overview of median COX-2 expression values depending on clinicopathological features. The expression was not statistically associated to either ascites or histological type. There was no difference between COX-2 expression in type I and type II cancers (p=0.6720). The COX-2 expression was higher in patients who developed the recurrence within 6 months since the end of first line chemotherapy (p=0.0337).

Table I. Correlation between the COX-2 immunostaining intensity and the clinicopathological factors (Mann-Whitney test).

Characteristics (Number of patients)	Median COX-2 expression	p-value
Histologic type		
serous (34)	6.0	0.1832
non-serous (31)	4.0	
Grading		
G1,G2 (34)	6.0	0.0041*
G3 (22)	3.0	
Ovarian cancer type		
I (23)	6.0	0.6720
II (42)	4.0	
Age		
≤50 (34)	5.0	0.9522
>50 (31)	5.0	
Ascites		
Yes (20)	6.0	0.5690
No (45)	4.0	
Time to recurrence		
≤ 6months (28)	6.0	0.0337*
> 6months (37)	4.0	

The median COX-2 expression value in grade 1/2 group was 6.0, whereas in grade 3 it was 3.0 (p=0.0041). There was no correlation between COX-2 expression and CA125 level (Spearman r = - 0.156, p=0.278).

The relationships between COX-2 expression and overall survival time (OS) as well as progression-free survival time (PFS) were assessed by means of Kaplan-Meier analysis (log-rank test). Both OS (p=0.0369; HR 2.211; 95%CI 1.049-4.658) (Figure 2A) and PFS (p=0.0218; HR 2.293; 95%CI 1.077-4.883) (Figure 2B) were shorter in patients with high expression of COX-2. In patients with high COX-2 expression the median OS and PFS were equal to 28 and 9 months, respectively. Whereas in a group with low COX-2 expression the median OS and PFS were 32 and 11 months.

OS and PFS were also estimated within groups demonstrating one particular clinicopathological factor (Table II). We have found an association between high COX-2 expression and decreased OS as well as PFS in following groups: non-serous histologic type (p=0.0475 for PFS), G1/G2 grade (p=0.0417 for OS), age <50 (p=0.0024 for OS; p=0.0149 for PFS), optimal cytoreduction (p=0.0007 for OS; p=0.0049 for PFS). The observed relationships are presented at the figures 3-4. We have found no relationships between COX-2 expression and either OS or PFS within groups with serous histologic type, G3 grade, type I, type II ovarian cancer, age >50, elevated CA125, the presence of the ascites.

In multivariate analysis COX-2 expression was an independent prognostic factor associated with unfavourable PFS (p=0.048; HR 1,632; 95%CI 0.958-2.779), however did not influence OS (p=0.691; HR 1.224; 95%CI 0.452-3.316).

Discussion

Our research has revealed an increased expression of COX-2 in ovarian cancer tissue. The staining was observed in nearly all cancer samples. This finding has been corraborated by a number

Table II. High COX-2 expression as a prognostic factor within subgroups divided according to clinicopathological traits (Cox proportional hazard model).

	High COX-2 expression (IRS 6-12)			
	Overall survival		Progression free survival	
	p	HR (95%CI)	p	HR (95%CI)
Serous histologic type	0.2481	1.815 (0.6598 – 4.995)	0.9476	1.026 (0.4734 – 2.225)
Non-serous histologic type	0.1069	2.527 (0.8188 - 7.797)	0.0475*	2.314 (1.009 - 5.303)
G1/G2 grade	0.0417*	3.032 (1.042 - 8.822)	0.7394	1.135 (0.5391 – 2.388)
G3 grade	0.3819	1.758 (0.4962 - 6.232)	0.2702	1.856 (0.6181 - 5.574)
Type I	0.1121	2.986 (0.7745 to 11.51)	0.5046	1.472 (0.4727 – 4.585)
Type II	0.8489	1.066 (0.5409 - 2.123)	0.5134	1.203 (0.6802 - 2.266)
Age <50	0.0024*	5.030 (1.769 - 14.30)	0.0149*	2.606 (1.206 - 5.634)
CA125 elevated	0.5147	1.431 (0.4871 - 4.203)	0.0868	2.489 (0.8766 - 7.066)
Presence of ascites	0.2294	2.066 (0.6328 - 6.748)	0.2120	1.822 (0.7102 - 4.677)
Optimal cytoreduction	0.0007*	10.92 (2.752 - 43.36)	0.0049*	4.880 (1.618 - 14.72)

of studies, concerning different types of neoplasms [12, 13]. In the analysis conducted by Denkert on the material from 117 patients, COX-2 expression was revealed in 42% of 86 malignant tumors and 37% of 19 borderline tumors. They did not observe expression of this protein in 12 cystadenomas and in 2 specimen of healthy ovarian tissues [14]. This work pointed that COX-2 expression is present only in certain group of ovarian cancers. Seo et al. also reports that COX-2 expression is markedly increased in non-mucinous histological tumor types compared to mucinous [15]. In our study mucinous ovarian cancer had in two cases high expression and in one case no expression. However this group is too small to draw any conclusions. In one of the previous studies the Northern Blot analysis revealed no mRNA for COX-2 in 12 cases of mucinous ovarian cancer [16]. Moreover, in patient with non-mucinous ovarian cancer, COX-2 expression was related with staging ($p<0.001$) and the presence of ascites ($p=0.036$) [15]. Klimp et al. confirmed COX-2 expression in 15 from 18 cases of ovarian cancers and in 10 from 15 borderline tumors [17]. Thus, COX-2 expression in ovarian cancer patients is significantly related to neoplasm histology.

COX-2 expression appeared to be strongly related to the grading of a neoplasm. Well differentiated (G1,G2) tumors have higher COX-2 concentration than those described as poorly differentiated (G3). Such a pattern has been observed in colorectal, lung and hepatocellular cancer [18-20]. What is more, COX-2 expression was detected in precancerous conditions such as colon adenomas [18]. It has been thus suggested that COX-2 is involved in early stages of tumorigenesis. It is worth to stress, however, that the models of neoplastic development in these types of cancer are based on the transition from low grade to high grade lesions. For this reason it is hardly reproducible to ovarian cancer biology, as current data imply an existence of two distinct pathophysiological pathways leading to ovarian cancer emergence [1, 2, 21]. The genetic profiling suggests that low-grade cancers (mainly type I) develop in a completely different mechanism from high grade tumors (mainly type II) [1]. These studies imply that the transition of low-grade into high grade lesion is not probable. The type I and type II tumors in our investigation, however, do not differ in terms of COX-2 expression.

The assessment of COX-2 expression turned out useful in the division of tumors with respect to response to chemotherapy. Platinum refractory and resistant ovarian cancers (recurrence before 6 months) have enhanced COX-2 expression compared to partly sensitive and sensitive tumors (recurrence after 6 months). This finding has potential clinical implications. A more intense surveillance over the group of patients with increased COX-2 expression in tumor tissue seems therefore reasonable. These patients could especially benefit from the maintenance therapy, as well. Given the link between COX-2 and angiogenesis an antiangiogenic therapy could be considered. Ferrandina et al. was the first who mentioned about relationship between increased COX-2 expression and resistance to chemotherapy in ovarian cancer. The two patient groups were compared, after optimal cytoreduction and after explorative laparotomy. It was proved that in both groups the percentage of COX-2 positive cases was significantly higher among the patients resistant for platinum than among patients sensitive to treatment. From the analyzed clinic-pathological factors, only increased COX-2 expression and the age above 60 were independent prognostic factors of worse treatment response [22]. It has been suggested that COX-2 may

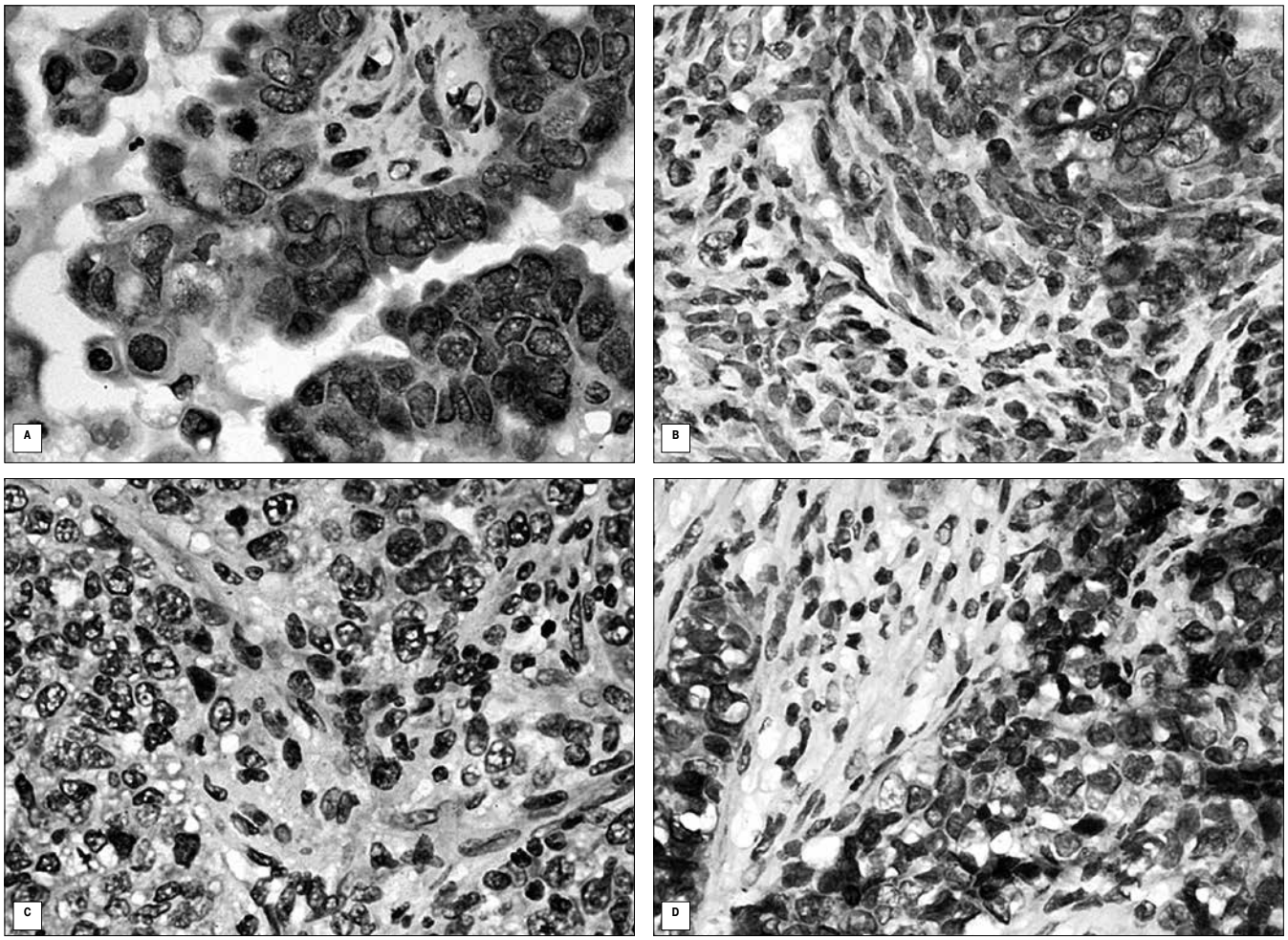

Figure 1 A-D. Immunohistochemical localization of COX-2 expression in ovarian carcinoma cells. Cytoplasmic reaction.
A – IRS 9, **B** – IRS 6, **C** – IRS 4, **D** – IRS 3 (hematoxylin, x400).

increase the expression of proteins responsible for multidrug resistance phenomenon, especially the resistance to cisplatin [23].

In multivariate analysis the COX-2 expression appeared to be an independent prognostic factor. A high expression is related to low overall survival and progression free survival. Given the heterogeneity of ovarian tumors, an analysis of the prognostic value of COX-2 expression has been conducted in the stratified subgroups. Its usefulness in assessing prognosis has been observed especially in patients with well differentiated tumors, at the age below 50 years and in those who had undergone an optimal cytoreduction. Again, it proves that the tumor differentiation decides about a distinct cancer biology. G1 ovarian cancers are usually related to better survival. An assessment of COX expression may indicate the group of patients vulnerable to recurrence in spite of low grading. It could be especially useful in stage FIGO I when the level of differentiation among others decides about the administration of chemotherapy. The unfavorable prognostic value of COX-2 expression has been corraborated by the meta-analysis [24].

The use of non-steroid anti-inflammatory drugs has appeared effective in prevention of colon cancer [25]. A number of studies has been conducted to confirm this activity with respect to ovarian cancer. Their results, however, remain inconclusive. It has been shown by some researchers that regular NSAID intake decrease the risk of ovarian cancer [26-30]. The others suggest that there is no relationship between these drugs administration and ovarian cancer incidence [31].

The mechanisms responsible for the worse prognosis in cases with increased COX-2 expression ovarian cancer are still unknown. Some functions induced by COX-2 were described in the biology of different neoplasms: increased cells proliferation, apoptosis inhibition, angiogenesis stimulation as well as inhibition of immune control mechanisms [3]. In the colon cancer COX-2 expression was unfavourable prognostic factor and correlated with the level of tumor vascularization [32]. COX-2 expression in stomach cancer was related to the tumor invasion into the lymph vessels as well as metastases to the lymph nodes [33]. COX-2 expression can be related to metastasis formation. In lung adenocarcinoma COX-2 expression was increased in metastatic tumors compared to the primary cancer focus [34]. Therefore it may be hypothesised that ovarian cancer cases with increased COX-2 expression have elevated potential for metastases formation and thus worse prognosis comparing to COX-2 negative tumors. Thus, an overexpression of COX-2 in ovarian cancer cannot be only a coexisting phenomenon but rather a mechanism important in the way the tumor progress.

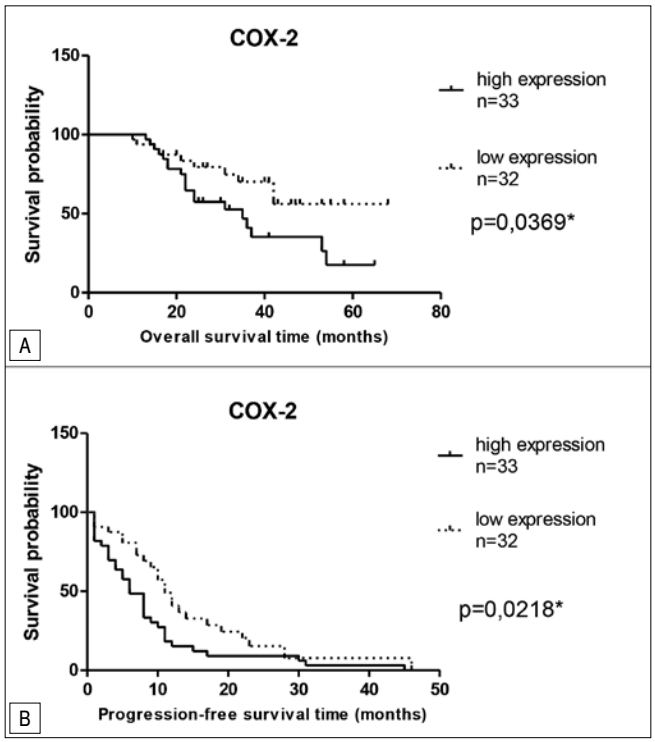

Figure 2. Kaplan Meier curves for A – overall survival time and COX-2 expression; B – progression-free survival time and COX-2 expression.

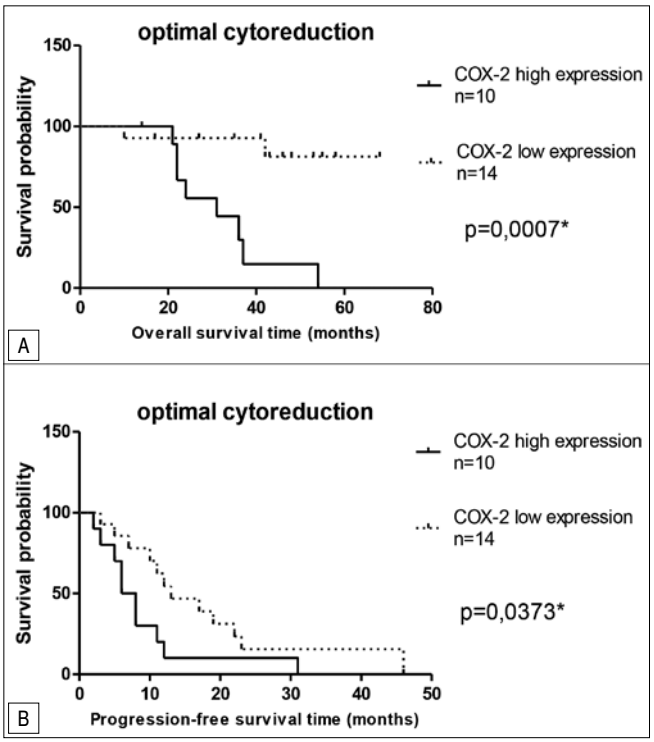

Figure 2. Kaplan Meier curves for A – overall survival time and COX-2 expression; B – progression-free survival time and COX-2 expression.

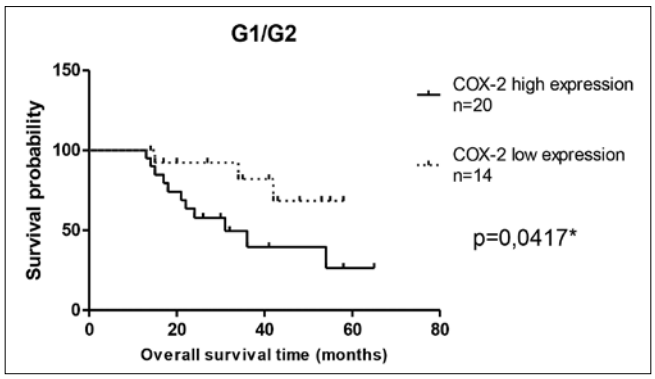

Figure 3. Overall survival time was significantly shorter in cases with high COX-2 expression and G1/G2 grade.

Conclusions

COX-2 expression is an unfavorable prognostic factor for progression-free survival and overall survival in ovarian cancer. (Ovarian cancer patients with elevated COX-2 expression may have shorter time to reccurence and shorter survival time). There is no relationship between COX-2 expression in ovarian cancer tissue and the examined model of ovarian cancer pathogenesis.

Oświadczenie autorów

1. Magdalena Magnowska – zebranie materiału, analiza statystyczna wyników, opracowanie wyników badań, przechowywanie dokumentacji, przygotowanie manuskryptu i piśmiennictwa – autor zgłaszający i odpowiedzialny za manuskrypt.
2. Mikołaj Zaborowski – współautor tekstu pracy, współautor protokołu, korekta i aktualizacja literatury.
3. Paweł Surowiak – uzyskanie funduszy na realizację badań laboratoryjnych, opracowanie koncepcji i założeń badań, wykonanie badań laboratoryjnych.
4. Ewa Nowak-Markwitz – autor założeń pracy, analizy i interpretacji wyników, przygotowanie, korekta i akceptacja ostatecznego kształtu manuskryptu.
5. Maciej Zabel – autor koncepcji i założeń pracy, przygotowanie manuskryptu.
6. Marek Spaczyński – ostateczna weryfikacja i akceptacja manuskryptu.

Źródło finansowania: Badania statutowe Kliniki Onkologii Ginekologicznej, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu.

Konflikt interesów: Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

References

1. Shih I, Kurman RI. Ovarian tumorigenesis. A proposed model based on morphological and molecular genetic analysis. *Am J Pathol.* 2004, 164 (5), 1511-1518.
2. Kurman RI, Shih I. The origin and pathogenesis of epithelial ovarian cancer – a proposed unifying theory. *Am J Surg Pathol.* 2010, 34 (4), 433-443.
3. Gasparini G, Longo R, Sarmiento R, Morabito A. Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? *Lancet Oncol.* 2003, 4 (10), 605-615.
4. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene.* 1999, 18 (55), 7908-7916.
5. Surowiak P, Materna V, Denkert C, [et al.]. Significance of cyclooxygenase 2 and MDR1/P-glycoprotein coexpression in ovarian cancers. *Cancer Lett.* 2006, 235 (2), 272-280.
6. Surowiak P, Materna V, Matkowski R, [et al.]. Relationship between the expression of cyclooxygenase 2 and MDR1/P-glycoprotein in invasive breast cancer and their prognostic significance. *Breast Cancer Res.* 2005, 7 (5),R862-870.

7. Surowiak P, Pawełczyk K, Maciejczyk A, [et al.]. Positive correlation between cyclooxygenase 2 and the expression of ABC transporters in non-small cell lung cancer. *Anticancer Re.* 2008, 28, 2967-2974.

8. Szczuraszek K, Materna V, Halon A, [et al.]. Positive correlation between cyclooxygenase-2 and ABC-transporter expression in non-Hodgkin's lymphomas. *Oncol Rep.* 2009, 22,1315-1323.

9. www.cancerresearchuk.org/cancer-help/type/ovarian-cancer/treatment/stages-of-ovarian-cancer

10. Shimizu Y, Kamoi S, Amada S, [et al.]. Toward the development of a universal grading system for ovarian epithelial carcinoma: testing of a proposed system in a series of 461 patients with uniform treatment and follow-up. *Cancer.* 1998, 82 (5), 893-901.

11. Remmele W, Stegner H. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. *Pathologe.* 1987, 8, 138-140.

12. Krawczyk-Rusiecka K, Lewiński A. Cyclooxygenase-2 expression and its association with thyroid lesions. *Arch Med Sci.* 2010, 6, 5, 653-657.

13. Klimek M, Urbański K, Kojs Z, [et al.]. Role of cyclooxygenase-2 in cervical cancer. *Arch Med Sci.* 2009, 5, 3, 303-307

14. Denkert C, Kobel M, Pest S, [et al.]. Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. *Am J Pathol.* 2002, 160, 893-903.

15. Seo SS, Song Y, Kang DH, [et al.]. Expression of cyclooxygenase-2 in association with clinicopathological prognostic factors and molecular markers in epithelial ovarian cancer. *Gynecol Oncol.* 2004, 92 (3), 927-935.

16. Ristimaki A, Honkanen N, Jankala H, [et al.]. Expression of cyclooxygenase-2 in human gastric carcinoma. *Cancer Res.* 1997, 57 (7), 1276-1280.

17. Klimp AH, Hollema H, Kempinga C, [et al.]. Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. *Cancer Res.* 2001, 61 (19), 7305-7359.

18. Eberhart CE, Coffey RJ, Radhika A, [et al.]. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology.* 1994, 707, 1183-1188.

19. Wolff H, Saukkonen K, Anttila S, Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res.* 1998, 58 (22), 4997-5001.

20. Koga H, Sakisaka S, Ohishi M, [et al.]. Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology.* 1999, 29 (3), 688-696.

21. Nowak-Markwitz E, Spaczyński M. Ovarian cancer – modern approach to its origin and histogenesis. *Ginekol Pol.* 2012, 83 (06),454-457.

22. Ferrandina G, Lauriola L, Zannoni G, [et al.]. Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients. *Ann Oncol.* 2002, 13 (8), 1205-1211.

23. Patel V, Dunn M, Soroki A. Regulation of MDR1 (P-glycoprotein) by cyclooxygenase-2. *J Biol Chem.* 2002, 227, 38915-38920.

24. Lee J-Y, Myung S-K, Song Y-S. Prognostic role of cyclooxygenase-2 in epithelial ovarian cancer: A meta-analysis of observational studies. Gynecol Oncol. 2013, http://dx.doi.org/10.1016/j.ygyno.2013.02.011

25. Flossmann E, Rothwell PM. British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet.* 2007, 369, 1603-1613

26. Schildkraut JM, Moorman PG, Halabi S, [et al.]. Analgesic drug use and risk of ovarian cancer. *Epidemiol.* 2006, 17 (1), 104-107.

27. Lo-Ciganic WH, Zgibor JC, Bunker CH, [et al.]. Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, or acetaminophen and risk of ovarian cancer. *Epidemiol.* 2012, 23 (2), 311-319.

28. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, [et al.]. Aspirin and epithelial ovarian cancer. *Prev Med.* 2001, 33 (6), 682-687.

29. Tavani A, Gallus S, La Vecchia C, [et al.]. Aspirin and ovarian cancer: an Italian case-control study. *Ann Oncol.* 2000, 11, 1171-1173

30. Rosenberg L, Palmer JR, Rao RS, [et al.]. A case-control study of analgesic use and ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2000, 9, 933-937.

31. Meier CR, Schmitz S, Jick H. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. *Pharmacotherapy.* 2002, 22,303-309.

32. Masunaga R, Kohno H, Dhar D, [et al.]. Cyclooxygenase-2 expression correlates with tumor neovascularization and prognosis in human colorectal carcinoma patients. *Clin Cancer Res.* 2000, 6 (10), 4064-4068.

33. Murata H, Kawano S, Tsujii S, [et al.]. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. *Am J Gastroenterol.* 1999, 94 (2), 451-455.

34. Hida T, Yatabe Y, Achiwa H, [et al.]. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res.* 1998, 58 (17), 3761-3764.