

Plasma and Peritoneal Fluid Annexin A2 Levels in Patients with Endometriosis

Michał Zygarowicz^{1,*}, Joanna Kacperczyk-Bartnik^{2,3,*}, Janusz Sierdzinski⁴, Cezary Wojtyła^{5,6}, Piotr Pierzynski^{5,6}, Grzegorz Manka⁷, Mariusz Kiecka⁷, Robert Z Spaczynski⁸, Piotr Piekarski⁹, Beata Banaszewska¹⁰, Artur Jakimiuk^{11,12}, Tadeusz Issat¹³, Wojciech Rokita^{14,15,†}, Jakub Młodawski^{14,15}, Maria Szubert^{3,16,17}, Piotr Sieroszewski^{16,18}, Grzegorz Raba^{19,20}, Kamil Szczupak^{19,20}, Tomasz Kluz²¹, Marek Kluza²¹, Michał Lipa^{3,22}, Damian Warzecha^{5,22}, Mirosław Wielgos^{23,24}, Ewa Koc-Zorawska^{25,26}, Marcin Zorawski^{26,27}, Piotr Laudanski^{5,6,28}

¹Students' Scientific Group Affiliated to the Department of Obstetrics, Gynecology and Gynecological Oncology, Medical University of Warsaw, Warsaw, Poland; ²II Department of Obstetrics and Gynaecology, Medical University of Warsaw, Warsaw, Poland; ³Club 35. Polish Society of Gynecologists and Obstetricians, Wrocław, Poland; ⁴Department of Medical Informatics and Telemedicine, Medical University of Warsaw, Warsaw, Poland; ⁵OVIklinika Infertility Center, Warsaw, Poland; ⁶Women's Health Research Institute, Calisia University, Kalisz, Poland; ⁷Angelus Provita Hospital, Katowice, Poland; ⁸Center for Gynecology, Obstetrics and Infertility Treatment Pastelova, Poznan, Poland; ⁹Division of Infertility and Reproductive Endocrinology, Department of Gynecology, Obstetrics and Gynecological Oncology, Poznan University of Medical Sciences, Poznan, Poland; ¹⁰Chair and Department of Laboratory Diagnostics, Poznan University of Medical Sciences, Poznan, Poland; ¹¹Department of Obstetrics and Gynecology, Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland; ¹²Center of Reproductive Health, Institute of Mother and Child in Warsaw, Warsaw, Poland; ¹³Department of Obstetrics and Gynecology, Institute of Mother and Child in Warsaw, Warsaw, Poland; ¹⁴Collegium Medicum Jan Kochanowski University in Kielce, Kielce, Poland; ¹⁵Clinic of Obstetrics and Gynecology, Provincial Combined Hospital in Kielce, Kielce, Poland; ¹⁶Department of Gynecology and Obstetrics Medical University of Lodz, Lodz, Poland; ¹⁷Department of Surgical Gynecology and Oncology, Medical University of Lodz, Lodz, Poland; ¹⁸Department of Fetal Medicine and Gynecology, Medical University of Lodz, Lodz, Poland; ¹⁹Clinic of Obstetrics and Gynecology, Provincial Hospital, Przemysl, Poland; ²⁰Department of Obstetrics and Gynecology, University of Rzeszow, Rzeszow, Poland; ²¹Department of Gynecology, Gynecology Oncology and Obstetrics, Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland; ²²Department of Obstetrics and Gynaecology, Warsaw Southern Hospital, Warsaw, Poland; ²³Premium Medical, Warsaw, Poland; ²⁴Faculty of Medicine, Lazarski University, Warsaw, Poland; ²⁵II Department of Nephrology, Hypertension and Internal Medicine with Dialysis Unit, Medical University of Białystok, Białystok, Poland; ²⁶The Academy of Applied Medical and Social Sciences, Elbląg, Poland; ²⁷Department of Cardiology, Lipidology and Internal Medicine with Cardiac Intensive Care Unit, Medical University of Białystok, Białystok, Poland; ²⁸Department of Obstetrics, Gynecology and Gynecological Oncology, Medical University of Warsaw, Warsaw, Poland

[†]Professor Wojciech Rokita passed away on March 18, 2020

*These authors contributed equally to this work

Correspondence: Piotr Laudanski, Department of Obstetrics, Gynecology and Gynecological Oncology, Medical University of Warsaw, Kondratowicza Street 8, Warsaw, 03-242, Poland, Tel +48 22 326 53 80, Email piotr.laudanski@wum.edu.pl

Introduction: Endometriosis is an inflammatory-related reproductive age disease characterized by the presence of endometrial cells outside the uterine cavity. Current laboratory practice does not provide specific markers for detecting and assessing the advancement of endometriosis in either plasma or peritoneal fluid. The severity of disease is assessed in stages from I to IV based on the results of laparoscopic inspection. The protein annexin A2 (ANXA2) has been reported to be associated with inflammatory processes.

Aim of the Study: The study aimed to investigate and compare ANXA2 protein concentration using the ELISA method in plasma and peritoneal fluid in a group of women with endometriosis compared to controls.

Materials and Methods: Biological material was collected during a multicenter, cross-sectional study, which was conducted at eight departments during elective laparoscopy from 53 women with and 40 women without endometriosis. Patients were divided by endometriosis stage and infertility status, and then compared with subgroups. Analysis included the Chi-square test for categorical variables, Mann–Whitney *U*-test and two-sided Wilcoxon rank-sum test for continuous variables.

Results: Women with endometriosis had significantly elevated plasma ANXA2 levels compared to women without endometriosis (mean concentrations 28.69 vs 19.61 ng/L, $p=0.01$). Differences in peritoneal fluid ANXA2 levels were statistically insignificant (mean concentrations of 23.7 vs 22.97 ng/L, $p=0.06$). Plasma concentrations in patients with stage III and IV endometriosis were significantly higher compared to controls (mean concentrations of 24.19 vs 19.71 ng/L, $p=0.03$). No such differences were observed in plasma when comparing stages I–II vs III–IV, and stages I–II vs controls (mean concentrations of 33.82 vs 24.19 ng/L, $p=0.72$ and 33.82 vs 19.71 ng/L, $p=0.12$, respectively). Comparison of samples from patients with or without infertility, primary or secondary infertility, endometriosis with

or without infertility, and non-endometriosis with or without infertility showed no significant differences in the plasma nor in the peritoneal fluid concentrations.

Conclusion: ANXA2 is possibly involved in the pathogenesis of endometriosis, especially in advanced stages. Due to the limited group of tested samples, further studies are needed to confirm its role.

Keywords: annexin A2, endometriosis, infertility

Introduction

Endometriosis is a benign, chronic inflammatory disease characterized by the presence of endometrial tissue outside the uterus, most commonly in the pelvic peritoneum, the ovaries, and the rectovaginal septum.¹ It affects about 10% of women in the general population of reproductive age, but among women suffering from infertility it affects about 50%.¹⁻⁴ Endometriosis is most often manifested by chronic pelvic pain, infertility, dysmenorrhea and pain during sexual intercourse. The severity of the disease is assessed on a scale from I to IV, but the severity of symptoms varies considerably and does not always correlate with endometriosis stage.¹⁻³

Endometriosis is an estrogen-dependent disease, which means that estrogen causes endometriosis cells to develop and survive, which then induces inflammation of the peritoneum. Chronic pro-inflammatory effects cause adhesions of intraperitoneal tissues, changes in the composition of the peritoneal fluid and, as a consequence, pain, infertility and difficulties maintaining pregnancy. Chronic inflammation creates conditions similar to those during the development of many cancers.⁵ The presence of endometriosis also increases the overall risk of developing malignancies, especially of the pelvis.⁶

The etiology of endometriosis is still not fully explained. There are numerous theories trying to illustrate this condition.^{4,7-13} Sampson's theory is the most popular explanation for the pathogenesis and states that endometriosis is the result of retrograde menstruation, during which endometrial cells implant in the peritoneum.¹² Moreover, the role of the immune response in endometriosis pathogenesis may be particularly extensive.^{14,15}

As the gold standard, final diagnosis of endometriosis is based on histopathological examination of lesion samples.¹⁶ Unfortunately, this method is invasive and requires visible macroscopic lesions.

Non-specific clinical presentation causes delays in diagnosis, often exceeding 7 years.^{17,18} A great hope for the development of diagnostics is associated with research on endometriosis-specific biomarkers enabling detection of the disease at an early stage.^{19,20}

Many potential biomarkers that may have an impact on pathogenesis of endometriosis are being studied, among which are metalloproteinases, growth factors, cytokines, chemokines, miRNAs and adhesion molecules.^{3,7-9,19,21}

Annexin family proteins are known to be involved in the interaction of membrane structures that expose these proteins.²²⁻²⁵ Annexin A2 (ANXA2) has been shown to be specific to endometriosis several times in scientific publications, but final conclusions differ in terms of whether it is significantly increased in stages I-IV or only in stages III and IV.^{26,27} The divergent results show that further studies are needed involving larger groups of patients.

ANXA2 protein is also markedly increased in malignant diseases, including lung cancer, gastric cancer, pancreatic ductal adenocarcinoma, colorectal cancer, hepatocellular carcinoma, breast cancer, cervical cancer, endometrial cancer, ovarian cancer, renal cell carcinoma, high-grade prostate cancer, glioblastoma, multiple myeloma, acute lymphoblastic leukemia, and acute promyelocytic leukemia.²⁸⁻³²

In addition, ANXA2 is a protein markedly present in prostasomes (secretory vesicles of the prostate epithelium of men) and is part of seminal fluid.²² Prostasomes, together with spermatozoa, enter the uterine cavity, and then combine with spermatozoa in the fallopian tube, presumably to participate in the process of fertilization of the ovum.³³ Other ANXA family proteins are known to function in a similar way to ANXA2.³⁴ ANXA1 is also present in sperm (not only in prostasomes). Together with ANXA5, both are reported to participate in fertilization through local immunomodulatory effects on women's lymphocytes.^{22,35} Prostasomes regulate sperm motility, capacitation and acrosomal reaction.³³ ANXA2 is involved in actin remodeling, protein assembly, transcription, mRNA transport, and DNA replication and repair.³⁶

The aim of this study was to examine ANXA2 protein levels in plasma and peritoneal fluid in a group of women with endometriosis compared to a control group. Secondary aims included analysis of ANXA2 levels in women at different endometriosis stages (I-IV) and in infertile patients.

Materials and Methods

Biological material was collected during a multicenter, cross-sectional study conducted in eight Obstetrics and Gynecology Departments in Poland between 2018 and 2019 (project number: 6/6/4/1/NPZ/2017/1210/13522, financed by the Polish Ministry of Health): Department of Obstetrics and Gynecology, Medical University of Warsaw; Angelius Provita Hospital, Katowice; Department of Gynecology, Division of Infertility and Reproductive Endocrinology, Obstetrics and Gynecological Oncology at Poznan University of Medical Sciences; Department of Obstetrics and Gynecology, Central Clinical Hospital of the Ministry of Interior in Warsaw; Clinic of Obstetrics and Gynecology, Provincial Combined Hospital, Kielce; Department of Surgical Gynecology and Oncology, Medical University of Lodz; Department of Gynecology and Obstetrics, Provincial Hospital, Przemyśl; and Department of Gynecology, Gynecology Oncology, and Obstetrics, Institute of Medical Sciences, Medical College of Rzeszow University.³⁷

Patients qualified for elective laparoscopic procedures if they had non-malignant indications, such as: idiopathic infertility, chronic pelvic pain syndrome, ovarian cysts or suspected endometriosis. Infertility was defined as the inability to become pregnant during at least 12 months of regular unprotected intercourse in women under the age of 35 and at least 6 months in other women.³⁸

Exclusion criteria were irregular menstruation (over 35 days or less than 25 days), age under 18 or over 40 years, hormonal therapy in the preceding 3 months before laparoscopy, malignant or suspected malignant disease, autoimmune disease, previous and/or current pelvic inflammatory disease, any previous pelvic surgery, uterine fibroids or polycystic ovaries.

The cycle phase was calculated based on previous menstruation dates and average length of menstrual cycle. In addition, the phases of the menstrual cycle in women with and without endometriosis were determined based on histological dating of eutopic endometrial samples collected simultaneously with pathological changes.

Each patient was evaluated based on the American Fertility Society revised classification of endometriosis, along with histological examination of the biopsies.³⁹

All patients completed the clinical questionnaire of the World Endometriosis Research Foundation.⁴⁰ Written informed consent was obtained from all patients. The study was approved by the Independent Ethics Committee of the Medical University of Warsaw (KB/223/2017).

Patients fasted 6 to 12 hours before surgery. Prior to the procedure and before anesthesia induction, blood samples were collected in 10 mL ethylenediaminetetraacetic acid tubes (Sarstedt) in order to assess ANXA2 plasma levels. Peritoneal fluid was aspirated with a Veress needle under direct visual control at the beginning of laparoscopy to avoid blood contamination. Each procedure was performed in accordance with the standard operating procedures of the Endometriosis Phenome and Biobanking Harmonization Project.⁴¹ The collection of biological material had no influence on the medical treatment of the patients and was completed in accordance with the Declaration of Helsinki. The aspirated peritoneal fluid was centrifuged in all centers at 1000 x g for 10 minutes at 4°C. The supernatant was transferred to a fresh 10 mL tube (Sarstedt). The same types of tube were used in all blood and peritoneal fluid collection centers. The time lapse between sample collection (both peritoneal fluid and plasma) and processing was under 45 minutes. All centers centrifuged blood samples at 2500 x g for 10 minutes at 4°C. All samples were stored at -80°C.

Based on the results of laparoscopic inspection, patients were assigned to the appropriate endometriosis stage subgroup (I–IV). Patients without visible endometriosis lesions were recruited to the control group. Later, we also divided the patients with endometriosis into two separate groups because of the similarities in disease activity between stages within the groups: (1) grade I or II endometriosis or (2) grade III or IV endometriosis. Information about endometriosis stage was not provided by participating centres in the case of three patients.

Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was performed in order to assess levels of ANXA2 in collected plasma and peritoneal fluid samples. ELISA is a quantitative method that has been used for decades for detection and quantification of specific substances.^{42,43} It has also been widely performed in order to detect ANXA2 in human biological samples.^{20,44–46} Human ANXA2 ELISA kits (SunRedBio, Shanghai, China, catalogue number 201-12-1089) were used with a sensitivity of 0.135 ng/L and an assay range of 0.3–40 ng/L. Quantitative ANXA2 level analyses were financed by an internal grant from the Medical University of Warsaw (project number: 1W51/1/M/MB/N/

21). Study protocol was approved by the Institutional Review Board at the Medical University of Warsaw (approval number: AKBE/245/2021).

Statistical analysis was performed with SAS v. 9.4 (SAS Institute, Cary, NC, USA) and Statistica v. 13.3 software (StatSoft Inc., Kraków, Poland). The groups were compared by Chi-square test for categorical variables. Mann–Whitney *U*-test and two-sided Wilcoxon rank-sum test were performed for continuous variables depending on the distribution of variables after testing for normal distribution using the Shapiro–Wilk test. Generation of a receiver-operating characteristics (ROC) curve and calculation of the area under the curve (AUC) and 95% confidence intervals (95% CI) were used to assess the predictive power of ANXA2 concentration in the plasma and peritoneal fluid. The level of statistical significance was set at $p < 0.05$. Datasets are available and will be shared by the corresponding author upon request.

Results

Table 1 and Table 2 show the characteristics and results of Annexin A2 levels in the study and control groups (women with and without endometriosis). There was a significantly higher mean level of plasma (28.69 vs 19.61 ng/L) and higher median (12.31 vs 7.85 ng/L) ANXA2 concentrations in patients with endometriosis compared to patients without endometriosis ($p=0.01$). There was also a higher mean level of peritoneal fluid (23.7 vs 22.97 ng/L) and higher median (15.66 vs 9.42 ng/L) ANXA2 concentrations in patients with endometriosis compared to patients without endometriosis, but the differences were not statistically significant ($p=0.06$).

Table 3 presents the results of ANXA2 levels of patients at different endometriosis stages and women without endometriosis. There was a statistically significant higher mean level of plasma (24.19 vs 19.71 ng/L) and higher median (14.69 vs 7.91 ng/L) ANXA2 concentrations in patients at stage III and IV compared to patients without endometriosis ($p=0.03$). There was also a higher mean level of peritoneal fluid (26.64 vs 22.92 ng/L) and higher median (19.7 vs 9.65 ng/L) ANXA2 concentrations in patients at stages III and IV compared to patients without endometriosis; however, the differences were not statistically significant ($p=0.07$).

Additional analyses, as shown in Table 4, examined the association between ANXA2 levels and infertility. A tendency towards a higher mean level of plasma (30.40 vs 18.45 ng/L) and median (12.06 vs 8.34 ng/L) ANXA2 levels were characteristic of a history of infertility, but no statistically significant differences regarding ANXA2 plasma levels were

Table 1 Group Characteristics and Annexin A2 Levels Detected in Plasma of Patients with and without Endometriosis

	Patients with Endometriosis (n=53)	Patients without Endometriosis (n=40)	p
Mean age (years)	33 (SD±4.99)	31 (SD±6.07)	0.13
Mean day of menstrual cycle	13.76 (SD±7.12)	11.52 (SD±6.02)	0.12
Stage I endometriosis	16 (30%)	Not applicable	–
Stage II endometriosis	8 (15%)	Not applicable	–
Stage III endometriosis	19 (36%)	Not applicable	–
Stage IV endometriosis	7 (13%)	Not applicable	–
Infertility	29 (55%)	21 (53%)	0.75
Primary infertility	25 (47%)	15 (38%)	0.44
Secondary infertility	4 (8%)	6 (15%)	0.22
Endometrial ovarian cysts	30 (57%)	Not applicable	–
Mean Annexin A2 concentration (ng/L)	28.69 (SD±36.41)	19.61 (SD±37.04)	0.01
Median Annexin A2 concentration (ng/L)	12.31 (Range: 3.19–151.5)	7.85 (Range: 3.93–189.1)	

Table 2 Group Characteristics and Annexin A2 Levels Detected in Peritoneal Fluid of Patients with and without Endometriosis

	Patients with Endometriosis (n=42)	Patients without Endometriosis (n=37)	p
Age (years)	32 (SD±4.5)	30 (SD±5.5)	0.1
Mean day of menstrual cycle	14.78 (SD±6.19)	12.08 (SD±5.69)	0.07
Stage I endometriosis	10 (24%)	Not applicable	–
Stage II endometriosis	6 (14%)	Not applicable	–
Stage III endometriosis	17 (40%)	Not applicable	–
Stage IV endometriosis	6 (14%)	Not applicable	–
Infertility	23 (55%)	17 (46%)	0.51
Primary infertility	20 (48%)	10 (21%)	0.03
Secondary infertility	3 (7%)	7 (19%)	0.13
Endometrial ovarian cysts	26 (62%)	Not applicable	–
Mean Annexin A2 concentration (ng/L)	23.7 (SD±28.06)	22.97 (SD±35.76)	0.06
Median Annexin A2 concentration (ng/L)	15.66 (Range: 4.59–181.8)	9.42 (Range: 4.52–168.9)	

Table 3 Comparison of Plasma and Peritoneal Fluid Annexin A2 Levels Depending on Endometriosis Stage

Plasma Annexin A2 Concentrations (ng/L)			
	Mean (±SD)	Median (Range)	p
Patients with endometriosis stages I and II (n=24)	33.82 (±45)	11.48 (3.19–151.5)	0.72
Patients with endometriosis stages III and IV (n=26)	24.19 (±28)	14.69 (3.32–123.9)	
Patients with endometriosis stages I and II (n=24)	33.82 (±45)	11.48 (3.19–151.5)	0.12
Patients without endometriosis (n=41)	19.71 (±37)	7.91 (3.96–189.1)	
Patients with endometriosis stages III and IV (n=26)	24.19 (±28)	14.69 (3.32–123.9)	0.03
Patients without endometriosis (n=41)	19.71 (±37)	7.91 (3.96–189.1)	
Peritoneal fluid Annexin A2 concentrations (ng/L)			
	Mean (±SD)	Median (Range)	p
Patients with endometriosis stages I and II (n=15)	18.26 (±14)	13.13 (4.59–47.73)	0.35
Patients with endometriosis stages III and IV (n=24)	26.64 (±35)	19.7 (6.29–181.8)	
Patients with endometriosis stages I and II (n=15)	18.26 (±14)	13.13 (4.59–47.73)	0.32
Patients without endometriosis (n=38)	22.92 (±35)	9.65 (4.52–168.9)	
Patients with endometriosis stages III and IV (n=24)	26.64 (±35)	19.7 (6.29–181.8)	0.07
Patients without endometriosis (n=38)	22.92 (±35)	9.65 (4.52–168.9)	

Table 4 Annexin A2 Levels Detected in Plasma and Peritoneal Fluid of Patients with and without Infertility

Plasma Annexin A2 Concentrations (ng/L)			
	Mean (\pm SD)	Median	p
Patients with infertility (n=50)	30.40 (\pm 43)	12.06 (3.19–189.1)	0.12
Patients without infertility (n=42)	18.45 (\pm 26)	8.34 (3.32–123.9)	
Patients with primary infertility (n=40)	34.77 (\pm 48)	12.31 (3.19–189.1)	0.35
Patients with secondary infertility (n=10)	12.93 (\pm 7)	11.1 (3.93–24.27)	
Endometriosis patients with infertility (n=29)	31.19 (\pm 39)	12.31 (3.19–151.5)	0.43
Endometriosis patients without infertility (n=23)	25.47 (\pm 33)	14.34 (3.32–123.9)	
Non-endometriosis patients with infertility (n=21)	28.16 (\pm 50)	10.74 (3.93–189.1)	0.23
Non-endometriosis patients without infertility (n=19)	9.95 (\pm 7)	6.92 (4.66–25.39)	
Peritoneal fluid Annexin A2 concentrations (ng/L)			
	Mean (\pm SD)	Median (Range)	p
Patients with infertility (n=40)	22.08 (\pm 25)	13.65 (4.52–120.3)	0.69
Patients without infertility (n=38)	20.87 (\pm 29)	11.00 (6.27–181.8)	
Patients with primary infertility (n=30)	25.61 (\pm 28)	14.70 (4.52–120.3)	0.06
Patients with secondary infertility (n=10)	11.52 (\pm 5)	8.69 (–5.9–21.15)	
Endometriosis patients with infertility (n=23)	19.10 (\pm 13)	13.92 (4.6–47.73)	0.5
Endometriosis patients without infertility (n=19)	29.27 (\pm 39)	19.26 (6.29–181.8)	
Non-endometriosis patients with infertility (n=17)	26.13 (\pm 36)	10.75 (4.52–120.3)	0.34
Non-endometriosis patients without infertility (n=19)	12.47 (\pm 10)	8.90 (6.27–43.56)	

detected ($p=0.12$). A similar trend was noticed in the peritoneal fluid. We also observed a higher mean level of peritoneal fluid (25.61 vs 11.52 ng/L) and higher median (14.70 vs 8.69 ng/L) ANXA2 concentrations in patients with primary infertility compared to patients with secondary infertility, yet the differences were not significant ($p=0.06$).

Comparison of plasma and peritoneal fluid ANXA2 concentrations within the endometriosis group did not show any statistically significant differences between women with and without infertility issues (Table 4).

The discriminatory ability of ANXA2 concentrations in the plasma and in the peritoneal fluid in the total population analyzed were evaluated by ROC analysis. ROC curves are presented in Figure 1. The AUC value for ANXA2 plasma concentration was 0.654 (95% CI: 0.54–0.77, $p=0.008$). For ANXA2 concentrations in the peritoneal fluid, the AUC value was 0.622 (95% CI: 0.50–0.75, $p=0.059$). The diagnostic values of ANXA2 concentrations evaluation are summarized in Table 5. The overall model quality for the diagnosis of endometriosis exceeded 0.5 only for the ANXA2 plasma concentration and this was not achieved for ANXA2 concentration in the peritoneal fluid. The cutoff value of 6.941 ng/L in the plasma was associated with 83% sensitivity and 57.5% specificity.

Discussion

The main finding of our study was the higher level of ANXA2 plasma concentration detected in patients with endometriosis compared to women without endometriosis. There are several reports in the literature on elevated ANXA2 protein levels in women with endometriosis. Nazri et al examined the concentration of exosomes in the peritoneal fluid and the content of protein composition in isolated exosomes in a group of women with endometriosis compared to a control group.²⁶ In their results, they

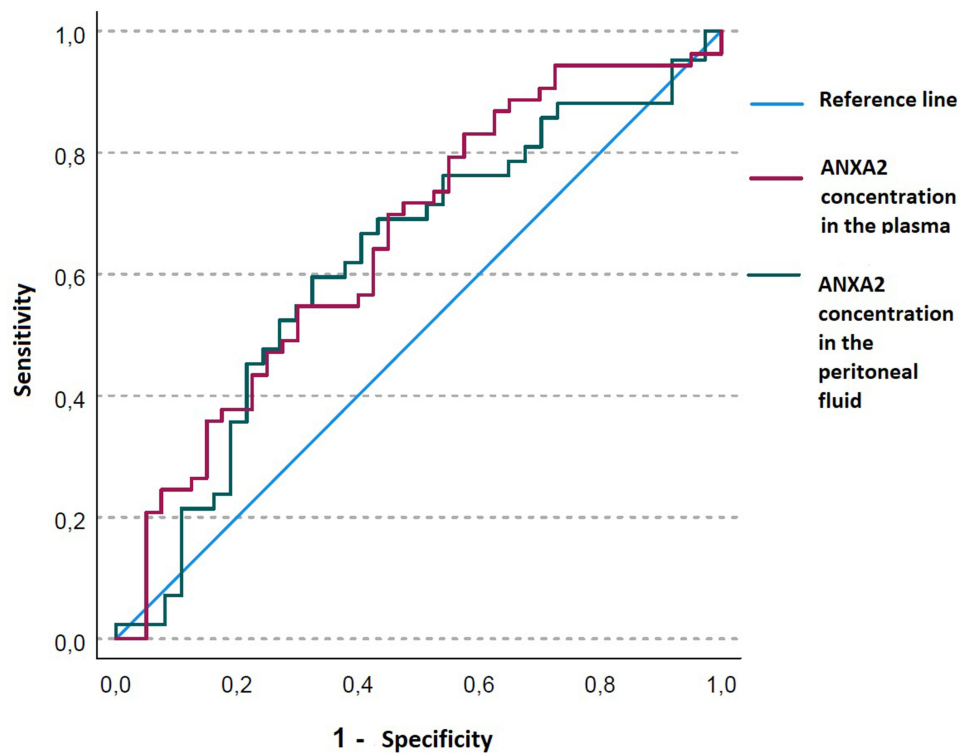


Figure 1 Receiver-operating characteristics (ROC) curves of ANXA2 concentration in the plasma and the peritoneal fluid of women with endometriosis and control women.

ranked ANXA2 among five proteins significantly specific for endometriosis. The authors explained that ANXA2 serves as an activator of matrix metalloproteinase (MMP-9) of peritoneal macrophages and participates in the degradation of the extracellular matrix of the endometrial tissue which flows through retrograde menstruation. In addition, simultaneous damage to the extracellular matrix of the peritoneal layer of mesothelial cells by ANXA2-containing exosomes may allow implantation and development of ectopic endometrial lesions.⁴⁷ The exact cellular source of the increased ANXA2 production is not clear, but the focus is on ectopic endometrial tissue. This idea is supported by a certain similarity between endometriosis and metastatic cancer, especially since the elevated level of ANXA2 protein is already known to be typically present in many cancers, including

Table 5 Receiver-Operating Characteristics (ROC) Analysis and Diagnostic Value of ANXA2 Concentration in the Plasma and in Peritoneal Fluid of Women with Endometriosis and Control Women

	ANXA2 Plasma Concentration	ANXA2 Peritoneal Fluid Concentration
Area under the curve (AUC)	0.654	0.622
Standard error (SE)	0.058	0.064
95% confidence interval (95% CI)	0.540–0.767	0.496–0.748
Asymptomatic significance (p)	0.008	0.059
Cutoff value (ng/L)	6.941	13.065
Sensitivity (95% CI)	0.830	0.595
Specificity (95% CI)	0.575	0.324
Overall model quality	0.54	0.5

endometrial cancer.^{48–52} These authors also consider monocytes to be cells that secrete ANXA2 in exosomes. In the same study it was also stated that endometriosis in stages I and II may differ in its pathogenesis from that in stages III and IV, resulting in a difference in ANXA2 concentrations, and this may create the use of ANXA2 as a biomarker differentiating endometriosis stages. In another study, Kaya et al also showed significantly higher serum concentrations of ANXA2 in women at stage III and IV endometriosis compared with those at stage I and II and in the control group without endometriosis.²⁷ Our study showed no significant differences between these groups in either plasma or peritoneal fluid; however, this could be caused by the sample size. We observed that plasma ANXA2 levels are significantly higher in patients at stage III–IV endometriosis compared with women without endometriosis. There was no similar observation for patients at stage I–II endometriosis in comparison to the control group (Table 3).

In a study by Sun et al, ANXA2 levels in ectopic (study group) and eutopic (control group) endometrial tissues were compared.⁵³ The results are inconsistent with previously cited studies, as ANXA2 concentration was reduced in the study group. This may be due to the fact that the control group included mostly endometrial tissues from patients with grade II–III cervical intraepithelial neoplasia. Considering the numerous reports of elevated ANXA2 in cancer, this may be the reason for the increased ANXA2 in the control group in relation to the study group. Another explanation for this finding may be the fact that endometrial tissue is evaluated rather than peritoneal fluid or plasma, which can give contradictory results. Thus, the results of Sun et al only seem to confirm that the process of carcinogenesis promotes the expression of ANXA2 in tissues, including the endometrium, which was particularly demonstrated by Zou et al with the example of endometrial cancer.⁵⁴

A different cross-sectional study, by Volpato et al, applied the Western blotting method to measure Annexin A1 expression in peritoneal samples of women with (n=22) and without (n=18) endometriosis.⁵⁵ Annexin A1 was detected in both the study and control groups, but its expression was lower in women with endometriosis. The same study also evaluated cytokine expression in the peritoneal fluid using the ELISA method and observed that women with endometriosis were characterized by higher IL-6 concentration, but the IL-1 β levels were comparable with the control group. The authors concluded that reduced inflammatory resolution mediators could cause development of inflammatory endurance and aggravation of endometriosis. Contradictory results were presented in a study by Li et al,⁵⁶ which analyzed eutopic endometrium samples of 25 patients with endometriosis and 16 healthy women. Additionally, peritoneal fluid of ten women with endometriosis was examined. The authors observed Annexin A1 overexpression in both the eutopic endometrium and peritoneal fluid of endometriosis patients. Paula et al compared the expression of Annexin A1, mast cell proteases and formyl peptide receptor 1 (FPR1) in the ectopic endometrium of 18 women with eutopic endometrium and 10 women without endometriosis.⁵⁷ Histology results of ectopic endometriotic lesions presented an undifferentiated glandular pattern with inflammatory cells and altered mast cell heterogeneity. This finding was also associated with elevated ANXA1 and FPR1 expression in the epithelial cells. It was concluded that simultaneous upregulation of mast cell chymase, ANXA1 and FPR1 is involved in development of ectopic endometriotic lesions.

Hsu et al demonstrated the positive effect of ANXA2 on motility and the angiogenic potential of endometrial stromal cells *in vitro*.⁵⁸ The authors examined the function of ANXA2 present in small extracellular vesicles produced by ectopic endometrial tissue. Change in cell activity is caused by upregulation of the ERK/STAT3 pathway. As a result, ANXA2 creates heterotetramer with S100 A10 calcium-binding protein (S100A10), translocates to the cell surface, activates the plasminogen, ERK1/2, and degrades the extracellular matrix. This process is required for the initiation of cell migration, invasion, and angiogenesis. ANXA2 is present in small extracellular vesicles from ectopic endometrial stromal cells and is absent in those from eutopic endometrial stromal cells, which is consistent with the findings reported by Nazri et al.²⁶ They concluded that retrograde menstrual tissue might be one of the sources of small extracellular vesicles within the peritoneal fluid and thus promotes the development of endometriotic lesions. However, our study showed that it is not so easy to detect the difference in the concentration of this protein in the peritoneal fluid using only ELISA without isolation of small extracellular vesicles. Our negative results regarding the lack of observation of significant differences in the concentration of ANXA2 in the peritoneal fluid between patients with and without endometriosis and between patients at different stages of endometriosis do not exclude the possibility of ANXA2 involvement in the pathogenesis of endometriosis. Kajdos et al performed flow cytometry and compared plasma and peritoneal fluid samples of patients with ovarian endometriosis (n=23) and teratoma (n=7) to evaluate the presence of extracellular microvesicles.⁵⁹ The authors reported an association between endometriosis and a significantly higher plasma level of Annexin V-positive microvesicles.

It is also worth noting that large variations in ANXA2 values were observed in our relatively homogeneous group of patients. This suggests that multiple factors may be responsible for the levels of ANXA2 detected and questions the possibility of using ANXA2 concentration in peritoneal fluid as a biomarker. Zhou et al reported overexpression of ANXA2 in adenomyosis and described many cellular processes in which this protein is involved, including endocytosis, exocytosis, and cellular adhesion.⁶⁰ ANXA2 can bind to membrane phospholipids in a Ca²⁺-dependent manner, which provides a link between Ca²⁺ signaling and membrane functions. It also forms networks on the cell surface, which organize membrane domains and membrane recruitment platforms for interacting proteins. This demonstrates multiple possible levels of ANXA2-mediated pathogenesis of endometriosis. Similar findings were reported in a study by Liu et al.²⁰ Overexpression of ANXA2 was detected using the immunohistochemical method in both ectopic and eutopic endometrium tissues from 30 patients with adenomyosis compared to 15 patients with uterine fibroids. No statistically significant differences in ANXA2 levels were observed between eutopic and ectopic samples of patients with adenomyosis. Additionally, these authors observed a positive correlation between severity of dysmenorrhea and expression of ANXA2. Saher et al explored the expression of potential biomarkers for ovarian endometriomas.⁶¹ In this case-control study, the authors performed differential proteomic analysis of eutopic and ectopic tissue of six patients with ovarian endometriosis. As Annexin A5 is responsible for apoptosis inhibition, cell migration and invasion, the molecular analysis showed its overexpression in the ectopic ovarian endometriomas compared with the eutopic endometrial tissue.

One of the limitations of our study was the size of the study group, which may have resulted in insignificant results regarding the difference in ANXA2 concentrations in the peritoneal fluid of patients with and without endometriosis. In order to overcome this limitation, further cooperation and research by a multi-center working group on endometriosis is planned. The strength of the work is the analysis of ANXA2 concentrations in samples collected from the population of women diagnosed with endometriosis and infertility, as so far there are few published reports on this subject, and most of them focus on oncology.

Conclusions

Our study mainly showed significantly higher plasma levels of ANXA2 in women with endometriosis compared to the control group without endometriosis. Undoubtedly, this is a reason for further research on the ANXA2 protein in the context of its use as a biomarker of endometriosis, perhaps also in combination with other biomarkers to increase the sensitivity of the test. Accurate results according to stage of endometriosis showed that this protein was much more useful in detecting stages III and IV than I and II. The lack of statistically significant results in infertility comparisons directs further ANXA2 research more towards elucidating a role in the pathogenesis of endometriosis, but not infertility in general.

Abbreviations

ANXA2, Annexin A2; AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; FPR1, peptide receptor 1; ROC, receiver-operating characteristics.

Disclosure

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