SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Predicting the primary surgical outcome of ovarian cancer and examining the effect of tumor reduction on phagocytic function

by Anna Rebeka Kovács MD

Supervisor: Rudolf Lampé MD PhD

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By: Anna Rebeka Kovács MD

Doctoral School of Clinical Medicine, University of Debrecen

Supervisor: Rudolf Lampé MD, PhD

Head of the Defense Committee:	Prof. Gabriella Szűcs MD, PhD, DSc
Reviewers:	Kálmán András Kovács MD, PhD
	Péter Attila Gergely MD, PhD
Members of the Defense Committee:	Zsuzsanna Besenyi MD, PhD
	István Szegedi MD, PhD

The PhD Defense takes place at the Lecture Hall of Building "A", Department of Internal Medicine, Faculty of Medicine, University of Debrecen
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1. Introduction

1.1. Ovarian cancer

Ovarian cancer is currently considered the leading cause of death among malignant gynecological tumors. Globally, it is the third most common gynecologic malignancy, with 314,000 new cases diagnosed in 2020, and responsible for approximately 207,000 deaths. Among women, it ranks fifth in cancer-related mortality. The high mortality rate is partly due to the fact that ovarian cancer remains asymptomatic for a long time and most often causes symptoms only at an advanced stage, which are not specific to the disease even then. The most commonly observed symptoms include abdominal discomfort, early satiety, nausea, bloating, changes in bowel habits, back pain, weight loss, and fatigue, which can often persist for months before diagnosis. The tumor may press against surrounding organs, causing abnormal urinary or bowel urgency or constipation. As the disease progresses further, ascites and cachexia may develop.

Several histological types of malignant ovarian tumors are known, the most common of which is epithelial ovarian cancer (EOC). The five-year survival rate for this type is only around 25%. The exact pathogenesis of ovarian epithelial tumors is not clear, and numerous theories have been proposed regarding their etiology. According to the so-called "incessant ovulation" hypothesis, the number of ovulations occurring during a woman's fertile years plays a role in the development of the disease. According to the theory, during ovulation, the surface epithelium of the ovaries is damaged, leading to increased cell proliferation for the purpose of epithelial repair. It is assumed that there is a greater chance for damage to the genes regulating this process, which may initiate tumor development.

Another hypothesis suggests that multiple inflammatory pathways involved in the ovulation process may also play a role in the development of EOC. During ovulation, certain inflammatory processes are activated, leading to the release of cytokines and the activation of immune cells, which contribute to tissue regeneration. These theories may explain why a lower number of ovulations during a woman's lifetime (e.g., due to pregnancy or polycystic ovary syndrome) reduces the risk of tumor development. Consistent with these theories, factors that increase the risk of EOC include a high number of ovulations over a lifetime (e.g., nulliparity, early menarche, and late menopause), smoking, and the previous occurrence of certain benign gynecological conditions. A positive family history is also considered a risk factor, and the genetic background of the disease is the subject of extensive research.

Early diagnosis of ovarian cancer is challenging. Physical examination and transvaginal ultrasound can suggest the possibility of a malignant tumor, but an accurate diagnosis can only be made through histological examination. There is no effective screening method for the disease: the cancer antigen 125 (Ca-125) tumor marker, which is typically elevated in cases of EOC, is not sensitive enough for early tumor detection. In the absence of adequate screening strategies, EOC is typically detected at an advanced stage (in approximately 80% of cases), when the tumor has already spread within the abdominal cavity.

Its incidence increases with age, and most EOC patients are diagnosed between the ages of 60 and 64. According to the staging system of the International Federation of Gynecology and Obstetrics (FIGO), the disease can be classified into stages I-IV based on the size and extent of the tumor.

The primary treatment for ovarian cancer is cytoreductive surgery (debulking), aimed at leaving less than 1 cm of residual tumor tissue after the procedure (optimal tumor reduction). According to the latest professional guidelines, the goal should be complete tumor reduction, meaning no visible tumor tissue remains after surgery. Following surgical treatment, current guidelines recommend platinum-based adjuvant chemotherapy (Carboplatin or Cisplatin) combined with taxane compounds (Paclitaxel). If optimal tumor reduction is unlikely due to the extent and/or localization of the tumor, neoadjuvant chemotherapy (NACT) followed by interval laparotomy after appropriate downstaging is the preferred approach. The effect of primary debulking surgery has been compared with the effect of NACT followed by interval debulking surgery (IDS) on progression-free survival (PFS) and overall survival (OS). The latter treatment did not increase survival and, in fact, the OS was likely lower in stage IIIC patients. It has been proven that the extent of tumor reduction achieved during primary surgery is a strong predictor of prognosis, as there is a direct correlation between the size of the post-surgical tumor residual and PFS, as well as OS. An additional theoretical advantage of surgical cytoreduction is that removing the large tumor mass may enhance the body's immune competence, thereby reducing the likelihood of developing resistance to chemotherapy.

Several attempts have been made to predict the degree of tumor reduction achieved during primary surgery in ovarian cancer patients. Some studies have used the predictive value of preoperative laboratory parameters, while others have utilized preoperative imaging or the results of diagnostic laparoscopy. The predictive value of the tumor markers human epididymis protein 4 (HE4) and Ca-125 has also been examined. *Feng et al.* analyzed the prognostic role of age, preoperative serum Ca-125, and HE4 levels, finding them useful for predicting surgical outcomes. They also examined the prognostic role of computed tomography (CT) imaging and Ca-125 levels. Despite these findings, no single convincing method is known for predicting the feasibility of complete tumor reduction.

1.2. The role of the innate immune system in carcinogenesis

During carcinogenesis, progenitor cells must overcome several control processes and their physiological balance, which can be achieved in various ways. These include independence from growth signals, insensitivity to growth-inhibitory signals, unlimited cell proliferation, avoidance of apoptosis, development of angiogenesis, and the ability of invasion and metastases formation. From an immunological perspective, the macroscopic appearance of a tumor indicates that the immune system's anti-tumor effectiveness is inadequate ("tumor escape").

The innate and adaptive immune system can have a dual role in the development of malignant tumors: firstly, a defensive role, known as traditional "immunosurveillance" (the immune system's ability to recognize non-self antigens, including abnormal tumor cells that have developed in the body, and destroy them), and secondly, they can also promote tumor development. It has been observed that the anti-tumor immune response is often suppressed by cells with immunosuppressive effects that appear within tumors. The metabolites of immune cells can create an optimal environment for tumor growth by enhancing the proliferation and survival of tumor cells, inducing angiogenesis, and increasing the permeability of blood vessels, thereby facilitating the supply of nutrients and oxygen to the tumor cells. Proteases produced by immune cells degrade the extracellular matrix, which, combined with extensive vascularization, promotes the detachment, dissemination, and metastasis formation of tumor cells.

The innate immune system constitutes the first line of non-specific defense against infections and malignant cell transformations. Monocytes and neutrophil granulocytes are phagocytic cells of innate immunity that play a role in eliminating tumor-transformed cells, thereby providing protection against tumor development. The purpose of phagocytosis is to destroy ingested microbes, apoptotic or tumor cells, and to activate the cells of the adaptive immune system through antigen presentation. Phagocytosis is induced by the binding of an antigen to a phagocytic receptor, during which interaction the cell membrane engulfs the particle into a phagosome. This phagosome then fuses with a lysosome containing degradative enzymes and antimicrobial substances. In the resulting phagolysosome, the pathogen is broken down. During this process, phagocytes become activated and induce an immune response through the secretion of pro-inflammatory cytokines and by facilitating antigen presentation on Major Histocompatibility Complex (MHC) class I and class II molecules.

Healthy tissues are capable of avoiding elimination by phagocytosis. One way they achieve this is through the cell surface expression of anti-phagocytic molecules known as "do not eat me" signals. However, tumor cells also heavily rely on the expression of these "do not eat me" signals, which include molecules such as CD47, programmed death-ligand 1, or β2-microglobulin. These molecules bind to receptors on phagocytes, preventing the phagocytosis of the cells that express them. While alterations in the phagocytic function of peripheral monocytes and neutrophil granulocytes have been observed in several diseases, we currently have limited information about the phagocytic function of these cells in the context of EOC.

1.3. Objectives

1.3.1. Investigation of the prognostic significance of preoperative laboratory parameters for the outcome of primary surgery

The prediction of the feasibility of primary cytoreductive surgery is of paramount importance for patient survival. Therefore, the aim of our study was to investigate the preoperative laboratory parameters of patients with high-grade serous EOC, addressing the following questions:

- Do the preoperative laboratory values of EOC patients who undergo primary cytoreductive surgery differ depending on whether optimal tumor reduction was achieved during surgery?
- Can differences in PFS and OS values between the two groups of patients (optimal vs. incomplete tumor reduction) be demonstrated in our patient population based on surgical outcomes?
- Can an optimal threshold be determined for laboratory parameters that show significant differences between the two patient groups, which could have predicted the surgical outcome preoperatively?
- Among the laboratory parameters with threshold values, which have significant, independent predictive value for the outcome of primary surgery?

- Do variables with significant, independent predictive value correlate with the PFS and OS values of the patients studied?

1.3.2. Investigation of the phagocytic function of monocytes and neutrophil granulocytes

Our aim was to examine the changes in the function of the innate immune system and the effects of surgical treatment in patients with EOC. To this end, we studied the phagocytic function of monocytes and neutrophil granulocytes isolated from peripheral blood samples of patients with advanced-stage ovarian cancer, patients with benign gynecological tumors, and healthy control individuals. We sought to answer the following questions:

- Is there a difference in the phagocytic function of peripheral monocytes and neutrophil granulocytes between healthy control individuals and patients diagnosed with EOC?
- Is there a difference in the phagocytic function of these immune cells in advanced-stage EOC patients before and after complete surgical removal of the tumor?
- Does the phagocytic function of monocytes and neutrophil granulocytes isolated from the blood samples of EOC patients differ from the results observed in healthy individuals after surgery?
- Does surgical treatment alone affect the examined function, i.e., is there a difference in the phagocytic function of cells in patients who underwent surgery for benign gynecological tumors before and after surgery, and do these differ from those observed in healthy individuals?

2. Materials and methods

2.1. Investigation of the prognostic significance of preoperative laboratory parameters for the outcome of primary surgery

With the approval of the Ethics Committee of the University of Debrecen, we examined ovarian cancer patients who underwent primary cytoreductive surgery as a first-line treatment at the Obstetrics and Gynecology Clinic of the University of Debrecen Clinical Center, between 2006 and 2013. The inclusion criterion was the histological type of the tumor being high-grade serous EOC. The patients were divided into two groups based on whether optimal tumor reduction was achieved during surgery or not.

The parameters we examined were age, body mass index (BMI), disease stage (FIGO I-IV), total white blood cell count (WBC), absolute neutrophil count (Neu#), absolute lymphocyte count (Ly#), absolute monocyte count (Mono#), platelet count (PLT), neutrophil-to-lymphocyte ratio (NLR = Neu#/Ly#), monocyte-to-lymphocyte ratio (MLR = Mono#/Ly#), platelet-to-lymphocyte ratio (PLR = PLT/Ly#), mean platelet volume (MPV), platelet distribution width (PDW), and Ca-125 level. Additionally, we observed the patients' PFS and OS.

Statistical calculations were performed using the SPSS 25.0 software. To test for normality, we used the Kolmogorov-Smirnov and Shapiro-Wilk tests. For comparing the data between the two patient groups (optimal vs. incomplete tumor reduction), we used an independent samples t-test for normally distributed data and a non-parametric Mann-Whitney U test for non-normally distributed data. For parameters showing significant differences in preoperative blood sample results between the two groups, we examined their correlation with surgical outcomes (optimal vs. incomplete tumor reduction) using Receiver Operating Characteristic (ROC) curve analysis. For variables with a significant correlation and an appropriate area under the curve (AUC), we determined optimal threshold values based on the Youden index, with corresponding sensitivity and specificity values. Using these threshold values, we dichotomized the examined parameters across the entire patient cohort and assessed the independent predictive value of potential predictors for achieving optimal tumor reduction using a binary logistic regression model with the Backward method. The PFS and OS values of the two patient groups were compared using Kaplan-Meier analysis. A p-value of <0.05 was considered significant

2.2. Investigation of the phagocytic function of monocytes and neutrophil granulocytes

With the approval of the Ethics Committee of the University of Debrecen and the informed consent of the participants, we examined peripheral blood samples from patients who underwent surgery for ovarian cancer and benign gynecological tumors (surgical controls) at the Obstetrics and Gynecology Clinic of the University of Debrecen Clinical Center between 2017 and 2019. Inclusion criteria for ovarian cancer patients included FIGO stage IIIC high-grade serous EOC. Another inclusion criterion was complete tumor reduction achieved during primary surgery. Patients were excluded from the study if the tumor was found to be unresectable during surgery or if the postoperative histological examination revealed a different type. As a control group, we examined peripheral blood samples from healthy women with no history of cancer. None of the participants had diabetes, immunosuppressive diseases, or obesity, did not regularly take medication, had not received hormonal treatment within the past 6 months, and did not have any internal medical conditions requiring treatment or a history of gynecological surgery. For blood sampling, we used Vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) (*Becton-Dickinson, Cedex, France*) in a closed system. Blood samples were collected on the day of surgery and on the 7th day post-surgery.

The separation of monocytes and granulocytes from the participants was performed as previously described in the literature. Blood samples collected from EOC and benign gynecological tumor patients before and after surgery, as well as from healthy individuals, were examined in the same manner. First, the samples were diluted to twice their volume with Hanks' solution (pH: 7.4), then layered onto Ficoll density gradients (1.119 g/cm^3 and 1.077 g/cm^3). After layering, the samples were centrifuged at 400 g, 24°C, for 30 minutes, resulting in the separation of mononuclear cells (monocytes and lymphocytes) and polymorphonuclear cells (granulocytes) at the phase boundary of the different density Ficoll layers. The separated cells were collected into separate tubes and washed twice with Hanks' solution. The viability of the cells was assessed with trypan blue staining, which was found to be 96-98% in all cases. Morphological assessment determined that the granulocyte proportion in the cell suspension was 95-98%.

The opsonization and fluorescent labeling of zymosan particles were performed as previously described in the literature. Zymosan-A particles (10^8/ml) were incubated for 60 minutes at 37°C in carbonate buffer (pH: 9.6) containing 0.01 mg/cm^3 fluorescein isothiocyanate (FITC), and then the particles were washed three times with Hanks' solution. The opsonization of the zymosan particles was carried out in Hanks' solution containing 50% human AB serum for 30 minutes at 37°C. The fluorescently labeled and opsonized zymosan-A particles (FITC-OZ) were washed three times with Hanks' solution, suspended in Hanks' solution to a concentration of 3×10^7 FITC-OZ particles per milliliter, frozen, and stored at –20°C until use.

The examination of phagocytic function was carried out according to methods previously described in the literature. After separation, the mononuclear cells and granulocytes were adhered to a cell culture chamber using Hanks' solution containing 5% heat-inactivated human AB serum (for 30 minutes at room temperature). Each chamber received 300 μl of a suspension containing 10^6 cells, and the chambers were then washed three times with Hanks' solution to remove non-adherent cells. FITC-OZ particles were added to the cells as targets for phagocytosis. The Hanks' solution containing FITC-OZ was further diluted after thawing (1.5:1 Hanks' solution: FITC-OZ). From this solution, 300 μl was added to each chamber, and the chambers were incubated at 37°C in 5% CO_2, 95% humidity for 60 minutes. The fluorescence of non-phagocytosed zymosan-A particles was quenched with trypan blue staining. After removing the chambers, the monocytes and granulocytes were fixed onto slides with 4% paraformaldehyde solution for 30 minutes.

For the identification of monocytes, we used indirect immunofluorescent labeling. First, monoclonal anti-human CD14+ antibodies produced in mice were added to the cells, followed by secondary antibodies conjugated with Dylight 594 fluorescent dye against mouse immunoglobulin G. Finally, the nuclei of both monocytes and granulocytes were stained with a mounting medium containing 4,6-diamidino-2-phenylindole (DAPI)

The number of FITC-OZ particles phagocytosed by the cells was determined using an Axioplan fluorescence microscope (*Zeiss Oberkochen, Germany*). During the evaluation, the zymosan-A particles phagocytosed by the cells were counted in randomly selected fields of view, and the phagocytic index (PI) was calculated: *PI = (Number of particles phagocytosed by 100 cells)/100*. The PI represents the average number of phagocytosed FITC-OZ particles per cell.

Statistical calculations were performed using the SPSS 25.0 software. The normality of data distribution was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For normally distributed data, we used one-way analysis of variance (ANOVA) to compare the PI values of different groups and to analyze the clinical data of the study subjects. If the data distribution differed from normal, we used the non-parametric Kruskal-Wallis H test. The comparison of preoperative and postoperative PI values was performed using Student's paired t-test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. The prognostic significance of preoperative laboratory parameters for the outcome of primary surgery

3.1.1. Clinical data of the participants

During the study period, we performed primary tumor reduction surgery on 150 patients with high-grade serous EOC histological findings. Complete or optimal tumor reduction was achieved in 67 patients, while tumor reduction was incomplete in 83 patients. The average age of the patients was 57.11 years (SD = 12.40); 45 patients were classified as FIGO stage I, 8 patients as FIGO stage II, 72 patients as FIGO stage III, and 25 patients as FIGO stage IV. Overall, 67.6% of the patients (97/150) had advanced-stage (FIGO III-IV) disease.

Among the parameters examined, the Kolmogorov-Smirnov and Shapiro-Wilk tests indicated that the age and BMI values of the patients followed a normal distribution, while the WBC, Neu#, Ly#, Mono#, PLT, NLR, MLR, PLR, PDW, MPV, and Ca-125 values deviated from the normal distribution. For comparing the two patient groups—optimal vs. incomplete tumor reduction—we used an independent samples t-test for age and BMI values, and a non-parametric Mann-Whitney U test for WBC, Neu#, Ly#, Mono#, PLT, NLR, MLR, PLR, PDW, MPV, and Ca-125 values.

According to these results, the following preoperative values were significantly lower in patients who later achieved optimal tumor reduction compared to those with incomplete tumor reduction: age ($p < 0.01$), WBC [U = 1838.00, Z = -2.12, $p < 0.05$], Neu# [U = 1627.00, Z = -2.89, $p < 0.01$], Mono# [U = 1499.00, Z = -3.16, $p < 0.01$], PLT [U = 1697.00, Z = -2.82, $p < 0.01$], NLR [U = 1343.50, Z = -4.12, $p < 0.001$], MLR [U = 1262.00, Z = -4.23, $p < 0.001$], PLR [U = 1245.00, Z = -4.55, $p < 0.001$], and Ca-125 [U = 573.50, Z = -3.87, $p < 0.001$]. There was no significant difference between the two patient groups regarding BMI ($p = 0.179$) and MPV [U = 2189.00, Z = -0.72, $p = 0.46$], while Ly# [U = 1470,50, Z = (-3,57); p <0,001] and PDW [U = 1850.00, Z = -2.17, $p < 0.05$] was significantly higher in those who underwent optimal tumor reduction.

Survival curves were plotted using Kaplan-Meier analysis, and the PFS and OS of the two patient groups were compared using the log-rank test. The results indicated that both the 5-year PFS ($p < 0.001$) and OS ($p < 0.001$) were significantly higher in the optimal tumor reduction group.

3.1.2. ROC curve analysis

Optimal thresholds for surgical outcomes were determined using ROC curve analysis. Variables with an AUC greater than 0.7 were used as predictive models. Among the parameters examined, threshold values could be established for Ca-125, NLR, MLR, and PLR for predicting the outcome of primary surgery. According to the ROC curve analysis results, the optimal threshold for Ca-125 (AUC = 0.734, 95% confidence interval [CI]: 0.629-0.840, $p < 0.001$) was 169.15 kU/l (sensitivity = 77.78%, specificity = 67.5%). The optimal threshold for NLR was 3.362 (AUC = 0.707, 95% CI: 0.620-0.793, $p < 0.001$, sensitivity = 70.89%, specificity = 63.79%), for MLR it was 0.305 (AUC = 0.714, 95% CI: 0.627-0.801, $p < 0.001$, sensitivity = 64.5%, specificity = 74.1%), and for PLR it was

199.371 (AUC = 0.728, 95% CI: 0.645-0.811, $p < 0.001$, sensitivity = 69.62%, specificity = 68.97%). For age, the AUC was 0.666 (95% CI: 0.579-0.753, $p < 0.001$) with a threshold of 57.5 years (sensitivity = 61.4%, specificity = 68.7%). Based on the area under the curve, WBC [AUC = 0.606 (95% CI: 0.507-0.704)], Ly# [AUC = 0.679 (95% CI: 0.590-0.768)], Neu# [AUC = 0.645 (95% CI: 0.549-0.741)], Mono# [AUC = 0.660 (95% CI: 0.567-0.753)], PLT [AUC = 0.640 (95% CI: 0.548-0.732)], and PDW [AUC = 0.608 (95% CI: 0.511-0.706)] were not suitable for determining threshold values.

3.1.3. Binary logistic regression

Using a binary logistic regression model, we examined the independent predictive value of variables that could potentially predict incomplete tumor reduction. In the statistical model, the variables were dichotomized according to the thresholds obtained from the ROC curve analysis; the parameters we considered were: age (>57.5 years), FIGO stage (I-II vs. III-IV), NLR (>3.362), MLR (>0.305), PLR (>199.371), and Ca-125 (>169.15 kU/l). The final step of the Backward model includes only the parameters with independent predictive value, excluding non-significant and dependent factors. Based on the logistic regression results, the stage (Odds Ratio [OR] = 5.102, 95% CI: 1.659-15.693, $p < 0.01$), MLR (OR = 5.028, 95% CI: 1.561-16.201, $p < 0.01$), and Ca-125 (OR = 4.671, 95% CI: 1.540-14.167, $p < 0.01$) had significant independent predictive value for the likelihood of achieving tumor reduction during primary surgery.

Following primary surgery, both the 5-year PFS ($p < 0.001$, log-rank test) and OS ($p < 0.001$, log-rank test) were significantly higher in patients with FIGO stage I-II compared to those with stage III-IV. The 5-year PFS and OS rates were also significantly higher in cases with MLR ≤ 0.305 ($p < 0.01$ and $p < 0.01$) and Ca-125 ≤ 169.15 kU/l ($p < 0.001$ and $p < 0.001$).

Based on the ROC curve analysis using the combination of MLR, Ca-125, and FIGO stage as independent variables, the AUC value was 0.853 (95% CI: 0.768-0.937, $p < 0.001$).

3.2. Phagocytic function of monocytes and neutrophil granulocytes

3.2.1. Clinical data of the participants

Primary complete tumor reduction surgery (no visible tumor tissue remained after surgery) was performed as the initial treatment in a total of 20 patients with stage IIIC high-grade serous EOC. Five patients were excluded from the study group due to incomplete cytoreductive surgery (n = 3), different FIGO stage (n = 1; FIGO stage IV), or different histological type (n = 1; mucinous EOC). In the surgical control group, laparotomy was performed in 14 patients due to uterine leiomyoma and in 2 patients due to benign ovarian cysts. The healthy control group consisted of 14 healthy women.

According to the Kolmogorov-Smirnov and Shapiro-Wilk tests, the age and BMI values of the patients, as well as the PI values of the monocytes and neutrophil granulocytes, followed a normal distribution. The values for gravidity and parity deviated from the normal distribution. The average age [± standard deviation (SD)] of the EOC patients was 62.25 (± 10.80) years, with an average BMI of 26.21 (± 3.73) kg/m², and the median (range) values for gravidity and parity were 2 (0–5) and 1 (0–3), respectively. The average age of the surgical control group was 55.00 (± 12.44) years, with an average BMI of 25.42 (± 4.13) kg/m², and the median (range) values for gravidity and parity were 1 (0–3) and 1 (0–2), respectively. The average age of the healthy controls was 53.43 (± 13.18) years, with an average BMI of 27.50 (± 4.39) kg/m², and the median (range) values for gravidity and parity were 2.5 (0–9) and 2 (0–3), respectively. There was no significant difference in age ($p = 0.077$), gravidity ($p = 0.109$), parity ($p = 0.514$), and BMI ($p = 0.378$) at the time of blood sampling between the EOC patients, healthy women, and surgical control group patients.

3.2.2. Phagocytic function of monocytes

The preoperative PI values of monocytes from EOC patients (2.14 ± 0.72) were significantly lower compared to healthy controls (3.88 ± 1.54; $p < 0.001$). The postoperative PI values of monocytes from EOC patients (3.63 ± 0.64) showed a significant increase ($p < 0.001$) compared to the preoperative values, reaching a level similar to that of the healthy controls ($p = 0.700$). In the surgical control group, there was no significant difference between the preoperative (3.40 ± 0.50) and postoperative (3.36 ± 0.52) PI values of monocytes isolated from blood samples ($p = 0.567$), and these values did not significantly differ from the PI values of monocytes from healthy controls either (preoperative: p = 0.361 and postoperative samples: $p = 0.303$). The preoperative PI values of monocytes isolated from EOC patients were significantly lower compared to the preoperative ($p < 0.01$) and postoperative values ($p < 0.01$) of the surgical controls.

3.2.3. Phagocytic function of neutrophil granulocytes

The preoperative PI values of neutrophil granulocytes isolated from EOC patients (2.37 ± 0.79) were significantly lower compared to those measured in healthy controls (3.99 ± 1.18; $p < 0.001$). The postoperative PI values of neutrophil granulocytes isolated from EOC patients (3.99 ± 0.75) were significantly higher ($p < 0.001$) compared to the preoperative values, reaching a level similar to that measured in the healthy control group ($p = 0.991$). In the surgical control group, there was no significant difference between the preoperative (3.37 ± 0.56) and postoperative (3.47 ± 0.49) PI values of neutrophil granulocytes ($p = 0.542$), and they did not significantly differ from the PI values of neutrophil granulocytes isolated from the healthy control group either (preoperative: $p = 0.150$; postoperative: $p = 0.235$). The preoperative PI values of neutrophil granulocytes from EOC patients were significantly lower compared to those isolated from the surgical control group before ($p < 0.01$) and after surgery ($p < 0.01$).

4. Discussion

4.1. Investigation of the prognostic value of preoperative laboratory parameters for the outcome of primary cytoreductive surgery

It is known that the inflammatory response and elevated levels of inflammatory markers are associated with the prognosis of malignant tumors, which is why these can be considered prognostic markers. According to the results of several studies, absolute neutrophil, monocyte, lymphocyte, and platelet counts, as well as their ratios, such as NLR, MLR, and PLR values, are associated with the prognosis of various gynecological malignancies, including ovarian cancer. Elevated pre-treatment NLR values (which correlate with elevated Ca-125 levels) are associated with a worse prognosis, and high PLR is associated with decreased OS and PFS. These values can predict not only tumor progression but also therapeutic response, as literature data suggest that NLR and PLR observed at diagnosis can predict tumor platinum resistance.

Despite advances in drug treatment, ovarian cancer still has a very high mortality rate. The extent of cytoreduction achieved during the primary debulking surgery, performed as the first treatment following the suspected diagnosis, is one of the main prognostic factors for survival. If the size of the residual tumor tissue is less than 1 cm after surgery, the patients' OS and PFS improve significantly. This is so true that if more than 1 cm of tumor tissue remains after surgery, neither OS nor PFS increases. In other words, in the case of suboptimal tumor reduction (i.e., if more than 1 cm of tumor tissue remains after surgery), the patient's prognosis does not improve, whereas if NACT treatment is chosen instead of surgery, both PFS and OS might have improved more favorably. Furthermore, the risk of complications is increased with full median laparotomy, and postoperative recovery alone can take a significant amount of time, potentially delaying the start of drug treatment. As a result, in a more debilitated patient, it might even occur that chemotherapy does not start within the optimal therapeutic window. When certain oncological surgical criteria are met, where complete removal of the EOC would be incompatible with life (e.g., mesenteric infiltration, celiac trunk infiltration, etc.), i.e., when the tumor is unresectable, the chosen approach should be NACT followed by complete tumor reduction (IDS) made possible by downstaging. However, selecting patients suitable for complete tumor reduction requires significant caution, as suboptimal tumor reduction negatively impacts survival. Furthermore, incomplete surgical treatment of tumors, typically diagnosed at an advanced stage in elderly patients, can cause unnecessary burden with prolonged recovery times, potentially leading to delayed treatment initiation and thereby affecting survival. In advanced stages, ovarian cancer forms a significant tumor mass, which can occur throughout the entire abdominal cavity. Therefore, its removal may require not only surgery on the pelvic organs but also bowel surgery and upper abdominal surgery. As a result, the tumor can be unresectable beyond the previously mentioned criteria, depending on the surgical situation. Thus, predicting the feasibility of complete tumor reduction remains a critically important task to this day.

Supporting the literature data, our own results also showed a significant difference in the 5-year PFS and OS of ovarian cancer patients who underwent primary debulking surgery, depending on whether optimal tumor reduction was achieved during the primary surgery. Therefore, accurately predicting the likelihood of complete/optimal or incomplete tumor reduction is of paramount importance for the patients we treat. This requires considering the clinical picture, laboratory, and imaging results collectively.

According to the scoring system developed by *Gu et al.*, predictive parameters associated with a high risk of suboptimal cytoreduction include age over 60 years, Ca-125 levels above 800 U/ml, and specific findings on preoperative CT scans. Some research groups have developed machine learning algorithms to predict surgical outcomes, identifying the five most important factors as Ca-125 and albumin levels, diaphragmatic involvement, age, and the presence/amount of ascites. Despite numerous attempts, there is still no reliable system for predicting the success of surgical treatment. Therefore, any scientific observations related to this topic can be valuable, as they may lay the foundation for an algorithm that aids in precise prediction.

Our study aimed to assess whether routine preoperative blood test parameters could be used to predict the outcome of primary debulking surgery. According to our results, there was a significant difference between the two patient groups (optimal vs. incomplete tumor reduction) in terms of age, WBC, and certain calculated values such as NLR, PLR, MLR, and Ca-125 levels. Despite these significant differences, the ROC curve analysis indicated that optimal thresholds could be determined for only a few parameters. We found that higher age significantly correlates with a higher risk of incomplete tumor reduction, with a threshold of 57.5 years. Additionally, we were able to determine optimal thresholds for other variables regarding the surgical outcome: 3.362 for NLR, 0.305 for MLR, 199.371 for PLR, and 169.15 kU/l for Ca-125. Exceeding these thresholds significantly increases the likelihood of not achieving optimal tumor reduction during primary debulking surgery.

Further analyzing our results using binary logistic regression, we found that in addition to disease stage, MLR (with a threshold of 0.305, often calculated as LMR: 3.28 in the literature) and Ca-125 (with a threshold of 169.15 kU/l) have significant predictive value in predicting incomplete tumor reduction. Our results are similar to those observed by Eo et al. (2016), who found that age, Ca-125, and LMR were the strongest predictors of suboptimal tumor reduction using multivariate logistic regression. However, their study included patients with various histological types of ovarian cancer, which have fundamentally different presentations, surgical treatments, adjuvant therapies, and prognoses. In contrast, our study focused exclusively on the most common histological type, high-grade serous EOC. In our study, we also performed ROC curve analysis using the combined application of the three parameters obtained from binary logistic regression (FIGO stage, preoperative MLR, and Ca-125). As a result, the AUC was higher than when these variables were analyzed individually. Based on this, we can conclude that the combined use of these parameters can provide additional information for estimating surgical outcomes.

We also examined the PFS and OS of the dichotomized patient groups based on the parameters obtained from binary logistic regression using Kaplan-Meier analysis (log-rank test). This analysis showed significant differences in the 5-year PFS and OS for patients with serous EOC for all three parameters. Specifically, if the preoperative MLR was higher than 0.305, Ca-125 levels were above 169.15 kU/l, and the disease was at an advanced stage (FIGO III-IV), the PFS and OS values were less favorable. These data are consistent with previous research findings, which suggest that a low LMR can predict decreased OS and PFS and correlate with the presence of malignant ascites, lymph node metastases, and resistance to chemotherapy.

4.2. Investigation of the phagocytic function of monocytes and neutrophil granulocytes

Neutrophil granulocytes are the most abundant leukocytes in the bloodstream and form the first line of defense against infections as part of the innate immune system. One of their main functions is phagocytosis and the destruction of ingested microbes. The entry of microorganisms into the body induces an inflammatory response, causing neutrophils to migrate from the circulation to the tissues. There neutrophil granulocytes can destroy microorganisms through several pathways, including phagocytosis, the release of antimicrobial substances, and the formation of neutrophil extracellular traps (NETs).

They may also play an important role in the defense against malignant tumors. Their antitumor effect can manifest in several ways: they can destroy cancer cells with the help of antimicrobial and cytotoxic substances released from their granules, and through the production of certain cytokines and chemokines they can attract other cells involved in the fight against cancer. By producing reactive oxygen species, they can directly kill tumor cells and induce their apoptosis through direct contact. Their most effective antitumor mechanism is antibody-dependent cell-mediated cytotoxicity. Monocytes are considered the third most common immune cell population in peripheral blood, following neutrophils and lymphocytes. Monocytes, along with macrophages and dendritic cells, belong to the mononuclear phagocyte system. Their main function is to destroy pathogens through phagocytosis and to produce biologically active mediators. They are involved in antigen processing and presentation, as well as in the elimination of tumor cells.

Phagocytosis is a multi-step cellular process that involves the recognition of target cells, their engulfment, and subsequent lysosomal digestion. Each of these steps is meticulously regulated by receptor-ligand interactions between phagocytes and their targets. A decrease in the phagocytic function of monocytes and neutrophil granulocytes has been observed in several diseases. For example, it has been shown that the phagocytic index of monocytes and neutrophil granulocytes isolated from peripheral blood samples is significantly reduced in healthy pregnancies compared to non-pregnant values. However, in preeclamptic pregnancies, the phagocytic function of these cells is even lower than the values measured in healthy pregnancies. When examining the phagocytic function of monocytes and neutrophil granulocytes isolated from peripheral blood samples of patients with periodontitis, reduced function was observed compared to healthy control subjects. Although changes in the phagocytic function of peripheral monocytes and neutrophil granulocytes have been observed in other conditions (e.g., endometriosis), the current literature provides limited and often contradictory information on this topic.

In our research, we investigated the phagocytic function of these immune cells to better understand the pathogenesis of ovarian cancer and its relationship with the immune system, as well as to assess the impact of surgical treatment. To this end, we conducted our experiments on patients with advanced-stage high-grade serous epithelial ovarian cancer and described the phagocytic function of the examined cells before and after primary cytoreductive surgery. Based on our results, it can be concluded that the phagocytic function of peripheral monocytes and neutrophil granulocytes in ovarian cancer patients is significantly reduced compared to the values of the corresponding cells in healthy controls. This is consistent with literature data where the phagocytic function of monocytes isolated from peripheral blood samples of breast cancer patients was also examined, and reduced function was observed compared to the values of healthy control subjects. Additionally, in advanced-stage breast cancer patients, the decrease in phagocytic function of monocytes was more significant, especially in those patients who had already developed metastases. Based on these findings, it was hypothesized that in ovarian cancer, the phagocytic function of monocytes and/or neutrophil granulocytes might also change during disease progression, which could weaken the defense against tumor cells.

According to the results of our further investigations, the phagocytic function of the cells normalizes if the complete surgical removal of the EOC is achieved. A good example of the related contradictions is the study by *Arsenijević et al.*, who found reduced phagocytic function of peripheral monocytes in breast cancer patients compared to preoperative values following surgical treatment. During their research, a further decrease in phagocytic capability was observed during chemotherapy, while three months after the last chemotherapy cycle, the phagocytic function of monocytes showed an increase but was still significantly lower than the values of healthy controls.

It was suggested that the surgical intervention involving laparotomy itself, regardless of the underlying disease, might have an impact on the phagocytic function of the cells. Therefore, we also examined the cells of patients who underwent laparotomy for benign gynecological tumors (surgical control group). Based on our results, the surgical intervention does not affect the phagocytic function of the cells, as there was no significant difference in the phagocytic function of monocytes and neutrophils from postoperative blood samples in the surgical control group compared to preoperative values, and there was no significant difference when compared to the values of cells from healthy control subjects either. The effect of tumors on the phagocytosis of various immune cells has been previously investigated, indicating that it either decreased or did not change during surgery and in the days following surgery compared to preoperative values. Based on previous research results, we hypothesized that in ovarian cancer patients, the observed increase in the phagocytic function of neutrophil granulocytes and monocytes following complete tumor reduction is due to the complete removal of the malignant tumor. This may indicate the reversible immunosuppressive effect of the tumor on the immune system.

In recent years, the connection between the development and progression of ovarian cancer and the dysregulation observed in certain elements of the immune system has become clear. The immune system and the inflammatory response play an important role in various stages of carcinogenesis, such as initiation, invasion, propagation, and metastasis formation. By examining various cytokines in blood samples taken from ovarian cancer patients, it was found that the serum levels of Ca-125, interleukin (IL)-6, IL-7, IL-8, IL-10, and transforming growth factor (TGF)-β in patients with epithelial ovarian carcinoma were significantly higher compared to the values in healthy individuals and patients with benign ovarian tumors.

Overall, it can be assumed that malignant tumors should be interpreted as organ-like structures in which complex and often pathologically regulated interactions occur between tumor cells and tumor-associated cells. In relation to ovarian cancer, it has been observed that tumor cells can induce various changes in the tumor stroma, transforming it into a pathological environment known as the tumor microenvironment (TME). The composition of this environment and the pathological interactions that occur within it are major facilitators of the progression of malignant tumors. The TME is primarily composed of endothelial cells, tumor-associated fibroblasts, adipocytes, and various immune cells that often exhibit altered functions in malignancies. These immune cells include myeloid-derived suppressor cells, natural killer cells, tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and tumor-infiltrating lymphocytes.

The communication within the TME is mediated by cytokines, growth factors, inflammatory mediators, and matrix-remodeling enzymes, which are regulated in a pathologically and complex manner. The interaction networks of these components are only partially understood. Inflammatory mediators secreted by epithelial cells that have been pathologically altered in number and quality due to the influence of cancer cells further enhance the formation of an inflammatory environment. This can reprogram surrounding cells, leading to the development of the TME. The components that make up the TME directly contribute to tumor survival by suppressing antitumor immunity and promoting tumor growth and metastasis formation. The cytokines involved in immunosuppression include IL-10, IL-6, TGF-β, vascular endothelial growth factor (VEGF), prostaglandin E-2, IL-35, IL-1, IL-8, and certain metalloprotease enzymes. As a result, the TME creates an optimal immune environment for the proliferation of tumor cells, which can subsequently lead to the appearance of clinical symptoms of the disease.

4.3. New scientific findings

4.3.1. Investigation of the prognostic value of preoperative laboratory parameters for the outcome of primary cytoreductive surgery

- Age, preoperative peripheral blood leukocyte, neutrophil, monocyte, and platelet counts, NLR, MLR, PLR, and Ca-125 levels are significantly higher, while lymphocyte count and PDW values are significantly lower in those advanced ovarian cancer patients for whom optimal tumor reduction could not be achieved during primary surgery.
- Using ROC curve analysis, optimal cutoff values for Ca-125, MLR, NLR, PLR, and age can be determined, which can aid in predicting the risk of incomplete tumor reduction. The cutoff values are 3.362 for NLR, 0.305 for MLR, 199.371 for PLR, and 169.15 kU/l for Ca-125.
- Based on a binary logistic regression model, the stage (FIGO III-IV), MLR, and Ca-125 have significant, independent predictive value for the outcome of primary debulking surgery.

4.3.2. Investigation of the phagocytic function of monocytes and neutrophil granulocytes

- The phagocytic function of monocytes and neutrophil granulocytes isolated from the peripheral blood samples of advanced-stage EOC patients is significantly reduced compared to the values of healthy women.
- In the case of complete surgical removal of EOC, the reduced phagocytic function observed in patients normalizes by the 7th postoperative day, reaching levels similar to those of healthy women, suggesting that the tumor's immunosuppressive effect appears to be reversible.

- The surgical intervention itself does not affect the phagocytic function of monocytes and neutrophil granulocytes.

5. Summary

In our study of the outcome of primary surgical treatment, which is the strongest determinant of ovarian cancer prognosis, we found that age, as well as preoperative white blood cell count, neutrophil count, monocyte count, and platelet count from peripheral blood samples, along with NLR, MLR, PLR, and Ca-125 values, were significantly higher, while lymphocyte count and PDW values were significantly lower in patients with serous EOC who had suboptimal outcomes from primary debulking surgery. Using ROC curve analysis, we were able to estimate optimal cut-off values for Ca-125, MLR, NLR, and PLR, as well as age, to predict surgical outcomes. Among these, stage, MLR, and Ca-125 had significant independent predictive value based on a binary logistic regression model. The significance of our results lies in the fact that blood tests are simple and inexpensive procedures performed on all patients during preoperative routine examinations. Although our conclusions alone are insufficient for accurately determining surgical outcomes, they may play a role in developing a multifactorial model to predict and potentially avoid unnecessary or unfavorable surgical interventions, and in determining the optimal first-line treatment strategy.

In our research on phagocytic function, we found that the phagocytic function of peripheral monocytes and neutrophil granulocytes is reduced in patients with advanced-stage, high-grade serous EOC compared to healthy controls. After complete tumor reduction, the phagocytic function of these cells shows a significant increase, reaching levels similar to those of healthy controls. Since there was no significant difference in the pre- and postoperative phagocytic function of monocytes and neutrophil granulocytes in the surgical control group and no difference compared to healthy controls, we can conclude that changes in phagocytic function are not solely a consequence of surgical intervention, but may be an effect of ovarian cancer. Based on the normalization of phagocytic function observed after complete primary cytoreductive surgery, we hypothesize that high-grade serous EOC and/or its microenvironment may produce factors that reduce the phagocytic function of peripheral monocytes and neutrophil granulocytes, and that the production of these factors likely decreases or ceases after complete surgical removal of the tumor. Our results highlight the importance of the quality of surgical care, as the immunological impact of complete surgical removal of ovarian cancer may contribute to a more favorable prognosis.

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7. List of publications

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List of publications related to the dissertation

1. **Kovács, A. R.**, Sulina, A., Kovács, K. S., Lukács, L., Török, P., Lampé, R.: Prognostic Significance of Preoperative NLR, MLR, and PLR Values in Predicting the Outcome of Primary Cytoreductive Surgery in Serous Epithelial Ovarian Cancer.
   Diagnostics. 13 (13), 1-13, 2023.
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2. **Kovács, A. R.**, Lukács, L., Pál, L., Szűcs, S., Kovács, K. S., Lampé, R.: Recovery of the Decreased Phagocytic Function of Peripheral Monocytes and Neutrophil Granulocytes following Cytoreductive Surgery in Advanced Stage Epithelial Ovarian Cancer.
   Medicina (Kaunas). 59 (9), 1-10, 2023.
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List of other publications

3. Lukács, L., **Kovács, A. R.**, Pál, L., Szűcs, S., Lampé, R.: Evaluating the Phagocytic Index of Peripheral Leukocytes in Endometriosis by Plasma Experiments.
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   Nőgyógy. Onkol. 23, 54-58, 2018.

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