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Authors: Andrzej Majdan, Maria Majdan, Magdalena Dryglewska, Patrycja Ziober-Malinowska, Jan Kotarski, Ludmiła Grzybowska-Szatkowska

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The presence of particular criteria and non-criteria antiphospholipid antibodies in patients with uterine malignancies

Authors:

Andrzej Majdan¹, Maria Majdan², Magdalena Dryglewska², Patrycja Ziober-Malinowska¹, Jan Kotarski¹, Ludmila Grzybowska-Szatkowska³

1. Department of Gynecological Oncology and Gynecology, Medical University of Lublin, Lublin, Poland

2. Department of Rheumatology and Connective Tissue Diseases, Medical University of Lublin, Lublin, Poland

3. Department of Radiotherapy, Medical University of Lublin, Lublin, Poland

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Correspondence should be directed to:

Andrzej Majdan, MD, Ph D

Dept of Gynecological Oncology and Gynecology, Medical University of Lublin ul. Stanisława Staszica 16, 20-400 Lublin, Poland. Phone: +48 81 532 78 47

E-mail: amajdan@cozl.eu

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What’s new?

There is limited data on the occurrence of criteria and non-criteria antiphospholipid antibodies (aPLs) in patients with malignancies of the female reproductive tract. Our observations confirmed the frequent criteria and non-criteria aPLs positivity in patients with uterine malignancies (UM). We found that there are significant differences concerning the occurrence of the particular aPLs between patients with UM and patients with non-cancerous gynecological diseases (NCGD). The non-criteria aPLs (against phosphatidic acid, phosphatidylserine, annexin V and prothrombin) are found more frequent in patients with UM than in patients with NCGD. Whereas, the criteria aPLs are not significantly different between UM and NCGD groups.
Abstract

Introduction

Currently, there is limited data about the presence of antiphospholipid antibodies (aPLs) in patients with uterine malignancies (UM).

Objectives

We aim to determine whether criteria and non-criteria aPLs are present and associated with thrombotic risk in patients with UM compared to patients with non-cancerous gynecological diseases (NCGD).

Patients and methods

The study involved 151 female patients scheduled for gynecological surgery. The patients were divided into: UM group – 70 patients and NCGD group – 81 patients. The Antiphospholipid 10 Dot assay was used to detect criteria and non-criteria aPLs before surgery. Patients were considered ‘positive’ for thrombosis if they exhibited signs of thrombosis within the two-year observation period following surgery.

Results

The positive results for aPLs were obtained in 17/70 (24.3%) patients with UM and in 6/81 (7.4%) patients with NCGD (p=0.008). Particular non-criteria aPLs (anti-phosphatidic acid, anti-phosphatidylserine, anti-annexin V, anti-prothrombin antibodies) but no criteria aPLs (anti-cardiolipin, anti-β2 glycoprotein I antibodies) were found more frequently in patients with UM than in the patients with NCGD. Thrombosis was diagnosed in 9/70 (12.9%) patients in the UM group and in 3/81 (3.7%) patients in NCGD group (p=0.03).
Conclusions

Antiphospholipid antibodies are present at significant levels in patients with UM. Non-criteria aPLs, but no criteria aPLs are found frequently in patients with UM than in patients with NCGD. The incidence of thrombosis is significantly higher in patients (pts) with UM.

Keywords: criteria and non-criteria antiphospholipid antibodies, thrombosis, uterine malignancies
Introduction

Thrombosis is a common complication observed in patients with malignancies [1-3]. Several factors responsible for the development of thrombosis have been identified. Interactions between cancer cells, coagulation mechanisms, and the immune system may play an essential role in initiating thrombotic processes accompanying tumors [4-9].

Women with reproductive tract malignancies, including uterine malignancies (UM) are at high risk for thromboembolic complications also due to comorbidities, advanced clinical stage of the disease at the time of diagnosis, the duration, and scope of the surgery (laparotomy/laparoscopy) and the need for long-term postoperative immobilization [10].

The role of the immune system in neoplasia and anti-tumor defense is well-established [11-15]. Furthermore, the literature describes many cases of thrombotic complications associated with the presence of the criteria antiphospholipid antibodies (aPLs) in cancer patients [16-19]. Although the exact relationship between aPLs and malignancies is unclear, the presence of aPLs in cancer patients may contribute to increased thromboembolic risk. There is limited data on the presence of aPLs and their association with thrombosis accompanying female reproductive tract tumors [16, 20].

Antiphospholipid antibodies are serological markers of immunization and thrombotic risk in patients with antiphospholipid syndrome (APS). The diagnosis of APS usually involves the detection of criteria aPLs, including classes immunoglobulin M (IgM) and immunoglobulin G (IgG) of anti-cardiolipin antibodies (aCL), anti-β2 glycoprotein I antibodies (aβ2GPI), lupus anticoagulant (LA) and thrombotic complications [21].

The literature emphasizes supplementing the risk assessment of thrombotic complications with the detection of non-criteria aPLs, including anti-annexin V and anti-
phosphatidylserine/prothrombin complex (anti-PS/PT), the presence of which may be associated with increased risk of thrombosis [5, 16, 22]. The role of non-criteria aPLs in the pathogenesis of thrombosis in the course of gynecological malignancies is not yet known.

In our study, we aimed to determine whether criteria and non-criteria aPLs are present and associated with thrombotic risk in patients with UM, compared to patients with non-cancerous gynecological diseases (NCGD).

**Patients and methods**

Our research involved 151 women admitted to the Department of Oncological Gynecology and Gynecology in 2015–2017. Patients were admitted for the diagnosis and treatment of female reproductive organ lesions suspicious for cancer or non-malignant lesions of the adnexa. All patients were qualified for surgical treatment.

The day before their scheduled surgery, a blood sample from each patient was collected in a clot tube. Each blood sample was centrifuged at 1008 relative centrifugal force for 10 minutes, and then the serum was frozen at -70 degrees C and stored for immunoassays for selected aPLs.

Surgery (via laparotomy or laparoscopy) was performed on 151 women, and final diagnosis for each was based on postoperative histological examination of the specimens. The postoperative histological diagnoses of the investigated patient groups are shown in additional data in supplementary material, table S1 and table S2.

The patients were divided into:

UM group: women with diagnosed uterine malignancies (n=70)

NCGD group: women with diagnosed non-malignant genital organ pathology (n=81)
The mean age of patients in UM group was 59.8 (SD 12.6) whereas in NCGD group the mean age was 45.1 (SD 14.7), \( P<0.001 \).

The analysis of coexisting comorbidities and behavioral characteristics of the patients is shown in table 1.

Table 1

In the patients with UM hypertension, obesity, diabetes type 2, heart failure - the characteristic features of metabolic syndrome (MetS) were recognized significantly more frequent than in patients with NCGD.

Patients were monitored for 24 months following surgery and were considered ‘positive’ for thrombosis if they exhibited clinical signs of a thrombotic process within this observation period, such as deep vein thrombosis confirmed by Doppler ultrasound examination, and/or a clinical event involving pulmonary or other organ embolism confirmed by radiological examination.

aPL determination

Anti-Phospholipid 10 Dot test sets supplied by Generic Assays were used to determine the aPLs. The nitrocellulose membranes with primary antibody were incubated with patients sera for 20 minutes. After washing with tris-buffered saline (TBS), the binding of aPL was detected with secondary IgG and IgM antibodies. Finally, the membranes were washed, dried, and the membranes were read by Canon Cano Scan LiDE 120 scanner. The intensity of the membrane readings was determined in a semi-quantitative manner by the GA DotBlot Analyzer. Specifically, the intensity of the spotting on the membranes was calculated concerning the intensity of the control spotting. The categories of semi-quantitative readings applied by the software interpreting the scanner readings were:
- extremely positive: >80 IgM antiphospholipid units/mL (MPL) or IgG antiphospholipid units/mL (GPL)
- strongly positive: 60 – 80 MPL/GPL
- positive: 40 – 59 MPL/GPL
- barely positive: 20 – 39 MPL/GPL
- negative: below 20 MPL/GPL

The DotBlot method was used to detect the following classes of aPLs from each patient’s frozen serum:

- **Non-criteria** antiphospholipid antibodies (IgM and IgG class):
  - Anti - phosphatidic acid
  - Anti - phosphatidylcholine
  - Anti - phosphatidylethanolamine
  - Anti - phosphatidylglycerol
  - Anti - phosphatidylinositol
  - Anti - phosphatidylserine
  - Anti - annexin V
  - Anti - prothrombin

- **Criteria** antiphospholipid antibodies (IgM and IgG class):
  - Anti - cardiolipin
  - Anti - β2 glycoprotein I

A total of 3020 immunoassays (20 antibody types in 151 patients) were performed. The software assessed a result of 20 or above as positive. The numerical values of the spotting intensity of the membranes were automatically recorded by the software in a spreadsheet file for further statistical analysis.

Statistical analysis
The clinical parameters and laboratory test results were subjected to statistical analysis. The values of the analyzed parameters were characterized using the R programming language. Statistical analysis was carried out at the significance level of $\alpha = 0.05$. The null hypothesis was rejected and an alternative hypothesis adopted when $P < 0.05$.

The chi2 statistic test was applied to check association between categorical variables. In the case of too few observations, to fulfill the criteria of the chi-square test, the Monte-Carlo method was used (for description of comorbidities and behavioral characteristics of the groups). For data presentation of the aPLs occurrence, we used chi2 Pearson and Fisher exact tests.

Data were described by number and percentage for categorical variables and as mean and standard deviation (SD) or median (first and third quartiles) for continuous variables.

Ethics

The study was approved by the ethics committee - the number of protocol: KE-0254/265/2014. The patients were provided written, informed consent to participate in the study.

Results

Statistically significant differences were observed between UM and NCGD groups for the presence of positive aPLs in the DotBlot test. We have found statistically significantly more patients (with at least one aPL detection value $\geq 40$) in the UM group compared to NCGD group (17/70 - 24.3% vs. 6/81 - 7.4%, $P=0.004$, respectively).

The double-positive aPL status was present in 3 patients of the UM group and one patient from NCGD; there was only one case of multipositivity in the UM group (4 positive results in the DotBlot test for MPL and GPL $\geq 40$).
The particular non-criteria aPLs (anti-phosphatidic acid IgM, anti-phosphatidylserine IgM, anti-annexin V IgM, anti-prothrombin IgM and IgG) were found more frequently in patients with UM than in patients with NCGD. There were no significant differences between the detection of criteria aPLs between the groups of patients (see tables 2-7).

Tables 2-7

Assessment of the thrombotic complication frequency in individual groups indicates significant differences in the incidence of thrombosis between the UM and NCGD groups: (UM 9 out of 70 pts vs. NCGD 3 out of 81 pts, chi2 P=0.04).

Discussion

Our study found that thrombotic complications are relatively common in patients with UM. Significant levels of particular criteria and non-criteria aPLs were present in patients with UM. Selected non-criteria aPLs: anti-phosphatidic acid, anti-phosphatidylserine, anti-annexin V, and anti-prothrombin were significantly more common in patients with UM than in patients with NCGD.

Our observations confirmed the frequent criteria and non-criteria aPLs positivity in patients with UM. The presence of selected non-criteria aPLs in patients with UM appears to be a risk factor for thrombosis. However, the causal relationships between criteria and non-criteria aPLs and thrombosis in UM patients remains unclear. They were not clearly related to thrombotic complications in patients with UM.

Patients with UM are at a higher risk of venous thromboembolism (VTE) than the general population [10]. The malignancy itself, the treatment modalities, including medication and surgery, and the increased levels of leukocytes, platelets, and tissue factor-positive microvesicles, contribute to this increased risk [23-25]. Several authors have shown that aPLs
can be detected in the peripheral blood of patients with malignancies [16-22, 26-29]; however, whether or not aPLs can induce thrombosis in patients with UM is unknown.

Further studies should improve the understanding of aPL role in thrombotic complications in patients with cancer.

The pathomechanism by which aPLs are generated in patients with malignancies remains unclear. Several mechanisms have been suggested to explain the association between aPLs and cancer, including [16, 30-33]:

- production of antibodies as a response to tumor antigens
- secretion of aCL antibodies from tumor cells
- production of monoclonal immunoglobulins with lupus anticoagulant activity

The autoantibodies present in serum may be a direct consequence of tumor presence [16, 30, 31], may result from specific cancer treatment or various infections [16, 32].

We speculated that in our group of UM patients the selected non-criteria aPLs (against phosphatidic acid, phosphatidylserine, annexin V and prothrombin) could be potential biomarker for malignancy.

APS developed during chemotherapeutic and immunotherapeutic treatment of different cancers [16, 34 - 36], and further investigations indicated that aPL-positive IgG from patients with autoimmune disease accelerates cancer angiogenesis and growth through a tissue factor-mediated mechanism [16, 36]. There are multiple mechanisms - through platelet activation, endothelial activation, and tissue factor expression - which may cause hypercoagulation in cancer patients by disrupting the coagulation pathway and fibrinolysis [16, 37-39]. With aPLs present, all of these mechanisms contribute to the development of thrombotic complications in APS [16, 40, 41].
Seronegative APS is defined as clinical manifestations suggestive of APS without the presence of criteria aPLs in the serum [16, 42-44]. The detection of non-criteria aPLs in seronegative APS patients may indicate an increased thrombotic risk [44]. Our study found that non-criteria aPLs occur more often in patients with UM than in those with NCGD – in particular (according to the study results): anti-phosphatidic acid IgM, anti-phosphatidylserine IgM, anti-annexin V IgM, anti-prothrombin IgM and IgG. One of the patients of the UM group with multipositive aPL status had been earlier diagnosed for APS, and she died 12 months after the surgery because of heart infarction. Therefore, screening for non-criteria aPLs in patients with UM may be useful as a prognostic factor for thrombotic/cardiovascular complications.

It is not yet known how high values of aPL detection should be considered as a „positive” prognostic factor. For instance, even the low (<20) MPL/GPL values may play an important role in possible thromboembolic complications in pregnant women [45].

In our study in patients with UM hypertension, obesity, type 2 diabetes - the characteristic features of metabolic syndrome (MetS) were recognized significantly more frequent than in patients with NCGD.

It is known that there are associations between MetS and endometrial cancer and that MetS increases the risk of venous thromboembolism [46-48]. MetS was often observed in UM patients and might have had an impact on higher frequency of thrombotic complications than in NCGD group.

Thrombosis in UM pts is also determined by underlying factors not related to aPL such as age and MetS, therefore the causality between the criteria and non-criteria aPL in pts with UM and thrombosis is unclear.
Our study was a pilot study; therefore we tried to determine the presence of aPL only in patients with malignancies, but without the APS diagnosed. Another limitation of our study was that we measured the aPL levels only once.

Conclusions

Antiphospholipid antibodies are present at significant levels in patients with UM. Contrary to expectations, the non-criteria aPLs (against phosphatidic acid, phosphatidylserine, annexin V and prothrombin) are found more frequent in patients with UM than in patients with NCGD. Whereas, the criteria aPLs are not significantly different between UM and NCGD groups. The incidence of thrombosis in UM patients is significantly higher than in patients with NCGD but there is not enough evidence yet to establish a direct causal relationship between aPLs presence and thrombosis in UM patients.

Further conclusions from the study which may constitute the basis for future research on immunological conditions of thrombosis in cancer:

- there is a possibility that non-criteria aPL-mediated mechanisms contribute to increased thrombosis in cancer patients

- high and average reading values for certain non-criteria aPLs (against phosphatidic acid, phosphatidylserine, annexin V and prothrombin) may indicate their future usefulness as novel cancer biomarkers.

Contribution statement

AM conceived the idea for the study. AM, MM, LGS, and JK contributed to the study design. AM, MD, and PZ were involved in data collection. AM, MM, and MD analyzed the data and prepared the manuscript. MM coordinated funding for the project. All authors edited and approved the final version of the manuscript.
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Table 1. Comparison of comorbidities and behavioral characteristics of the patients (*Pearson chi² test*).

| Comorbidity or characteristics of behavior | UM (n=70) | NCGD (n=81) | Statistical significance |
|-------------------------------------------|-----------|-------------|-------------------------|
| **Hypertension**                          | 42 (60%)  | 22 (27.2%)  | *P=0.001*               |
| **Obesity (BMI >30)**                     | 24 (34.3%)| 15 (18.5%)  | *P=0.043*               |
| **Diabetes type 2**                       | 10 (14.3%)| 4 (5%)      | *P=0.047*               |
| **Heart failure**                         | 16 (22.9%)| 5 (6.2%)    | *P=0.007*               |
| **Miscarriages**                          | 6 (8.6%)  | 7 (8.6%)    | *P=1.00*                |
| **Smoking**                               | 8 (11.4%) | 16 (19.8%)  | *P=0.24*                |
| **Another neoplasm**                      | 4 (5.7%)  | 2 (2.5%)    | *P=0.42*                |
| **Oral contraception**                    | 2 (2.9%)  | 9 (11.1%)   | *P=0.07*                |
| **Long-term immobilization (over 72 hours) prior the operation** | 9 (12.9%) | 4 (4.9%)    | *P=0.15*                |

* Monte Carlo simulation; bold indicates significant differences (*P<0.05*)
Table 2. DotBlot test results for anti-phosphatidic acid antibodies.

| Patient Group | Anti-phosphatidic acid IgM | Anti-phosphatidic acid IgG |
|---------------|-----------------------------|----------------------------|
|               | Number of results           | Number of results          |
|               | <20 (%) | ≥20 and <40 (%) | ≥40 (%) | <20 (%) | ≥20 and <40 (%) | ≥40 (%) |
| UM n = 70     | 58 (82.6%) | 11 (15.71%) | 1 (1.43%) | 69 (98.57%) | 0 (0%) | 1 (1.43%) |
| NCGD n = 81   | 78 (96.23%) | 3 (3.7%) | 0 (0%) | 81 (100%) | 0 (0%) | 0 (0%) |

**CHI² (Fisher) P – value with Monte Carlo simulations (based on 2000 replicates)**

| UM vs NCGD | UM vs NCGD |
|------------|------------|
| *P*=0.007  | *P*=0.46   |

IgM - immunoglobulin M, IgG - immunoglobulin G, **bold** indicates a statistically significant value, UM- uterine malignancies, NCGD- non-cancerous gynecological diseases
| Patient Group | Anti-phosphatidylserine IgM | Anti-phosphatidylserine IgG | CHI² (Fisher) P – value with Monte Carlo simulations (based on 2000 replicates) |
|---------------|-----------------------------|-----------------------------|--------------------------------------------------------------------------------|
|               | Number of results (%)       | Number of results (%)       |                                                                                |
| UM n = 70     | 58 (82.86%)                 | 70 (100%)                  | UM vs NCGD P=0.041                                                            |
|               | 12 (17.14%)                 | 0 (0%)                     |                                                                                |
|               | 0 (0%)                      | 2 (2.47%)                  |                                                                                |
| NCGD n = 81   | 76 (93.83%)                 | 79 (97.53%)                 |                                                                                |
|               | 5 (6.17%)                   | 0 (0%)                     |                                                                                |
|               | 0 (0%)                      | 0 (0%)                     |                                                                                |

IgM - immunoglobulin M, IgG - immunoglobulin G, **bold** indicates a statistically significant value, UM- uterine malignancies, NCGD- non-cancerous gynecological diseases.
Table 4. DotBlot test results for anti-annexin V antibodies.

| Patient Group | Anti-annexin V IgM | Anti-annexin V IgG |
|---------------|--------------------|--------------------|
|               | Number of results  | Number of results  |
|               | <20 (%))           | ≥20 and <40 (%)    |
|               | ≥40 (%)            | ≥40 (%)            |
| UM n = 70     | 28 (40%)           | 36 (51.43%)        |
|               | 6 (8.57%)          | 62 (88.57%)        |
| NCGD n = 81   | 52 (64.2%)         | 27 (33.3%)         |
|               | 2 (2.47%)          | 79 (97.53%)        |

**CHI² (Fisher) P – value with Monte Carlo simulations (based on 2000 replicates)**

|                | UM vs. NCGD | UM vs. NCGD |
|----------------|-------------|-------------|
| _P_=0.007      |             | _P_=0.06    |

IgM - immunoglobulin M, IgG - immunoglobulin G, **bold** indicates a statistically significant value, UM- uterine malignancies, NCGD- non-cancerous gynecological diseases.
Table 5. DotBlot test results for anti-prothrombin antibodies.

| Patient Group | Anti-prothrombin IgM | Anti-prothrombin IgG |
|---------------|----------------------|----------------------|
|               | Number of results    | Number of results    |
|               | <20 (%)              | ≥20 and <40 (%)      | ≥40 (%)              |
|               | ≥20 (%)              | ≥40 (%)              |<20 (%)              |
| UM n = 70     | 12 (17.14%)          | 54 (77.14%)          | 4 (5.71%)            |
|               |                      |                      | 51 (72.86%)          |
|               |                      |                      | 18 (25.71%)          |
|               |                      |                      | 1 (1.43%)            |
| NCGD n = 81   | 35 (43.21%)          | 43 (53.1%)           | 3 (3.7%)             |
|               |                      |                      | 73 (90.12%)          |
|               |                      |                      | 8 (9.88%)            |
|               |                      |                      | 0 (0%)               |

**CHI² (Fisher) P – value with Monte Carlo simulations (based on 2000 replicates)**

UM vs. NCGD  
**P=0.002**

UM vs. NCGD  
**P=0.01**

IgM - immunoglobulin M, IgG - immunoglobulin G, **bold** indicates a statistically significant value, UM - uterine malignancies, NCGD- non-cancerous gynecological diseases
Table 6. The value of the DotBlot test results in each patient group, for anti-cardiolipin antibodies.

| Patient Group | Anti-cardiolipin IgM |ANTI-cardiolipin IgG |
|---------------|----------------------|----------------------|
|               | Number of results    | Number of results    |
|               | <20 (%)              | ≥20 and <40 (%)      | ≥40 (%)              |
|               | ≥40 (%)              | ≥20 and <40 (%)      | ≥40 (%)              |
| UM n = 70     | 59 (84.29%)          | 10 (14.29%)          | 1 (1.43%)            |
|               | 61 (87.14%)          | 8 (11.43%)           | 1 (1.43%)            |
| NCGD n = 81   | 77 (95.06%)          | 4 (4.94%)            | 0 (0%)               |
|               | 74 (91.36%)          | 7 (8.64%)            | 0 (0%)               |

**CHI² (Fisher) P – value with Monte Carlo simulations (based on 2000 replicates)**

| UM vs. NCGD  | UM vs. NCGD  |
|--------------|--------------|
| *P=1.00*     | *P=1.00*     |

IgM - immunoglobulin M, IgG - immunoglobulin G, bold indicates a statistically significant value, UM- uterine malignancies, NCGD- non-cancerous gynecological diseases
Table 7. The value of the DotBlot test results in each patient group, for anti-β2glycoprotein I antibodies.

| Patient Group | Anti-β2GPI IgM | Anti-β2GPI IgG |
|---------------|----------------|----------------|
|               | Number of results | Number of results |
|               | <20 (%)          | ≥20 and <40 (%) | ≥40 (%) |
|               | ≥20 (%)          | ≥20 and <40 (%) | ≥40 (%) |
| UM n = 70     | 19 (27.14%)      | 44 (62.86%)     | 7 (10%) |
|               | 32 (45.71%)      | 38 (54.3%)      | 0       |
| NCGD n = 81   | 43 (53.08%)      | 36 (44.4%)      | 2 (2.47%) |
|               | 57 (70.37%)      | 24 (29.63%)     | 0       |

**CHI² (Fisher) P – value with Monte Carlo simulations (based on 2000 replicates)**

**UM vs. NCGD**

\[ P = 0.109 \]

**UM vs. NCGD**

\[ P = 0.127 \]

IgM - immunoglobulin M, IgG - immunoglobulin G, **bold** indicates a statistically significant value, UM - uterine malignancies, NCGD - non-cancerous gynecological diseases