

Anti-ovarian antibodies in sera of patients with ovarian tumors

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ABSTRACT

Aim of paper: The purpose of this study was to assess the presence of anti-ovarian autoantibodies in sera of patients with ovarian tumors.

Materials and methods: The study group consisted of 82 patients treated at the Division of Gynecological Surgery, Poznan University of Medical Sciences, Poland in 2007–2011. 46 patients with malignant ovarian tumors and 36 patients diagnosed with benign ovarian tumor were included into the study. Age-matched healthy control groups consisted of 15 women and 19 men. Anti-ovarian autoantibodies in serum were assessed with the use of indirect immunofluorescence. The presence of anti-ovarian autoantibodies in serum was correlated with clinical and histopathological features of the disease.

Results: Serum anti-ovarian autoantibodies were found in 59% (27/46) of patients with malignant ovarian tumors, compared to 55% (20/36) and 26% (4/15) of patients with benign ovarian tumors and healthy female controls respectively. The difference was statistically significant ($P=0.04$). All serum samples from the male controls were negative. Anti-ovarian antibodies occurred more often among advanced stage (III and IV stage according to FIGO, $P=0.037$) and grade 3 ($P=0.049$) ovarian cancers, however, there were no differences in median progression-free survival ($P=0.388$). The presence of anti-ovarian antibodies was neither influenced by histopathological type of the tumor, menopausal status, presence of ascites nor CA125 levels. Seropositivity for anti-ovarian antibodies was correlated positively with patients' age and negatively with tumor size.

Conclusions: Anti-ovarian autoantibodies develop with higher frequency in ovarian cancer comparing to healthy controls, however with similar proportion to benign ovarian tumors patients. The presence of antibodies against normal ovarian tissue correlates with ovarian cancer aggressiveness.

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1. Introduction

Epithelial ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer-related death among women in United States [1]. The main cause of high mortality related to ovarian cancer is lack of early symptoms and unavailable screening tests leading to delayed diagnosis. Currently, about 70% of patients are diagnosed at III and IV FIGO clinical stage [2].

Recently, the role of autoantibodies to tumor-associated antigens (TAAs) in the diagnosis of cancer has been widely investigated [3,4]. Ovarian cancer is regarded as strongly immunogenic neoplasm and significant proportion of TAAs have been associated with ovarian cancer development [5,6]. Moreover, in sera of patients with ovarian cancer also the antibodies targeting normal ovarian

tissue were revealed [7]. However, their clinical significance has not been elucidated in ovarian cancer. Thus, the main purpose of this study was to assess the incidence of anti-ovarian autoantibodies in sera of patients with ovarian tumors and to correlate their presence with clinical and histopathological features of the disease.

2. Materials and methods

Serum samples were collected from 82 consecutive patients treated due to ovarian tumor in Division of Gynecological Surgery Poznan University of Medical Sciences between 2007 and 2011. Patients were divided into two groups: (1) malignant ovarian tumors ($n=46$), including 6 cases of borderline ovarian tumors, and (2) benign ovarian tumors ($n=36$). The group of healthy age-matched controls included 15 women and 19 men. None of subjects from control group had any acute or chronic autoimmune or inflammatory condition at the inclusion or in history. No clinical or laboratory signs of the disease was observed. One control subject had history of cholecystectomy and one of appendectomy.

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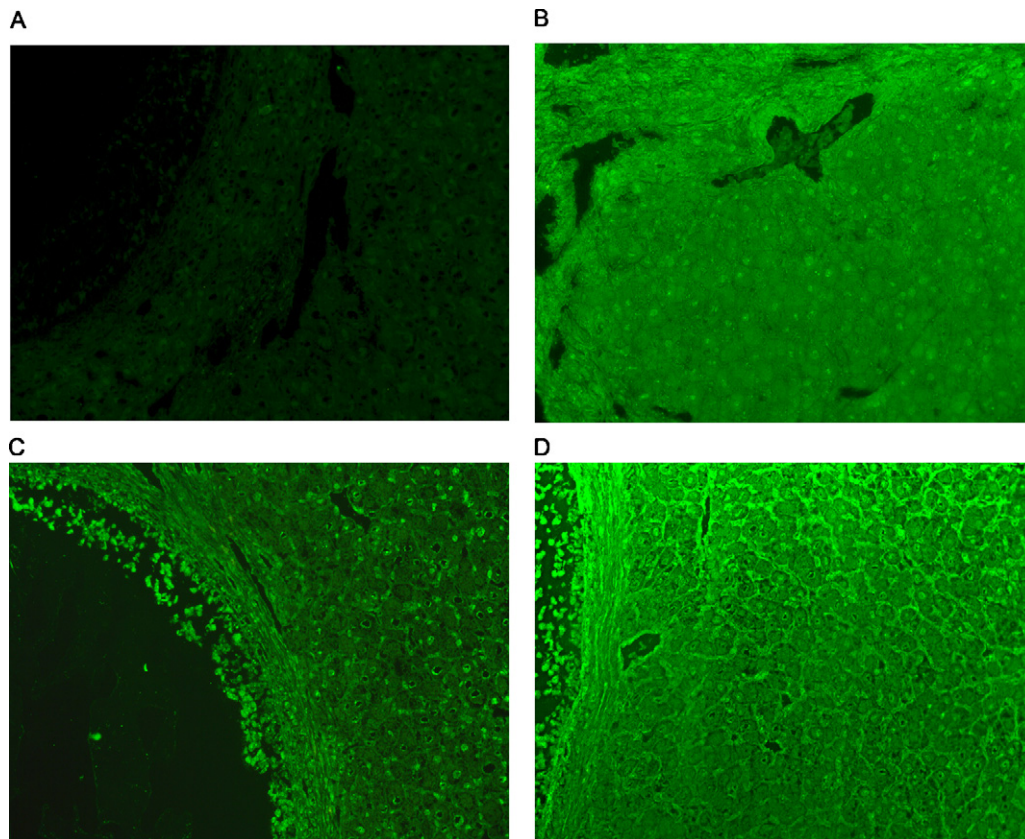


Fig. 1. Examples of immunofluorescence reactions in selected patients. (A) 43-years-old patient diagnosed with mucinous cystadenoma. The serum was negative for anti-ovarian autoantibodies, (B) 53-years-old patient diagnosed with serous adenocarcinoma, (C) 39-years-old patient with mucinous borderline tumor, and (D) 62-years-old patient diagnosed with secondary ovarian cancer (metastasis from colorectal cancer). Pictures B–D present positive reactions between patients' sera and ovarian antigens.

An anamnesis was taken and routine gynecological examination with two-dimensional transvaginal ultrasonography was performed within one to five days before laparotomy or laparoscopy. Each ovarian tumor was examined histopathologically with assessment of histological grade. Ovarian cancers and borderline tumors were staged according to current International Federation of Gynecology and Obstetrics (FIGO) classification. Due to small number of patients, malignant ovarian tumors group was divided into early-stage ovarian cancer (including FIGO I and II stages) and advanced-stage ovarian cancer (FIGO III and IV stages). Progression of cancer disease was assessed according to RECIST [8] and GCI [9] criteria.

Serum samples were collected preoperatively and stored at -82°C . The evaluation of the presence of anti-ovarian antibodies in sera was performed by means of indirect immunofluorescence with the use of the commercial diagnostic kit (EUROIMMUN, Germany). The EUROIMMUN company establishes and applies a quality management system that fulfills the requirements certified by EN ISO 9001:2008 (the certificate is valid until 2017). EUROIMMUN warrants design, development, production and services of immunofluorescence systems for in vitro diagnosis in humans (certificate of TÜV Rheinland Products GmbH-registration number SY 60076866 0001). The test uses monkey ovarian sections as a substrate applied in BIOCHIP technology. Frozen monkey ovarian sections are fixed to the glass surface by covalent bonding, that significantly (>100 times) increases adhesion and prevents the substrates from being detached. Afterwards cover glasses coated with monkey ovarian sections are cut in the automated system into millimeter-sized fragments (BIOCHIPS). Thus BIOCHIP technology assures high-quality and homogenous preparations of tissue section. Moreover the kit utilizes TITERPLANETM technique in which samples and reagents

are applied to the reaction fields of a reagent tray. Patients sera diluted 1:10 with phosphate buffered salt–Tween (PBS–Tween) are placed onto BIOCHIP slides localized in the recesses of the reagent tray. Because the fluids are confined in a closed space, such a technology do not requires humidity chamber. Next, secondary antibody – goat anti-human immunoglobulins (IgA, IgM, IgG) conjugated with IFCC are applied. After required incubation and washing steps the samples were evaluated in fluorescent microscope (EUROStar, Zeiss). Fig. 1 demonstrates examples of performed investigations. Each series of samples (10 cases) was preceded by testing of positive and negative control sera supplied with the kit. The specificity reaching 100% was observed in German population. No cross-reactivity of tested antibodies was reported.

Statistical analyses were carried out with MedCalc 10.4.0.0, GraphPad InStat 3.06 and StatXact-9, version 9.0.0 software.

3. Results

The presence of anti-ovarian antibodies in ovarian cancer group was age-dependent. Seropositive ovarian cancer patients were significantly older than seronegative patients ($56.4, \text{SD} \pm 10.4$ years vs. $47.83, \text{SD} \pm 13.6$ years, $P=0.022$). While, there were no differences in patients age between seropositive and seronegative subjects among benign ovarian tumors group ($39.6, \text{SD} \pm 13.5$ years vs. $38.9, \text{SD} \pm 13.8$, respectively, $P=0.886$). However, the seropositivity for anti-ovarian antibodies was not influenced by menopausal status ($P=0.142$). Demographic characteristic of studied patients was presented in Table 1.

Sera obtained from 27 (59%) patients diagnosed with ovarian cancer were positive for anti-ovarian antibodies. Similarly, 20 (55%) patients with benign ovarian tumor were seropositive, while, only

Table 1
Demographic characteristic of studied patients.

Median patients age (minimum and maximum)			
Ovarian cancer group	Benign ovarian tumor group	Female healthy controls	Male healthy controls
53 (21–85)	36 (20–74)	51 (43–67)	54 (22–75)
Menopausal status – number of patients (%)			
	Ovarian cancer group	Benign ovarian tumor group	Female healthy controls
Premenopausal	20 (43.5%)	29 (80.6%)	8 (53.3%)
Postmenopausal	26 (56.5%)	7 (19.4%)	7 (46.3%)

4 (26%) patients among female controls were positive for anti-ovarian antibodies. When three groups were taken into analysis, the difference in presence of antibodies among groups were statistically significant ($P=0.04$). All serum samples from the male controls were negative for the presence of anti-ovarian antibodies. The distribution of anti-ovarian antibodies within different histopathological types of malignant and benign ovarian tumors was shown in Tables 2 and 3, respectively. There was no statistically significant difference regarding histopathological type of cancer for the presence of anti-ovarian antibodies among analyzed groups ($P=0.363$). Similarly, we did not reveal differences in seropositivity for anti-ovarian antibodies regarding histopathological findings in benign ovarian tumors group ($P=0.235$). However, as high as 72% (13/18) of patients with benign endometrial cysts were seropositive. This was significantly higher than in subjects with benign serous tumors ($P=0.0007$) (Table 3). While in malignant tumors the relation was opposite (Table 2).

We have found statistically significant difference in the presence of anti-ovarian antibodies between different histological grades of the cancer ($P=0.049$). We have not found any seropositive cancer patients with grade 1 (G1) tumor, while, anti-ovarian antibodies were found in sera of majority (68%) of patients with grade 3 (G3) cancers. Moreover, there was statistically significant difference ($P=0.037$) in presence of anti-ovarian antibodies between early and advanced-stage ovarian cancer patients. At the advanced-stage of ovarian cancer 71.4% (20/28) of patients were seropositive in comparison to 38.9% (7/18) within early stage ovarian cancer patients.

Table 2
Histopathological findings and distribution of anti-ovarian antibodies among patients with malignant ovarian tumors.

Group of malignant ovarian tumors					
	Serous tumors	Mucinous tumors	Undifferentiated carcinomas	Endometrioid adenocarcinomas	Others ^a
Seropositive	11 ^b	3	7	2	4
Seronegative	5	5	3	4	2
Total	16	8	10	6	6

^a The group of other malignant tumors included: 3 clear cell carcinomas, 1 metastatic ovarian tumor of colorectal origin, 1 case of malignant hemangiopericytoma and 1 borderline adenofibroma. Among group of serous and mucinous tumors there were 2 and 3 cases of borderline tumors.

^b $P=0.0007$ – seropositive serous tumors vs. seropositive endometrioid.

Table 3
Distribution of anti-ovarian antibodies among patients with benign ovarian tumors.

Group of benign ovarian tumors					
	Serous tumor	Mucinous tumor	Endometrioid tumors	Adult teratomas	Others ^a
Seropositive	2	1	13 ^b	4	0
Seronegative	2	2	5	5	2
Total	4	3	18	9	2

^a The group of other tumors included: 1 case of functional ovarian cyst and 1 case of theca cell tumor.

^b $P=0.0007$ – seropositive serous tumors vs. seropositive endometrioid.

Table 4
The correlation between occurrence of anti-ovarian antibodies in patients sera depending on several clinical features of ovarian cancer disease.

Median progression free survival – months (standard error – %)		
Seropositive	14.2 (±16%)	P=0.388
Seronegative	21.4 (±12%)	
Mean tumor diameter – mm (SD)		
Seropositive	102.25 mm (±45.01 mm)	P=0.026
Seronegative	134.68 mm (±46.64 mm)	
Median CA125 serum concentration – U/ml (25th and 75th percentile)		
Seropositive	1128.3 U/ml (419.8–1806.3)	P=0.109
Seronegative	237.4 U/ml (85.1–747.6)	
Development of ascites – number of patients (%)		
Seropositive	9/26 (20%)	P=0.765
Seronegative	8/20 (17%)	

The presence of anti-ovarian antibodies did not affect median progression – free survival during at least one year follow-up available in 27 cases. Mean tumor diameter in seropositive ovarian cancer patients was 102.25 mm (SD ± 45.01 mm), and it was significantly lesser than diameter in seronegative ovarian cancer patients, 134.68 mm (SD ± 46.64 mm, $P=0.026$). Median CA125 serum concentrations in ovarian cancer patients and proportion of patients presented with ascites did not differ between seropositive and seronegative cases (Table 4).

4. Discussion

In this study we have observed in more than half of ovarian cancer patients the humoral immunological response represented by the production of autoantibodies against normal ovarian tissue detected by means of indirect fluorescence. Our observation supports the results of the previous study involving ovarian tumor patients and focused on immune reaction to antigens expressed in normal tissue [7]. In that study of Barua et al., anti-ovarian antibodies were found by means of Western blotting in 81% of ovarian cancer patients. We have found 55% of benign ovarian tumor patients to be seropositive in comparison to 36% in the study of Barua et al. When sera of healthy controls were examined for the

presence of anti-ovarian antibodies, similarly to Barua's study [7], we found them less frequently than in sera of patients with malignant and benign tumors. We have not found anti-ovarian antibodies in the sera of healthy male controls. This may suggest that positive results obtained in the group of healthy women are rather linked with subclinical adnexal inflammatory event than false positive results.

However, contrary to Barua et al. study [7] our results showed, that progression of the disease is related to the production of anti-ovarian autoantibodies, which are also associated with moderately- and poorly-differentiated ovarian cancers. Whereas, the seropositivity was inversely correlated with tumor size. To conclude we may suggest, that the presence of anti-ovarian antibodies is associated with aggressive type of the cancer, not with the extent of the disease.

However, it awaits elucidation, whether humoral immunologic response acts as ally or enemy in the host of the cancer. Nevertheless, several data suggest that humoral anti-tumor response can favor tumor growth. For instance, enhanced humoral response and suppressed cell-mediated immunity was shown to be associated with several cancers and premalignant lesions [10,11]. Depletion of B-lymphocytes in clinical [12] and experimental [13–15] studies resulted in reduction of tumor burden. Several humoral response related anti-inflammatory cytokines, like interleukin-10 and -13, were implicated in cancer growth [16,17]. Also development of immune complexes and humoral response against tumor-associated antigens were shown to promote tumor invasion and metastasis [15,18].

Recently, α -actinin 4 (α ACTN4), heat shock 70 protein 5 (HSPA5) and β -actin (ACTB), have been identified as autoantigens in patients with idiopathic premature ovarian failure [19]. Previously, anti-heat shock 70 protein (Hsp70) antibodies were also observed in endometrioid endometrial cancer patient with paraneoplastic cancer-associated retinopathy [20]. Chaperon proteins which are ubiquitous, highly conserved phylogenetically molecules with an important role in stress survival may be potential antigens. Heat shock 27 protein, heat shock 70 protein and heat shock 90 protein are candidates indicated as target antigens for autoimmune reaction in cancer patients [21,22] and anti-ovarian immunity [23]. Anti-Hsp90 antibodies were found in late stages of ovarian cancer [22], thus because we have observed higher percentage of seropositivity among advanced-stage of ovarian cancer patients further studies on anti-Hsp90 are reasonable.

Consistent results were obtained in several studies concerning antibodies against tissue associated antigens (TAA) in ovarian cancer patients. Vogl et al. [24] evaluated the presence of anti-p53 antibodies in serum of 113 patients with ovarian cancer, 15 patients with borderline tumors and 117 patients with benign tumors. Seropositivity for anti-p53 antibodies was correlated with tumor stage and grade and it was associated with shortened overall and relapse-free survival. In the study of Angelopoulou et al. [25], the presence of anti-p53 antibodies was related to tumor grade, with more frequently presence in G2 and G3 cancers, but it was not dependent on stage of the disease. On the other hand, Høgdall et al. [26] did not found association between anti-p53 antibodies and clinical stage, histological subtype and survival. Valuable results were obtained in the study of Gnjatic et al. [5], where the presence of antibodies against several ovarian cancer antigens (ANXA2, FAM131B, NY-ESO-1) was associated with favorable prognosis, while antibodies against different TAAs (ERFFI1, PHLDB1, TRH, TRUB1) indicated shorter survival. These results suggest that influence of humoral response on the course of ovarian cancer is a complex process. Despite of association between the presence of anti-ovarian antibodies with advanced stage and poor cancer differentiation, we did not reveal differences in progression-free survival

depending on development of anti-ovarian antibodies during one year follow-up.

Investigation of antibodies produced in the course of cancer may be attractive issue in cancer screening, because it spares time and funds. Antibodies against TAA were identified even 6 years before diagnosis of cancer [27] and the progression of the disease from preinvasive to invasive stage is associated with occurrence of new antibodies [28,29]. In general, the presence of antibodies against specific TAA were found in less than 1/3 of cancer patient resulting in low sensitivity in cancer diagnosis, while, the percentage of false-positive results is less than 1% [4,30,31]. Thus, make use of diagnostic panels evaluating presence of several antibodies seem more valuable [32]. Chatterjee et al. [33], developed the panel of 65 ovarian cancer antigens for detection of specific immunoglobulins in patients sera. The panel reached sensitivity of 55% and specificity of 98%. Due to small difference in the presence of anti-ovarian antibodies between ovarian cancer and benign ovarian tumor patients, we have shown, that method identifying spectrum of anti-ovarian antibodies has limited utility in diagnosis of ovarian cancer and we may suggest that evaluation of specific anti-ovarian antibodies could be more valuable.

Although, the exact cause of endometriosis is not elucidated, autoimmunity seems to be involved [34]. In the present study, similarly to previous reports [35,36], anti-ovarian antibodies were found in half or more of patients with endometriosis.

In conclusion, more than half of patients with ovarian cancer were seropositive for antigens expressed in normal ovarian tissue. Anti-ovarian antibodies were found especially in aggressive types of ovarian cancer and in older subjects. The proportion of seropositive cases is similar in ovarian cancer and benign tumors patients, however higher in malignant tumors than healthy subjects.

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