LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Jonas Ulevičius

DYSREGULATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN OVARIAN CANCER PATIENTS FOLLOWING SURGICAL TRAUMA

Doctoral Dissertation
Medical and Health Sciences,
Medicine (M 001)

Kaunas, 2025

This dissertation was prepared from 2019 to 2024 at the Surgical Gastroenterology Laboratory, Institute for Digestive Research, Medical Academy of the Lithuanian University of Health Sciences.

Scientific Supervisor:

Prof. Dr. Saulius Paškauskas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Consultant:

Prof. Dr. Žilvinas Dambrauskas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

This dissertation is defended at the Medical Research Council of the Lithuanian University of Health Sciences:

Chairperson:

Prof. Dr. Elona Juozaitytė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Members:

Assoc. Prof. Dr. Lina Poškienė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);
Prof. Dr. Reda Žemaitienė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);
Assoc. Prof. Dr. Vilius Rudaitis (Vilnius University, Medical and Health Sciences, Medicine – M 001);
Assoc. Prof. Dr. Ivane Abiatari (Ilia State University, Medical and Health Sciences, Medicine – M 001).

This dissertation will be defended at the open session of the Medical Research Council of the Lithuanian University of Health Sciences on 21 May 2025 at noon in the Great Auditorium of the Department of Obstetrics and Gynaecology of the Lithuanian University of Health Sciences.
Address: Eivenių 2, LT-50161 Kaunas, Lithuania.

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS

Jonas Ulevičius

KIAUŠIDŽIŲ VĖŽIU SERGANČIŲ PACIENČIŲ PERIFERINIO KRAUJO VIENBRANDUOLIŲ LĄSTELIŲ POKYČIAI PO CHIRURGINĖS TRAUMOS

Daktaro disertacija
Medicinos ir sveikatos mokslai,
medicina (M 001)

Kaunas, 2025

Disertacija rengta 2019–2024 metais Lietuvos sveikatos mokslų universiteto Medicinos akademijos Viškinimo sistemos tyrimų instituto Chirurginės gastroenterologijos laboratorijoje.

Mokslinis vadovas

prof. dr. Saulius Paškauskas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Konsultantas

prof. dr. Žilvinas Dambrauskas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija ginama Lietuvos sveikatos mokslų universiteto medicinos mokslo krypties taryboje:

Pirmininkė

prof. dr. Elona Juozaitytė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Nariai:

doc. dr. Lina Poškienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
prof. dr. Reda Žemaitienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Vilius Rudaitis (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Ivane Abiatari (Ilja valstybinis universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija bus ginama viešajame Lietuvos sveikatos mokslų universiteto Medicinos mokslo krypties tarybos posėdyje 2025 m. gegužės 21 d. 12.00 val. Lietuvos sveikatos mokslų universiteto Akušerijos ir ginekologijos klinikos Didžiojoje auditorijoje.
Disertacijos gynimo vietos adresas: Eivenių g. 2, LT-50161 Kaunas, Lietuva.

CONTENTS

ABBREVIATIONS

AKT – protein kinase B
BMI – body mass index
CAFs – cancer-associated fibroblasts
CAR – chimeric antigen receptor
CBC – complete blood count
CD3+ T cells – T lymphocytes
CD4+ T cells – T helper lymphocytes
CD8+ T cells – cytotoxic T lymphocytes
CD19+ B cells – B lymphocytes
CD20+ B cells – mature B lymphocytes
CD4/CD8 ratio – CD4+ T cell to CD8+ T cell ratio
CTLA-4 – cytotoxic T lymphocyte-associated protein 4
DCs – dendritic cells
DAMPs – damage-associated molecular patterns
cDNA – complementary deoxyribonucleic acid
ECM – extracellular matrix
ELISA – enzyme-linked immunosorbent assay
FCM – flow cytometry
FIGO – International Federation of Gynecology and Obstetrics
GAPDH – glyceraldehyde-3-phosphate dehydrogenase
HGSC – high-grade serous carcinoma
HIPEC – hyperthermic intraperitoneal chemotherapy
IDS – interval debulking surgery
IFN-γ – interferon γ
IL-1 – interleukin 1
IL-1β – interleukin 1β
IL-4 – interleukin 4
IL-6 – interleukin 6
IL-10 – interleukin 10
IL-12 – interleukin 12
IL-23 – interleukin 23
IQR – interquartile range
JAK – janus kinase
LAG-3 – lymphocyte activation gene-3
LGSC – low-grade serous carcinoma
LMR – lymphocyte-to-monocyte ratio
LPS – lipopolysaccharide
MDSCs – myeloid-derived suppressor cells
MFI – mean fluorescence intensity
mRNA – messenger RNA
mTOR – mammalian target of rapamycin
MUC16 – mucin 16
M1/M2 ratio – M1-to-M2 monocyte ratio
NACT – neoadjuvant chemotherapy
NETs – neutrophil extracellular traps
NK cells – natural killer cells

NLR – neutrophil-to-lymphocyte ratio
NO – nitric oxide
OC – ovarian cancer
OS – overall survival
PARP – poly ADP-ribose polymerase
PBMCs – peripheral blood mononuclear cells
PDS – primary debulking surgery
PD-1 – programmed cell death protein 1
PD-L1 – programmed death-ligand 1
PI3K – phosphatidylinositol 3-kinase
PFS – progression-free survival
PLR – platelet-to-lymphocyte ratio
RBCs – red blood cells
RNA – ribonucleic acid
ROS – reactive oxygen species
RT-PCR – real-time polymerase chain reaction
SDS – secondary debulking surgery
STAT – signal transducer and activator of transcription
TAMs – tumor-associated macrophages
TBHP – tert-butyl hydroperoxide
TGF-β – tumor growth factor β
Th1 – type 1 T helper lymphocytes
Th2 – type 2 T helper lymphocytes
TILs – tumor-infiltrating lymphocytes
TIM-3 – T cell immunoglobulin and mucin domain-containing protein 3
TME – tumor microenvironment
TNF-α – tumor necrosis factor α
TNF-β – tumor necrosis factor β
Tregs – regulatory T lymphocytes
VEGF – vascular endothelial growth factor
WBCs – white blood cells

INTRODUCTION

Ovarian cancer (OC) is the eighth most common cancer among women worldwide, with over 324,000 new cases and nearly 207,000 deaths annually. It is considered to be the most fatal gynecologic malignancy [1, 2]. In 2022 global OC incidence rate was 8.3 per 100,000 women and mortality rate was 5.3 per 100,000 women [3]. Projections from the Global Cancer Observatory suggest that without significant advancements in detection and treatment, new cases are expected to rise by 55%, and deaths from OC by nearly 70% by 2050 [4]. In Lithuania, the situation is particularly concerning. In 2022, there were 366 new cases of OC, with an incidence rate of 25.6 per 100,000 women and mortality rate of 16.9 per 100,000 women [3]. These statistics highlight the importance of the topic and the need for development of more effective management.

Currently, cytoreductive surgery and chemotherapy are the two main treatment methods for OC [5, 6]. When combined adequately, these methods can improve patient prognosis; however, the overall 5-year survival rate remains around 49% [2, 6]. The relatively low survival rate is primarily due to the fact that most OC cases are diagnosed at late stages, and in many cases, tumors are resistant to chemotherapeutic agents [7, 8]. Ongoing biomedical research has revealed new potential treatment methods, including targeted therapy and immunotherapy. While early data on the efficacy of these agents and their combinations are promising, further evaluation is still needed [5, 9–12]. Despite the novel OC treatment strategies, cytoreductive surgery currently remains the core treatment method for OC patients. Complete cytoreductive surgery, which aims to remove all visible tumors, is associated with improved outcomes, including progression-free survival (PFS) and overall survival (OS) [12, 13]. More fundamental knowledge of OC progression is still needed to create successful treatment methods.

Peripheral blood mononuclear cells (PBMCs) are key regulators of anticancer immunity [14, 15]. This heterogeneous population includes T lymphocytes (CD3+ T cells), B lymphocytes (CD19+ B cells), natural killer (NK) cells, monocytes, and dendritic cells (DCs), each with distinct functional profiles. PBMCs interact with tumor cells both directly through cytotoxic activity and indirectly via cytokine secretion, antigen presentation, and regulatory functions [15, 16]. In OC patients, significant changes in PBMC-derived cell populations, cytokine production, and other immune functions have been well documented, highlighting their role in tumor progression [15–17]. However, studies investigating postoperative immune responses in OC patients are limited, heterogeneous, and primarily focus on the later postoperative period,

presenting varied findings [18–20]. Data on the early postoperative function of PBMCs and anticancer immunity in OC patients remain limited.

In addition to cellular alterations, studying cytokines and immune-related proteins is essential for understanding anticancer immunity. Cytokines modulate immune responses by regulating communication between immune cells, influencing their ability to recognize and attack tumor cells, thus affecting tumor growth [15, 16, 21]. Immune-related proteins like checkpoint molecules also play a critical role in shaping the tumor microenvironment (TME), either promoting or inhibiting cancer progression [22, 23]. While their roles in the TME and their serum profiles in OC patients are well researched [15, 16, 24], little is known about their expression in PBMCs or the dynamics in the early postoperative period. Given the role of these proteins in OC progression, understanding their dynamics postoperatively is important for a thorough understanding of OC development.

While cytoreductive surgery offers clear benefits for OC patients, it also induces significant tissue trauma, which has systemic effects on the patient [25, 26]. Trauma triggers the release of damage-associated molecular patterns (DAMPs), the production of neutrophil extracellular traps (NETs), and the activation of myeloid-derived suppressor cells (MDSCs), leading to the dysregulation of immune effector and suppressor cells, consequently affecting immune function [25, 27]. These alterations may promote metastasis formation, a phenomenon observed in patients undergoing surgery for breast, lung, pancreatic, colorectal, and other cancers [28–30]. However, specific data on the impact of these changes in OC patients remain limited. Given the relationship between the immune system, the TME, and the progression of OC [15, 16], it is reasonable to speculate that surgical intervention may also impact the course of OC.

We propose the hypothesis that OC patients experience immune dysregulation involving PBMCs during the early postoperative period. This study focuses on analyzing changes in PBMC subpopulations, their functional activity, immune-related protein production, and serum levels in the early postoperative period. Our findings may offer insights that contribute to improving OC treatment strategies in the future.

1. AIM AND OBJECTIVES OF THE STUDY

1.1. The aim of the study

To investigate the alterations in the immune response of peripheral blood mononuclear cells (PBMCs) following surgical treatment for ovarian cancer (OC).

1.2. Objectives of the study

1. To assess changes in the peripheral blood cell population composition in OC patients.
2. To evaluate changes in PBMC subpopulations caused by OC and surgical trauma.
3. To assess the functional changes in PBMCs caused by OC and surgical trauma.
4. To evaluate changes in the expression of immune-related proteins in PBMCs caused by OC and surgical trauma.
5. To assess changes in immune-related protein serum levels caused by OC and surgical trauma.

1.3. The novelty of the study

The novelty of our study can be assessed from several perspectives. While the role of the immune system in OC progression is well known, most existing research focuses on the local immune composition within OC tissue and its components. In contrast, our study emphasizes PBMCs, a less explored but more accessible way to investigate anticancer immunity in OC patients.

As part of our background analysis, we examined preoperative PBMC-related immunity in OC patients. Although this approach is not entirely unique, data on specific assays, such as cytokine messenger ribonucleic acid (mRNA) levels in PBMCs, monocyte subpopulations, and monocyte cytokine expression in OC patients, remain scarce or non-existent.

The most significant aspect of our study's novelty is its focus on the early postoperative period. While the impact of surgery on the immune system in cancer patients has been studied, most research in OC patients examines the later postoperative periods, highlighting the long-term positive effects of surgical treatment. Studies investigating the early postoperative immune response in OC patients are limited and often narrow in scope. By targeting this underexplored timeframe and employing a wide range of assays, many of which have not been previously applied to this patient group, our study provides unique insights into immune dynamics during the early postoperative period in OC patients.

2. THE LITERATURE REVIEW

2.1. Incidence and mortality of OC

OC remains a significant public health challenge globally, with incidence and mortality rates varying across different regions. Although OC is the eighth most common cancer among women, accounting for over 324,000 new cases annually, it stands as the most lethal gynecological malignancy, leading to approximately 207,000 deaths each year [1, 2]. The global incidence rate is approximately 8.3 per 100,000 women, while the mortality rate is about 5.3 per 100,000 women [3].

Europe carries the highest burden, with incidence rates reaching 18.0 per 100,000 and a mortality rate of 12.0 per 100,000 women. North America ranks second, with incidence and mortality rates of 13.0 and 8.3 per 100,000 women, respectively. Detailed information on the incidence and mortality rates across European countries is presented in Fig. 2.1.1. Notably, Lithuania ranks second in Europe and globally, with incidence and mortality rates of 25.6 and 16.9 per 100,000 women, respectively, following Latvia, which reports the highest rates (29.9 and 18.1 per 100,000 women, respectively) [3]. Incidence rates differ significantly across different regions, especially between high- and low-income countries. In high-income regions like Europe and North America, risk factors such as lower parity, later age at the first childbirth, and the use of hormone replacement therapy contribute to higher incidence rates. In contrast, countries with lower socioeconomic status, including regions in Asia and Africa, report lower incidence rates [31, 32].

Mortality rates show less variability across regions and remain relatively close to the incidence rates. High mortality remains a global issue due to challenges in early diagnosis and effective treatment, as most women are diagnosed at advanced stages of OC. The early stages of OC are often asymptomatic, and when combined with limited access to healthcare, particularly in low-income countries, this significantly worsens the mortality rate [7, 33].

Despite global advancements in healthcare, the age-standardized incidence rates of OC have changed little over the past decades. Between 1990 and 2019, the global age-standardized incidence rates increased slightly from 6.5 to 6.9 per 100,000 women [34]. While high-income countries have had stable or declining rates, with some European countries reporting a drop of 5–10%, low-income countries in Asia and Africa have experienced a 10–20% increase in incidence, mostly due to rising obesity rates, delayed childbirth, and poorer access to healthcare [32, 35]. Mortality rates have decreased in many high-income countries, largely due to improved treatment and early detection efforts [32].

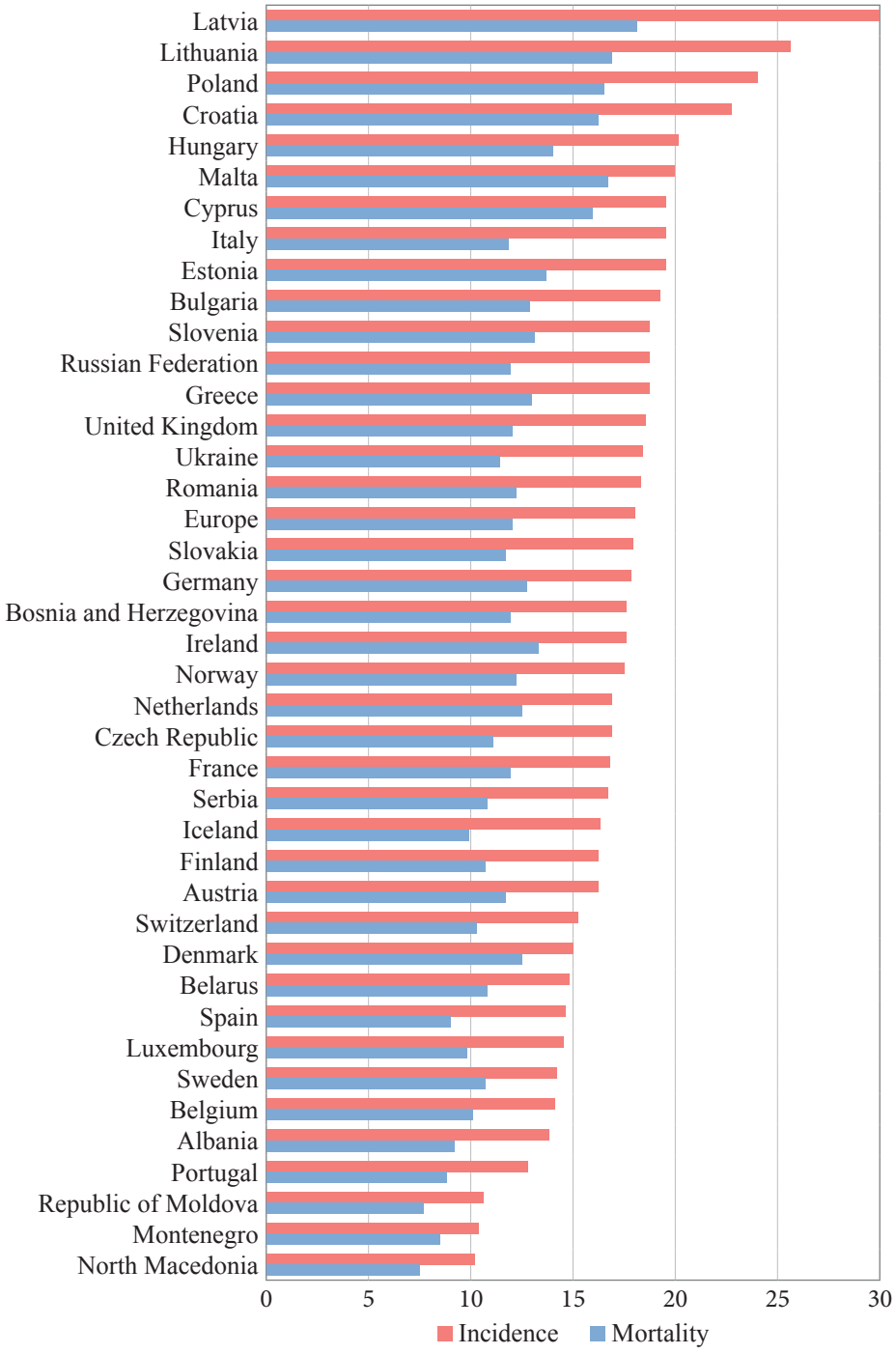


Fig. 2.1.1. *Incidence and mortality rates of ovarian cancer (OC) in Europe [3]*

In Lithuania, between 1977 and 2020, the incidence rate of OC remained stable, but the mortality rate dropped by about 30%, reflecting improvements in care and treatment.

These statistics highlight the need for global and regional initiatives to further improve early detection of OC, access to advanced treatments, and the creation of new management strategies.

2.2. Histological classification of OC and clinical features

OC is a histologically heterogenous disease, and malignant ovarian tumors are categorized into four primary histological types based on their cell of origin: epithelial, sex cord-stromal, germ cell, and mesenchymal tumors. Rare OC types are classified under the miscellaneous category. This classification is outlined in the 5th edition of the World Health Organization classification of female genital tumors, which is the current standard [36]. Each category is further divided into subtypes with different characteristics [37, 38].

Epithelial tumors are the most common ovarian malignancies, accounting for approximately 90% of cases. They are divided into five main subtypes: high-grade serous carcinoma (HGSC), low-grade serous carcinoma (LGSC), endometrioid carcinoma, clear cell carcinoma, and mucinous carcinoma [36, 38]. Historically, ovarian carcinomas were treated as a single disease and classified only by stage. However, research has shown that epithelial OCs are a heterogeneous group, differing in precursors, genetic mutations, pathophysiology, immune evasion mechanisms, dissemination patterns, treatment responses, and subsequently prognoses [12, 38, 39].

HGSC is the most prevalent subtype, comprising approximately 70% of epithelial OCs [38, 40]. It mainly originates from the fallopian tube epithelium, with serous tubal intraepithelial carcinoma being the precursor lesion. Clinically, HGSC often involves both ovaries and can grow to extensive sizes. The prognosis is poor, with a five-year survival rate of 30–40% for advanced stages, despite aggressive surgical and chemotherapeutic treatment [40].

LGSC accounts for 3–5% of epithelial OC and differs significantly from HGSC in its molecular and clinical characteristics [38, 40]. It usually arises from serous borderline tumors, progresses slowly, and is often diagnosed at an earlier stage. LGSC is frequently unilateral and is notably resistant to conventional chemotherapy. Its prognosis is more favorable than HGSC, with five-year survival rates exceeding 50% for early-stage disease [40].

Endometrioid carcinoma constitutes 10–15% of epithelial OC and is strongly associated with endometriosis and Lynch syndrome [38, 40]. It tends to be diagnosed at earlier stages compared to HGSC, resulting in better outcomes. The five-year survival rate exceeds 80% for early-stage disease, but decreases markedly in advanced stages [40].

Clear cell carcinoma accounts for approximately 10% of epithelial OC and is also strongly linked to endometriosis [38, 40]. Clinically this type often presents at an early stage, but advanced disease is associated with poor outcomes due to resistance to platinum-based chemotherapy. Five-year survival rates are high (> 80%) for early-stage tumors, but are significantly lower in advanced stages [40].

Mucinous carcinoma accounts for less than 5% of epithelial OCs and is characterized by large, unilateral cystic masses [38, 40]. It originates from mucinous borderline tumors and exhibits signs of gastrointestinal differentiation. Mucinous carcinoma often presents as a large symptomatic abdominal mass, and early-stage disease has an excellent prognosis, with five-year survival rates exceeding 90% [40].

Sex cord-stromal tumors make up approximately 2–6% of OCs and arise from the ovarian stroma [38, 40]. These include granulosa cell tumors and Sertoli-Leydig cell tumors. They frequently produce estrogen, leading to symptoms such as abnormal uterine bleeding or endometrial hyperplasia. Prognosis is generally favorable, although recurrences can occur decades after treatment [40].

Germ cell tumors comprise 2–3% of OCs and include dysgerminomas, yolk sac tumors, and immature teratomas [38, 40]. These tumors mainly affect younger women and have an excellent prognosis, especially when diagnosed at an early stage [40].

Mesenchymal tumors are rare, constituting about 1% of OC. These tumors, which include leiomyosarcomas, endometrioid stromal sarcomas, and undifferentiated sarcomas, originate from the ovarian stroma. They tend to be aggressive and are associated with poor outcomes [40].

2.3. Current OC treatment and perspectives

The standard treatment for OC has evolved over recent years. Current treatment methods include surgery, chemotherapy, and more recently targeted therapy and immunotherapy. Despite significant achievements, the prognosis of advanced OC remains poor [12]. New treatment strategies are continually being investigated with the aim to improve outcomes for OC patients [5, 41, 42].

2.3.1. Surgical treatment in OC

Surgery remains the cornerstone of OC treatment. Its primary purpose is complete cytoreduction – the removal of all visible tumor tissue [12]. It is especially important in advanced-stage OC, where the presence of residual tumor tissue after surgery is related to poorer prognosis [13]. Classically cytoreductive surgery can be categorized as complete (no residual tumor tissue), optimal (residual tumor nodules < 1 cm in diameter), and suboptimal (residual tumor nodules > 1 cm in diameter) [43]. Currently it is accepted that complete cytoreduction is associated with significantly improved OS [12].

Current treatment strategies rely on the International Federation of Gynecology and Obstetrics (FIGO) staging system [44]. Complete surgical staging is mandatory in all OC cases to accurately assess the spread of the disease and establish the correct stage, which helps to guide further management [12]. In early-stage OC (FIGO stages I and II), surgery is typically curative, even with fertility-sparing options for younger patients when necessary [12]. In advanced-stage disease (FIGO stages III and IV), surgery is often combined with neoadjuvant chemotherapy (NACT) to shrink the tumor tissue when achieving complete cytoreduction is unlikely with primary debulking surgery (PDS). The use of NACT followed by interval debulking surgery (IDS) has shown comparable outcomes to PDS, particularly in cases where complete cytoreduction is unlikely to be achieved by PDS. Both methods have their advantages if selected properly [12, 45].

Surgery may not be an option in cases of extensive disease spread or in patients with poor performance status. In such cases, palliative care, including chemotherapy and targeted therapies, becomes the priority [12].

2.3.2. Chemotherapy in OC

Chemotherapy is another critical component of OC treatment, especially in advanced-stage and recurrent cases. The first-line chemotherapy regimen typically consists of a platinum-based agent (cisplatin or carboplatin) and paclitaxel. This combination significantly prolongs PFS and OS in OC patients [12]. However, one of the major challenges in OC treatment is chemoresistance. Primary resistance to platinum-based agents occurs in approximately 25% of OC patients, and 75–80% of patients develop resistance to platinum therapy at some point during treatment, leading to relapse and death [46].

Platinum-sensitive patients generally respond well to subsequent chemotherapy, while platinum-resistant patients face a much poorer prognosis and have fewer treatment options. In these cases, other chemotherapeutic agents

(e.g. pegylated liposomal doxorubicin, gemcitabine) or biological therapy (e.g. bevacizumab) are used [12, 46].

2.3.3. Established targeted therapies for OC

Among the most significant advances in OC treatment are targeted therapies, which have transformed OC management. One example is the class of poly ADP-ribose polymerase (PARP) inhibitors, which are now well-established in OC treatment. PARP inhibitors (e.g., olaparib, niraparib, rucaparib) have shown efficacy in extending PFS, especially as maintenance therapies following chemotherapy for patients with breast cancer gene (BRCA) mutations or homologous recombination deficiency-positive patients [12, 47].

Bevacizumab, an anti-angiogenic agent targeting vascular endothelial growth factor (VEGF), has also been integrated into standard treatment protocols. When combined with chemotherapy, bevacizumab improves PFS; however, its effect on OS is modest. It is often used in recurrent and platinum-resistant OC cases [5, 12].

2.3.4. Investigational and emerging therapies for OC

While targeted therapies like PARP inhibitors and bevacizumab are now part of routine care, many other investigational therapies are in various stages of clinical trials, including immunotherapy and novel targeted treatments. Immunotherapy, particularly immune checkpoint inhibitors, has shown promising results in OC treatment. Immune checkpoint blockade targets regulatory proteins, such as programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1), which tumors use to suppress the immune response and escape elimination [16]. PD-1/PD-L1 checkpoint inhibitors, such as pembrolizumab and nivolumab, are currently being evaluated for OC treatment. Early-phase trials have shown some benefits, particularly in combination with other therapies, such as PARP inhibitors or bevacizumab. While single-agent checkpoint inhibitor therapies have not shown strong efficacy in OC, a combination of these therapies may be required for optimal results [48].

Targeting the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) and Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways, which are frequently dysregulated in OC, is another area of ongoing research [5]. Inhibitors of these pathways have shown early signs of efficacy in preclinical and clinical trials [49, 50]. As these pathways are integral to cancer cell survival and proliferation, their inhibition could offer new opportunities for treating OC, especially in resistant cases [51].

Recent studies have also focused on adoptive cell therapies, such as tumor-infiltrating lymphocytes (TILs) therapy and chimeric antigen receptor (CAR) T cell therapy, although these remain in the early stages of development for OC. However, existing data show that adoptive cell therapies are a highly promising treatment approach and will likely take part in future personalized OC treatment [41, 52].

Hyperthermic intraperitoneal chemotherapy (HIPEC) has recently regained attention and is an emerging treatment option for OC, particularly for patients undergoing IDS [12]. In this approach, heated chemotherapy is delivered directly into the abdominal cavity during surgery, with the goal of eliminating microscopic residual disease [12, 53]. Current clinical trials have shown that HIPEC may improve OS by nearly 12 months in advanced-stage OC [54, 55]. However, its use is still limited to selected centers and specific scenarios, and more evidence is still needed to clarify its role in OC treatment [12, 56].

Antibody-drug conjugates are another emerging class of therapeutics in OC treatment. These agents consist of an antibody specific to cancer cells attached to a potent cytotoxic drug. This combination allows targeted delivery of chemotherapeutic drugs directly to cancer cells while minimizing systemic toxicity. Antibody-drug conjugates have the potential to improve the management and prognosis of OC, especially for those with platinum-resistant disease, as demonstrated by recent clinical trials and ongoing research [57].

In addition to the methods already discussed above, several other approaches for OC treatment are emerging. In addition to CAR T cell therapy, CAR NK cells are gaining attention due to their ability to target specific OC antigens, such as mucin-16 (MUC16) and folate receptor-1. Bispecific antibodies, like MUC16xCD3, are another promising development, designed to engage T lymphocytes in destroying cancer cells. Vaccine therapies targeting tumor-associated antigens, along with checkpoint inhibitors (targeting CTLA-4, TIM-3, and LAG-3), are also under investigation. Many of these therapies are currently in clinical trials [58].

The common aspect of these innovative approaches is the employment of the immune system against OC. Given the strong connection between OC and the immune response, these approaches are undoubtedly promising [15, 16]. As we await further validation from ongoing studies, the search for the optimal OC treatment continues.

2.4. OC and immune response

The immune response plays a pivotal role in shaping the TME and influencing OC progression. Both innate and adaptive immune cells interact with OC cells, either promoting tumor destruction or contributing to immune evasion and tumor growth [15, 16]. The balance between pro-inflammatory and immunosuppressive signals within the TME determines the effectiveness of the immune response in controlling OC growth. Knowledge about these interactions is essential for improving OC treatment and patient outcomes [16, 59].

Immune cells from peripheral blood are attracted to the TME by signals from the tumor tissue, where they influence TME formation. Recent studies confirm the importance of peripheral blood-derived immune cells in shaping the OC TME [60, 61]. The complex and multifactorial nature of these interactions makes OC progression difficult to understand and may explain why OC treatments often fail, even if the exact reasons remain unknown [62, 63].

2.4.1. Tumor microenvironment in OC

The TME in OC is composed of stromal cells, including cancer cells, immune cells, and extracellular matrix (ECM) components [64]. The TME serves as a medium where conditions for tumor growth, metastasis, and immune evasion are created. The immune cells within the TME are often manipulated by the tumor to create an environment that suppresses the antitumor immune response [16, 62]. Unlike tumors such as melanoma or non-small cell lung cancer, which have high levels of immune activity, the OC TME is highly immunosuppressive. This classifies OC as a “cold” tumor, despite significant immune cell infiltration [58].

In addition to cancer cells, tumor stroma includes cancer-associated fibroblasts (CAFs) and endothelial cells, which contribute to tumor progression through various mechanisms [16]. CAFs, originating from mesenchymal cells, play a role in remodeling the ECM and secrete proteins that support tumor growth [65]. They promote tumor progression by expressing metalloproteinases and cytokines such as C-X-C motif chemokine ligand 14 and C-X-C motif chemokine ligand 12, as well as through other immune suppression and angiogenesis enhancement mechanisms [65–67]. Endothelial cells, which line blood vessels, participate in angiogenesis. In response to pro-angiogenic factors like VEGF and fibroblast growth factor, they form new blood vessels that supply tumors with nutrients and oxygen [16]. Endothelial

cells also participate in immune evasion by regulating immune cell trafficking and immune checkpoint molecule expression [68].

2.4.2. Tumor microenvironment: immune cells

Immune cells in the TME include macrophages, DCs, neutrophils, mast cells, MDSCs and lymphocytes [15, 16].

The monocyte–macrophage cell lineage

The monocyte-macrophage lineage significantly contributes to OC progression, with tumor-associated macrophages (TAMs) being key components of the innate immune response [69]. TAMs are the most abundant immune cells in the OC TME, constituting up to 39% of all immune cells in OC tissue [70]. These cells originate from circulating monocytes, which are attracted to the tumor site by chemokines and growth factors secreted by tumor and stromal cells. After infiltrating the tumor, they differentiate further into macrophages [71].

Macrophages are classified into two main types based on the cytokine environment they encounter: classically activated (M1) and alternatively activated (M2) macrophages [72]. M1 macrophages, characterized by high levels of interleukin 12 (IL-12), interleukin 23 (IL-23), tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and interleukin 6 (IL-6), exhibit pro-inflammatory properties and are effective at eliminating microbes and tumor cells. In contrast, M2 macrophages express lower levels of interleukin 1β (IL-1β) and IL-23, but higher levels of interleukin 10 (IL-10), tumor growth factor β (TGF-β), and specific receptors such as mannose and scavenger receptors. M2 macrophages are anti-inflammatory, suppress cytotoxic T lymphocyte (CD8+ T cell) responses, promote angiogenesis, tissue remodeling, and facilitate tumor growth [69, 72].

Studies suggest that macrophages in OC tissue predominantly adopt the M2 phenotype and express associated markers [73]. In OC, TAM composition is reported as 25% non-classified (undifferentiated) macrophages, 20% M1, 51% M2, and 4% monocytes [70]. The balance between M1 and M2 macrophages has been linked to clinical outcomes not only in OC but also in other tumors, such as breast, colorectal, gastric, liver, and kidney cancers [74]. A higher M1-to-M2 macrophages ratio correlates with better PFS [75, 76]. Additionally, high overall infiltration of TAMs in OC tissue has been correlated with poor prognosis and resistance to therapies [75, 77].

TAMs' role in OC metastasis formation has also been a field of interest. Several mechanisms have been identified, including the secretion of tumor necrosis factor β (TNF-β), which activates the PI3K/AKT pathway to enhance cell migration and invasion; the production of immunosuppressive

factors such as IL-10 and TGF-β, which activate the STAT3 pathway to promote tumor survival and metastasis; and the release of matrix metalloproteinases, which degrade the extracellular matrix, facilitating tumor invasion [69, 78]. Moreover, TAMs contribute to chemotherapy resistance through various direct and indirect mechanisms [79].

In conclusion, TAMs play an undeniable role in OC progression. They promote immunosuppression within the TME, drive disease progression, contribute to chemoresistance, and facilitate metastasis formation.

TILs

TILs are lymphocytes that have left the bloodstream and migrated into the tumor. The presence of TILs within the tumor is generally associated with a more favorable prognosis, as they indicate that the immune system is attempting to target the tumor [15, 80, 81]. However, in OC, the function of TILs is often impaired due to the immunosuppressive TME [58]. TILs are a heterogeneous population, including CD3+ T cells, CD19+ B cells, and NK cells.

Among these, CD3+ T cells are a major component, making up about 29% of all immune cells in the TME [70, 82]. These cells develop in the thymus and are considered naïve before encountering their first antigen. Once exposed to an antigen, these naïve cells become activated and begin to differentiate into specialized T cells [83]. There are three main functionally distinct subsets: T helper lymphocytes (CD4+ T cells), CD8+ T cells, and regulatory T lymphocytes (Tregs), each contributing to the immune response against tumors [15, 81].

CD4+ T cells regulate immune responses by releasing cytokines that either promote or inhibit inflammation. These cells are further divided into T helper lymphocytes type 1 (Th1) and type 2 (Th2) subsets. Th1 cells produce pro-inflammatory cytokines that help CD8+ T cells and macrophages target and eliminate tumor cells. Th1 cells also promote the recruitment of dendritic cells and reinforce their antitumor function [16, 62]. In contrast, Th2 cells release anti-inflammatory cytokines, which can contribute to tumor progression by suppressing the immune response [16, 62]. Evidence links certain gene expression patterns in OC to Th2 cell signatures, which are associated with poorer prognosis and shorter OS [84]. Studies have also shown a survival benefit in OC patients with higher overall numbers of CD4+ T cells in the TME [85].

Another subpopulation of CD3+ T cells is CD8+ T cells. These cells recognize cancer-specific antigens presented by tumor cells and trigger local inflammation by releasing cytokines such as interferon γ (IFN-γ) and TNF-α. This response recruits other immune cells to enhance antitumor activity,

making CD8+ T cells essential for adaptive antitumor immunity [62]. The presence of CD8+ T cells among TILs has been shown to correlate with improved OS in OC patients [86, 87]. However, in OC, CD8+ T cells often become dysfunctional or "exhausted" and lack their primary function. This happens due to prolonged antigen exposure and the influence of immunosuppressive cytokines, such as IL-10 and TGF-β [15, 88].

Under normal conditions, Tregs suppress immune responses and maintain tolerance, preventing autoimmune reactions. They originate from the thymus or can be induced peripherally from naïve CD4+ T cells [15, 89]. In OC, high numbers of Tregs in the TME are generally linked to more aggressive tumor behavior, higher metastatic potential, and lower OS rates [90, 91]. This is because Tregs inhibit the immune system's ability to target the tumor by suppressing effector immune cells like CD8+ T cells [89]. Increased Tregs in the TME are also associated with decreased immune cell infiltration, downregulated immune checkpoint expression, and poorer responses to immunotherapy, allowing the tumor to grow and spread more easily [91].

Primary functions of CD19+ B cells include antigen recognition, antibody production, and immune memory formation. Additionally, they act as antigen-presenting cells, which activate adaptive immunity [92]. CD19+ B cells, specifically mature B lymphocytes (CD20+ B cells), are generally associated with favorable outcomes in OC [93]. The presence of these cells, along with CD8+ and CD4+ T cells, contributes to the formation of tertiary lymphoid structures in the tumor stroma, which are linked to improved prognosis [94]. Another study demonstrated a close relationship between B lymphocytes and other immune cells, showing that the presence of CD20+ B cells alongside CD8+ T cells in HGSC was associated with better patient survival compared to cases where only CD8+ T cells were present [95]. However, some data suggest that high CD19+ B cell infiltration can correlate with poor outcomes [96, 97]. This discrepancy highlights the importance of evaluating both the quantity and subtype of B lymphocytes to fully understand their clinical relevance in OC [62].

NK cells are another component of the innate immune system. Unlike CD3+ T cells and macrophages, which make up the majority of immune cells in the OC TME, NK cells represent only about 5% of all immune cells in OC tissue [15]. In addition to other functions, NK cells can eliminate tumor cells using perforin and granzymes, without prior sensitization [58, 63]. They are regulated by a balance of activating (e.g., CD16, NKp30, NKG2D) and inhibitory signals from major histocompatibility complex proteins on healthy cells. However, OC cells can evade NK cell-mediated lysis through the downregulation of stress ligands and the upregulation of inhibitory molecules

[63]. In OC, NK cells are primarily located in the tumor stroma and malignant ascites. Higher levels of NK cell infiltration are generally associated with improved prognosis for patients [98, 99]. Although conflicting data exist, this is most likely due to the different subtypes of NK cells. For example, higher infiltration of CD56+ NK cells correlates with better outcomes in OC patients [98, 100], while the presence of CD16+ NK cells is associated with a worse prognosis [97]. Despite the recognized importance of NK cells in OC progression, further research is needed to fully understand their role and therapeutic potential [63].

Neutrophils

Neutrophils are another part of the innate immune system. They are initially involved in defending the body against infections and tissue damage. They show significant plasticity, with both pro- and anti-tumor functions [63]. In the OC TME neutrophils can be divided into two subtypes: N1 and N2. N1 neutrophils support the immune response by attacking tumor cells, while N2 neutrophils promote immune suppression, tumor progression, and metastasis formation [101]. In OC, neutrophils contribute to disease progression by forming NETs, which enhance the seeding of tumor cells within the peritoneal cavity [102]. Their interactions with the TME can shift the immune response towards tumor promotion, especially in advanced stages of the disease [103]. Moreover, various immune cell ratios are studied to improve the management of OC patients. Neutrophils are also included in one of these ratios – the neutrophil-to-lymphocyte ratio (NLR). Numerous studies have demonstrated that a higher NLR in peripheral blood is associated with worse PFS and OS in OC patients [104].

DCs, MDSCs and mast cells

DCs are specialized immune cells responsible for antigen presentation to lymphocytes, thus initiating and controlling the adaptive immune response [105]. In OC, DCs play various roles depending on the stage and subtype of the tumor. In early tumor development, certain DC subsets activate CD8+ T cells, which promote antitumor activity. In later stages, DCs become more immunosuppressive, contributing to tumor progression [106]. However, DCs are not a homogeneous group, as they consist of multiple subtypes with distinct functions. For example, the presence of plasmacytoid DCs in OC tissue contributes to immunosuppression and is linked to worse outcomes [107]. In contrast, the presence of mature migratory DCs that activate CD8+ T cells is associated with a better prognosis [108]. Although DCs account for only about 6% of the immune cells in the OC TME [70], they are gaining attention as targets for DC-based vaccines aimed at strengthening long-lasting antitumor immune responses. Further research is needed to fully

understand the roles of different DC subtypes in OC to develop effective therapies [109].

MDSCs are a diverse population of myeloid cells that originate from bone marrow stem cells. Under physiological conditions, MDSCs regulate immune responses, playing protective roles in autoimmune diseases, allergies, and organ transplantation. However, in certain infectious diseases and cancers, including OC, MDSCs have a tumor-promoting role [110]. They achieve this by accumulating within the TME, inhibiting immune cells such as CD3+ T cells, and enhancing the activity of cancer stem cells [111]. As a result, in the past few years, MDSCs have been receiving considerable interest and have become a target for the design of new treatment options [112].

Mast cells, which also originate from bone marrow progenitor cells, migrate to peripheral tissues, including the OC TME, where they mature. Mast cells have been shown to have multiple roles during OC progression, but they predominantly exhibit tumor-inhibiting effects by slowing tumor growth, inhibiting cancer cell migration, and modulating the TME. However, some studies suggest that mast cells could have a pro-tumor role in OC, particularly in murine models, which highlights the need for further investigation into this field [113].

2.4.3. Tumor microenvironment: extracellular matrix

The ECM components in OC influence cancer cell behaviour, immune responses, therapeutic resistance and consequently tumor progression. The ECM is composed of fibrous proteins like collagens, elastin, fibronectin, laminins, and proteoglycans. Its structure is dynamic and constantly remodeled, providing mechanical and biochemical support to tumor cells, which allows their proliferation, migration, and invasion [114, 115]

Collagens, particularly collagen I and XI, are the main components of the ECM in OC. Collagen I supports cancer cell adhesion and migration, functioning as a guiding mechanism that promotes directed movement, especially in metastatic cells. It is also linked to chemoresistance, particularly in response to paclitaxel treatment [116, 117]. Collagen XI is associated with cancer progression and poor prognosis, especially in advanced stages of OC. Its overexpression strongly correlates with metastasis formation [116]. Other fibrous proteins, such as fibronectin and tenascin, also contribute to the invasive nature of OC by promoting cell adhesion, migration, and metastasis [116, 117].

ECM remodeling is one of the mechanisms aiding tumor progression. This process is mediated by enzymes such as matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, and lysyl oxidase. Matrix

metalloproteinases remodel the ECM, allowing cancer cells to invade surrounding tissues, while lysyl oxidase contributes to ECM stiffening, which supports tumor cells and enhances resistance to treatments [114, 116]. Detached tumor cells in the peritoneal cavity also interact with specific ECM components, like laminin and fibronectin, which help cancer cells adhere to surfaces. These proteins are overexpressed at metastatic sites [115].

Hypoxia within the TME further accelerates ECM remodeling. It triggers the expression of hypoxia-inducible factors and promotes the secretion of ECM modifying enzymes, creating a more aggressive and immune-evasive tumor environment [114, 118]. Tumor cells in hypoxic conditions also release micro ribonucleic acids (RNAs) that regulate ECM components and their remodeling enzymes, affecting cell-cell and cell-matrix interactions [118].

The ECM can modulate immune responses not only by physically restricting immune cell infiltration, but also by creating an immunosuppressive environment that prevents effective antitumor immunity. For instance, the ECM components directly influence TAMs, promoting an M2-like phenotype, which supports tumor progression and immune suppression [118].

Overall, the ECM in OC does not act only as a supportive structure but actively participates in tumor development as well. Given its role in tumor growth, metastasis formation, immune evasion mechanisms, and therapeutic resistance, it may become a target for new treatment strategies [119].

2.4.4. Molecular immune evasion mechanisms in OC

Immune evasion is one of the most significant characteristics of OC. Cancer cells use various mechanisms, including immune checkpoints, to suppress the immune response, allowing them to avoid elimination [15]. In healthy individuals, these immune evasion mechanisms are needed to prevent autoimmunity [120]. Among these, the PD-1/PD-L1 pathway is a significant immune checkpoint promoting immune tolerance within the TME and is currently the most studied immune evasion pathway in OC immunotherapy [121].

In the PD-1/PD-L1 pathway, immune suppression occurs when cancer cells express PD-L1 on their surface, which binds to PD-1 receptors on CD3+ T cells, leading to the suppression of T cell function and reducing their ability to eliminate tumor cells [22, 62]. Approximately 30% of OC tumors express significant levels of PD-L1 [58]. Blocking this pathway with inhibitory agents was initially considered a promising strategy for OC treatment, but so far, only limited efficacy has been observed, likely due to the complex nature of immunosuppressive mechanisms in OC [48, 121]. However, combinations of these inhibitory agents with other medications show more promising results [48].

In addition to the PD-1/PD-L1 pathway, OC immune evasion also involves other immune checkpoints and mechanisms. OC cells can upregulate inhibitory receptors, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte activation gene-3 (LAG-3), and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), further weakening T cell responses [58]. Physiologically, CTLA-4 suppresses both effector and regulatory immune cells to prevent excessive inflammation. CTLA-4 inhibitors have been developed to enhance T cell expansion and antitumor responses and are already utilized in certain cancer treatments [122, 123]. LAG-3 is a co-inhibitory receptor found on several immune cells, including activated CD4+ and CD8+ T cells, Tregs, NK cells, and DCs. When triggered LAG-3 signaling works together with the PD-L1 pathway and weakens CD4+ and CD8+ T cell functions, induces their apoptosis, and promotes Treg proliferation [58]. TIM-3 is another immune checkpoint receptor expressed on dysfunctional CD4+ and CD8+ T cells, Tregs, NK cells, M2 macrophages, and DCs [124]. It interacts with CD3+ T cells, causing T cell exhaustion and boosting the immunosuppressive activity of Tregs [125].

Despite of extensive knowledge about these immune evasion mechanisms and attempts to create novel therapeutics, they have not yet been proven to work effectively in OC treatment [48, 58].

2.4.5. Cytokines and OC

Cytokines are another inseparable part of the immune system, acting as mediators that regulate both pro-inflammatory and anti-inflammatory responses. In the context of OC, a variety of cytokines within the TME contribute to mechanisms of immune evasion and immune activation, which influence tumor growth and progression [15, 16]. While primarily secreted by immune cells, some cytokines can also be secreted by OC cells, leading to paracrine and autocrine effects [21].

Among all the cytokines implicated in OC progression, in our study we have focused on the most relevant ones. Cytokines can generally be categorized as either pro-inflammatory or anti-inflammatory based on their mechanism of action. The pro-inflammatory cytokines selected for our investigation include IL-1β, IL-6, IL-12, and TNF-α [15, 21]. The anti-inflammatory cytokines examined include interleukin 4 (IL-4) and IL-10 [15, 58].

Among the studied cytokines, IL-1β and IL-6 appear to create the strongest tumor-promoting effects in OC, although they originally have immune-activating properties [126-129]. Initially, TNF-α was believed to have tumor-inhibiting properties due to its ability to induce tumor cell apoptosis and activate immune responses [130]. However, recent studies in the context of OC

have shown that TNF-α primarily acts as a tumor-promoting cytokine [16, 62, 131–133].

IL-4 and IL-10 are anti-inflammatory and induce immune-suppressive effects that promote tumor growth. Their impact on tumor progression is more indirect, and the data are somewhat conflicting compared to IL-1β and IL-6. Therefore, in terms of their influence on OC progression, they may be classified as moderately tumor-promoting cytokines [15, 16, 72, 134, 135]. Finally, IL-12 has well-documented tumor-inhibiting effects in OC. As a pro-inflammatory cytokine, it promotes M1 macrophage polarization, enhances Th1 responses, activates CD8+ T cells and NK cells, and drives other tumor-inhibiting mechanisms [16]. The summary of the investigated cytokines, their origin, physiological effects, and roles in OC progression is listed in Table 2.4.5.1.

Table 2.4.5.1. *Cytokines participating in OC progression*

Cytokine	Origin cells	Physiological effect	Role in OC
IL-1β	Monocytes/macrophages, neutrophils [136]	Pro-inflammatory; acute phase response, fever control, lymphocyte activation [21]	Tumor-promoting [127, 128]
IL-4	Basophils, CD4+ T cells [136]	Anti-inflammatory; enhances CD19+ B cell proliferation, antibody secretion, Th2 differentiation, M2 macrophage activation; controls allergies [21, 137]	Moderately tumor-promoting [16, 72, 134]
IL-6	Monocytes/macrophages, CD19+ B cells [136]	Pro-inflammatory; acute phase response, fever control [137]	Tumor-promoting [126, 129]
IL-10	Monocytes/ macrophages, CD3+ T cells, CD19+ B cells [136]	Anti-inflammatory; suppresses immune responses, inhibits cytokine production, regulates immune tolerance [138]	Moderately tumor-promoting [15, 16, 135]
IL-12	DCs, monocytes/ macrophages [136, 139]	Pro-inflammatory; promotes Th1 differentiation, supports NK cell activity, M1 polarisation; stimulates cell-mediated immunity [15, 16, 139]	Tumor-inhibiting [16]
TNF-α	Monocytes/macrophages, CD3+ T cells [136]	Pro-inflammatory; acute phase response, enhances immune cells migration and macrophages adhesion, CD3+ T cell activation [131, 132]	Tumor-promoting [16, 62, 131–133]

IL-1β, interleukin 1β; IL-4, interleukin 4; IL-6, interleukin 6; IL-10, interleukin 10; IL-12, interleukin 12; TNF-α, tumor necrosis factor α; CD3+ T cells, T lymphocytes, CD4+ T cells, T helper lymphocytes; Th1, type 1 T helper lymphocytes; Th2, type 2 T helper lymphocytes; CD19+ B cells, B lymphocytes; NK cells, natural killer cells; DCs, dendritic cells; M1, classically activated monocytes/machropages; M2, alternatively activated monocytes/macrophages.

2.4.6. PBMCs – source of anticancer immunity

PBMCs are a heterogeneous group of immune cells circulating in the blood. This group of cells includes lymphocytes, monocytes and DCs. PBMCs are characterized by their single, round nuclei, distinguishing them from other blood cells, such as red blood cells (RBCs) and polymorphonuclear leukocytes (e.g., neutrophils), which have multi-lobed nuclei [140]. PBMCs have a unique density, which is different from that of the rest of blood cells. Thus, they can be isolated from whole blood using a technique called density gradient centrifugation, often with Ficoll-Paque [140, 141]. The majority of cells within the PBMC population are lymphocytes. Usually, lymphocytes make up 70–90%, monocytes account for 10–20%, and DCs account for 1–2% of all PBMCs. Among the lymphocytes, CD3+ T cells are the most prevalent (70–85%), followed by NK cells (2–20%) and CD19+ B cells (5–10%). Within the CD3+ T cell group, the ratio of CD4+ T cells to CD8+ T cells is generally around 2:1 [140].

These cells have been a focus for immune system evaluation in OC for a while. The TME of OC is well documented, with extensive data on its various components, including immune cells [15, 16, 62]. However, there is less data on the involvement and significance of peripherally obtained PBMCs in the progression of OC [142]. Additionally, most research on antitumor immunity tends to focus solely on either localized immune responses within the TME or broader systemic responses. Consequently, there is a notable gap in studies that integrate immune system analyses across blood, ascites, and tumor tissue [142]. Some studies investigating these aspects have found differences in cell ratios and activity when comparing peripheral lymphocytes and TILs. For instance, TILs appear to be more suppressed than their peripheral counterparts [143, 144]. Moreover, other studies even reported a lack of correlation between and differences in certain cell population sizes in blood and tumor tissue samples [144, 145].

Despite the discussed differences, PBMCs should not be dismissed as insignificant in the context of OC. It is evident that peripherally obtained immune cells differ in their quantities when comparing patients with OC and those with benign pathology [17]. Furthermore, PBMC levels have been shown to independently correlate with disease aggressiveness, patient survival, and other outcomes [17, 142, 146]. Specific immune ratios derived from PBMCs, such as the lymphocyte-to-monocyte ratio (LMR), NLR, CD4+ T cell to CD8+ T cell (CD4/CD8) ratio, platelet-to-lymphocyte (PLR), and M1-to-M2 monocyte (M1/M2) ratio, have also been associated with patient outcomes, highlighting their potential utility in OC management [75, 76, 104, 142, 147, 148].

Although there are differences between peripheral immune cells and those within the TME of OC, studies suggest that changes in PBMC proportions and ratios can often be interpreted in a similar way. Additionally, PBMCs are useful for predicting outcomes in other cancers as well [149]. Evidence suggests that both local and systemic immune mechanisms are essential for robust antitumor responses, so PBMCs should be taken independently as relevant participants in OC progression [142]. A summary of PBMCs' role in OC progression and their association with OC prognosis is listed in Table 2.4.6.1.

Table 2.4.6.1. *PBMC-derived immune cells' association with OC progression and prognosis*

Cells	Role in OC progression	Association with OC prognosis
CD3+ T cells	Function depends on subtype.	Tumor infiltration and peripheral blood levels are associated with better prognosis [81, 142, 150, 151].
CD4+ T cells	**Th1**: releases pro-inflammatory cytokines, recruits and activates immune cells (**tumor-inhibiting**). **Th2**: releases anti-inflammatory cytokines, indirectly contributes to immunosuppressive environment (**tumor-promoting**). **Tregs:** releases anti-inflammatory cytokines and other immune suppressive factors, inhibits CD8+ T cell activity (**tumor-promoting**) [15, 16, 62].	Th1 infiltration is associated with better prognosis. Th2 infiltration is associated with poorer prognosis in some cases (conflicting data). Treg infiltration and blood levels are generally associated with worse prognosis [142, 152, 153].
CD8+ T cells	Cytotoxic after tumor-specific sensitization; releases pro-inflammatory cytokines, recruits immune cells. Overall **tumor-inhibiting** [15, 16, 62].	High infiltration is associated with better prognosis [142].
CD19+ B cells	Produces antibodies, recruits immune cells, presents antigen. Roles vary by subtype [62].	Prognostic value depends on subtype; thus, tumor infiltration shows variable effects. Higher overall blood levels can be associated with better outcomes [62, 142].
NK cells	Cytotoxic without prior sensitization; releases pro-inflammatory cytokines. Overall **tumor-inhibiting** [15, 16, 62].	Tumor infiltration is associated with better prognosis; subtype-dependent effects observed. Low blood levels are associated with poor prognosis [100, 142].

Table 2.4.6.1. *continued*

Cells	Role in OC progression	Association with OC prognosis
Monocytes/ macrophages	**M1:** cytotoxic; releases pro-inflammatory cytokines and reactive oxygen species, strengthens T cell and NK cell activity. Overall **tumor-inhibiting.** **M2:** releases anti-inflammatory cytokines; promotes angiogenesis, tissue remodeling, immunosuppressive environment. Overall **tumor-promoting** [15, 16, 62].	Predominant M2 (but not overall TAMs) infiltration is generally associated with worse outomes [73]. High M1/M2 ratio in tumors and blood is associated with better prognosis [142].
DCs	Presents antigen, activates CD4+ and CD8+ T cells. Overall **tumor-inhibiting**. Tumor affected subtype – plasmacytoid DCs promotes Tregs and angiogenesis (tumor-promoting) [15, 62].	Overall DC infiltration is associated with better prognosis. High plasmocytoid DC infiltration and blood levels are associated with poor prognosis [62, 142].

PBMC, peripheral blood mononuclear cells; CD8+ T cells, cytotoxic T lymphocytes; TAMs, tumor associated macrophages; Tregs, regulatory T lymphocytes; M1/M2, M1-to-M2 monocyte ratio.

2.5. Impact of surgery on the immune response

Surgical removal of tumor tissue remains a cornerstone of solid cancer treatment, including OC [27]. In most cases, surgery is the primary treatment method, allowing subsequent therapies, such as chemotherapy, to be more effective. It is also an independent positive prognostic factor for OC patients, whether performed as PDS, IDS or secondary debulking surgery (SDS) [12].

Despite its benefits, surgical intervention disrupts the body's hormonal and immune balance, triggering a physiological stress response. These changes include the release of DAMPs, which activate immune responses and inflammation. The release of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, prostaglandin E2, initiates and sustains systemic inflammation and immunosuppression mechanisms. DAMPs and cytokines also attract a variety of immune cells to the surgical site. Surgical stress also activates the hypothalamic-pituitary-adrenal axis, increasing the production of cortisol and other stress hormones that suppress immune function. While such reactions are a natural part of the body's response to stress, they may also contribute to cancer progression [25, 27].

Surgical stress affects immune cell function in two main ways. First, it suppresses the activity of effector cells, which stimulate an active immune response or are a part of it [27]. Neutrophils, for example, change in subpopulation proportions and are attracted to the injury site, where they can form

NETs. Although NETs play a physiological role, they also support tumor cell survival and metastasis by creating a pro-tumorigenic environment. Surgical stress also alters macrophage behavior, shifting them to an M2 phenotype, and impairs NK cell functions, such as target recognition, cytokine production, and cytolytic activity. The quantity and activity of CD8+ T cells are similarly affected, showing increased exhaustion markers and reduced cytotoxic cytokine production [25, 27].

The second effect of surgical stress is the activation of immunosuppressive cells [27]. Tregs expand and show reduced apoptosis, leading to higher frequencies in peripheral blood. MDSCs also increase in numbers, impairing CD3+ T cell and NK cell function and promoting Treg activation [25, 27].

In addition to surgical trauma, several perioperative factors, including blood transfusions, hypothermia, postoperative complications, and anesthesia, can also influence the immune function [154]. Anesthesia plays a significant role in these changes. For example, opioid analgesics can suppress humoral and cellular immunity, particularly affecting NK cell activity and CD3+ T cell differentiation in a dose-dependent manner. They may also promote tumor growth by increasing angiogenesis. Similarly, volatile anesthetics like halothane and sevoflurane weaken immune responses by reducing NK cell function, increasing hypoxia-inducible factor 1-α expression, and promoting angiogenic factors like VEGF and TGF-β, which support tumor progression [155, 156]. While no clear advantage was found between intravenous and volatile anesthesia regarding cancer prognosis [157], a combination of general anesthesia and epidural analgesia was associated with improved outcomes in OC. This effect is probably due to the requirement for lower doses of general anesthetics [155].

The immunosuppressive state begins immediately after surgery and lasts for several days to several months. Shortly after surgery, cytokines such as IL-6, IL-8, and IL-10 increase, while IFN-γ levels decrease within a few hours. This leads to a reduced cellular immunity state that typically lasts about a week. While the immune system generally recovers within 14 days, some studies suggest that certain functions remain impaired for as long as six months [25]. A visualization of surgery-related immune dysregulation and benefits is presented in Fig. 2.5.1.

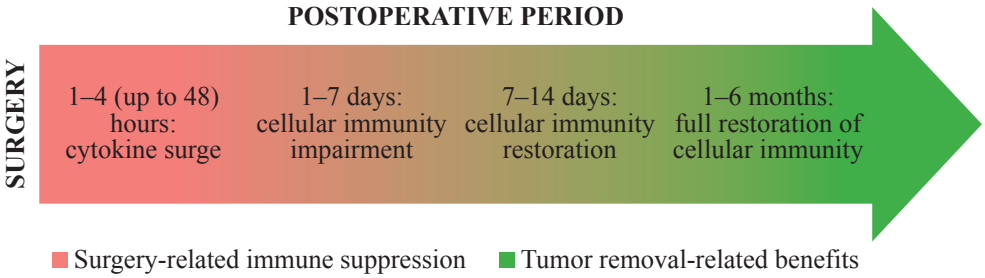


Fig. 2.5.1. *Immune dysfuncion following surgery*

The figure illustrates the sequence of immune alterations after major surgery in oncological patients. Cytokines IL-6, IL-8, and IL-10 increase, while IFN-γ levels decrease within the first 1–4 hours postoperatively and may remain altered for up to 48 hours. Cellular immunity impairment, characterized by reduced NK cell activity, lymphocyte function, and dendritic cell activity, peaks between 24–72 hours and persists for 1–7 days. Partial immune restoration begins within 7–14 days, with cytokine levels normalizing, NK cell and lymphocyte functions gradually recovering. Full recovery of cellular immunity, however, may take 1–6 months, depending on patient-specific factors. The transition from red to green in the figure represents the hypothetical shift from the period of surgery-related immune suppression to the period of benefits associated with tumor removal.

Many researchers support the theory of prolonged postoperative immune dysfunction, where some immune functions recover early, but the overall immune function may take longer to stabilize [25]. For example, studies have shown that phagocytic activity in monocytes and neutrophils can return to normal within 7–8 days after OC surgery [20, 158]. Napoletano et al. observed a reduction in Tregs and an increase in CD8+ T cells by the 15th day after surgery [18]. Brochner et al. reported that IL-6 and IL-10 levels, as well as NK cell function, improved but remained abnormal at 21 days post-surgery [159]. Wu et al. observed a decline in Tregs as early as two days postoperatively, but the positive effects were still unfolding for a month [160]. However, findings are inconsistent; one study even observed an increase in Treg proportions in cancer patients one week after surgery [161].

OC is characterized by its ability to relapse and metastasize [15, 16]. Generally, for cancer cells to metastasize, they must enter the bloodstream, evade immune defenses, and establish new tumors at distant sites. Surgery can inadvertently facilitate this process by causing tissue trauma, which increases the release of cancer cells into circulation. Elevated levels of circulating tumor cells before and during surgery are associated with a higher risk of cancer recurrence. Additionally, postoperative inflammation and immunosuppression create a supportive environment for circulating tumor cells to survive and colonize new tissues [156]. Animal studies and clinical data from other solid cancers, such as breast, lung, liver, pancreatic and colorectal

cancers, provide evidence for this mechanism. However, direct proof in OC is still lacking [28–30, 156, 162]

Surgical removal of the tumor ultimately has positive effects on the immune system. The extent of tumor removal correlates with the degree of immune restoration, with optimal cytoreduction being more effective at reversing immunosuppression than suboptimal surgeries. For instance, Nowak et al. demonstrated that optimal cytoreduction had a significantly stronger impact on improving the immune function [163]. Thus, surgical treatment of OC creates a complex, antagonistic interaction where the benefits of tumor removal are counteracted by the stress-related immune suppression.

Despite the availability of data on postoperative immune changes in OC, most studies focus on the recovery phase rather than the critical early postoperative period. Research varies widely in terms of patient selection, timing, and methods of evaluating immune responses, leading to inconsistent findings [161]. Critical questions remain, such as determining the peak period of postoperative immune suppression, its onset, and its significance for OC patients [25]. We propose that the early postoperative period in OC patients could represent a critical window for disease progression, despite the overall benefits of tumor tissue removal.

3. METHODS

3.1. Study design

This prospective observational study was conducted at the Laboratory of Surgical Gastroenterology, Institute for Digestive Research, Lithuanian University of Health Sciences, and Department of Obstetrics and Gynaecology, Hospital of the Lithuanian University of Health Sciences, Kaunas Clinics, between January 2021 and April 2023.

3.2. Ethics review

The study followed the guidelines of the Declaration of Helsinki and received approval from the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-16; approved on 5 February 2020).
Written informed consent was obtained from all participants.

3.3. Study population

Enrollment for the OC group began with patients who had a clinical diagnosis of OC, suspected to be at stage III–IV according to the FIGO staging system [44], and were scheduled for PDS. The complete inclusion and exclusion criteria for the OC group were as follows:

Inclusion criteria for OC group:

- Clinical or histological diagnosis of OC.
- OC stage III–IV according to FIGO [44].
- Planned surgical treatment.
- Age between 18 and 80 years.

Exclusion criteria for OC group:

- Histologically confirmed non-epithelial OC subtypes.
- Blood transfusion during the perioperative period or within the past month.
- Prior NACT.
- Active inflammation or infection requiring antibiotic therapy during the perioperative period or within the past month.
- Chronic inflammatory/autoimmune conditions (e.g., rheumatoid arthritis, systemic lupus erythematosus, etc.).
- Surgical intervention within the past month.

- History of malignancy of a different origin.
- Pregnancy.

The schematic overview of the OC patient inclusion process in the study is presented in Fig. 3.3.1.

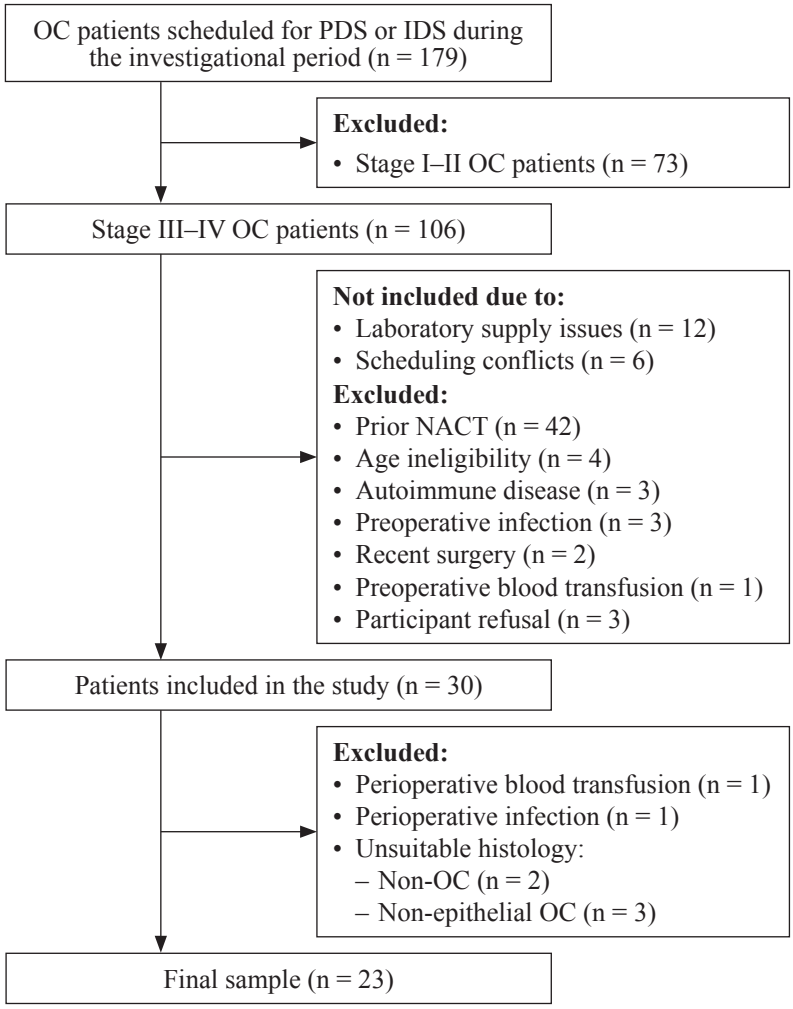


Fig. 3.3.1. *The flowchart of OC patient inclusion in the study*

The stages of OC are presented according to the 2021 FIGO staging system [44]. PDS, primary debulking surgery; IDS, interval debulking surgery.

The control group consisted of participants who were matched with the OC patients based on age and body mass index (BMI). The same exclusion criteria were applied to the control group. Additionally, controls were required to have no history of malignancy and no current suspicion of cancer.

However, non-malignant comorbidities (e.g., hypertension, type 2 diabetes) were permitted in both groups. An interview and a review of medical documentation were conducted to assess comorbidities and determine eligibility based on inclusion and exclusion criteria.

Before enrollment, all OC patients underwent a standardized diagnostic evaluation for OC, which included pelvic ultrasound, pelvic, abdominal, and chest computed tomography, and histological testing when necessary. All patients were confirmed to have OC through histopathological examination after PDS.

PDS procedures typically included a total hysterectomy with bilateral salpingo-oophorectomy, omentectomy, peritoneal biopsies, and, if necessary, pelvic and/or para-aortic lymph node biopsy or removal, as well as appendectomy, splenectomy, and resection of any visible tumor tissue. According to the residual visible tumor tissue after the surgery, PDS outcomes were classified as complete (no residual tumor tissue), optimal (residual tumor nodules < 1 cm in diameter), or suboptimal (residual tumor nodules > 1 cm in diameter) [43].

3.4. Sample size

Our study included 23 OC patients and 23 healthy participants. No prior sample size calculation was performed, primarily due to the difficulty in predicting the expected differences. Several factors contributed to this challenge. First, the study focused on the early postoperative period, which is poorly researched in OC patients. Second, our focus on peripherally obtained cells involved tests such as protein and mRNA expression in PBMCs, cytokine expression in monocytes, and analysis of monocyte subpopulations–fields with little to no prior documented data. This knowledge gap, combined with the study's primarily descriptive nature rather than a hypothesis-driven search for specific differences, made it difficult to estimate preliminary findings and determine a set sample size.

3.5. Blood sampling

Peripheral venous blood samples were collected from both OC patients and healthy controls. For OC patients, samples were taken before surgery and on days 1, 3, and 5 postoperatively. Insights from preliminary results led to the decision not to conduct certain tests on day 3. Blood sampling for healthy controls was a single event per participant, following the same assay protocol

as for the preoperative OC samples. A detailed schedule of blood sampling and tests performed for both groups is shown in Fig. 3.5.1.

Each blood sampling included either one 4 mL and three 10 mL vacutainers, or three 10 mL vacutainers, or one 10 mL vacutainer containing EDTA K2 (BD, Plymouth, UK), along with two 3.5 mL vacutainers with a serum clot activator (SST) from Weihai Hongyu (Weihai, China), depending on the specific day of sampling.

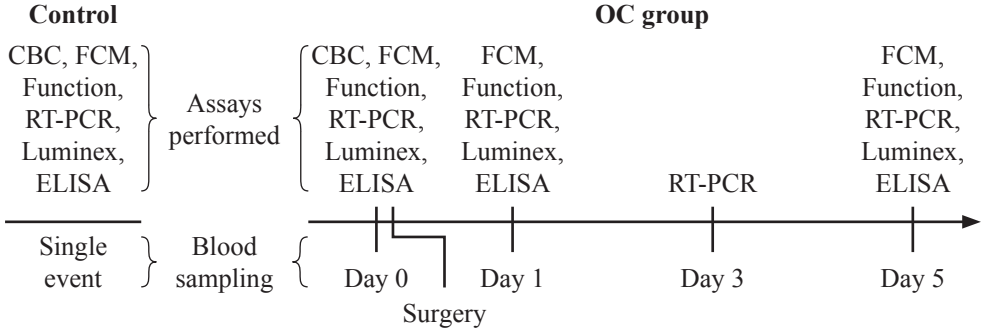


Fig. 3.5.1. *Blood sampling and assay schedule for study participants*

The lower part of the timeline shows the timing of blood collection for the control group and OC patients. The upper part outlines the assays performed at each sampling: CBC (complete blood count), FCM (flow cytometry), Function (PBMC functional and metabolic assessments), RT-PCR (real-time polymerase chain reaction), Luminex (assays performed by the Luminex system), and ELISA (enzyme-linked immunosorbent assay) for serum protein analysis.

3.6. Performed assays and laboratory procedures

3.6.1. Complete blood count assay

Venous blood samples drawn into 4 mL vacutainers containing K_2EDTA (BD, Plymouth, UK) were used for CBC analysis. The measurements were carried out using an automated hematology analyzer, UniCell DxH 800 (Beckman Coulter, Brea, CA, USA), following the manufacturer's guidelines. The analyzer provided data on CBC parameters, including red blood cell (RBC) count, hemoglobin concentration, platelet count, white blood cell (WBC) count, and differential leukocyte count.

3.6.2. PBMC isolation and serum preparation

Blood samples collected in 10 mL vacutainers containing K_2EDTA (BD, Plymouth, UK) were centrifuged at 2470 × g for 10 minutes to separate the plasma, which was carefully removed. PBMCs were then isolated using grad-

ient centrifugation with Ficoll-Paque PREMIUM medium (Cytiva, Uppsala, Sweden), following the manufacturer's instructions. The PBMC layer was carefully collected by pipetting and washed twice with phosphate-buffered saline (Sigma-Aldrich, Taufkirchen, Germany) at 250 × g for 5 minutes each.

After isolation from both OC patients and healthy controls, PBMCs were resuspended at a concentration of 1 million cells per mL and divided into 1 mL aliquots for further use. Separate aliquots of the PBMCs were used for PBMC functional evaluation by fluorometric or spectrophotometric assays and for flow cytometry (FCM). Another aliquot was preserved by mixing with RNAlater® (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −20 °C or −80 °C for real-time polymerase chain reaction (RT-PCR).

For serum preparation, blood collected in vacutainers with SST was allowed to clot for one hour. The samples were then centrifuged at 2500 × g for 20 minutes at 4 °C. The serum, located above the gel layer, was carefully transferred into cryogenic tubes and stored at −80 °C until analysis.

3.6.3. FCM analysis of lymphocyte and monocyte subsets

For lymphocyte immunophenotyping, FCM was performed using the BD Multitest 6-color TBNK kit (BD, Franklin Lakes, NJ, USA). The analysis identified key lymphocyte subsets, including CD3+ (T lymphocytes), CD3+/CD4+ (T helper lymphocytes), CD3+/CD8+ (cytotoxic T lymphocytes), CD19+ (B lymphocytes), and CD3−/CD16+CD56+ (NK cells).

Aliquots of PBMCs, each containing 1 million cells, were incubated with a mixture of monoclonal antibodies, including CD3-FITC (Leu-4), CD4 PE-Cy™7 (Leu-3a), CD8 APC-Cy™7 (Leu-2a), CD16 PE (Leu-11c), CD19 APC (Leu-12), CD56 PE (Leu-19), and CD45 PerCP-Cy5.5 (2D1), following the manufacturer's instructions. After staining, the cells were washed to remove excess antibodies and analyzed on a FACSLyric 10-color flow cytometer (BD, San Jose, CA, USA). For each sample, data were acquired for up to 10,000 events. Lymphocyte populations were gated based on their granularity (measured by side scatter) and CD45 expression. This approach allowed precise quantification of cells expressing specific antigens, enabling accurate identification of lymphocyte subsets.

To differentiate monocyte and macrophage subsets, PBMCs were stained with a more detailed panel of monoclonal antibodies, including CD3 PE (UCHT1), CD14 BV510 (MφP9), CD16 APC (B73.1), CD80 APC-H7 (L307.4), CD86 PE-Cy7 (2331 (FUN-1)), CD163 BV605 (GHI/61), CD206 FITC (19.2), HLA-DR PerCP (L243), CD19 PE (HIB19), CD56 PE (555516) and CD66b PE (G10F5). All antibodies were obtained from BD Biosciences (San Jose, CA, USA).

3.6.4. PBMC metabolic and functional activity assessment

Preparation of PBMCs and metabolic activity assessment

After isolation, PBMC samples were cultured in RPMI 1640 medium (Gibco Life Technologies Limited, Paisley, UK), which was free of phenol red and supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (both from Gibco Life Technologies Limited, Paisley, UK). Samples were incubated for 30 minutes at 37 °C before being distributed into dark and clear 96-well plates, with each used well receiving 90,000 cells suspended in the medium. Following a 3-hour activation with different effectors, plates were used in fluorometric and spectrophotometric assays to evaluate PBMC functional activity, specifically assessing phagocytosis and the production of reactive oxygen species (ROS) and nitric oxide (NO).

In parallel, cell viability (metabolic activity) was assessed by measuring resazurin metabolism. Clear well plates were used for metabolic activity evaluation using the AlamarBlue® assay (Thermo Fisher Scientific, Waltham, MA, USA). Comparable to the functional assays, three types of wells were prepared: those containing specific activators (as for PBMC function assays), negative controls, and media controls. The AlamarBlue® assay was conducted 3 hours post-activation, in line with the manufacturer's protocol, followed by spectrophotometric measurements using the Sunrise Absorbance Reader (Tecan, Grodig, Austria). Baseline cell viability was determined by measuring resazurin metabolism in wells without activators.

Phagocytosis assessment

Phagocytosis is a fundamental immune process in which cells, particularly macrophages and DCs, engulf and eliminate foreign particles or pathogens. Evaluating phagocytic activity gives valuable insights into immune cell function, particularly in the context of anticancer immunity, as it plays a role in clearing cancer cells and debris, thus creating an antitumor immune response [164, 165]. Lipopolysaccharide (LPS) (Sigma-Aldrich, Rehovot, Israel) was used to induce phagocytic activity in PBMCs. LPS was added to three wells of a dark 96-well plate, with each well receiving 10 µL of a 5 µg/mL solution. Negative controls were set up in three other wells using 10 µL of LPS diluent, while the remaining three wells acted as media controls, containing only RPMI and 10 µL of 5 µg/mL LPS without cells. After a 2-hour incubation period to stimulate phagocytic function, the plate was centrifuged, and the supernatant was carefully removed. Then, 100 µL of pHrodo Green Zymosan Bioparticles (Invitrogen, Eugene, OR, USA) was added to each well, followed by a 1-hour incubation at 37 °C. Fluorescence was measured using a Fluoroskan Ascent fluorometer (Thermo Fisher Scien-

tific, Waltham, MA, USA) with excitation and emission set to 510 nm and 538 nm, respectively.

ROS production assessment

The production of ROS is an important indicator of immune cell activity, as ROS play a role in signaling, inflammation, and pathogen defense. In anti-cancer immunity, ROS are significant due to their ability to mediate cytotoxic effects on tumor cells [164, 166]. To assess ROS production, PBMCs were activated with tert-butyl hydroperoxide (TBHP), an organic peroxide that induces oxidative stress. Cells were stained with 2,7 dichlorodihydrofluorescein diacetate (DCFDA) from the Cellular ROS Assay Kit (Abcam, Cambridge, UK) and subsequently treated with 10 µL of 2.5 mM TBHP (Abcam, Cambridge, UK), following the manufacturer's protocol. Using dark 96-well plates TBHP was added to three wells, while three additional wells served as negative controls, receiving 10 µL of TBHP diluent. Other three wells were designated as media controls, containing RPMI and 10 µL of 2.5 mM TBHP without cells. After a 3-hour incubation at 37 °C, fluorescence was measured using a Fluoroskan Ascent fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), with an excitation of 485 nm and an emission of 538 nm.

NO production assessment

NO plays a dual role in oxidative stress regulation, acting as a free radical and as a mediator of immune responses. Its involvement in immune reactions against cancer cells includes direct cytotoxic effects and the modulation of antitumor immunity [164, 167, 168]. NO production was assessed in clear 96-well plates after a 3-hour PBMCs activation with 10 µL of 5 µg/mL LPS (Sigma-Aldrich, Rehovot, Israel) and 100 mM L-arginine (Sigma-Aldrich, Tokyo, Japan). Three wells received the LPS and L-arginine mixture, three served as negative controls with the diluent, and three acted as media controls, containing RPMI with 10 µL of 5 µg/mL LPS and 100 mM L-arginine without cells. Following a 2.5-hour incubation at 37 °C, 100 µL of Griess reagent (Invitrogen, Eugene, OR, USA) was added to each well, and the plate was incubated at room temperature for 30 minutes. Absorbance was measured at 550 nm using the Tecan Sunrise spectrophotometer (Tecan, Grodig, Austria).

3.6.5. RNA extraction and RT-PCR

Total RNA was extracted from preprepared PBMC samples using an RNA extraction kit (Abbexa, Cambridge, UK), following the manufacturer's protocol. The quantity and purity of the isolated RNA were then measured by UV spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). To synthesize complementary deoxyribonucleic acid (cDNA),

2 μg of RNA was reverse-transcribed using the High/Medium-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) and processed on the 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA).

The amplification of specific RNA targets was carried out in a 20 μL reaction volume, containing 2 μL of template cDNA, 1 μL of primers, 10 μL of TaqMan™ Universal PCR Master Mix (Applied Biosystems, Waltham, MA, USA), and 7 μL of nuclease-free water. The primers used, all sourced from Applied Biosystems (Waltham, MA, USA), were as follows: IL-1β (Hs001555410), IL-4 (Hs00174122), IL-6 (Hs00174131), IL-10 (Hs00961619), PD-1 (Hs05043241), PD-L1 (Hs00204257), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs02786624). To verify amplification specificity, primer efficiency and melting curve analyses were conducted. Each experiment was run in triplicate, with additional repeats performed as required to confirm the accuracy of results.

3.6.6. FCM analysis of monocyte cytokine expression

To assess cytokine production within monocytes, specific monoclonal antibodies were used, targeting intracellular proteins: IL-1β (H1b-98), IL-4 (MP4-25D2), IL-6 (MQ2-13A5), IL-10 (JES3-19F1), IL-12 (C8.6), and TNF-α (MAb11). All antibodies were obtained from BD Biosciences (San Jose, CA, USA). Intracellular cytokine detection was performed using the BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit, along with the BD GolgiStop™ protein transport inhibitor, which contained monensin to facilitate cytokine retention within the cells. Multicolor staining followed the manufacturer's detailed protocol.

The samples were analyzed using a FACSLyric flow cytometer (BD, San Jose, CA, USA). PMT voltage and compensation settings were calibrated using BD™ CompBeads Anti-Mouse Ig, κ/Negative Control Compensation Particles Set (BD, San Jose, CA, USA). Data acquisition aimed for up to 30,000 events per sample. The M1 macrophage subset, identified by CD45+/CD80+/CD86+ markers, was distinguished from the M2 subset, characterized by CD45+/CD163+/CD206+ markers, and were quantified as a percentage of cells within the lymphocyte gate. Cytokine expression levels for each subset were measured and reported as mean fluorescence intensity (MFI) values, reflecting the degree of cytokine production.

3.6.7. Serum cytokine analysis via Luminex

Serum levels of free IL-1β, IL-4, IL-6, IL-10 and IL-12 were measured using magnetic bead-based multiplex assays (Human Cytokine Premixed Multi-Analyte Kit from R&D Systems, Minneapolis, MN, USA) and a Luminex® 100 analyser (Luminex Corporation, Austin, TX, USA). Frozen serum samples were thawed and centrifuged at 4 °C and 16,000 × g for 4 minutes to clear any debris. Following the manufacturer's protocol, analyte-specific antibodies pre-coated onto magnetic beads containing distinct fluorophores were dispensed into wells with standards and samples, allowing antibodies to capture their target cytokines. After unbound substances were washed away, biotinylated detection antibodies and streptavidin–phycoerythrin were added as reporters. Additional washes removed any unbound streptavidin–phycoerythrin, and the beads were suspended in buffer for analysis on the Luminex® 100.

Using laser excitation, the Luminex instrument identified each bead region and detected its assigned analyte. A second laser measured the phycoerythrin signal intensity, proportional to the analyte concentration bound to each bead. Multiple readings of MFI were taken per bead region to ensure accurate measurement. Cytokine concentrations were determined by comparing the MFI values to a standard curve correlating MFI with known protein concentration.

3.6.8. Serum protein analysis via enzyme-linked immunosorbent assay (ELISA)

Concentrations of PD-1 and PD-L1 in serum samples were measured using ELISA kits specific for PD-1 (ab252360) and PD-L1 (ab277712) (Abcam, Cambridge, MA, USA), following the manufacturer's instructions. Optical density measurements were taken at specified wavelengths to determine the levels of PD-1 and PD-L1, and concentrations were calculated by interpolating the values against standard curves established for each protein.

3.7. Statistical analysis

Statistical analyses were conducted using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 9.5.1; GraphPad Software Inc., La Jolla, CA, USA).

Due to the sample size and the non-normal distribution of most variables (estimated via Shapiro–Wilk test), the Mann–Whitney test was applied for comparisons between independent variables. For multiple dependent variab-

les, we used the Friedman rank test, and when significant differences were observed, the Wilcoxon test was used for further pairwise comparisons.

The Chi-square or Fisher's exact test was employed to compare nominal variables between the two groups. To examine relationships between variables, Spearman's correlation coefficient (rs) was calculated. Quantitative results are presented as median values with interquartile ranges (IQR), defined from the first to the third quartile, unless stated otherwise. Statistical significance was set at $p < 0.05$.

PBMC activity was assessed by calculating the ratio of fluorometric or spectrophotometric signals between wells containing activators and those with negative controls. Resazurin metabolism in non-activated PBMCs was evaluated by fluorometric assay and normalized to control group levels.

Relative transcription levels of the analyzed proteins were normalized to GAPDH and calculated using the $2^{-\Delta\Delta CT}$ method [169]. Protein changes were shown relative to controls or preoperative OC patient values, which were set to 1.

Outlier removal was conducted in two stages. First, data points evidently inconsistent with realistic ranges were removed, followed by statistical screening. For statistical outlier evalutation, the Modified Z-Score method was used with formula: Modified Z-score = 0.6745 (Xi − Median) / MAD (where Xi is the data point, Median is the data set median, and MAD is the median absolute deviation). Modified Z-scores beyond ± 3.5 were flagged as outliers [170]. This approach was selected due to its suitability for small sample sizes and to account for potential artificial data variability from multistep laboratory procedures. Although a minimal number of points was excluded, this process improved data clarity and interpretability.

4. RESULTS

4.1. Study population

There were 46 participants in the final sample, with 23 in the OC group and 23 in the control group. The median age of the study group, 58 years (47–61), did not differ significantly from that of the control group. BMI was also comparable between the groups. The incidence of comorbidities was similar, with hypertension being the most common condition in both groups. OC histologic types distribution was consistent with that typically observed in the general population [38]. In the OC group, two-thirds (65.1%) of surgeries achieved complete cytoreduction. Detailed clinicopathologic characteristics of participants are presented in Table 4.1.1.

Table 4.1.1. *Clinicopathological characteristics of the participants*

Characteristic	OC group (n = 23)	Control group (n = 23)	p value
Age (years), median (IQR)	58 (47–61)	59 (49–61)	0.84
BMI (kg/m^2), median (IQR)	24.0 (22.3–29.8)	23.7 (21.9–28.7)	0.60
Comorbidities, n (%)			
Hypertension	9 (39.1)	10 (43.5)	1.0
Ischaemic heart disease	1 (4.4)	2 (8.7)	1.0
Heart failure	1 (4.4)	2 (8.7)	1.0
Type II diabetes	1 (4.4)	1 (4.4)	1.0
Other	6 (26.1)	4 (17.4)	0.72
No comorbidities	12 (52.2)	12 (52.2)	1.0
Stage of OC, n (%)			
IIIA	4 (17.4)	NA	NA
IIIB	4 (17.4)	NA	NA
IIIC	7 (30.4)	NA	NA
IVA	1 (4.4)	NA	NA
IVB	7 (30.4)	NA	NA
Histological type of OC, n (%)			
LGSC	1 (4.4)	NA	NA
HGSC	15 (65.1)	NA	NA
Endometrioid carcinoma	2 (8.7)	NA	NA
Clear cell carcinoma	1 (4.4)	NA	NA
Mucinous carcinoma	1 (4.4)	NA	NA
Serous endometrioid carcinoma	3 (13)	NA	NA

Table 4.1.1. *continued*

Characteristic	OC group (n = 23)	Control group (n = 23)	p value
Cytoreduction outcome, n (%)			
Complete	15 (65.1)	NA	NA
Optimal	5 (21.9)	NA	NA
Suboptimal	3 (13)	NA	NA

The stages of OC are presented according to the 2021 FIGO staging system [44]. BMI, body mass index; IQR, interquartile range; n, number of cases; LGSC, low-grade serous carcinoma; HGSC, high-grade serous carcinoma; NA, not applicable.

4.2. Alterations in peripheral blood cell counts and ratios

CBC analysis was performed for the control group and preoperative OC patients. Results showed that OC patients had elevated WBC counts compared to controls, largely due to a 1.5-fold increase in neutrophil levels. Additionally, OC patients demonstrated higher platelet counts and a tendency toward reduced RBC counts. Although RBC counts were not significantly different, hemoglobin levels were lower in OC patients, measuring 124 g/L (115–130), compared to 135 g/L (126–140) in controls (p = 0.01). Further analysis revealed higher NLR and PLR in the OC group. A comprehensive summary of the CBC data is provided in Table 4.2.1.

Table 4.2.1. *Comparison of peripheral blood cell counts and ratios between the control group and preoperative OC patients*

Cell type / ratio	OC group (n = 23)	Control group (n = 23)	p value
WBCs	6.9 (5.2–8.2)	5.6 (4.4–6.4)	0.02
Neutrophils	4.7 (3.6–5.6)	3.1 (2.3–3.5)	< 0.001
Eosinophils	0.1 (0.05–0.2)	0.1 (0.1–0.1)	0.87
Basophils	0.04 (0.03–0.07)	0.04 (0.02–0.04)	0.26
Lymphocytes	1.4 (1.2–2.2)	1.9 (1.3–2.3)	0.2
Monocytes	0.6 (0.3–0.9)	0.4 (0.4–0.5)	0.26
RBCs	4.3 (4.1–4.5)	4.5 (4.4–4.7)	0.06
Platelets	367 (324–402)	267.5 (234–289)	< 0.001
NLR	3.2 (2.2–4.1)	1.7 (1.3–1.9)	< 0.001
PLR	250 (177–305)	156 (110–202)	< 0.001

WBCs, white blood cells; RBCs, red blood cells; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio. RBC counts are expressed in units of $\times 10^{12}$/L; other cell counts are expressed in units of $\times 10^{9}$/L. Cell counts are displayed as median values with IQR.

4.3. Alterations in PBMC subpopulations and related ratios

After isolating PBMCs, we analyzed subpopulation changes in OC patients. Preoperatively, OC patients showed significantly lower levels of CD3+ T cells and CD8+ T cell subsets, along with a higher proportion of CD19+ B cells (Fig. 4.3.1 A). Postoperative analysis indicated some fluctuations in PBMC subpopulations, though none were statistically significant when comparing postoperative values to the preoperative baselines in OC patients. However, there were notable differences between the control and postoperative OC groups. Specifically, CD4+ T cell counts significantly increased 1 day after surgery, with values of 36.1% (35.3–37.1) *vs.* 40.9% (37.7–46.8), respectively ($p < 0.001$). Another notable change was in CD19+ B cells, which peaked at 6.7% (5.7–7.9) one day postoperatively, compared to the control level of 4.1% (3.8–4.6) ($p < 0.001$). In contrast, CD3+ T cell and CD8+ T cell proportions remained stable postoperatively, showing no significant fluctuations from their preoperative levels.

Monocyte subpopulation analysis revealed similar proportions of M1 monocytes between controls and OC patients both before and after surgery (Fig. 4.3.1 B). However, M2 monocytes were approximately 2.5 times higher in OC patients preoperatively and continued to increase postoperatively, reaching more than three times the control level at 7.5% (6.7–7.8) compared to 2.3% (1.4–2.8) in controls ($p < 0.001$). In contrast, non-classified monocytes were lower in preoperative OC patients than in controls and declined further 1 day postoperatively to 4.4% (4.1–4.8) *vs.* 9.6% (8.4–12.1) in controls ($p < 0.001$). While the most pronounced changes in M2 and non-classified monocytes occurred 1 day after surgery, these shifts were not significant when compared to preoperative levels.

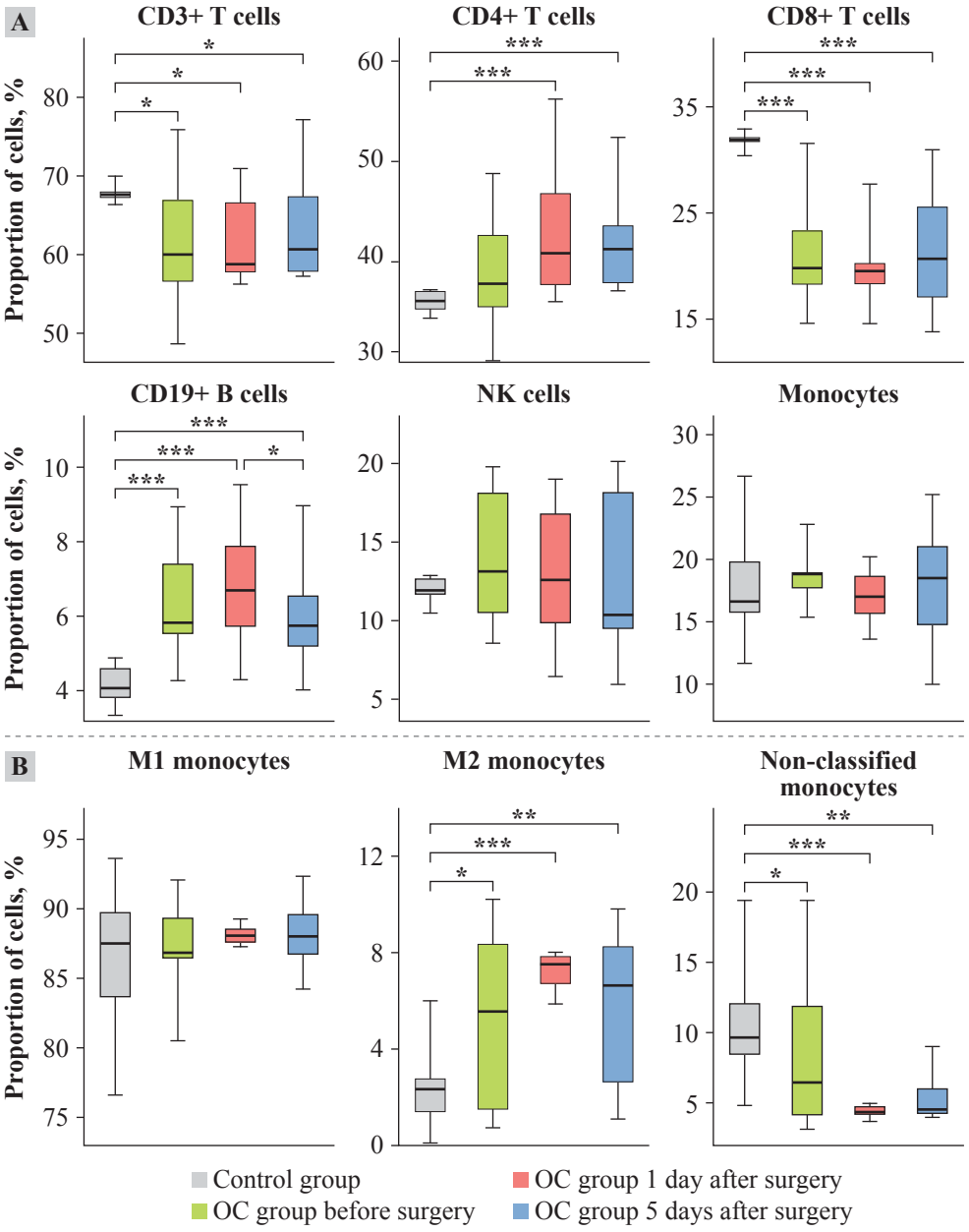


Fig. 4.3.1. *Changes in PBMC subpopulations in control and OC groups*

The upper graphs **(A)** show median proportions of PBMC subpopulations. The lower graphs **(B)** display median proportions of monocyte subsets within all monocytes. Data are presented for the control group and OC patients preoperatively and at 1 and 5 days after surgery. Boxes indicate the IQR with the median marked by a line, and error bars extend to the minimum and maximum values. All p-values between groups are > 0.05 unless stated otherwise. *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Sample sizes: OC group n = 13, control group n = 8 (PBMCs without monocytes) or n = 23 (monocytes).

We further analyzed PBMC-related ratios to explore their dynamics across the investigated groups. In the preoperative OC group, there was a significantly higher CD4/CD8 ratio and a markedly decreased M1/M2 ratio among OC patients. However, the LMR was comparable between the investigated groups. Postoperative analysis of PBMC ratios in OC patients did not reveal any additional alterations when compared to either the control group or the preoperative OC group levels (Fig. 4.3.2).

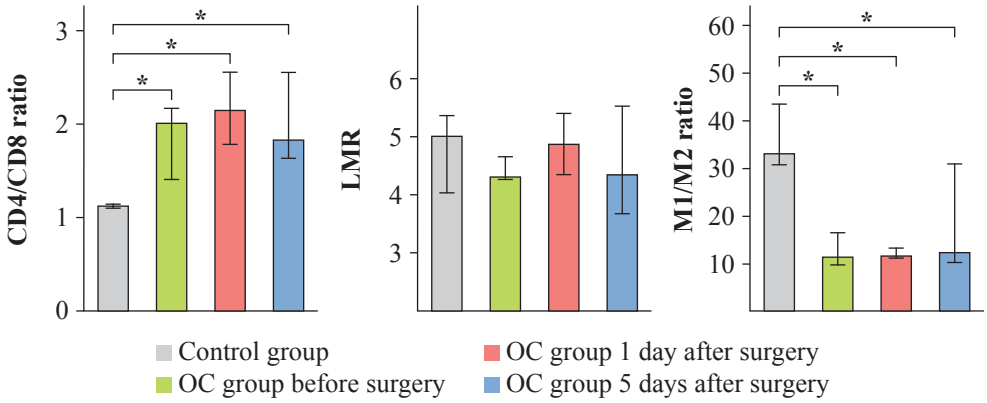


Fig. 4.3.2. *Changes in PBMC-related ratios in control and OC groups*

Data are presented for the control group and OC patients preoperatively and at 1 and 5 days after surgery. Graphs indicate median values with IQR. All p-values between groups are > 0.05 unless stated otherwise. *p ≤ 0.001. CD4/CD8, CD4+ T cell to CD8+ T cell ratio; LMR, lymphocyte-to-monocyte ratio. Sample sizes: OC group n = 13, control group n = 8 (CD4/CD8 ratio) or n = 23 (LMR and M1/M2 ratio).

To explore the interrelationships among the investigated ratios and peripheral blood cells, we performed a correlation analysis. Fig. 4.3.3 presents a correlation matrix displaying associations between these ratios, blood cell counts, and PBMCs subpopulation proportions in preoperative OC patients. Notably, a strong correlation was observed between the CD4/CD8 ratio and neutrophils ($r_s = -0.81$; $p = 0.01$) and between the M1/M2 ratio and the CD4+ T cell proportion within PBMCs ($r_s = -0.71$; $p = 0.047$).

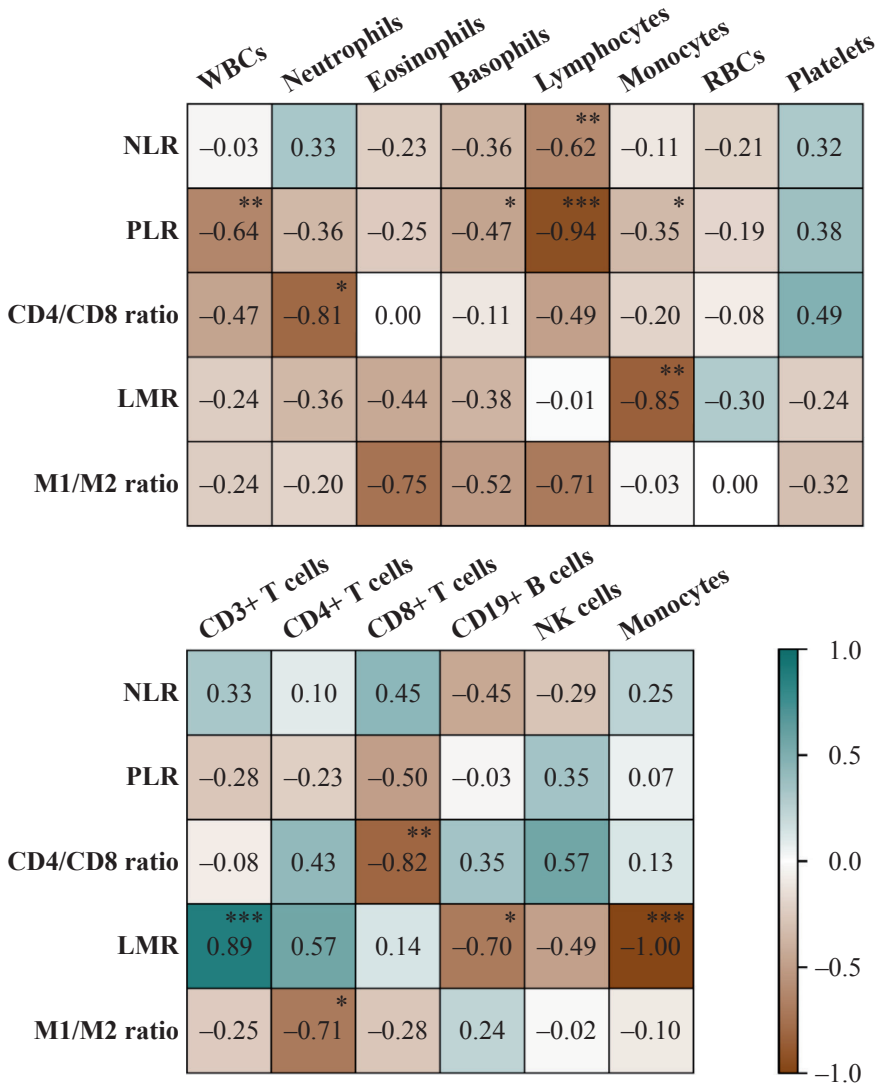


Fig. 4.3.3. *Correlation between peripheral blood cell ratios and counts in preoperative OC patients*

The upper graph shows the correlation between blood cell ratios and their absolute counts. The lower graph displays correlation between the ratios and proportions of PBMC subsets among all PBMCs. Spearman's correlation coefficients (rs) are indicated, with colors ranging from brown (rs = −1) to teal (rs = 1). All correlation p values are > 0.05 unless stated otherwise. *p < 0.05; **p ≤ 0.01; ***p ≤ 0.001.

4.4. Alterations in PBMC function

Analysis of PBMC metabolic and functional activity revealed no significant differences between healthy participants and preoperative OC patients. Only a slight non-significant decline in most metrics was observed in the preoperative OC group compared to controls, as shown in Fig. 4.4.1.

No significant changes in activity were evident in postoperative OC patients compared to their preoperative levels. However, one day after surgery, there was a marked reduction in both baseline metabolic activity and phagocytic activity relative to the control group. By day five postoperatively, PBMC activity partially rebounded, with increased phagocytic activity and a trend toward higher metabolic activity in response to LPS activation. Additionally, functional activity demonstrated an opposite shift one day post-surgery, with a significant increase in ROS production, which returned to preoperative levels by day five.

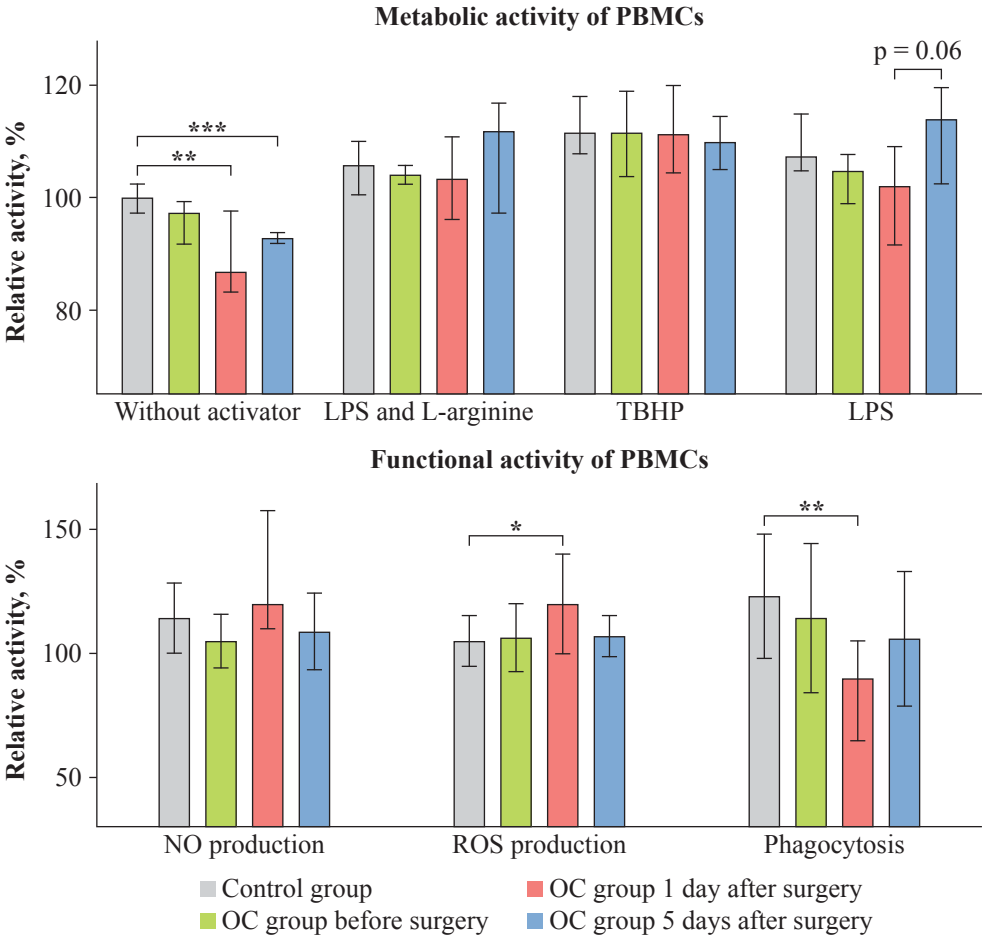


Fig. 4.4.1. *PBMC activity in healthy controls and OC patients pre- and postoperatively*

The upper graph shows PBMC viability assessed via the resazurin metabolism assay (AlamarBlue) without activators and after stimulation with LPS (lipopolysaccharide), L-arginine or TBHP (tert-butyl hydroperoxide). The lower graph presents functional activity, including nitric oxide (NO), reactive oxygen species (ROS) production, and phagocytic activity. Relative activity was calculated by comparing results with and without activators, or normalized to the control group for baseline resazurin metabolism. All p-values between groups are > 0.05 unless stated otherwise. *p = 0.02; **$p \leq 0.01$; ***p = 0.001. Sample sizes: OC group n = 13; control group n = 17.

4.5. Alterations in immune-related protein expression in PBMCs

We thoroughly analyzed the expression of immune-related proteins in PBMCs, beginning with mRNA expression levels. As presented in Fig. 4.5.1, the preoperative OC group showed a significant reduction in the expression of nearly all studied proteins compared to healthy controls, with IL-6 being the most downregulated. Notably, IL-10 mRNA expression in OC patients did not differ significantly from that of controls.

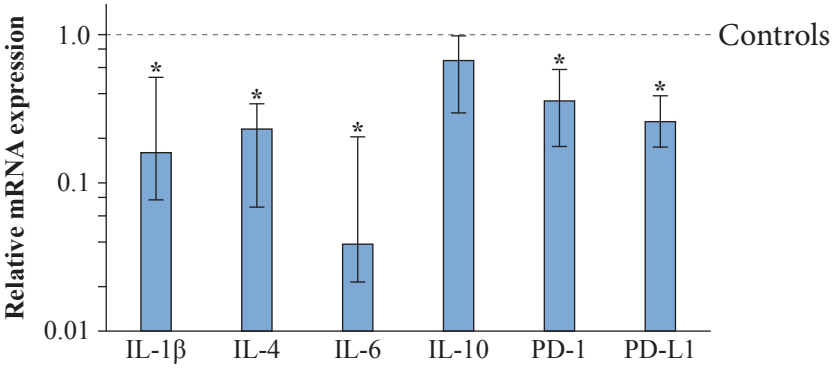


Fig. 4.5.1. *Preoperative messenger RNA (mRNA) expression of immune-related proteins in PBMCs in OC patients*

Bars represent the relative median mRNA expression levels of IL-1β, IL-4, IL-6, IL-10, PD-1 (programmed death-1 receptor), and PD-L1 (programmed death ligand-1), compared to healthy controls, with values normalized to one. All marked differences were significant (*$p \leq 0.001$). Sample size: OC group, n = 23; control group, n = 20.

In addition to the nearly complete downregulation of immune-related protein mRNAs in PBMCs preoperatively, further changes in mRNA expression occurred during the postoperative period (Fig. 4.5.2). Two interleukins, IL-1β and IL-6, were downregulated postoperatively, reaching their lowest levels on day 3 after surgery. Specifically, IL-1β mRNA expression dropped to 52% (21–85) one day postoperatively and further decreased to 12% (2–27) by day 3 after surgery, compared to preoperative levels ($p = 0.01$ and $p < 0.001$, respectively). IL-6 mRNA expression declined to 36% (4–103) on day 3 postoperatively ($p = 0.01$), with both interleukins showing a rebound in mRNA expression by day 5 after surgery.

Meanwhile, the majority of other proteins studied exhibited their most pronounced alterations immediately following surgery. IL-4 mRNA expression dropped to 41% (29–94), PD-L1 mRNA expression decreased to 50% (34–78), while IL-10 mRNA expression increased to 221% (113–356) compared to preoperative levels ($p = 0.01$, $p = 0.001$, and $p = 0.002$, respectively). Notably, IL-10 was the only protein with increased mRNA expression during the postoperative period. PD-1 showed a continuous decline in expression throughout the postoperative period, although these changes were not statistically significant.

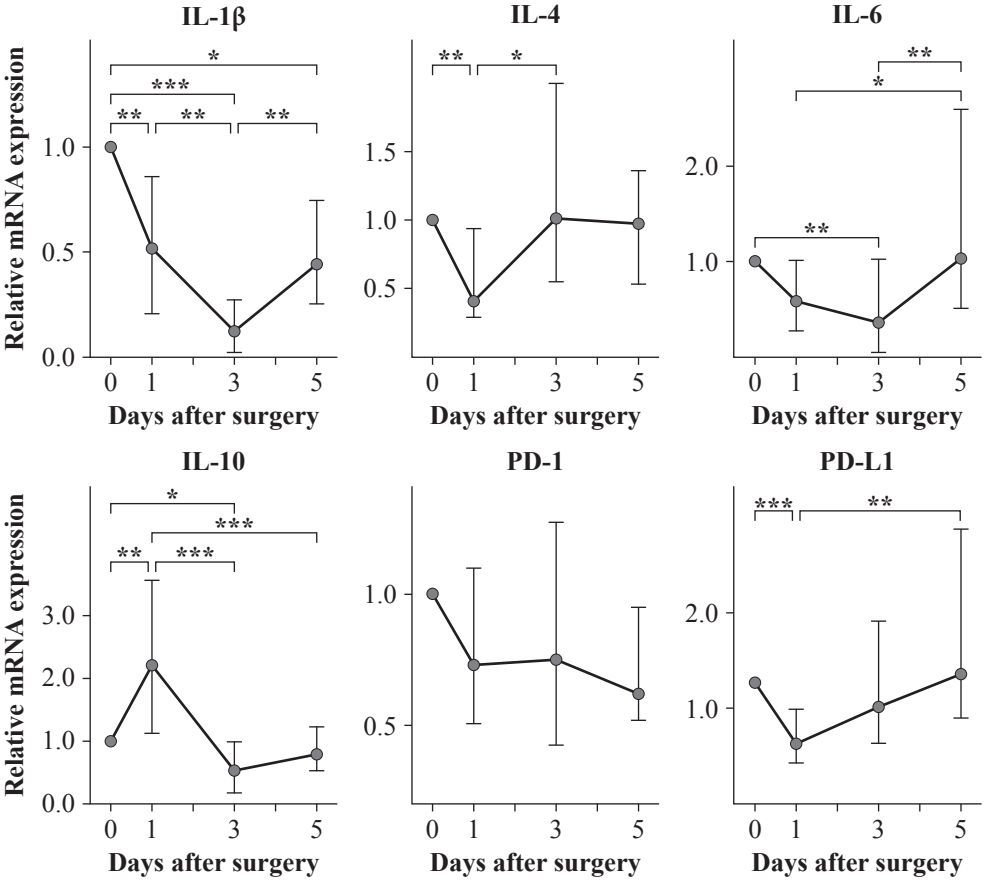


Fig. 4.5.2. *Postoperative mRNA expression of immune-related proteins in PBMCs in OC patients*

Relative expression levels of immune-related protein mRNAs are shown for days 1, 3, and 5 after surgery compared to the preoperative baseline. All p-values between groups are > 0.05 unless stated otherwise. *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Sample size n = 23.

We further analyzed cytokine expression in monocytes isolated from PBMCs by measuring intracellular cytokine accumulation. Despite cytokine alterations in preoperative OC patients, no statistically significant changes were observed when comparing postoperative cytokine expression to preoperative levels. However, alterations were apparent when comparing postoperative cytokine expression to that of the control group as detailed in Fig. 4.5.3.

IL-6 expression in M2 monocytes showed the most noticeable fluctuations. It was elevated in preoperative OC patients and increased further on the first postoperative day, reaching levels 1.5 times higher than those in controls (MFI values 2602 (2296–2850) *vs.* 3894 (3196–4028), respectively, $p < 0.001$). By the fifth postoperative day, IL-6 expression showed a trend toward returning to control levels.

TNF-α expression tended to be higher in M1 monocytes in preoperative OC patients. One day post-surgery, this trend became significant, with TNF-α expression in M1 monocytes reaching 1.24 times that of controls (MFI values 634 (449–663) *vs.* 789 (633–845), $p = 0.02$). In contrast, TNF-α expression in M2 monocytes, which was lower in OC patients preoperatively, returned to levels comparable to controls on the first postoperative day (MFI values of 535 (398–683) *vs.* 487 (298–621), $p = 0.64$). IL-10 expression in M2 monocytes followed a similar pattern, aligning with control levels by the fifth postoperative day, with MFI values of 640 (426–729) *vs.* 645 (363–701), respectively ($p = 0.53$).

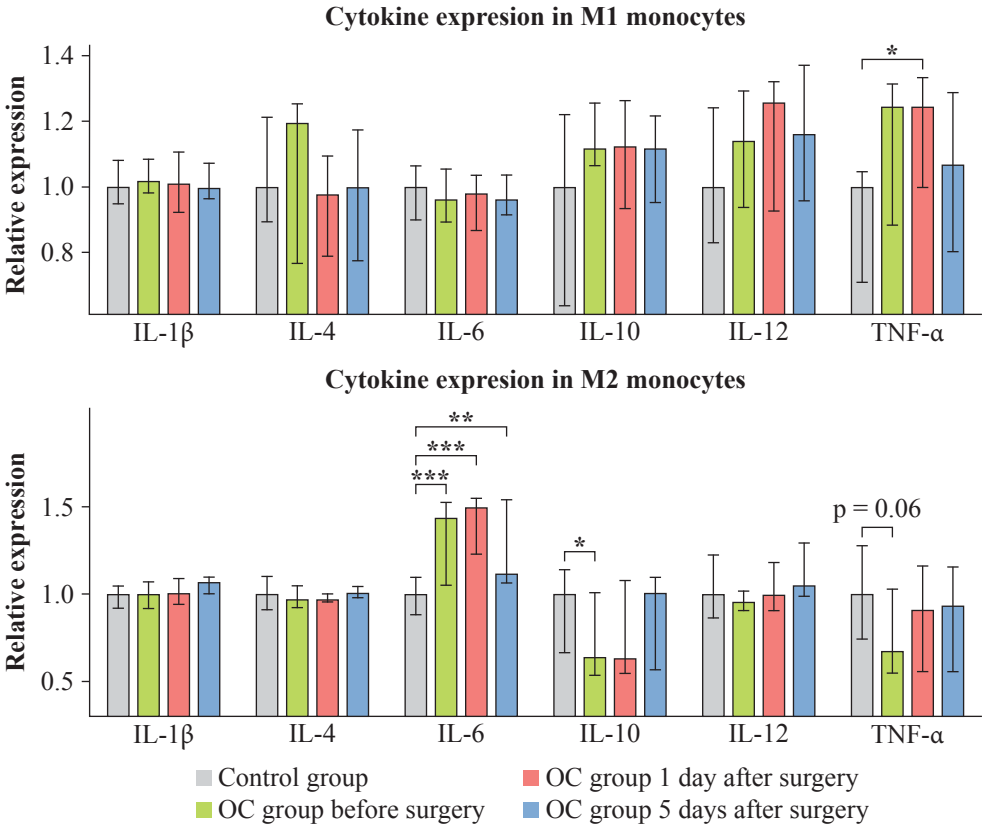


Fig. 4.5.3. *Changes in cytokine expression in monocytes of pre- and postoperative OC patients*

The upper graph shows the fold change in cytokine expression in M1 monocytes, while the lower graph displays cytokine expression in M2 monocytes. Columns represent the fold change of mean fluorescence intensity (MFI) values for preoperative and postoperative OC patients on days 1 and 5 after surgery, normalised to control levels. All p-values between groups are > 0.05 unless stated otherwise. *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Sample sizes: OC group, n = 13; control group, n = 23.

4.6. Alterations in immune-related protein serum levels

For the final evaluation of immune-related protein expression, we measured serum concentrations in controls and OC patients. In terms of serum interleukin levels, no significant differences were observed between controls and the preoperative OC group (Fig. 4.6.1).

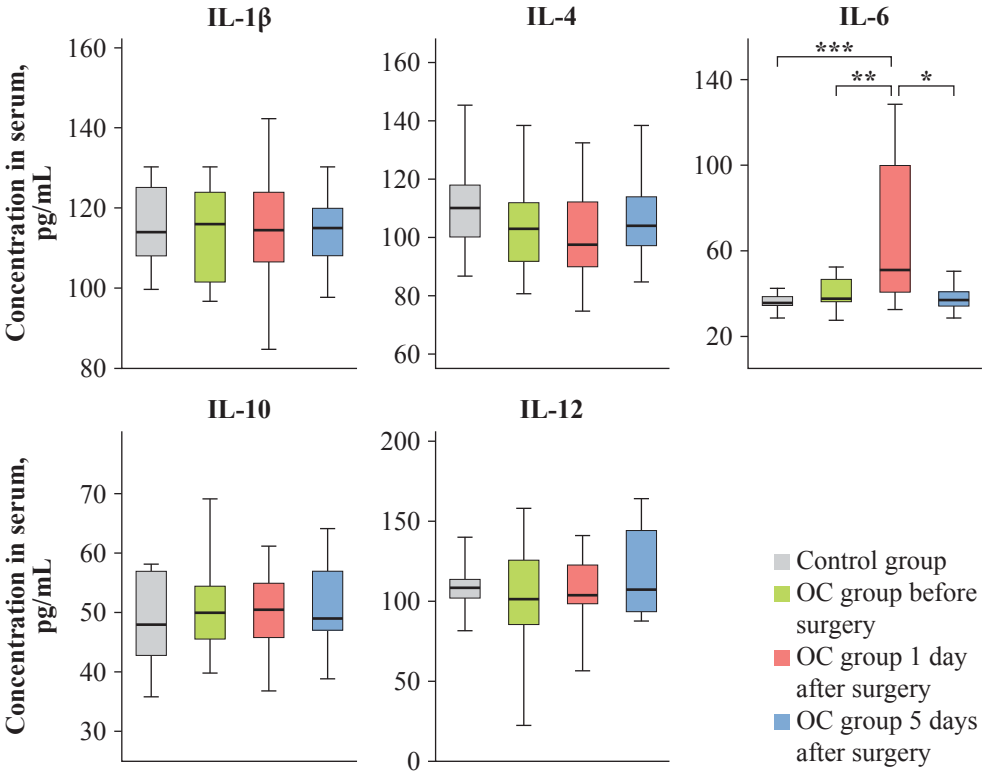


Fig. 4.6.1. *Changes in serum interleukin levels in pre- and postoperative OC patients*

Serum cytokine levels were measured using a Luminex assay. All p-values between groups are > 0.05 unless stated otherwise. *p < 0.05; **p ≤ 0.01; ***p ≤ 0.001. Sample sizes: OC group, n = 18; control group, n = 17.

The same was true for postoperative OC patients, with the exception of IL-6, which increased by 36% one day after surgery compared to preoperative levels (37.5 pg/mL (36–46.8) preoperatively *vs.* 51 pg/mL (40.5–100) postoperatively; p = 0.003). From this elevated level IL-6 returned to preoperative levels by day 5 (37.5 pg/mL (36–46.8) *vs.* 37 pg/mL (34–41), respectively; p = 0.45).

Additionally, preoperative OC patients showed a trend toward lower serum levels of free PD-1 compared to controls (Fig. 4.6.2). However, no significant differences were found in free serum PD-1 or PD-L1 levels across other comparisons.

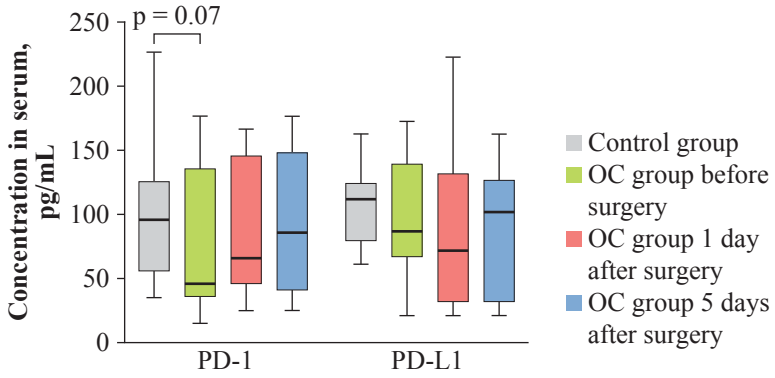


Fig. 4.6.2. *Changes in serum PD-1 and PD-L1 levels in pre- and postoperative OC patients*

Serum protein levels were measured using an ELISA assay. All p-values between groups are > 0.05 unless stated otherwise. Sample sizes: OC group, n = 11; control group, n = 15.

5. DISCUSSION

5.1. Peripheral blood cell counts and ratios in preoperative OC patients

As an initial analysis, we performed a CBC and compared the absolute cell counts in preoperative OC patients with controls. Cancer cells have a well described link to immune cells within the TME, which participate in tumor progression [15, 16]. However, this immune response is not only local but also has systemic effects [60, 61, 142]. Even in a basic test like CBC, we observed notable differences. In particular, our study found elevated WBC counts in preoperative OC patients, primarily due to increased neutrophils. Although neutrophils typically rise in response to infections, our strict inclusion criteria ruled out patients with infections or other inflammatory diseases that could influence WBC or neutrophil levels. Additionally, clinicopathological characteristics were similar between the OC and control groups. Our study findings also align with existing literature showing elevated WBC and neutrophil counts in OC patients [171, 172]. Absolute lymphocyte counts were lower in the OC group; however, the difference was not statistically significant, even though lower lymphocyte levels were anticipated [172]. Other differences included lower hemoglobin and higher platelet counts in OC patients, which have also been previously documented [171].

There is growing interest in identifying diagnostic and prognostic markers for OC using accessible tests like CBC. While neutrophils and platelets are nonspecific cell populations, certain platelet characteristics are being studied for their diagnostic potential in OC. A notable case-control study found that elevated platelet counts are linked to a higher incidence of cancers, including colorectal, lung, gastric, and OC [173]. Additionally, research on tumor-educated platelets has shown their potential as novel biomarkers for OC [174].

Platelets interact with tumor cells, releasing cytokines and growth factors that promote immune evasion and angiogenesis [63]. Neutrophils, meanwhile, are a diverse population with subtypes that have opposing functions and effects, but their role in OC progression is well documented. [63, 102]. Although changes in these individual cell counts do not directly reflect immune function alterations, studies have consistently shown that higher neutrophil and platelet counts are linked to poorer prognosis in OC [172, 175]. The elevated platelet and neutrophil levels observed in our study were, therefore, expected. Additionally, we found increased NLR and PLR, which are often elevated in OC, correlate with worse outcomes, and are increasingly recognized as valuable prognostic indicators [104].

Summary of the section

Though investigating CBC in preoperative OC patients and controls was not the main objective of our study, these results highlight two key points: first, our findings align with the existing literature, supporting the validity of our study; and second, shifts in neutrophils and platelets suggest a proinflammatory state in OC patients.

5.2. PBMC proportions and ratios in pre- and postoperative OC patients

Lymphocytes and related ratio alterations in preoperative OC patients

In the next part of our study, we focused on PBMCs as an essential indicator of anticancer immunity, given their critical role in OC progression [15, 16, 62]. Peripheral blood cells may not perfectly reflect the immune environment within the tumor, as differences in immune cell counts, ratios, and their functions between tumor tissue, ascites, and blood have been shown [143–145]. However, they still undergo significant changes in OC patients, and these alterations can be interpreted similarly to those in tumor tissue [17]. By choosing to investigate PBMCs in preoperative and postoperative OC patients, we aimed to first assess immune function and response, and second to explore a more accessible method for this evaluation.

Our analysis of lymphocyte subpopulations and related ratios revealed significant shifts in lymphocyte composition in preoperative OC patients compared to controls. Decreased proportions of CD3+ T cells and CD8+ T cells, alongside an increased proportion of CD19+ B cells and a higher CD4/CD8 ratio, suggest substantial immune alterations in OC patients, even though total lymphocyte counts were similar in both groups.

Most studies on lymphocyte analysis focus on TILs and their role in the TME. However, data show that higher levels of lymphocytes – both within the TME [81, 150] and in peripheral blood (independent of TILs) [142, 151, 176] – are associated with better PFS and OS. CD3+ T cells constitute the largest portion of all PBMCs, particularly among lymphocytes [140]. Given the abundance of CD3+ T cells within the lymphocyte population, we may draw an indirect implication that lower levels of CD3+ T cells indicate a tumor-promoting environment in OC patients as well. However, this is merely speculation.

In particular, the reduction in CD3+ T cells in preoperative OC patients appears to be largely due to a significant decrease in CD8+ T cells, which were nearly 40% lower than in controls. Similar findings have been reported by several other authors as well [17, 18, 177]. The interpretation of such shifts

is more straightforward. CD8+ T cells are known for their cytotoxic role in targeting and eliminating cancer cells [15, 16, 62]. Although direct evidence linking peripheral CD8+ T cell levels to OC prognosis is lacking, high levels of tumor-infiltrating CD8+ T cells are clearly associated with better outcomes [142]. Given this context, the reduction in peripheral CD8+ T cells most likely indicates impaired anticancer immunity in OC patients.

The LMR is a well-known prognostic marker used in OC. In OC patients, the LMR is generally lower than in healthy controls [172], with a reduced LMR associated with poorer OS and PFS [148]. While our study observed a slight decrease in LMR in preoperative OC patients, this reduction was not statistically significant.

The CD4/CD8 ratio has also been studied as a potential prognostic marker in OC, with findings indicating that a higher CD4/CD8 ratio in OC tissue correlates with poorer prognosis [142, 147]. However, data on the CD4/CD8 ratio in peripheral blood as a prognostic marker in OC are limited [142]. One study reported a higher CD4/CD8 ratio in OC patients compared to those with benign pathologies [17], while another found similar levels between OC patients and controls [163]. Additionally, Santin et al. demonstrated that the CD4/CD8 ratio is elevated in peripheral blood lymphocytes compared to tumor-associated lymphocytes or TILs [143]. Although the CD4/CD8 ratio alone does not directly reflect the immune status, the elevated CD4/CD8 ratio observed in preoperative OC patients could indicate a tumor-promoting environment, highlighting the need for further research into its use as a prognostic marker.

The observed increase in CD19+ B cells in preoperative OC patients is more challenging to interpret. While some data suggest that higher levels of intratumoral and peripheral CD19+ B cells correlate with improved prognosis in OC, other studies report conflicting results [62, 142]. The underlying reason for these discrepancies lies in the different effects of CD19+ B cell subtypes [62], leaving the interpretation of our findings inconclusive.

We also analyzed the correlations between the investigated ratios and CBC counts or PBMC proportions in OC patients. While this analysis is beyond the main focus of our study, it provides useful insights for developing new ratios that could help in managing OC patients. As expected, some absolute cell counts and proportions correlated with the ratios of which they are a part. However, we also found some new correlations. It is important to note that the sample size for such an analysis is small, so we cannot make firm conclusions. Nonetheless, these findings provide a background for future, more comprehensive investigations.

Summary of the section

Our analysis of lymphocyte subpopulations revealed significant preoperative reductions in CD3+ and CD8+ T cells, along with an increased CD4/CD8 ratio and a higher proportion of CD19+ B cells. While the relevance of changes in CD3+ T cells and CD19+ B cells may be debated, the decrease in CD8+ T cells and increase in CD4/CD8 ratio clearly indicate an immunosuppressive state in preoperative OC patients.

Lymphocytes and related ratios alterations in postoperative OC patients

Surgery has been shown to influence the immune system, potentially creating a temporary immunosuppressive state that can support metastatic spread [25, 156, 162]. In light of this knowledge, we further analyzed changes in PBMC proportions during the early postoperative period. Notably, we observed an increase in CD4+ T cells following surgery. Although direct data on early postoperative changes in CD4+ T cells are sparse, Nowak et al. found no significant change in CD4+ T cell levels seven days postoperatively [163], while Napoletano et al. observed a decrease in overall proportion of CD4+ T cells 15 days post-surgery [18]. Together, these findings are not contradicttory but rather complementary, suggesting a dynamic shift in CD4+ T cell proportions from the early postoperative period to the later stages after surgery.

Early postoperative shifts in specific subsets of CD4+ T cells, such as Tregs, have been documented. Studies show a beneficial decrease in Tregs as early as two to three days after surgery [18, 160]. Conversely, insufficient removal of tumor tissue leads to an unfavorable increase in Tregs seven days postoperatively [163]. Another interesting finding presented by Napoletano et al. was the previously mentioned decrease in the overall proportion of CD4+ T cells 15 days post-surgery; however, a detailed analysis of subpopulations revealed an increase in effector CD4+ T cells within the CD4+ population [18]. These examples serve as a reminder that the CD4+ T cell population is heterogeneous, with subsets like Th1, Th2, and Tregs playing different roles in OC progression [15, 16, 62]. Consequently, the interpretation of CD4+ T cell changes in our study remains inconclusive.

Certain PBMC subpopulations remained relatively stable during the early postoperative period. CD3+ T cells, CD8+ T cells, and the CD4/CD8 ratio showed no significant changes after surgery. This finding somewhat aligns with a study by Nowak et al., who observed no changes in CD3+ T cells, CD8+ T cells, or the CD4/CD8 ratio postoperatively. However, their observation was made seven days after surgery [163]. Similarly, Napoletano et al. found no differences in CD3+ and CD8+ T cell populations 15 days postoperatively compared to preoperative levels. However, they noted an

increase in effector CD8+ T cells 15 days after surgery [18]. Alhough we cannot support this from our data, the existing literature suggests that the most dynamic changes occur in smaller subpopulations of lymphocytes, while larger populations may remain relatively unchanged.

Notably, the LMR remained stable throughout the postoperative period. Studies have not examined postoperative LMR dynamics [148, 178], as this ratio has generally been considered a prognostic marker rather than an indicator of immune function change postoperatively. However, the stability of LMR suggests that LMR measured in the early postoperative period may still have prognostic value. Although this is speculative, further research is needed to validate the use of postoperative LMR.

In addition to these stable patterns, CD19+ B cells showed a temporary increase on the first day after surgery. Given the unclear role of CD19+ B cells in OC progression, interpreting this fluctuation is challenging.

Summary of the section

The increase in CD4+ T cells and CD19+ B cells observed on the first postoperative day is difficult to interpret clearly. However, the stable postoperative proportions of CD8+ T cells and the unchanged CD4/CD8 ratio suggest that the preoperative immunosuppressive environment persisted into the early postoperative period.

Monocyte and M1/M2 ratio alterations in pre- and postoperative OC patients

Macrophages are the most abundant cells in OC tissue, making up over a third of immune cells in OC tissue [70]. While macrophages typically serve protective roles in pathogen elimination and inflammation control within the OC TME, they can paradoxically contribute to immunosuppression, drive disease progression, support chemoresistance, and promote metastasis [69, 72]. Therefore, understanding their role in the OC TME is essential.

Originating primarily from peripheral monocytes, macrophages differentiate into TAMs upon entering tumor tissue [71]. TAMs exhibit two main phenotypes, M1 and M2, depending on cytokine signals in their environment. M1 macrophages are pro-inflammatory and work to inhibit tumor growth, while M2 macrophages possess anti-inflammatory properties that favor tumor progression [69, 72]. In our study, we observed significant differences in the M2 monocyte population, while the overall monocyte proportion within PBMCs and the M1 monocyte levels among all monocytes remained relatively stable across the investigated groups.

Specifically, the M2 monocyte population was 2.5 times higher in preoperative OC patients than in controls. One day after surgery, the M2 monocyte levels increased to more than three times compared to those in controls.

While this postoperative increase was not statistically significant relative to preoperative levels, it demonstrated a greater divergence from control values, with reduced data variability and stronger statistical significance. Considering the small sample size, this shift should be interpreted as an independent rise in M2 proportions one day after surgery. Given the role of M2 monocytes/macrophages in promoting OC progression, this shift suggests that OC patients experience a tumor-promoting environment preoperatively, which becomes more pronounced immediately after surgery.

Non-classified monocytes, whose final phenotype remains undetermined, showed precisely the opposite dynamics compared to M2 monocyte changes. Although these monocytes do not yet serve a specific immune function, their fluctuations offer additional insights. Monocyte/macrophage polarization is not final; on the contrary, it is a dynamic process [69, 179]. Studies in animal models even show that survival can be extended by reprogramming M2 monocytes into M1 types via immunotherapy [180]. Thus, the stable absolute monocyte counts and consistent proportions of overall monocytes and M1 monocytes in controls and preoperative OC patients in our study suggest that the observed rise in M2 monocytes occurred at the expense of non-classified monocytes, which further decreased one day after surgery. These findings underscore the dynamic nature of monocyte/macrophage phenotypes and their ability to shift toward specific subtypes upon changes in environmental conditions.

There are certain peculiarities when analysing the relationship between TAMs and OC prognosis. Most TAMs in the OC TME are of the M2 phenotype [73], so higher monocyte infiltration into OC tissue might be expected to correlate with worse outcomes. Interestingly, overall macrophage infiltration does not necessarily correlate with prognosis in OC [75]. However, once again, the critical insight lies in the subpopulations. A higher density of M2 macrophages and a lower M1/M2 ratio in OC tissue are associated with poorer OS, PFS, and resistance to therapies [75–77].

In our study, the peripheral M1/M2 monocyte ratio was significantly reduced in preoperative OC patients. While prior studies focused on intratumoral macrophage levels and ratios, peripheral monocytes and their postoperative dynamics remain minimally explored. This raises the question: were these changes anticipated? Some data exist regarding peripheral monocyte levels and their subpopulations in cancer patients. Shibutani et al. found a correlation between peripheral monocyte levels and TAM infiltration in colorectal cancer patients [181], while Fadhil et al. observed an elevated M2/M1 monocyte ratio in breast cancer patients [182]. Taken together, these findings suggest that a low M1/M2 monocyte ratio was expected, at least in preoperative OC patients, and likely reflects an immunosuppressive environment,

characteristic of OC. Furthermore, the M1/M2 ratio remained unchanged postoperatively, indicating that this feature of a tumor-promoting environment persists into the early postoperative period.

Monocytes are undoubtedly critical to cancer progression, acting as precursors to TAMs, DCs, and MDSCs. The programming and functional shifts of monocytes during cancer treatment are beginning to receive significant attention. Surgery, as a major cancer therapy, influences monocyte profiles, with evidence from some cancer types showing decreased monocyte functionality immediately post-surgery, followed by gradual recovery. However, findings are highly variable across cancer types, with limited studies addressing monocyte dynamics specifically in OC surgery. This leaves us with virtually no knowledge about monocyte dynamics after OC surgery, not to mention the unexplored early postoperative period [183].

Summary of the section

To conclude this section, we found a marked increase in the M2 monocyte proportion and a decreased M1/M2 ratio, both indicative of a tumor-promoting environment in OC patients. Although the M1/M2 ratio remained stable postoperatively, the elevation of the M2 proportion one day after surgery points to an even more pronounced tumor-promoting environment in the early postoperative period.

5.3. PBMC function in pre- and postoperative OC patients

As previously demonstrated, OC patients experience an immunosuppressive state, evident not only in the tumor microenvironment but also in peripheral immune cells such as PBMCs [60, 61]. It is a closed-loop mechanism, where interactions between OC cells and immune cells suppress the immune system. Consequently, this suppression creates a tumor-promoting environment, allowing cancer to progress [15, 16].

Changes in PBMC subpopulations provide useful insights into the immune system's status, but are rather indirect and do not fully reflect immune functionality. To assess anticancer immunity more directly, we analyzed PBMC activity by measuring their metabolic activity, ROS, NO production, and phagocytic capacity. While there are various methods to evaluate immune functionality, we consider these tests to be reliable indicators of the overall effects on PBMCs.

Comparing preoperative OC patients to a control group, multiple studies have shown reduced activity in various PBMC subsets. For instance, monocytes/macrophages in OC patients display impaired migration [184], and cytotoxic PBMC components, such as CD8+ T cells and NK cells, express

reduced granzyme B levels, compromising their ability to mediate cytotoxicity [185]. Additionally, PBMC cytotoxicity and phagocytic activity have been shown to be lower in OC patients, with decreased cell lysis and phagocytosis observed in experimental assays [20, 186]. However, in our study, both metabolic and functional activities of PBMCs in preoperative OC patients showed no significant differences compared to the control group. This lack of statistical significance was rather unexpected and was most likely due to the small sample size.

Postoperative PBMC activity revealed more dynamic changes. Metabolic activity, assessed via resazurin metabolism, was most suppressed on the first postoperative day, rebounding by day 5 (as observed following stimulation with LPS). This assay primarily measures cell viability but also indirectly reflects the suppression or activation of cellular metabolism [187].

Functional assays showed increased ROS production and decreased phagocytosis on day 1 postoperatively, with a tendency toward reversal by day 5. Phagocytosis is a rather straightforward and crucial function of monocytes/macrophages, which directly correlates with antitumor immunity. Reduced phagocytic activity indicates impaired immune response [15, 16, 62]. ROS production, however, presents a more complex picture. While ROS contribute to cytotoxic effects against tumor cells [164, 166], their overproduction can promote an immunosuppressive environment. Elevated ROS levels impair T cell activation, promote Treg expansion, polarize macrophages toward an immunosuppressive M2 phenotype, suppress NK cell activity, and inhibit dendritic cell function [15, 188]. Thus, in the context of OC, ROS overproduction is more likely to cause tumor progression than to induce antitumor effects [188, 189].

Surgery is an independent positive prognostic factor for OC patients due to tumor removal [12]. However, its immediate impact on the immune system creates a highly suppressive environment that may contribute to cancer progression and metastasis formation [25, 27]. It is important to acknowledge the limited number of studies investigating the postoperative immune response in OC patients, with most focusing on the later postoperative period. For example, Kovacs et al. reported increased monocyte phagocytic activity seven days postoperatively compared to preoperative levels [20]. Brøchner et al. observed significant suppression in NK cell numbers and activity one day postoperatively, with incomplete recovery even after 21 days [159]. Additionally, Napoletano et al. highlighted early postoperative benefits, such as a reduction in Tregs two days after surgery, with these positive effects becoming more evident by day 15 [18]. While positive immune effects may appear shortly after OC surgery, they are mostly counteracted by strong immunosuppressive effects in the early postoperative period. The exact

timing of when the negative immune suppression induced by surgery is outweighed by the positive effects of tumor removal remains uncertain but is estimated to occur two to four weeks after surgery [25]. However, our data, supported by the existing literature, indicate that this balance is not achieved in the early postoperative phase, which instead represents a period of significant immune suppression.

It is also worth noting that while the negative impact of surgery on immune suppression and its contribution to metastasis formation has been well-documented in various cancers, there is limited evidence directly addressing these effects in OC [27–30, 156, 161]. This gap underscores the need for further research to better understand these immune responses and tumor progression dynamics in the context of OC.

Summary of the section

Our analysis of PBMC activity did not reveal significant changes in OC patients preoperatively, but the postoperative period showed marked suppression of metabolic activity and phagocytosis, alongside increased ROS production on day 1 after surgery. These alterations indicate impaired PBMC function and a heightened tumor-promoting environment in the early postoperative period, with trends toward recovery by day 5 postoperatively.

5.4. Expression of immune-related proteins in PBMCs of pre- and postoperative OC patients

Immune, cancer, and other cells communicate either through direct contact via surface molecules or indirectly through signaling molecules. Cytokines are central mediators in this communication, shaping inflammation and immune responses by either suppressing or activating immunity. Their imbalance can promote tumor progression by creating an immunosuppressive environment or can enhance antitumor immunity [15, 16, 21]. Similarly, immune checkpoint molecules regulate immune cell activity and often enable tumors to evade immune surveillance [15, 22]. Investigating cytokines and other immune-related molecule dynamics helps to understand the immune response in OC more thoroughly.

Immune-related protein mRNA expression in PBMCs

For the initial analysis of immune-related protein production in PBMCs we measured mRNA levels of two pro-inflammatory cytokines (IL-1β and IL-6), two anti-inflammatory cytokines (IL-4 and IL-10), and immune checkpoint proteins (PD-1 and PD-L1). All studied proteins, except IL-10 mRNA, were downregulated in preoperative OC patients compared to controls. Since the other three cytokines [16, 126–129, 134] and the PD-1/PD-L1 checkpoint

[22, 62] have tumor-promoting roles, this downregulation could suggest strengthened antitumor immunity. However, such an interpretation is overly simplistic. Research has shown that mRNA expression does not always correlate with final protein production due to regulatory mechanisms in post-transcriptional and translational processes [190, 191]. Our findings further support this discrepancy, as monocyte cytokine expression and serum cytokine levels in our study did not align with mRNA expression in PBMCs. Few studies have directly investigated cytokine mRNA levels in OC patients. However, one study reported results that were inconsistent with our findings [192]. Nevertheless, other research using different approaches consistently has shown suppressed PBMC activity in OC patients [184-186]. The overall downregulation of mRNA expression for the studied proteins most likely reflects suppressed PBMC activity [190].

Postoperative analysis of immune-related protein mRNA expression revealed various changes. Except for IL-10 and PD-1 mRNA, all other studied proteins showed further downregulation on either day 1 or day 3 postoperatively, followed by a rebound by day 5. Similar to preoperative downregulation, this postoperative decrease likely reflects further suppression of PBMC activity. Most of the observed alterations were most pronounced on day 1 after surgery, with significant alterations also noted on day 3.

Summary of the section

We cannot draw definitive conclusions about the implications of each protein's mRNA expression dynamics, as mRNA levels do not always correlate with the final protein expression. However, the data show that the mRNA expression of the investigated proteins was downregulated preoperatively and further suppressed during the early postoperative period. These changes likely reflect PBMC suppression preoperatively and additional suppression after surgery in OC patients.

Cytokine expression in monocytes

Monocytes and macrophages are the most abundant immune cells in the TME [70] and are responsible for a variety of cytokine production [136, 139]. For our further analysis of cytokine expression in PBMCs, we focused on monocytes. Using FCM, we measured intracellular cytokine accumulation, which reflects the final step of cytokine production at the protein level. Studies specifically analyzing monocyte cytokine expression in preoperative or postoperative OC patients are limited.

Preoperative evaluation of cytokine production in monocytes showed no significant changes in intracellular levels of IL-1β, IL-4, or IL-12. It is worth mentioning that IL-4 is predominantly produced by basophils, and IL-12 secretion is primarily attributed to DCs, with monocytes contributing only

minimally to these interleukins' secretion [136, 139]. IL-10 was significantly downregulated and TNF-α showed a tendency toward downregulation in M2 monocytes before surgery. Although IL-10 has multifunctional roles, it is often associated with tumor-promoting effects [15, 16, 135]. TNF-α, although recognized for its antitumor properties, has been shown to indirectly support tumor progression under certain conditions [16, 62, 131–133]. These changes in IL-10 and TNF-α expression may indicate a less supportive environment for tumor progression. Conversely, IL-6 was markedly overexpressed in M2 monocytes from preoperative OC patients. Given IL-6's established role in facilitating tumor growth and progression [126, 129], this upregulation suggests a more pronounced tumor-promoting immune environment.

Postoperative cytokine expression in monocytes exhibited further changes. TNF-α (in M1 monocytes) and IL-6 (in M2 monocytes) levels were elevated on the first day after surgery compared to controls but began trending toward control levels by day 5. Meanwhile, IL-10 levels, which had been reduced preoperatively, returned to levels comparable to those observed in healthy controls. These results indicate that, in addition to the preoperative imbalance, cytokine expression in monocytes shifts further during the postoperative period. Given the established roles of TNF-α, IL-6, and IL-10 in OC progression [15, 16, 62, 126, 129, 131–133, 135], the postoperative rise in cytokine levels may reflect an enhanced state of tumor-promoting environment.

Summary of the section

Overall, our findings in preoperative OC patient monocytes indicate dysregulated cytokine expression. Clear conclusions are challenging due to the dual roles of some cytokines in cancer biology and differing interpretations of the observed changes. However, postoperative alterations in monocyte cytokine expression more clearly suggest a shift toward a tumor-promoting environment.

5.5. Immune-related protein serum levels in pre- and postoperative OC patients

To gain a broader understanding of the immune response in OC patients, we analyzed serum levels of the investigated cytokines and PD-1/PD-L1 proteins. In preoperative OC patients, only minor and statistically insignificant changes in cytokine levels were observed. While increases in cytokines like IL-10 or IL-6 were anticipated in this group, findings such as ours are not uncommon. Variations across studies often arise due to differences in cutoff values and measurement methods [193, 194].

Postoperative cytokine analysis revealed a significant change: a marked increase in IL-6 levels. On the first day after surgery, the median IL-6 level rose 1.5-fold compared to preoperative levels, with the third quartile reaching nearly three times the preoperative value. This rise in IL-6 aligns with expectations given the extensive cytoreductive surgery OC patients undergo [195]. Such a postoperative surge in IL-6 is known to foster a tumor-promoting environment, contributing to OC progression [126, 129].

PD1/PD-L1 is one of the main immune checkpoints that participates in immune evasion in OC patients [23]. PD-L1 is expressed on tumor cells, while PD-1 is found on immune cells. Their interaction suppresses antitumor immunity, and higher cell surface expression levels are associated with worse prognoses [22, 196]. Although PD-1 and PD-L1 are primarily cell surface proteins, they can also be detected in serum in a soluble form, making them potentially convenient prognostic markers. Higher plasma levels of PD-1 and PD-L1 have been identified as negative prognostic indicators for HGSC patients [197]. In our study, we noted a trend toward lower PD-1 levels in preoperative OC patients, which faded after surgery, possibly reflecting an increased tumor-promoting effect postoperatively. Fluctuations in PD-L1 levels were also observed, though these were insignificant. However, these findings should be interpreted cautiously due to the wide data dispersion in serum PD-1 and PD-L1 levels.

Summary of the section

Among the investigated cytokines, no significant serum level changes were observed preoperatively in OC patients, while a significant postoperative increase in IL-6 was detected. This rise can undoubtedly be interpreted as a tumor-promoting factor. Additionally, despite some fluctuations, we did not find any significant alterations in PD-1 and PD-L1 serum levels in pre- and postoperative OC patients.

5.6. Final summary and considerations

The primary goal of this study was to analyze the immune system in OC patients during the early postoperative period, which may be a critical time for OC progression. While we did not link our findings to long-term outcomes or patient prognoses, we aimed to draw attention to this largely unexplored period as a potential target for therapeutic intervention.

For the foundation of our study, we compared preoperative OC patients with healthy individuals. Our findings provide sufficient evidence, based on multiple levels of PBMC investigation, that anticancer immunity in OC patients is either suppressed or altered in a way that contributes to a tumor-

promoting environment. Although these findings align with prior research and were anticipated, they reaffirm the immunosuppressive state of OC patients.

Major surgery is well-documented to significantly impact the immune system, contributing to cancer progression in certain cases. However, such effects have not been thoroughly studied in OC patients. Existing research on postoperative immunity in OC typically focuses on later postoperative periods, concluding that the immune system recovers and that surgical treatment has a positive long-term effect. In contrast, our study highlights the early postoperative period, which remains largely unexplored in OC patients. Given the existing evidence, we anticipated immune suppression during this phase.

Our findings on postoperative PBMC-related immune alterations can be described at two levels. First, the preoperative immune suppression and tumor-promoting environment observed in preoperative OC patients persist into the early postoperative period and remain unresolved. Second, additional immune suppression and tumor-promoting features emerge during this critical time, as shown across multiple levels of PBMC investigation in our study.

Considering our focus on the early postoperative period in OC patients and the wide spectrum of assays performed, this study is unique. We demonstrated that despite the long-term positive effects of OC surgery, the early postoperative period is marked by significant immune suppression. Understanding and addressing the immune suppression observed during this critical early postoperative window could offer a transformative opportunity to improve patient outcomes and combat OC progression.

5.7. Study limitations

While our study gives us valuable insights, there are certain limitations that must be acknowledged. One of the main limitations is the relatively small sample size. This was primarily due to the focus on the early postoperative period, a less-studied period in OC management, and the involvement of a wide range of tests with various potential findings. These factors made it challenging to predict preliminary findings and calculate the required sample size. In this context, the absence of statistically significant differences in certain comparisons should not be interpreted as the absence of actual differences. Moreover, it is important to acknowledge that the global COVID-19 pandemic, along with certain clinical regulations, disrupted patient enrollment, which may have limited the sample size. A larger cohort might have been achievable under different circumstances during the study period.

Additionally, our study included only patients with advanced-stage OC. This limits the comparability of our findings across different disease stages and affects their generalizability. However, given the poor prognosis of advanced-stage OC patients [2, 6], we prioritized studying this group due to its clinical significance.

While we analyzed changes in PBMC subpopulations and cytokine production, we did not examine their direct impact on disease progression or clinical outcomes. Addressing this would require a longer study duration and is planned for future research.

Moreover, despite the wide range of tests conducted, we did not analyze smaller PBMC subpopulations or the relationships between the observed changes, which could provide a deeper understanding of postoperative immune suppression. To fully harness the potential of the early postoperative period in OC patients, further investigation into the specific immune alterations during this time is needed.

Another limitation is the lack of a control group comprising individuals with benign gynecologic conditions undergoing surgery. Without such a group, it is difficult to isolate the specific impact of OC on postoperative immune dysregulation. The complex nature of OC surgery makes selecting suitable control patients difficult. Despite this limitation, we believe our findings remain significant. Whether immune suppression is caused by surgery alone or in combination with OC, the key point is that surgery remains the cornerstone of OC treatment [12], and OC patients inevitably face postoperative immune suppression as part of their recovery process.

Lastly, as this is an observational study, we cannot establish cause-and-effect relationships. Further research is essential to uncover the mechanisms underlying immune changes in OC patients undergoing surgery.

CONCLUSIONS

Based on our PBMC analysis, OC patients exhibit significant immune suppression and alterations associated with a tumor-promoting environment. These immune changes persist in the early postoperative period, with additional tumor-promoting features emerging during this phase.

To provide a detailed summary aligned with our study aims, the key findings are as follows:

1. Preoperative OC patients were characterized by elevated WBC, neutrophil, and platelet counts, alongside lower hemoglobin levels. Both NLR and PLR were significantly higher in this group.
2. In preoperative OC patients, proportions of CD3+ and CD8+ T cells, non-classified monocytes, and the M1/M2 ratio were decreased, while proportions of CD19+ B cells, M2 monocytes, and the CD4/CD8 ratio were elevated. Postoperatively, most alterations persisted or became even more pronounced.
3. PBMC metabolic activity and function in preoperative OC patients were comparable to controls. One day after surgery, PBMC metabolic activity and phagocytosis decreased, while ROS production increased.
4. In OC patients, the mRNA expression of immune-related proteins in PBMCs was mainly downregulated preoperatively and further suppressed during the early postoperative period. Some monocyte cytokine expression in OC patients was dysregulated, with mixed expression patterns.
5. Among the serum levels of immune-related proteins in OC patients, only one significant alteration was noted – a marked increase in IL-6 levels one day after surgery.

PRACTICAL RECOMMENDATIONS

At this stage, no direct clinical recommendations can be made based on the findings of this study. However, our results underscore the need for further research. First, additional studies are needed to determine the clinical relevance of the observed immune changes in the early postoperative period and their potential impact on disease progression. Establishing these associations would provide a foundation for future clinical trials. Second, targeted clinical trials should explore strategies to counteract postoperative immune alterations, aiming to reduce immunosuppressive effects and improve patient outcomes. Notably, potential intervention periods may extend beyond the early postoperative phase to include intraoperative strategies as well.

SANTRAUKA

SANTRUMPOS

BKT	–	bendras kraujo tyrimas
CD3+ T ląstelė	–	T limfocitas
CD4+ T ląstelė	–	pagalbinis T limfocitas
CD8+ T ląstelė	–	citotoksinis T limfocitas
CD19+ B ląstelė	–	B limfocitas
CD4 / CD8	–	CD4+ T ir CD8+ T ląstelių santykis
CRO	–	citoredukcinė operacija
ELISA	–	fermentinis imunosorbentinis tyrimas (angl. *enzyme-linked immunosorbent assay*)
iRNR	–	informacinė ribonukleorūgštis
IL-1β	–	interleukinas 1β
IL-4	–	interleukinas 4
IL-6	–	interleukinas 6
IL-10	–	interleukinas 10
IL-12	–	interleukinas 12
IQR	–	tarpkvartilinis plotis (angl. *interquartile range*)
kDNR	–	komplementarioji deoksiribonukleorūgštis
KMI	–	kūno masės indeksas
KV	–	kiaušidžių vėžys
LPS	–	lipopolisacharidas
LMR	–	limfocitų ir monocitų santykis (angl. *lymphocyte-to-monocyte ratio*)
M1 / M2	–	M1 ir M2 monocitų santykis
NK ląstelė	–	natūralusis žudikas (angl. *natural killer cell*)
NO	–	azoto oksidas (angl. *nitric oxide*)
NLR	–	neutrofilų ir limfocitų santykis (angl. *neutrophil-to-lymphocyte ratio*)
PD-1	–	programuotos ląstelės žūties baltymas 1 (angl. *programmed cell death protein 1*)
PD-L1	–	programuotos ląstelės žūties baltymo ligandas 1 (angl. *programmed death-ligand 1*)
PKVL	–	periferinio kraujo vienbranduolės ląstelės
PLR	–	trombocitų ir limfocitų santykis (angl. *platelet-to-lymphocyte ratio*)
Proc.	–	procentai
RL PGR	–	realaus laiko polimerazės grandininė reakcija
RNR	–	ribonukleorūgštis
ROS	–	reaktyvios deguonies formos (angl. *reactive oxygen species*)
TBHP	–	tert-butil hidroperoksidas
TC	–	tėkmės citometrija
TNF-α	–	naviko nekrozės faktorius α (angl. *tumor necrosis factor α*)
VFI	–	vidutinis fluorescencijos intensyvumas

ĮVADAS

Kiaušidžių vėžys (KV) yra aštunta pagal dažnumą moterų onkologinė liga pasaulyje. Kiekvienais metais nustatoma daugiau nei 324 tūkst. naujų ligos atvejų ir beveik 207 tūkst. mirčių nuo KV [1, 2]. 2022 m. pasaulinis sergamumas KV siekė 8,3 atvejo 100 tūkst. moterų, o mirtingumas – 5,3 atvejo 100 tūkst. moterų [3]. Remiantis Pasaulinės vėžinių ligų stebėjimo duomenų bazės (angl. *Global Cancer Observatory*, GLOBOCAN) prognozėmis, neatradus geresnių diagnostikos ar gydymo metodų, naujų atvejų skaičius iki 2050 m. gali padidėti 55 proc., o mirčių nuo KV – beveik 70 proc. [4]. Situacija Lietuvoje ypač kelia nerimą: 2022 m. buvo diagnozuoti 366 nauji KV atvejai, sergamumas – 25,6 atvejo 100 tūkst. moterų, o mirtingumas – 16,9 atvejo 100 tūkst. moterų [3]. Šie duomenys atskleidžia KV problemos aktualumą bei būtinybę ieškoti efektyvesnių diagnostikos ir gydymo strategijų.

Dabartiniu metu KV gydymo pagrindas yra citoredukcinė operacija (CRO) ir chemoterapija [5, 6]. Tinkamai suderinus šiuos du gydymo metodus, pasiekiama geriausių įmanomų gydymo rezultatų, tačiau bendrasis 5 metų išgyvenamumas yra tik apie 49 proc. [2, 6]. Menką išgyvenamumą lemia tai, kad daugeliu atvejų KV diagnozuojamas vėlyvų stadijų ir daugelis navikų yra atsparūs chemoterapijai [7, 8]. Siekiant pagerinti KV gydymo rezultatus, nuolat ieškoma naujų gydymo būdų, pvz., taikinių terapija ar imunoterapija. Pirmieji naujų gydymo metodų veiksmingumo tyrimų rezultatai yra daug žadantys, bet būtini tolesni tyrimai [5, 9–12]. Nepaisant šių naujovių, CRO tebėra pagrindinis KV gydymo metodas. Visiška CRO, kurios tikslas – pašalinti visą matomą naviko audinį, yra siejama su geresniais išgyvenimo be ligos progresavimo bei bendrojo išgyvenamumo rodikliais [12, 13]. Vis dėlto, norint sukurti naujų veiksmingų gydymo metodų, būtina geriau suprasti KV vystymosi mechanizmus.

Periferinio kraujo vienbranduolės ląstelės (PKVL) yra pagrindinės imuninio atsako į vėžį ląstelės [14, 15]. Ši mišri ląstelių grupė apima T limfocitus (CD3+ T ląstelės), B limfocitus (CD19+ B ląstelės), natūraliuosius žudikus (angl. *natural killer cells*, NK ląstelės), monocitus ir dendritines ląsteles. CD3+ T ląstelių grupę sudaro pagalbiniai T limfocitai (CD4+ T ląstelės) ir citotoksiniai T limfocitai (CD8+ T ląstelės). PKVL su naviko ląstelėmis sąveikauja tiesiogiai darydamos citotoksinį poveikį ir netiesiogiai vykstant citokinų sekrecijai, antigenų pateikimui bei reguliacinėms funkcijoms [15, 16]. Iš PKVL kilusių ląstelių funkcija KV audinyje, citokinų gamybos pokyčiai ir jų įtaka naviko augimui yra žinomi ir gerai aprašyti [15–17]. Be to, paskelbta tyrimų, nagrinėjusių KV sergančių pacienčių pooperacinį imuninį atsaką, bet jie yra įvairūs, daugiausia tirtas vėlyvasis pooperacinis laikotarpis

ir gauti rezultatai skiriasi [18–20]. Duomenų apie ankstyvąjį KV sergančių pacienčių pooperacinį laikotarpį turima labai mažai.

Norint nuodugniai suprasti imuninį atsaką į vėžį, svarbu atsižvelgti ir į citokinų bei imuninio atsako baltymų vaidmenį. Citokinai – tai imuninį atsaką moduliuojantys baltymai, kurie dalyvauja imuninių ląstelių sąveikoje ir veikia imuninių ląstelių gebėjimą naikinti vėžio ląsteles [15, 16, 21]. Kita su imuniniu atsaku susijusių baltymų grupė, imuninės kontrolės taško molekulės, taip pat atlieka svarbų vaidmenį formuojant naviko mikroaplinką, skatindamos arba slopindamos naviko vystymąsi [22, 23]. Šios molekulės reikšmingai prisideda prie KV vystymosi būdamos tiek naviko audinyje, tiek kraujo serume [15, 16, 24], tačiau mažai žinoma apie jų raišką PKVL bei jų raiškos pokyčius ankstyvuoju pooperaciniu laikotarpiu.

Chirurginis KV gydymas neabejotinai yra svarbus gydymo būdas, tačiau operuojant smarkiai žalojami audiniai, o tai paveikia visą paciento organizmą [25, 26]. Audinių trauma lemia molekulinių mechanizmų pokyčius (angl. *damage-associated molecular patterns*), neutrofilų tarpląstelinių tinklų gamybą bei mieloidinės kilmės slopinančiųjų ląstelių suaktyvėjimą. Dėl to sutrinka aktyvinančiųjų bei slopinančiųjų imuninių ląstelių veikla ir imuninis atsakas [25, 27]. Šie pokyčiai gali skatinti metastazių susidarymą – tai įrodyta tiriant chirurgiškai gydytus krūties, plaučių, kasos, storosios žarnos ir kitų rūšių vėžiu sergančius pacientus [28–30]. Koks šių pokyčių poveikis KV atvejais, duomenų stokojama. Atsižvelgiant į glaudų ryšį tarp imuninės sistemos, naviko mikroaplinkos ir KV vystymosi [15, 16], galima daryti prielaidą, kad chirurginė intervencija turi įtakos ir KV progresavimui.

Iškelta hipotezė, kad ankstyvuoju pooperaciniu laikotarpiu KV sergančių pacienčių imuninės sistemos, susijusios su PKVL, veikla sutrinka. Šiame tyrime analizuotos PKVL populiacijos, jų funkcija, imuninio atsako baltymų raiška ir jų kiekis kraujo serume ankstyvuoju pooperaciniu laikotarpiu. Mūsų rezultatai gali padėti geriau suprasti KV vystymosi mechanizmus ir prisidėti prie naujų gydymo strategijų kūrimo.

Darbo tikslas

Ištirti su periferinio kraujo vienbranduolėmis ląstelėmis (PKVL) susijusio imuninio atsako pokyčius, atsirandančius po chirurginio kiaušidžių vėžio (KV) gydymo.

Tyrimo uždaviniai

1. Įvertinti KV sergančių pacienčių periferinio kraujo ląstelių sudėties pokyčius.
2. Įvertinti KV ir papildomos chirurginės traumos sukeliamus PKVL subpopuliacijų pokyčius.
3. Įvertinti KV ir papildomos chirurginės traumos sukeliamus PKVL funkcijos pokyčius.
4. Įvertinti KV ir papildomos chirurginės traumos sukeliamus imuninio atsako baltymų raiškos PKVL pokyčius.
5. Įvertinti KV ir papildomos chirurginės traumos sukeliamus imuninio atsako baltymų kiekio kraujo serume pokyčius.

Tyrimo naujumas ir aktualumas

Mūsų tyrimo naujumas gali būti vertinamas keliais požiūriais. Imuninės sistemos vaidmuo KV vystytis yra gerai žinomas, tačiau dauguma tyrimų analizuoja vietinę imuninės sistemos veiklą KV audinyje. Mūsų tyrimo ašis – PKVL, kurios yra mažiau ištirtos esant KV, tačiau yra lengviau pasiekiama imuninės sistemos dalis, palyginti su vėžio audiniu.

Atliekant pradinę analizę, buvo ištirtas su PKVL susijęs KV sergančių pacienčių imunitetas prieš operaciją. Nors KV pacienčių PKVL yra tyrinėjamos, tačiau tam tikrų tyrimų (pvz.: citokinų informacinės ribonukleino rūgšties (iRNR) raiškos PKVL, monocitų subpopuliacijų, citokinų raiškos monocituose) duomenų vis dar stinga arba jų visai nėra.

Svarbiausias mūsų tyrimo naujumo aspektas – dėmesys ankstyvajam pooperaciniam laikotarpiui. Nors operacijos poveikis KV sergančių pacienčių imuninei sistemai yra nagrinėtas, daugiausia tirtas vėlyvasis pooperacinis laikotarpis, pabrėžiant vėlyvąjį teigiamą chirurginio gydymo poveikį. Tyrimų, analizuojančių ankstyvąjį pooperacinį imuninį atsaką KV atvejais, atlikta nedaug, daugelis jų mažos apimties. Susitelkus į šį menkai ištirtą laikotarpį ir atlikus platų spektrą tyrimų, iš kurių daugelis šios grupės pacientėms dar nebuvo atlikti, gauta unikalių duomenų apie KV sergančių pacienčių imuninių procesų dinamiką ankstyvuoju pooperaciniu laikotarpiu.

METODAI

Tyrimo organizavimas

Šis perspektyvusis stebimasis tyrimas buvo atliekamas Lietuvos sveikatos mokslų universiteto Virškinimo sistemos tyrimų instituto Chirurginės gastroenterologijos laboratorijoje bei Lietuvos sveikatos mokslų universiteto ligoninės Kauno klinikų Akušerijos ir ginekologijos klinikoje nuo 2021 m. sausio mėn. iki 2023 m. balandžio mėn. Tyrimas atliktas vadovaujantis Helsinkio deklaracijos nuostatomis. Leidimas vykdyti tyrimą buvo gautas iš Kauno regioninio biomedicininių tyrimų etikos komiteto (Nr. BE-2-16; patvirtinta 2020 m. vasario 5 d.). Rašytinis informuoto asmens sutikimas buvo gautas iš visų tyrimo dalyvių.

Tiriamųjų atranka

Į tiriamųjų grupę įtrauktos pacientės, kurioms buvo įtariamas III–IV stadijos KV pagal Tarptautinę akušerijos ir ginekologijos federacijos (angl. *International Federation of Obstetrics and Gynaecology,* FIGO) klasifikaciją [44] ir numatyta pirminė CRO.

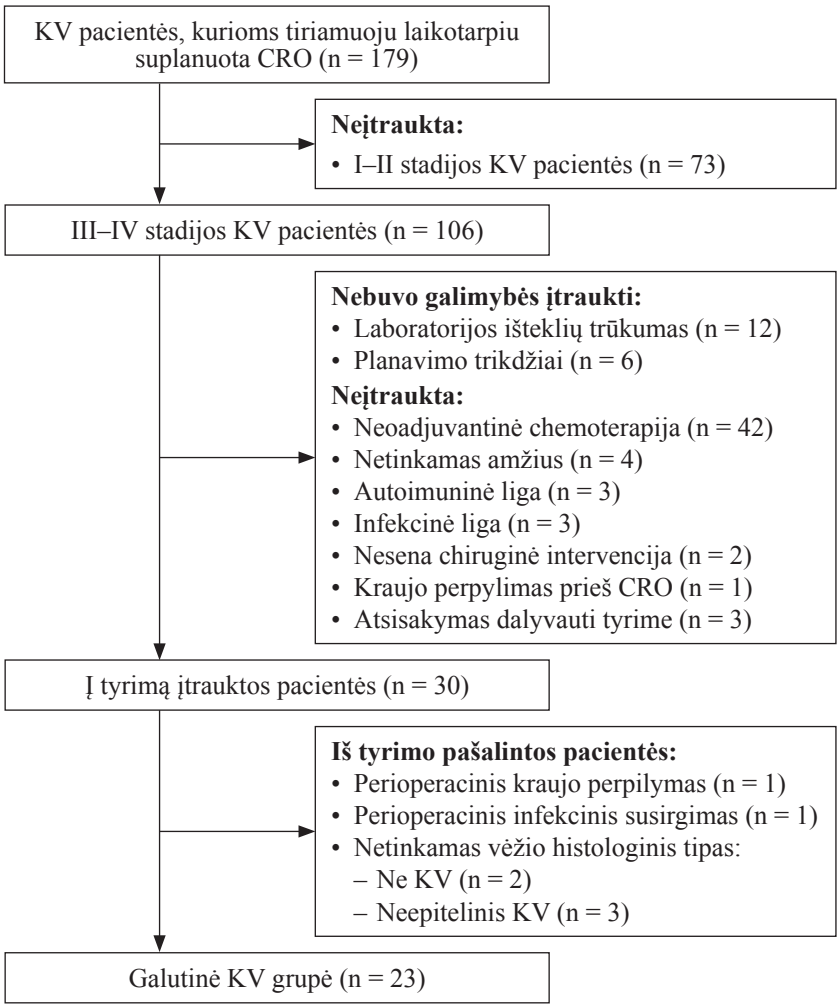
Įtraukimo kriterijai:

- Klinikinė arba histologinė KV diagnozė.
- III–IV KV stadija pagal FIGO [44].
- Planuojamas chirurginis gydymas.
- Amžius nuo 18 iki 80 metų.

Neįtraukimo kriterijai:

- Histologiškai patvirtintas neepitelinis KV.
- Atliktas kraujo perpylimas operacijos metu, po operacijos arba per pastarąjį mėnesį.
- Anksčiau taikyta neoadjuvantinė chemoterapija.
- Aktyvus uždegimas ar infekcija su poreikiu gydyti antibiotikais operacijos metu, po operacijos arba per pastarąjį mėnesį.
- Lėtinės uždegimo arba autoimuninės ligos (pvz.: reumatoidinis artritas, sisteminė raudonoji vilkligė ir kt.).
- Chirurginė intervencija per pastarąjį mėnesį.
- Onkologinė liga anamnezėje.
- Nėštumas.

KV grupės pacienčių įtraukimo į tyrimą schema pateikta 1 pav.



1 pav. *Kiaušidžių vėžio grupės pacienčių įtraukimo į tyrimą schema*

KV – kiaušidžių vėžys, CRO – citoredukcinė operacija.

Kontrolinę grupę sudarė moterys, parinktos atsižvelgiant į tiriamosios grupės pacienčių kūno masės indeksą (KMI) bei amžių. Kontrolinei grupei buvo taikomi tokie patys neįtraukimo kriterijai kaip ir KV pacientėms. Be to, kontrolinėje grupėje negalėjo dalyvauti moterys, sirgusios vėžiu iki tyrimo ar tyrimo metu, taip pat kurioms tyrimo metu vėžys buvo įtariamas. Gretutinės ligos, tokios kaip hipertenzija, 2 tipo cukrinis diabetas ir kt., buvo leidžiamos abiejose grupėse. Visos tyrimo dalyvės buvo apklausiamos, vertinama medi-

cininė dokumentacija išsiaiškinti gretutines ligas ir nustatyti tinkamumą dalyvauti tyrime pagal įtraukimo ir neįtraukimo kriterijus.

Prieš įtraukiant į tiriamąją grupę visoms pacientėms buvo atliktas standartinis KV išplitimo ištyrimas: vidinių genitalijų ultragarsinis tyrimas, pilvo, dubens bei krūtinės kompiuterinė tomografija ir, jei reikėjo, kiti tyrimai. KV diagnozė buvo patvirtinta po pirminės CRO, atlikus histologinį tyrimą.

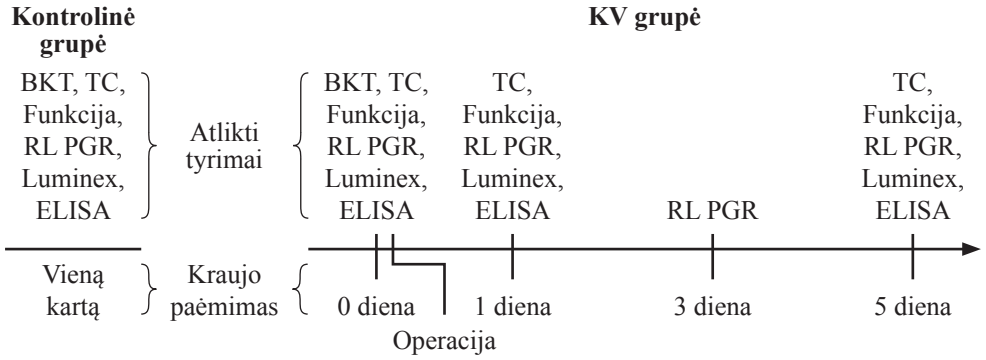
Pirminės CRO metu atliekama histerektomija su gimdos priklausinių pašalinimu, omentektomija, matomo navikinio audinio židinių pašalinimas, paimama pilvaplėvės biopsinės medžiagos ir, jei reikia, pašalinami dubens bei paraaortiniai limfmazgiai. Operuojant gali būti atliekama apendektomija ir splenektomija. Atsižvelgiant į likusį naviko audinį po CRO, operacijos rezultatai apibūdinami taip: visiška CRO (nėra likusio matomo naviko audinio), optimali CRO (likusio naviko audinio židiniai mažesni nei 1 cm dydžio) ar suboptimali CRO (likusio naviko audinio židiniai didesni nei 1 cm dydžio) [43].

Imties dydis

Į tyrimą įtrauktos 23 KV sergančios pacientės ir 23 sveikos moterys. Dėl suplanuotų įvairaus pobūdžio tyrimų bei sunkumų numatant preliminarius šių tyrimų rezultatus, imties dydžio skaičiavimas nebuvo atliktas. Taip pat svarbu, jog tyrimo tikslas buvo ištirti ankstyvąjį pooperacinį laikotarpį, kuris KV atveju dar nėra plačiai aprašytas. Išankstinių duomenų stoka ir tai, kad šis tyrimas yra aprašomojo pobūdžio, o ne skirtas ieškoti hipotezėmis grindžiamų specifinių skirtumų, lėmė sunkumus numatant tinkamą imties dydį.

Kraujo mėginių rinkimas

KV ir kontrolinės grupių tiriamosioms atlikti periferinio veninio kraujo ėminiai. KV grupės pacienčių kraujo buvo imama prieš operaciją ir 1-ą, 3-ią bei 5-tą dieną po operacijos. Atsižvelgiant į pirmųjų tyrimų rezultatus, trečią dieną po operacijos kai kurių tyrimų nuspręsta neatlikti. Kontrolinės grupės dalyvių kraujo buvo imama vieną kartą, tyrimai atlikti pagal tą patį protokolą kaip ir KV pacientėms prieš operaciją. Detalus kraujo ėminių ir atliekamų tyrimų planas pateiktas 2 pav. Kraujas buvo surenkamas į vieną 4 ml ir tris 10 ml vakuuminius mėgintuvėlius (mėgintuvėlius) arba tris 10 ml mėgintuvėlius, arba vieną 10 ml mėgintuvėlį su EDTA K2 (DB, Plymouth, Jungtinė Karalystė) bei du 3,5 ml serumo mėgintuvėlius su krešėjimo aktyvikliu (Weihai Hongyu, Weihai, Kinija) priklausomai nuo kraujo paėmimo dienos.



2 pav. *Kraujo ėminių ir tyrimų atlikimo planas*

Apatinėje laiko juostos dalyje nurodytas KV ir kontrolinės grupės tiriamųjų kraujo ėminių laikas, o viršutinėje dalyje išvardyti atlikti tyrimai: BKT – bendrasis kraujo tyrimas, TC – tėkmės citometrija, Funkcija – periferinio kraujo vienbranduolių ląstelių funkcinio ir metabolinio aktyvumo vertinimas, RL PGR – realaus laiko polimerazės grandininė reakcija, Luminex – tyrimai, atlikti naudojant Luminex sistemą, ELISA – fermentinis imunosorbentinis tyrimas (angl. *Enzyme-Linked Immunosorbent Assay*).

Atlikti tyrimai ir laboratorinės procedūros

Bendras kraujo tyrimas (BKT)

Veninio kraujo ėminiai, surinkti į 4 ml mėgintuvėlius su EDTA K_2 (DB, Plymouth, JK), buvo skirti atlikti bendrąjį kraujo tyrimą (BKT). Kraujas tirtas automatiniu hematologiniu analizatoriumi UniCell DxH 800 (Beckman Coulter, JAV), vadovaujantis gamintojo instrukcijomis. Analizatoriaus pateikti BKT duomenys: eritrocitų, hemoglobino, trombocitų, leukocitų koncentracija ir leukocitų populiacijų kiekis.

PKVL išskyrimas ir kraujo serumo paruošimas

Kraujo ėminiai, surinkti į 10 ml mėgintuvėlius su EDTA K2 (DB, Plymouth, JK), buvo centrifuguojami 2470 g greičiu 10 min. plazmos sluoksniui atskirti ir pašalinti. Vadovaujantis gamintojo instrukcijoms, PKVL buvo išskirtos naudojant Ficoll-Paque PREMIUM (Cytiva, Švedija) gradientinę izoliavimo terpę. Susidaręs PKVL sluoksnis buvo atsargiai surinktas ir nuplautas fosfato buferiniu tirpalu (Sigma-Aldrich, Taufkirchen, Vokietija) centrifuguojant 250 g greičiu 2 kartus po 5 min. Izoliuotos KV ir kontrolinės grupės tiriamųjų PKVL buvo pakartotinai praskiedžiamos iki 1 mln. ląstelių/ml koncentracijos ir padalytos į 1 ml tūrio dalinius mėginius tolesniems tyrimams. Šie mėginiai buvo naudojami PKVL funkcijos bei tėkmės citometrijos (TC) tyrimams atlikti. Papildoma PKVL dalis buvo sumaišyta su RNAlater® terpe (Thermo Fisher Scientific, Waltham, MA, JAV) ir laikoma

–20 °C ar –80 °C temperatūroje iki realaus laiko polimerazės grandininės reakcijos (RL PGR) atlikimo.

Kraujo serumui atskirti buvo naudojamas kraujas, surinktas į mėgintuvėlius su krešėjimo aktyvikliu (Weihai Hongyu, Weihai, Kinija). Paėmus kraują, buvo laukiama 1 val., kol susidarys krešulys, tada 4 °C temperatūroje atliktas 20 min. trukmės centrifugavimas 2500 g greičiu. Virš gelio sluoksnio esantis serumas buvo surenkamas, perkeliamas į kriogeninius mėgintuvėlius ir laikomas –80 °C temperatūroje iki tyrimų atlikimo.

Limfocitų ir monocitų imunofenotipavimas naudojant TC

Limfocitų imunofenotipavimas atliktas TC metodu su Multitest 6-color TBNK rinkiniu (BD, Franklin Lakes, NJ, JAV). Tyrimo metu buvo išskiriamos pagrindinės limfocitų grupės naudojant šiuos ląstelių žymenis: CD3+ (T limfocitams), CD3+ / CD4+ (pagalbiniams T limfocitams), CD3+ / CD8+ (citotoksiniams T limfocitams), CD19+ (B limfocitams) ir CD3– / CD16+ / CD56+ (NK ląstelėms). Daliniai mėginiai su 1 mln. PKVL buvo inkubuojami su monokloninių antikūnų mišiniu, kuriame buvo šių antikūnų: CD3-FITC (Leu-4), CD4 PE-Cy™7 (Leu-3a), CD8 APC-Cy™7 (Leu-2a), CD16 PE (Leu-11c), CD19 APC (Leu-12), CD56 PE (Leu-19) ir CD45 PerCP-Cy5.5 (2D1). Procedūra atlikta vadovaujantis gamintojo rekomendacijomis. Vėliau ląstelės buvo nuplautos, kad pasišalintų neprisitvirtinę antikūnai, ir mėginiai toliau analizuoti FACSLyric 10 spalvų tėkmės citometru (BD, San Jose, CA, JAV). Kiekvienam mėginiui parinkta 10 tūkst. ląstelių identifikavimo riba. Limfocitų pogrupiai limfocitų vartuose buvo identifikuoti įvertinus grūdėtumą ir CD45 žymens raišką.

Monocitų ir makrofagų imunofenotipavimui buvo naudojamas papildomas monokloninių antikūnų mišinys, sudarytas iš šių antikūnų: CD3 PE (UCHT1), CD14 BV510 (MφP9), CD16 APC (B73.1), CD80 APC-H7 (L307.4), CD86 PE-Cy7 (2331 (FUN-1)), CD163 BV605 (GHI/61), CD206 FITC (19.2), HLA-DR PerCP (L243), CD19 PE (HIB19), CD56 PE (555516) ir CD66b PE (G10F5). Antikūnų gamintojas BD Biosciences (San Jose, CA, JAV).

PKVL metabolinio ir funkcinio aktyvumo vertininimas

Išskirtos ir į dalinius mėginius padalytos PKVL buvo kultivuojamos RPMI 1640 terpėje (Gibco Life Technologies Limited, Paisley, JK) papildomai pridėjus 10 proc. embrioninio galvijų serumo (Gibco Life Technologies Limited, Paisley, JK) ir 1 proc. penicilino su streptomicinu (Gibco Life Technologies Limited, Paisley, JK). Po to mėginiai inkubuoti 30 min. 37 °C temperatūroje ir išskirstyti į šviesias bei tamsias 96 šulinėlių plokšteles. Į kiekvieną šulinėlį įdėta 90 tūkst. ląstelių su atitinkama terpe. Po 3 val. trukusio aktyvinimo skirtingais efektoriais plokštelės buvo naudojamos PKVL

funkciniam aktyvumui nustatyti. Atliekant fluorometrinius ir spektrofotometrinius tyrimus buvo vertinama PKVL fagocitozė, reaktyviųjų deguonies formų (angl. *reactive oxygen species,* ROS) ir azoto oksido (angl. *nitric oxide,* NO) gamyba.

Metabolinio aktyvumo nustatymas

Atliekant PKVL funkcijos tyrimus, kartu vertinant resazurino metabolizmą buvo nustatomas ir PKVL gyvybingumas (metabolinis aktyvumas). Metaboliniam aktyvumui nustatyti buvo naudojamos šviesios 96 šulinėlių plokštelės bei AlamarBlue® tyrimo paketas (Thermo Fisher Scientific, Waltham, MA, JAV). Taip pat kaip PKVL funkcijos tyrimams buvo paruošiami 3 tipų šulinėliai: su specifiniais aktyvikliais (kaip ir PKVL funkcijos tyrimams), neigiamos kontrolės ir kontroliniai terpės šulinėliai. AlamarBlue® tyrimas atliktas po 3 val. trukusio aktyvavimo, vadovaujantis gamintojo protokolu. Resazurino metabolizmas įvertintas spektrofotometriniais matavimais naudojant Sunrise Absorbance Reader spektrofotometrą (Tecan, Grodig, Austrija). Bazinis ląstelių metabolinis aktyvumas buvo nustatytas įvertinus resazurino metabolizmą šulinėliuose be aktyviklių.

Fagocitozės vertinimas

PKVL fagocitiniam aktyvumui sužadinti buvo naudojami lipopolisacharidai (LPS) (Sigma-Aldrich, Rehovot, Izraelis). Tyrimui atlikti naudotos tamsios 96 šulinėlių plokštelės ir ruošiami 3 tipų šulinėliai, kiekvienam tipui po tris. Į pirmuosius 3 šulinėlius su PKVL buvo dedama 10 µl 5 µg/ml koncentracijos LPS tirpalo. Į tris neigiamos kontrolės šulinėlius su PKVL buvo dedama tik 10 µl LPS skiediklio. Į likusius 3 kontrolinius terpės šulinėlius be PKVL buvo dedama tik RPMI 1640 terpės ir 10 µl 5 µg/ml koncentracijos LPS tirpalo. Po 2 val. trukusio inkubacijos periodo fagocitozei sužadinti plokštelė centrifuguota ir pašalintas paviršinis skystis paliekant ląsteles. Toliau į kiekvieną šulinėlį įdėta 100 µl pHrodo Green Zymosan biologinių dalelių (Invitrogen, Eugene, OR, JAV) ir papildomai inkubuota 1 val. Dalelių fagocitozė buvo įvertinta naudojant Fluoroskan Ascent fluorometrą (Thermo Fisher Scientific, Waltham, MA, JAV), nustačius 510 nm ekscitacijos bangos ilgį ir 538 nm emisijos bangos ilgį.

ROS gamybos vertinimas

ROS gamybos vertinimo procedūra pradėta nuo PKVL dažymo 2,7 dichlorofluoresceino diacetatu (DCFDA) naudojant Cellular ROS Assay rinkinį (Abcam, Cambridge, JK). PKVL aktyvinti naudotas tetr-butilo hidroperoksido (TBHP) tirpalas (Abcam, Cambridge, JK), į šulinėlius dedant po 10 µl 2,5 mM tirpalo. Šie veiksmai atlikti vadovaujantis gamintojo protokolu. Kaip ir fagocitozės funkcijos vertinimo procedūrai paruošti 3 tipų šulinėliai tam-

siose 96 šulinėlių plokštelėse: turintys PKVL ir TBHP, turintys PKVL ir TBHP skiediklio (neigiamos kontrolės šulinėliai) bei turintys RPMI 1640 terpės ir 10 µl 2,5 mM TBHP be PKVL (kontroliniai terpės šulinėliai). Po 3 val. inkubacijos 37 °C temperatūroje buvo matuojama PKVL fluorescencija Fluoroskan Ascent fluorometru (Thermo Fisher Scientific, Waltham, MA, JAV). Nustatytas 485 nm ekscitacijos bangos ilgis ir 538 nm emisijos bangos ilgis.

NO gamybos vertinimas

NO gamybai vertinti buvo naudojamos skaidrios 96 šulinėlių plokštelės po 3 val. aktyvinimo LPS (Sigma-Aldrich, Rehovot, Izraelis) 10 µl 5 µg/ml tirpalu ir 100 mM L-arginino (Sigma-Aldrich, Tokyo, Japonija). Kaip aprašyta anksčiau, paruošti 3 tipų šulinėliai: turintys PKVL ir LPS su L-argininu, neigiamos kontrolės ir kontroliniai terpės šulinėliai. Po 2,5 val. inkubacijos 37 °C temperatūroje į kiekvieną šulinėlį buvo įdėta 100 µl Griess reagento (Invitrogen, Eugene, OR, JAV) ir dar inkubuota kambario temperatūroje 30 min. Šviesos absorbcija buvo matuojama nustačius 550 nm bangos ilgį Tecan Sunrise spektrofotometru (Tecan, Grodig, Austrija).

iRNR išskyrimas ir RL PGR tyrimas

Bendras iRNR išskyrimas iš paruoštų PKVL mėginių atliktas naudojant RNR išskyrimo rinkinį (RNA extraction kit, Abbexa, Cambridge, JK), vadovaujantis gamintojo protokolu. Išskirtos RNR kiekis ir grynumas buvo matuojamas spektrofotometru NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, JAV). Komplementariosios deoksiribonukleorūgšties (kDNR) sintezei pasitelktas atvirkštinės transkripcijos metodas. Panaudota 2 µg išskirtos iRNR ir High/Medium-Capacity cDNA Reverse Transcription Kit rinkinys (Applied Biosystems, Waltham, MA, JAV). Procesas atliktas 7500 Fast Real-Time PCR System aparatu (Applied Biosystems, Waltham, MA, JAV).

Specifinių iRNR taikinių dauginimas atliktas 96 šulinėlių RL PGR plokštelėje. Kiekviename šulinėlyje paruoštas 20 µl mišinys, susidedantis iš 2 µl kDNR, 2 µl pradmenų, 10 µl TaqMan™ Universal PCR Master Mix mišinio (Applied Biosystems, Waltham, MA, JAV) ir 7 µl vandens. iRNR raiškai vertinti panaudoti pradmenys, pagaminti Applied Biosystems (Waltham, MA, JAV): Hs001555410 (interleukinui 1β, IL-1β), Hs00174122 (interleukinui 4, IL-4), Hs00174131 (interleukinui 6, IL-6), Hs00961619 (interleukinui 10, IL-10), Hs05043241 (programuotos ląstelės žūties baltymui 1, angl. *programmed cell death protein 1*, PD-1), Hs00204257 (programuotos ląstelės žūties baltymo ligandui 1, angl. *programmed cell death ligand 1*, PD-L1) ir Hs02786624 (gliceraldehido-3-fosfato dehidrogenazei, GAPDH). iRNR taikinių dauginimo specifiškumui patikrinti buvo atlikta pradmenų efekty-

vumo ir lydymosi kreivės analizė. Kiekvienas eksperimentas atliktas tris kartus, o prireikus kartotas papildomai, kad būtų užtikrintas rezultatų tikslumas.

Monocitų citokinų raiškos nustatymas naudojant TC

Citokinų gamybai monocituose įvertinti buvo naudojami specifiniai monokloniniai antikūnai: IL-1β (H1b-98), IL-4 (MP4-25D2), IL-6 (MQ2-13A5), IL-10 (JES3-19F1), interleukinui 12 (IL-12; C8.6) ir naviko nekrozės faktoriui α (TNF-α; MAb11). Visi antikūnai buvo gauti iš BD Biosciences (San Jose, CA, JAV). Tyrimo metu ląstelės viduje esančių antikūnų kiekis nustatytas naudojant BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit rinkinį kartu su BD GolgiStop™ baltymų pernašos inhibitoriumi. Daugiaspalvis dažymas atliktas vadovaujantis gamintojo protokolu.

Mėginiai buvo analizuojami FACSLyric tėkmės citometru (BD, San Jose, CA, JAV). Įrenginio (šviesos daugintuvo) įtampa sukalibruota ir kompensacija nustatyta naudojant BD™ CompBeads Anti-Mouse Ig, κ/Negative Control Compensation Particles Set rinkinį (BD, San Jose, CA, JAV). Kiekvienam mėginiui parinkta 30 tūkst. ląstelių identifikavimo riba. M1 makrofagų pogrupis identifikuotas pagal CD45+ / CD80+ / CD86+ žymenis, M2 – pagal CD45+ / CD163+ / CD206+ žymenis ir kiekybiškai įvertintas kaip ląstelių procentinė dalis limfocitų vartuose. Citokinų raiška kiekviename pogrupyje buvo matuojama ir pateikiama kaip vidutinio fluorescencijos intensyvumo (VFI) reikšmės.

Kraujo serumo citokinų analizė naudojant Luminex sistemą

Laisvųjų IL-1β, IL-4, IL-6, IL-10 ir IL-12 koncentracija serume buvo matuojama naudojant fluorescuojančių magnetinių sferinių mikrodalelių pagrindu veikiantį reagentų rinkinį Human Cytokine Premixed Multi-Analyte Kit (R&D Systems, Minneapolis, MN, JAV) ir Luminex® 100 analizatorių (Luminex Corporation, Austin, TX, JAV). Užšaldyti serumo mėginiai buvo atšildyti ir 4 min. centrifuguoti 4 °C temperatūroje 16 000 g greičiu nuosėdoms pašalinti. Vadovaujantis gamintojo protokolu, į reakcijos plokštelės šulinėlius įdėta serumo mėginių, mikrodalelių ir standartinių tirpalų.

Toliau mėginiai analizuoti Luminex® 100 prietaisu (Austin Tex, JAV). Citokinų koncentracijos buvo nustatomos lyginant gautas VFI reikšmes su standartine kreive, kurioje VFI reikšmės buvo susietos su žinoma baltymo koncentracija.

Kraujo serumo baltymų analizė naudojant fermentinį imunosorbentinį tyrimą

PD-1 ir PD-L1 koncentracijos kraujo serume buvo nustatytos fermentiniu imunosorbentiniu tyrimu (angl. *Enzyme-linked immunosorbent assay*, ELISA). Tyrimas atliktas naudojant ELISA rinkinius ab252360 (PD-1) ir ab277712 (PD-L1) iš Abcam (Cambridge, MA, JAV) pagal gamintojo instrukcijas. Baltymų koncentracijos buvo nustatomos matuojant optinį tankį pasirinkus PD-1 ir PD-L1 specifinius bangos ilgius ir gautus duomenis palyginus su standartinėmis baltymų koncentracijų kreivėmis.

Statistinė duomenų analizė

Statistinė duomenų analizė atlikta SPSS (22.0 versija; IBM Corp., Armonk, NY, JAV) bei GraphPad Prism (9.5.1 versija; GraphPad Software Inc., La Jolla, CA, JAV) statistine programine įranga. Atsižvelgiant į imties dydį ir daugeliui kiekybinių kintamųjų neatitinkant normaliojo skirstinio sąlygų (vertinimui taikytas Šapiro-Vilko kriterijus), nepriklausomi kintamieji lyginti pagal Mano-Vitnio kriterijų. Daugiau nei dviejų priklausomų kiekybinių kintamųjų palyginimui taikytas Frydmano kriterijus, o radus skirtumų, Vilkoksono kriterijus poriniams palyginimams.

Asociacijai tarp kategorinių kintamųjų vertinti taikytas Chi kvadrato (χ^2) ar Fišerio kriterijus. Koreliacijai tarp kintamųjų nustatyti naudotas Spirmeno koreliacijos koeficientas (rs). Jei nenurodyta kitaip, kiekybinių kintamųjų rezultatai pateikti kaip mediana su tarpkvartiliniu pločiu (angl. *interquartile ranges,* IQR), nurodant pirmąjį ir trečiąjį kvartilius. Skirtumas tarp tiriamųjų grupių laikytas statistiškai reikšmingu, kai reikšmingumo lygmuo $p < 0,05$.

PKVL aktyvumas buvo vertinamas skaičiuojant fluorometrinių ar spektrofotometrinių rodmenų santykį tarp neigiamos kontrolės ir aktyvintų PKVL šulinėlių. Resazurino metabolizmas neaktyvintose PKVL buvo įvertintas atliekant fluorometriją ir KV grupės rezultatus palyginus su kontrolinės grupės rezultatais.

Tirtų baltymų santykinė iRNR raiška PKVL buvo įvertinta rezultatus palyginus su GAPDH baltymo raiška ir taikant $2^{-\Delta\Delta CT}$ metodą [169]. Baltymų iRNR raiškos pokyčiai išreikšti santykiniu dydžiu palyginus KV ir kontrolinės grupių rezultatus arba pooperacinius ir priešoperacinius KV grupės rezultatus.

Surinktų duomenų išskirtys atpažintos ir pašalintos dviem etapais. Pirmuoju etapu pašalinti duomenys, akivaizdžiai neatitinkantys tikėtinų reikšmių. Antruoju etapu atlikta statistinė duomenų analizė, naudojant modifikuotą Z balą (angl. *Modified Z-Score*) ir formulę: modifikuotas Z balas = 0,6745 (Xi − mediana) / ANM (kur Xi − kintamojo reikšmė, mediana −

kintamojo reikšmių mediana, ANM − absoliutaus nuokrypio mediana). Jei kintamojo reikšmės modifikuotas Z balas viršijo ± 3,5, ji buvo pašalinta [170]. Toks išskirčių vertinimo metodas buvo pasirinktas dėl to, kad tinka mažoms imtims ir padeda pašalinti klaidingus duomenis, kurie atsiranda dėl daug etapų apimančių laboratorinių procedūrų. Pašalintas tik nedidelis išskirčių kiekis, bet duomenys tapo aiškesni ir lengviau interpretuojami.

REZULTATAI

Tiriamoji populiacija

Tyrime dalyvavo 46 pacientės: 23 KV grupėje ir 23 kontrolinėje. KV grupės tiriamųjų amžiaus mediana buvo 58 metai (47–61) ir reikšmingai nesiskyrė nuo kontrolinės grupės dalyvių amžiaus. KMI ir gretutinių ligų dažnumas taip pat nesiskyrė. Skirtingų KV histologinių tipų dažnumas buvo panašus į bendrosios populiacijos [38]. Išsamūs klinikiniai ir demografiniai duomenys pateikti 1 lentelėje.

1 lentelė. *Klinikiniai ir demografiniai dalyvių duomenys*

Rodiklis	KV grupė (n = 23)	Kontrolinė grupė (n = 23)	p vertė
Amžius (metais), mediana (IQR)	58 (47–61)	59 (49–61)	0,84
KMI (kg/m^2), mediana (IQR)	24,0 (22,3–29,8)	23,7 (21,9–28,7)	0,60
Gretutinės ligos, n (proc.)			
Hipertenzija	9 (39,1)	10 (43,5)	1,0
Išeminė širdies liga	1 (4,4)	2 (8,7)	1,0
Širdies nepakankamumas	1 (4,4)	2 (8,7)	1,0
2 tipo diabetas	1 (4,4)	1 (4,4)	1,0
Kita	6 (26,1)	4 (17,4)	0,72
Nėra gretutinių ligų	12 (52,2)	12 (52,2)	1,0
KV stadija, n (proc.)			
IIIA	4 (17,4)	–	–
IIIB	4 (17,4)	–	–
IIIC	7 (30,4)	–	–
IVA	1 (4,4)	–	–
IVB	7 (30,4)	–	–
Histologinis KV tipas, n (proc.)			
Žemo laipsnio serozinė karcinoma	1 (4,4)	–	–
Aukšto laipsnio serozinė karcinoma	15 (65,1)	–	–
Endometrioidinė karcinoma	2 (8,7)	–	–
Šviesių ląstelių karcinoma	1 (4,4)	–	–
Mucininė karcinoma	1 (4,4)	–	–
Serozinė endometrioidinė karcinoma	3 (13)	–	–

1 lentelės tęsinys

Rodiklis	KV grupė (n = 23)	Kontrolinė grupė (n = 23)	p vertė
CRO išeitys, n (proc.)			
Visiška CRO	15 (65,1)	–	–
Optimali CRO	5 (21,9)	–	–
Suboptimali CRO	3 (13)	–	–

KV stadijos grindžiamos 2021 m. FIGO klasifikacija [44]. IQR – tarpkvartilinis plotis (angl. *interquartile range*), KMI – kūno masės indeksas.

Periferinio kraujo ląstelių ir ląstelių santykių pokyčiai

Periferinio kraujo ląstelių kiekiui įvertinti kontrolinėje ir KV grupėje prieš operaciją tiriamosioms atliktas BKT. Nustatyta, kad KV pacienčių leukocitų koncentracija buvo didesnė, daugiausia dėl 1,5 karto padidėjusio neutrofilų kiekio. Be to, buvo didesnis trombocitų skaičius, ir pastebėta mažesnio eritrocitų skaičiaus tendencija. Nors eritrocitų skaičius reikšmingai nesiskyrė, hemoglobino koncentracija KV grupėje buvo mažesnė: 124 g/l (115–130), lyginant su 135 g/l (126–140) kontrolinėje grupėje (p = 0,01). Papildoma analizė atskleidė, kad KV grupėje neutrofilų ir limfocitų santykio (angl. *neutrophil-to-lymphocyte ratio,* NLR) bei trombocitų ir limfocitų santykio (angl. *platelet-to-lymphocyte ratio,* PLR) rodikliai buvo aukštesni. BKT rodmenų santrauka pateikta 2 lentelėje.

2 lentelė. *Kontrolinės grupės ir priešoperacinio KV grupės tiriamųjų periferinio kraujo ląstelių kiekio ir jų santykio lyginamieji duomenys*

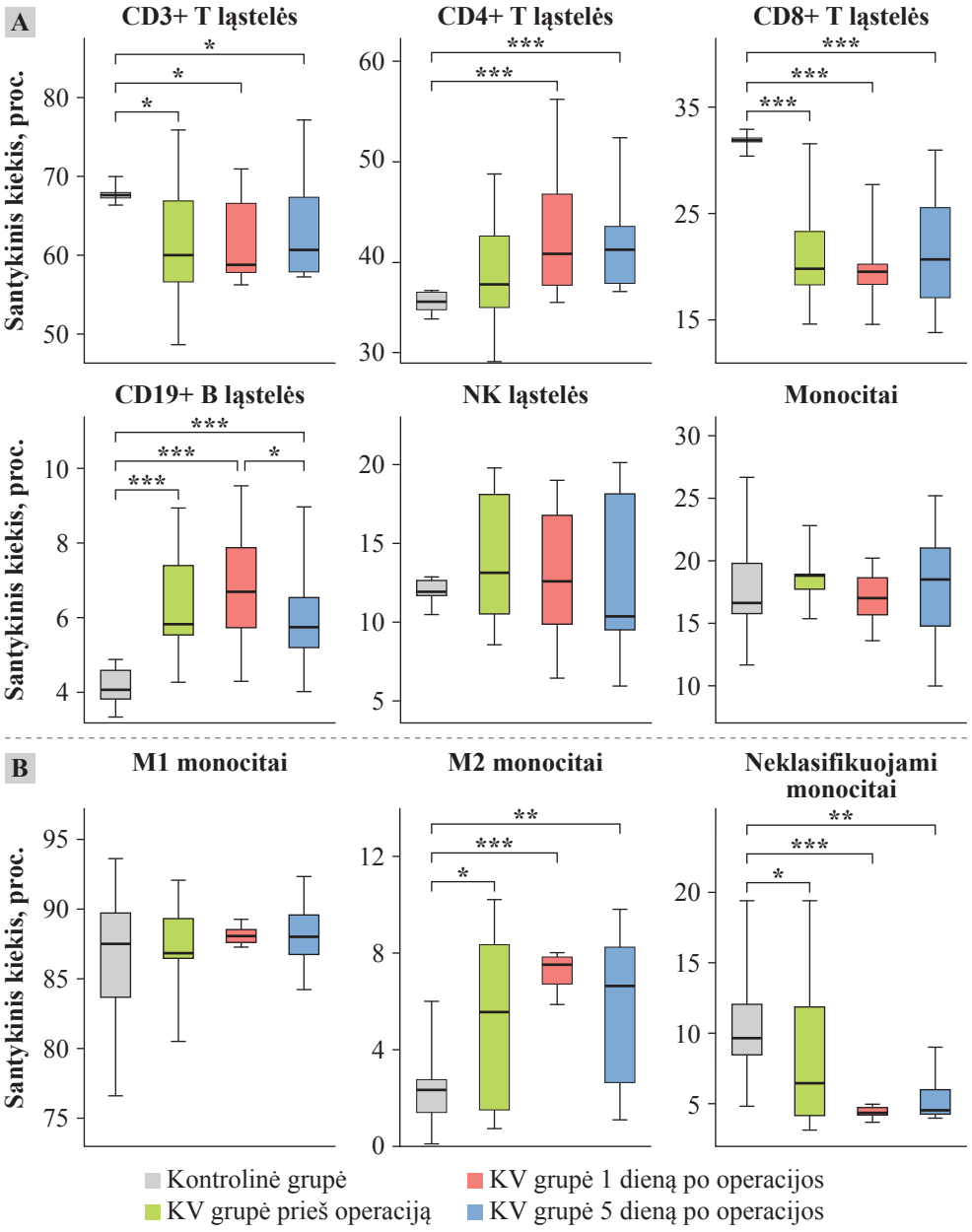
Ląstelės/santykis	KV grupė (n = 23)	Kontrolinė grupė (n = 23)	p vertė
Leukocitai	6,9 (5,2–8,2)	5,6 (4,4–6,4)	0,02
Neutrofilai	4,7 (3,6–5,6)	3,1 (2,3–3,5)	< 0,001
Eozinofilai	0,1 (0,05–0,2)	0,1 (0,1–0,1)	0,87
Bazofilai	0,04 (0,03–0,07)	0,04 (0,02–0,04)	0,26
Limfocitai	1,4 (1,2–2,2)	1,9 (1,3–2,3)	0,2
Monocitai	0,6 (0,3–0,9)	0,4 (0,4–0,5)	0,26
Eritrocitai	4,3 (4,1–4,5)	4,5 (4,4–4,7)	0,06
Trombocitai	367 (324–402)	267,5 (234–289)	< 0,001
NLR	3,2 (2,2–4,1)	1,7 (1,3–1,9)	< 0,001
PLR	250 (177–305)	156 (110–202)	< 0,001

NLR – neutrofilų ir limfocitų santykis (angl. *neutrophil-to-lymphocyte ratio*), PLR – trombocitų ir limfocitų santykis (angl. *platelet-to-lymphocyte ratio*). Eritrocitų skaičius išreiškiamas vienetais × 10^{12}/l, kitų ląstelių − vienetais × 10^{9}/l. Ląstelių skaičius pateikiamas kaip medianos reikšmė su IQR.

PKVL subpopuliacijų ir susijusių santykių pokyčiai

PKVL subpopuliacijų analize nustatyta, kad prieš operaciją KV pacienčių santykinis CD3+ T ir CD8+ T ląstelių kiekis buvo mažesnis, o CD19+ B ląstelių kiekis buvo didesnis, lyginant su kontroline grupe (3 pav. A). Palyginus pooperacinius KV grupės rezultatus su priešoperaciniais, reikšmingų skirtumų nenustatyta, tačiau KV grupės pooperaciniai rezultatai akivaizdžiai skyrėsi nuo kontrolinės grupės. CD4+ T ląstelių kiekis reikšmingai padidėjo praėjus 1 dienai po operacijos (atitinkamai 36,1 proc. (35,3–37,1 proc.) ir 40,9 proc. (37,7–46,8 proc.), $p < 0,001$). CD19+ B ląstelių didžiausias kiekis taip pat buvo 1 dieną po operacijos (4,1 proc. (3,8–4,6 proc.) ir 6,7 proc. (5,7–7,9 proc.), $p < 0,001$). CD3+ ir CD8+ T ląstelių kiekis po operacijos reikšmingai nepakito.

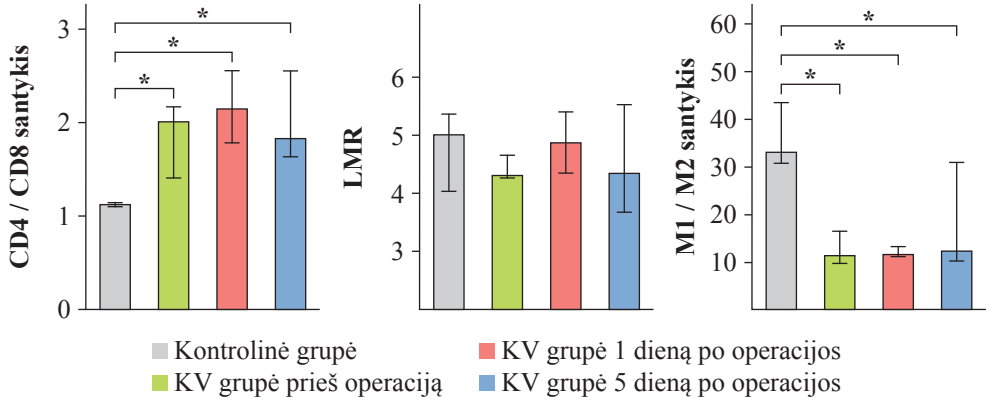
M1 monocitų proporcija visose grupėse buvo panaši, o M2 monocitų kiekis KV grupėje prieš operaciją buvo maždaug 2,5 karto didesnis ir po operacijos toliau didėjo, iki pasiekė daugiau nei tris kartus didesnę proporciją, lyginant su kontroline grupe (atitinkamai 7,5 proc. (6,7–7,8 proc.) ir 2,3 proc. (1,4–2,8 proc.), $p < 0,001$). Priešingai, neklasifikuojamų monocitų proporcija KV grupėje prieš operaciją buvo mažesnė nei kontrolinėje ir dar labiau sumažėjo pirmą dieną po operacijos (atitinkamai 4,4 proc. (4,1–4,8 proc.) ir 9,6 proc. (8,4–12,1 proc.), $p < 0,001$). Monocitų subpopuliacijų pokyčiai tiriamųjų grupėse pateikti 3 pav. B.



3 pav. *PKVL subpopuliacijų pokyčiai KV ir kontrolinėje grupėse*

(A) PKVL santykinių kiekių medianos, **(B)** monocitų subpopuliacijų santykinių kiekių (tarp visų monocitų) medianos. Pateikti kontrolinės grupės bei KV grupės duomenys prieš operaciją, 1-ą ir 5-ą dieną po operacijos. Paryškinta linija stačiakampės diagramos viduryje rodo medianą, pagrindas ir viršus – pirmą ir trečią kvartilius, o nuo jų besitęsiančios linijos – mažiausią ir didžiausią reikšmes. Visų skirtumų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: *$p < 0{,}05$, **$p \leq 0.01$; ***$p \leq 0.001$. Imties dydis: KV grupė n = 13, kontrolinė grupė n = 8 (PKVL be monocitų) arba n = 23 (monocitams).

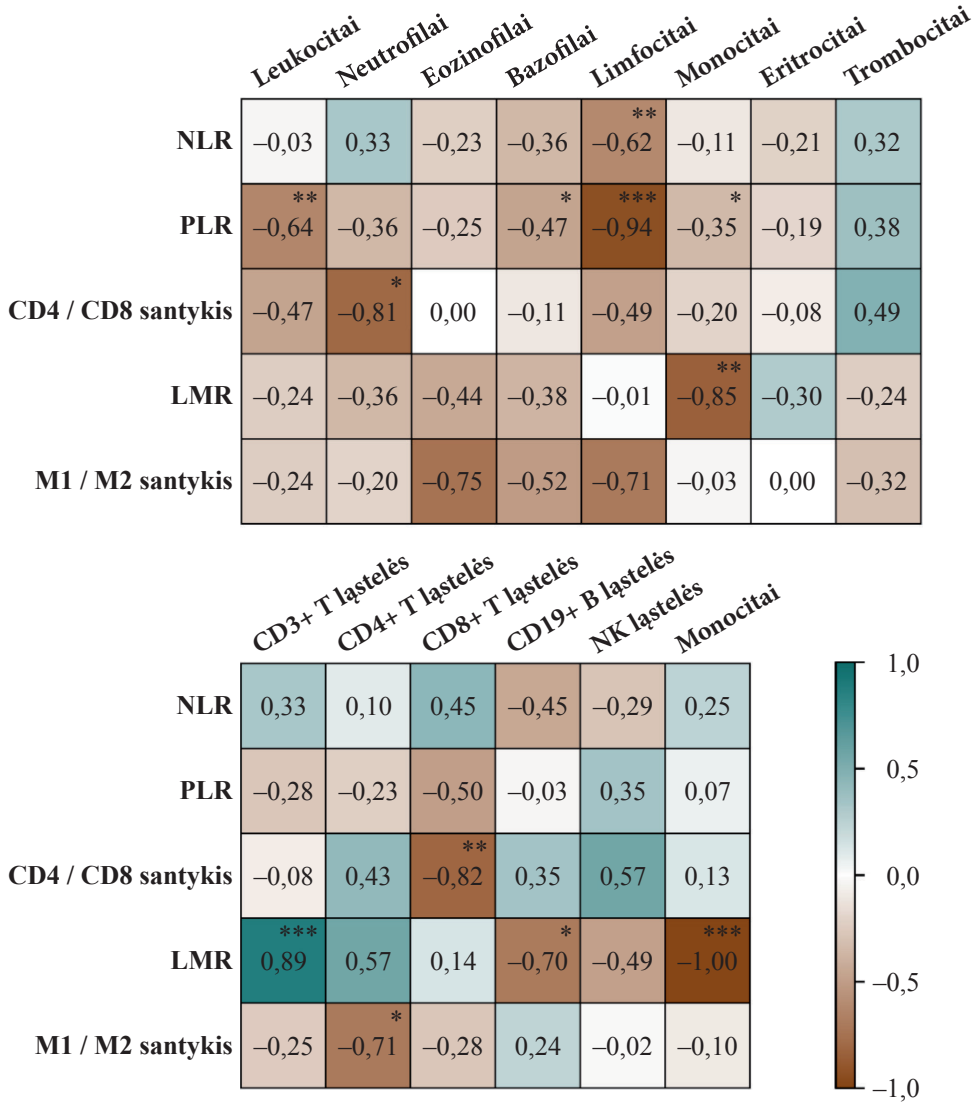
Toliau atlikta su PKVL susijusių santykių analizė. Nustatyta, kad KV grupėje prieš operaciją buvo didesnis CD4+ T ir CD8+ T ląstelių santykis (CD4 / CD8) ir gerokai mažesnis M1 ir M2 monocitų santykis (M1 / M2). Limfocitų ir monocitų santykis (angl. *lymphocyte-to-monocyte ratio,* LMR) tiriamose grupėse nesiskyrė. KV grupėje po operacijos minėti ląstelių santykiai ženkliau nekito (4 pav.).



4 pav. *Su PKVL susijusių santykių pokyčiai KV ir kontrolinėje grupėje*

Pateikti kontrolinės grupės duomenys bei KV grupės duomenys prieš operaciją, 1-ą ir 5-ą dieną po operacijos. Histogramos rodo medianą su IQR. Visų skirtumų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: *p < 0,001. CD4 / D8 – CD4+ T ir CD8+ T ląstelių santykis; LMR – limfocitų ir monocitų santykis (angl. *lymphocyte-to-monocyte ratio*), M1 / M2 – M1 ir M2 monocitų santykis. Imties dydis: KV grupė n = 13, kontrolinė grupė n = 8 (CD4 / CD8 santykiui) arba n = 23 (LMR bei M1 / M2 santykiui).

Norint ištirti ląstelių santykių ir periferinio kraujo ląstelių tarpusavio ryšius KV grupėje prieš operaciją, buvo atlikta koreliacinė analizė (5 pav.). Nors tam tikros koreliacijos numanytos, kai kurios buvo netikėtos. Pastebėta stipri koreliacija tarp CD4 / CD8 santykio ir neutrofilų (rs = –0,81; p = 0,01), taip pat koreliavo M1 / M2 santykis ir CD4+ T ląstelių santykinis kiekis (rs = –0,71; p = 0,047).

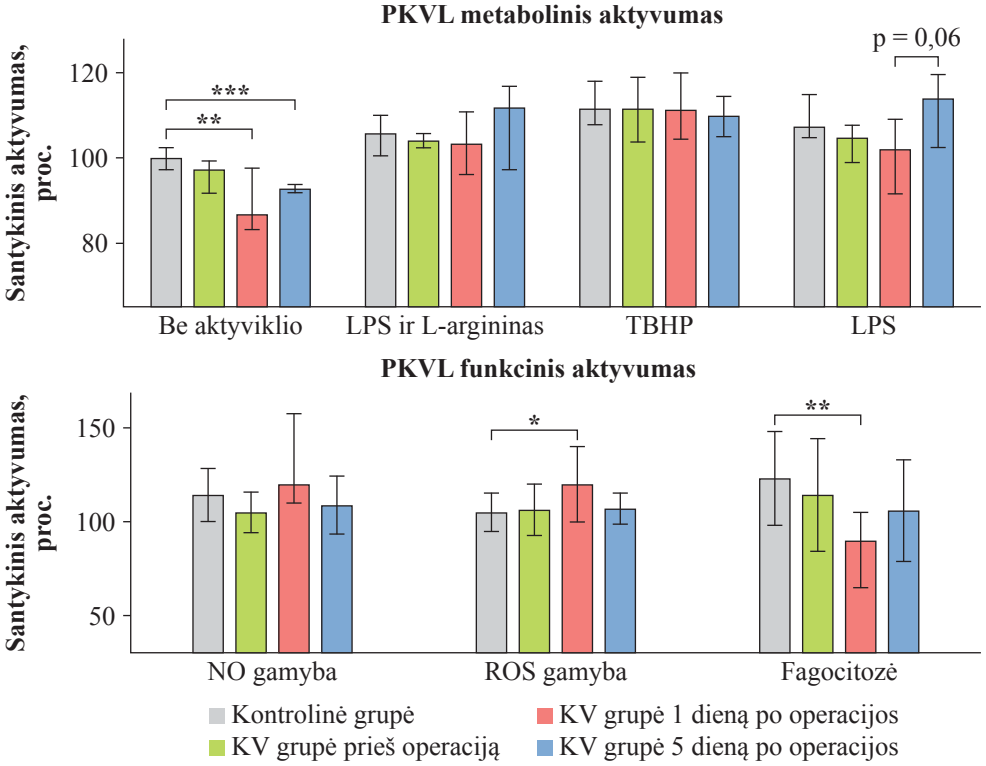


5 pav. *Koreliacija tarp periferinio kraujo ląstelių santykių ir jų kiekio KV grupėje prieš operaciją*

Viršutinėje matricoje pateikti ląstelių santykių ir absoliutaus ląstelių kiekio koreliacijos koeficientai, apatinėje – analizuojamų santykių ir PKVL subpopuliacijų santykinių kiekių koreliacijos koeficientai. Matricose pateikti Spirmano koreliacijos koeficientai (rs), o spalvos nuo rudos iki turkio spalvos rodo koreliaciją nuo –1 iki 1. Visų koreliacijų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: *p < 0,05; **p ≤ 0,01; ***p ≤ 0,001.

PKVL funkcijos pokyčiai

Ištyrus PKVL metabolinį ir funkcinį aktyvumą, nenustatyta jokių reikšmingų skirtumų tarp KV grupės prieš operaciją ir kontrolinės grupės (6 pav.). Nesiskyrė ir KV grupės pooperacinis ir priešoperacinis PKVL aktyvumas. Visgi pastebėtas reikšmingai sumažėjęs metabolinis aktyvumas ir susilpnėjusi fagocitozė KV grupėje pirmą dieną po operacijos, lyginant su kontroline grupe. Penktą dieną po operacijos PKVL aktyvumas iš dalies atsigavo – tai rodė didėjantis fagocitinis bei metabolinis aktyvumas, ypač aktyvuojant LPS. ROS aktyvumas, priešingai nei anksčiau aprašyti pokyčiai, KV grupėje buvo padidėjęs 1-ą dieną ir normalizavosi 5-ą dieną po operacijos.

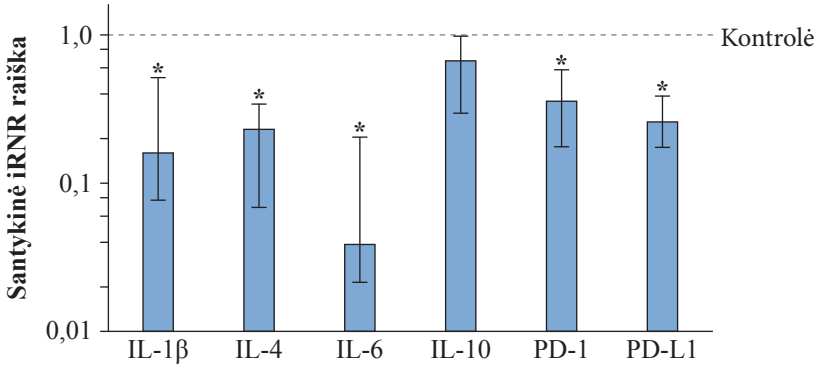


6 pav. *PKVL aktyvumas kontrolinėje ir KV grupėse*

Viršutinės histogramos rodo PKVL metabolinį aktyvumą tiriamose grupėse įvertinus resazurino metabolizmą be aktyviklio ir panaudojus lipopolisacharidą (LPS), L-argininą ar tert-butilo hidroperoksidą (TBHP). Apatinėse histogramose pateiktas PKVL funkcinis aktyvumas: azoto oksido (angl. *nitric oxyde*, NO), reaktyviųjų deguonies formų (angl. *reactive oxygen species*, ROS) gamyba bei fagocitozė. Santykinis aktyvumas apskaičiuotas palyginus aktyvumo rezultatus su aktyvikliu ir be jo arba normalizuotas pagal kontrolinės grupės bazinį resazurino metabolizmą. Visų skirtumų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: *p = 0,02; **p ≤ 0,01; ***p = 0,001. Imties dydis: KV grupė n = 13, kontrolinė grupė n = 17.

Baltymų, susijusių su imuniniu atsaku, raiškos PKVL pokyčiai

Imuninio atsako baltymų analizė pradėta nuo šių baltymų iRNR raiškos PKVL tyrimų. Kaip matyti 7 paveiksle beveik visų tiriamų baltymų iRNR raiška KV grupėje prieš operaciją buvo silpnesnė nei kontrolinėje grupėje. Labiausiai susilpnėjusi buvo IL-6 iRNR raiška, nuo kontrolinės grupės statistiškai reikšmingai nesiskyrė tik IL-10 iRNR raiška.



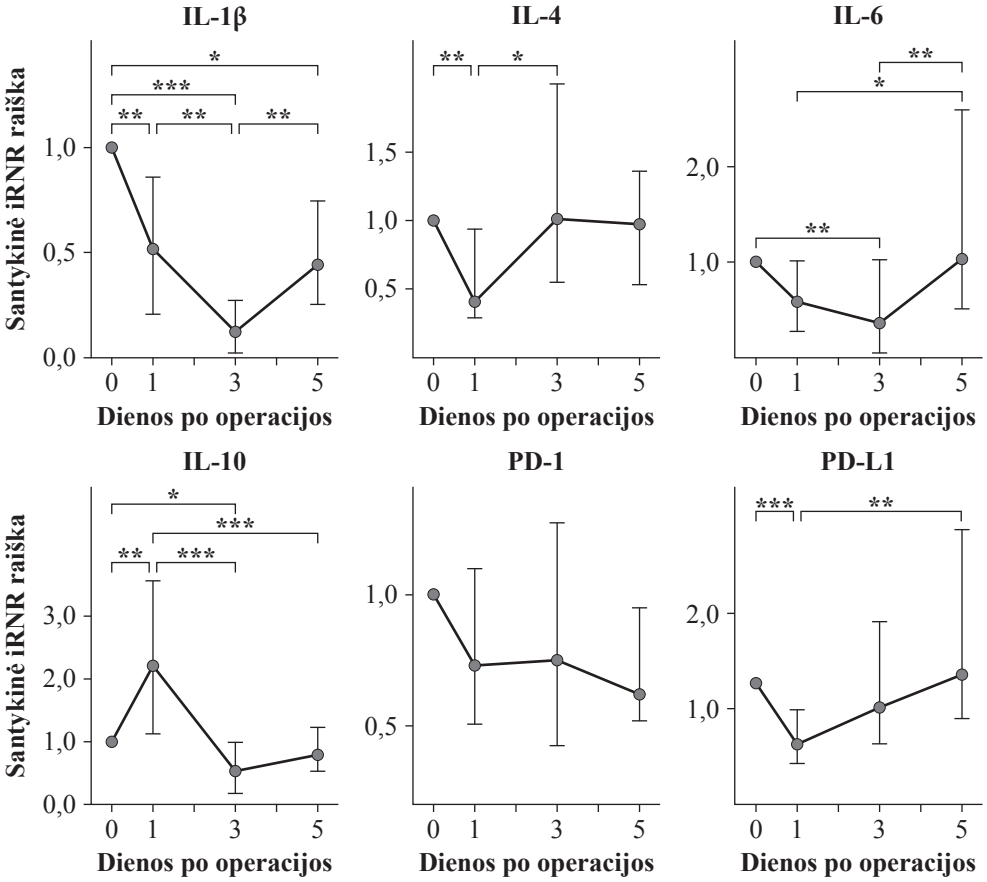
7 pav. *Imuninio atsako baltymų iRNR raiška PKVL KV grupėje prieš operaciją*

Histogramos rodo imuninio atsako baltymų santykinę iRNR raišką, lyginant su kontrolinės grupės baltymų raiška (prilygintai 1; taškinė linija). Tirti šie baltymai: interleukinas 1β (IL-1β), interleukinas 4 (IL-4), interleukinas 6 (IL-6), interleukinas 10 (IL-10), programuotos ląstelės žūties baltymas 1 (angl. *programmed cell death protein 1*, PD-1), programuotos ląstelės žūties baltymo ligandas 1 (angl. programmed death-ligand 1, PD-L1). Pažymėti skirtumai buvo statistiškai reikšmingi (*$p \leq 0{,}001$). Imties dydis: KV grupė n = 23, kontrolinė grupė n = 20.

Po operacijos šių baltymų iRNR raiška PKVL toliau kito (8 pav.). Dviejų interleukinų (IL-1β ir IL-6) raiška buvo toliau slopinama ir silpniausia nustatyta trečią dieną po operacijos. Pirmą dieną po operacijos IL-1β iRNR raiška susilpnėjo iki 52 proc. (21–85 proc.), o trečią – iki 12 proc. (2–27 proc.), lyginant su priešoperaciniais rodikliais (atitinkamai $p = 0{,}01$ ir $p < 0{,}001$). IL-6 raiška trečią dieną po operacijos susilpnėjo iki 36 proc. (4–103 proc.) ($p = 0{,}01$). Pastebėta, kad abiejų interleukinų raiška pradėjo atsistatyti penktą dieną po operacijos.

Daugumos kitų tirtų baltymų iRNR raiškos pokyčiai buvo ryškiausi iš karto po operacijos. IL-4 raiška susilpnėjo iki 41 proc. (29–94 proc.), PD-L1 – iki 50 proc. (34–78 proc.), o IL-10 raiška sustiprėjo iki 221 proc. (113–356 proc.), lyginant su buvusia prieš operaciją ($p = 0{,}01$, $p = 0{,}001$ ir $p = 0{,}002$). Pabrėžtina, kad IL-10 buvo vienintelis baltymas, kurio iRNR

raiška pooperaciniu laikotarpiu sustiprėjo. PD-1 iRNR raiška po operacijos nuolat silpnėjo, tačiau pokyčiai nebuvo statistiškai reikšmingi.



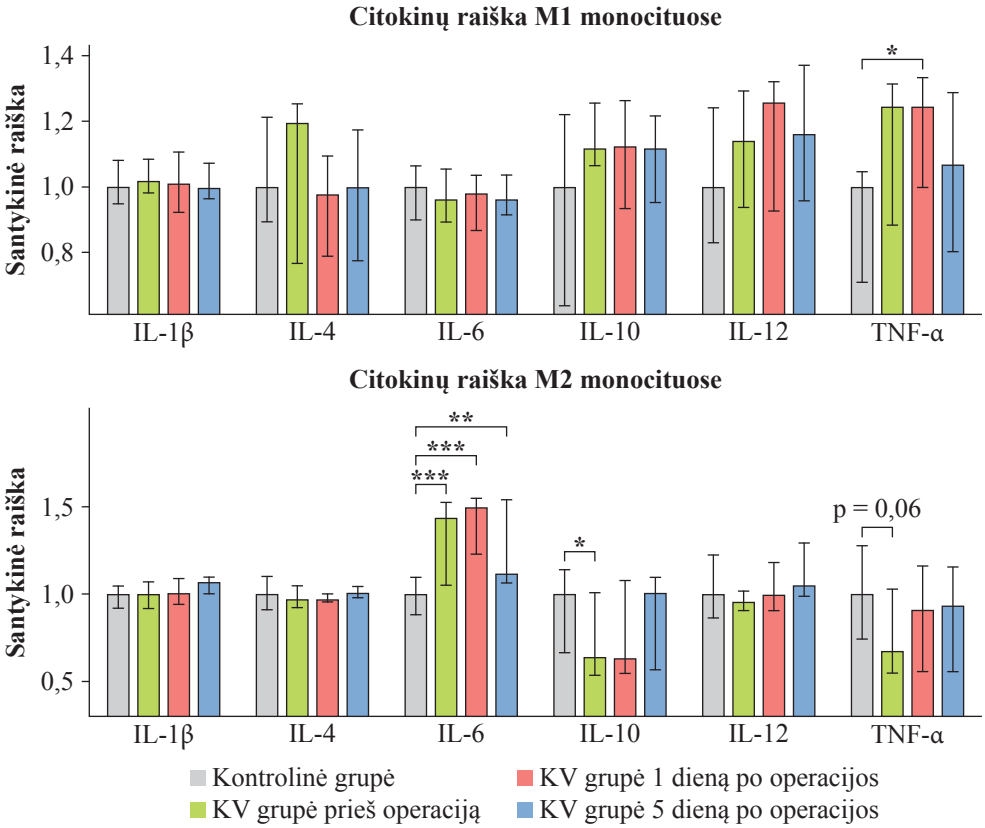
8 pav. *Imuninio atsako baltymų iRNR raiška PKVL KV grupėje po operacijos*

Grafikai rodo imuninio atsako baltymų santykinę iRNR raišką 1-ą, 3-ią ir 5-ą dieną po operacijos, lyginant su raiška prieš operaciją (prilyginta 1). Visų skirtumų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: *p < 0,05; **p ≤ 0,01; ***p ≤ 0,001. Imties dydis: n = 23.

Kad citokinų raiška būtų ištirta nuodugniau, pasirinkta išanalizuoti jų raišką monocituose, išskirtuose iš PKVL. Prieš operaciją KV grupės citokinų raiška skyrėsi nuo kontrolinės grupės, o palyginus pooperacinę ir priešoperacinę citokinų raišką, statistiškai reikšmingų skirtumų nenustatyta. Visgi pooperaciniai pokyčiai tapo reikšmingi juos palyginus su kontrolinės grupės duomenimis (9 pav.).

Ryškiausiai pakito IL-6 raiška M2 monocituose. KV grupėje IL-6 raiška buvo stipresnė prieš operaciją ir dar labiau išaugo pirmą pooperacinę dieną – pasiekė 1,5 karto didesnį rodiklį nei kontrolinėje grupėje. Penktą pooperacinę dieną pastebėta IL-6 raiškos silpnėjimo tendencija. Panašiai svyravo ir naviko nekrozės faktoriaus α (angl. *tumor necrosis factor* α, TNF-α) raiška M1 monocituose: jau prieš operaciją buvusi stipresnė TNF-α raiška pirmą dieną po operacijos nuo kontrolinės grupės skyrėsi statistiškai reikšmingai – padidėjo 1,24 karto.

Priešingai, TNF-α raiška M2 monocituose, kuri prieš operaciją KV grupėje buvo silpnesnė, pirmą pooperacinę dieną susilygino su kontrolinės grupės. Panašiai svyravo ir IL-10 raiška M2 monocituose: silpnesnė priešoperacinė raiška penktą pooperacinę dieną priartėjo prie kontrolinės grupės rodiklio.

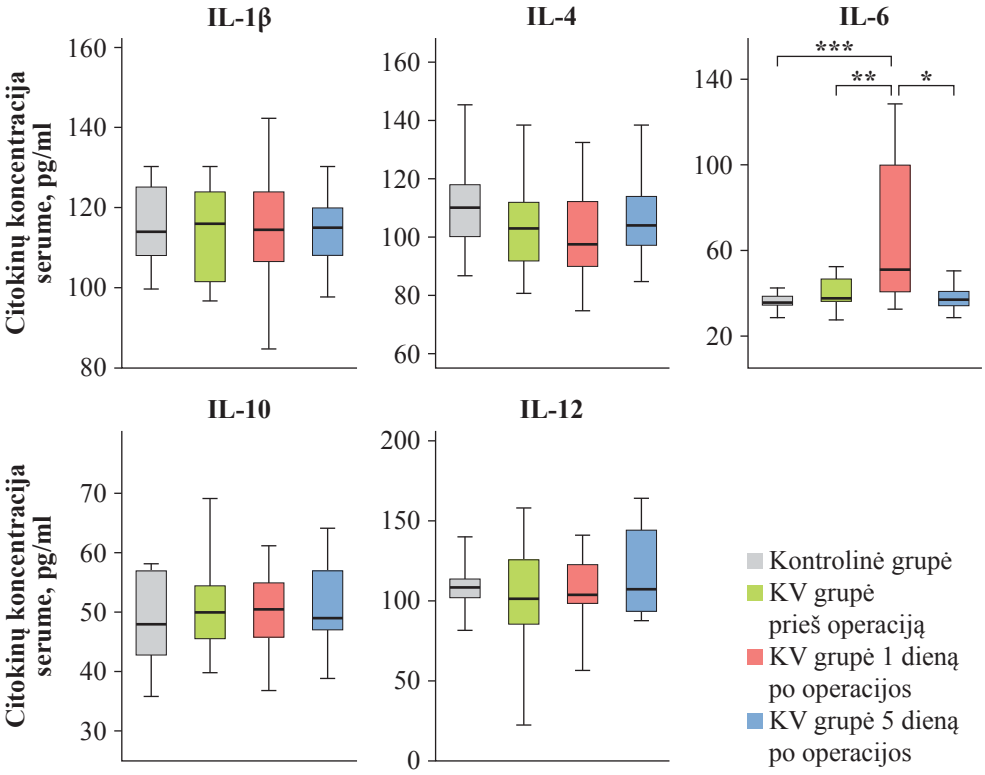


9 pav. *Citokinų raiškos pokyčiai monocituose KV grupėje prieš operaciją ir po jos*

Viršutinėje dalyje matyti santykinė citokinų raiška M1, o apatinėje – M2 monocituose. Histogramos rodo santykinius vidutinio fluorescencijos intensyvumo (VFI) pokyčius KV grupėje, rezultatus normalizavus pagal kontrolinę grupę (prilyginta 1). VFI atspindi viduląstelinių citokinų kiekį KV grupėje 1-ą ir 5-ą dieną po operacijos. Visų skirtumų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: * $p < 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$. Imties dydis: KV grupė n = 13, kontrolinė grupė n = 23. IL-12 – interleukinas 12, TNF-α – naviko nekrozės faktoriaus α (angl. *tumor necrosis factor α*).

Imuninio atsako baltymų pokyčiai kraujo serume

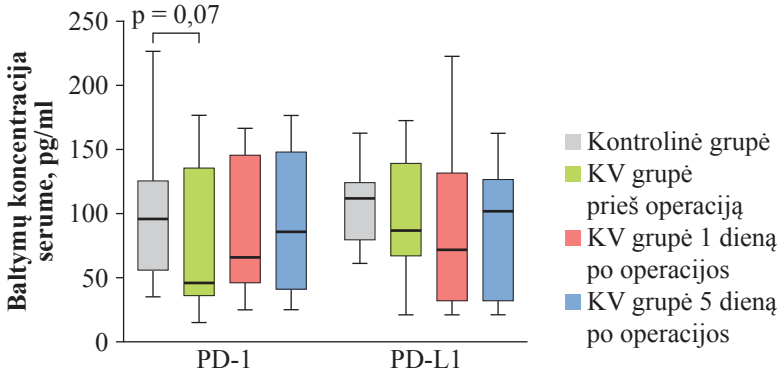
Galutiniam imuninio atsako baltymų įvertinimui buvo ištirta jų koncentracija tiriamųjų kraujo serume. Kraujo serumo interleukinų koncentracijos kontrolinėje grupėje ir KV grupėje prieš operaciją buvo vienodos (10 pav.). Skirtumų nerasta ir ištyrus KV grupę po operacijos, išskyrus IL-6 pokyčius. Pirmą dieną po operacijos IL-6 koncentracija padidėjo 36 proc., lyginant su priešoperacine (atitinkamai 37,5 pg/ml (36–46,8) ir 51 pg/ml (40,5–100), p = 0,003). Penktą dieną po operacijos IL-6 koncentracija mažėjo ir grįžo į priešoperacinį lygį (atitinkamai 37,5 pg/ml (36–46,8) ir 37 pg/ml (34–41), p = 0,45).



10 pav. *Kraujo serumo interleukinų koncentracijos pokyčiai kontrolinėje ir KV grupėje prieš operaciją ir po jos*

Visų skirtumų tarp grupių p reikšmės > 0,05, nebent nurodyta kitaip: *p < 0,05; **p ≤ 0,01; ***p ≤ 0,001. Imties dydis: KV grupė n = 18, kontrolinė grupė n = 17.

KV grupėje prieš operaciją pastebėta mažesnės PD-1 koncentracijos kraujo serume tendencija, lyginant su kontroline grupe (11 pav.). Kitų reikšmingų skirtumų tarp tiriamų grupių kraujo serumo PD-1 ir PD-L1 koncentracijų nenustatyta.



11 pav. *Kraujo serumo PD-1 ir PD-L1 koncentracijos pokyčiai kontrolinėje ir KV grupėje prieš operaciją ir po jos*

Visų skirtumų tarp grupių p reikšmės > 0,05. Imties dydis: KV grupė n = 11, kontrolinė grupė n = 15.

REZULTATŲ APTARIMAS

Galutinė rezultatų interpretacija

Pagrindinis šio tyrimo tikslas – įvertinti KV sergančių pacienčių imuninės sistemos pokyčius ankstyvuoju pooperaciniu laikotarpiu, kuris gali būti svarbus KV vystytis. Šiame tyrime nebuvo ieškoma imuninės sistemos funkcijos sutrikimo sąsajų su vėlyvaisiais rezultatais, pacienčių ligos prognoze, tačiau siekta daugiau sužinoti apie šį mažai ištirtą laikotarpį, kuris gali būti tinkamas naujiems gydymo būdams pritaikyti.

Pirmoji tyrimo kryptis buvo palyginti KV sergančių pacienčių ir sveikų moterų imuninės sistemos veiklą. Tyrimo rezultatai pakankami įrodyti, kad KV pacienčių imuninis atsakas į vėžį yra silpnesnis arba rasti pokyčiai prisideda prie naviko augimą skatinančios aplinkos [15, 16, 62, 72, 75, 126, 142, 190]. Šią išvadą patvirtina sumažėjęs santykinis CD8+ T ląstelių kiekis, sumažėjęs M1 / M2 monocitų santykis, apskritai sumažėjusi tiriamų baltymų iRNR raiška PKVL, padidėjęs CD4 / CD8 santykis, santykinis M2 monocitų kiekis ir sustiprėjusi IL-6 raiška monocituose. Nors šie rezultatai tik patvirtina panašius anksčiau atliktų tyrimų duomenis [20, 184–186], jie dar kartą įrodo, kad KV pacienčių imuninė sistema esti prislopinta.

Tyrimai ir apžvalgos rodo, kad didelės apimties chirurginė intervencija reikšmingai veikia imuninę sistemą ir tam tikrais atvejais prisideda prie vėžio progresavimo [25–28]. Vis dėlto šis poveikis KV atvejais nėra išsamiai ištirtas. Tyrimai, skirti KV ligonių pooperaciniam imunitetui įvertinti, daugiausia orientuoti į vėlesnį pooperacinį laikotarpį, jų duomenimis, imuninė sistema atsikuria, o chirurginio gydymo vėlyvasis poveikis yra teigiamas [18, 20, 159]. Šiame tyrime analizuota KV sergančių pacienčių imuninė sistema menkai ištirtu ankstyvuoju pooperaciniu laikotarpiu.

Mūsų atskleisti pooperaciniai imuninės sistemos pokyčiai gali būti apibūdinti dvejopai. Pirmiausia, KV sergančių pacienčių imuninės sistemos funkcija ankstyvuoju pooperaciniu laikotarpiu išlieka sutrikusi, dar nespėjus pasireikšti teigiamam chirurginio gydymo poveikiui [15, 16, 62, 72, 126, 142]. Tai patvirtina šie duomenys: išlikęs sumažėjęs santykinis CD8+ T ląstelių kiekis bei M1 / M2 monocitų santykis ir padidėjęs CD4 / CD8 santykis bei sustiprėjusi IL-6 raiška monocituose. Antra, prie jau esamo susilpnėjusio KV sergančių pacienčių imuniteto, ankstyvuoju pooperaciniu laikotarpiu prisideda papildomas imuninės sistemos slopinimas bei pokyčiai, galintys sudaryti sąlygas KV vystytis [15, 16, 62, 72, 126, 188]. Tokią išvadą pagrindžia šie duomenys: padidėjęs M2 monocitų santykinis kiekis, sumažėjęs PKVL metabolinis aktyvumas bei fagocitozė ir padidėjusi ROS gamyba, dar labiau susilpnėjusi daugumos analizuotų baltymų iRNR raiška PKVL ir padidėjusi IL-6 serumo koncentracija ankstyvuoju pooperaciniu laikotarpiu.

Atsižvelgiant į tai, kad šiame tyrime analizuotas ankstyvasis KV pacienčių pooperacinis laikotarpis ir plačią tyrimų apimtį, galima laikyti, kad šis tyrimas unikalus. Atskleista, kad, nepaisant aprašyto teigiamo vėlyvojo KV operacijos poveikio, ankstyvuoju pooperaciniu laikotarpiu būdingas reikšmingas imuninės sistemos slopinimas bei pokyčiai galintys prisidėti prie KV progresavimo. Išsamus šio laikotarpio imuninės sistemos pokyčių suvokimas gali padėti atrasti naujų KV gydymo metodų, kurie pagerintų KV sergančių pacienčių gydymo rezultatus ir prognozę.

Tyrimo trūkumai

Nors atliktas darbas suteikia vertingų įžvalgų ir pagrindo tolesniems tyrimams, reikia pripažinti, jog jis turi trūkumų. Vienas pagrindinių – nedidelė tiriamųjų imtis. Tyrimo metu daugiausia dėmesio buvo skiriama ankstyvajam pooperaciniam laikotarpiui, kuris yra mažai ištirtas KV atveju. Be to, numatytam tikslui pasiekti pasirinktas platus kraujo tyrimų spektras. Dėl paminėtų priežasčių buvo sunku iš anksto numatyti preliminarius atliekamų tyrimų rezultatus ir reikalingą imties dydį. Taip pat svarbu pripažinti, jog pasaulinė COVID-19 pandemija, lėmusi tam tikrus klinikinio darbo

apribojimus, sutrikdė pacientų įtraukimą į tyrimą. Kitokiomis aplinkybėmis numatytu tyrimo laikotarpiu būtų buvę galima surinkti didesnę tiriamųjų imtį.

Svarbu pabrėžti, jog į tyrimą įtrauktos tik tos pacientės, kurioms diagnozuotas paženges KV, o tai riboja rezultatų pritaikomumą visai KV sergančių pacienčių populiacijai. Kadangi pažengusiu KV sergančių pacienčių prognozė yra prasta [2, 6], tyrimo objektu buvo pasirinktos tik šios pacientės.

Šiame tyrime neanalizuotos PKVL subpopuliacijų ir citokinų gamybos pokyčių sąsajos su KV eiga, vėlyvaisiais gydymo rezultatais. Tokiai analizei reikalingas ilgesnis tyrimo laikotarpis ir tai yra planuojamų būsimų tyrimų subjektas.

Analizuojant PKVL subpopuliacijas, netirti smulkių PKVL subpopuliacijų pokyčiai, kas galėtų suteikti papildomos vertingos informacijos apie KV sergančių pacienčių imuninės sistemos pokyčius. Šiam tikslui įgyvendinti reikalingi papildomi tyrimai.

Dar vienas trūkumas – tyrime nebuvo kontrolinės grupės, sudarytos iš pacienčių, chirurgiškai gydytų nuo nepiktybinės ginekologinės patologijos. Dėl to nebuvo įmanoma nustatyti, kurie pooperaciniai imuninės sistemos pokyčiai yra specifiški KV sergančioms pacientėms. Kadangi operuojant KV intervencija yra didelės apimties, atrinkti į kontrolinę grupę analogiškos apimties chirurginį gydymą patyrusias pacientes, nesergančias KV, yra sunkiai įgyvendinama užduotis. Nepaisant to, mūsų rezultatai reikšmingi – nesvarbu, ar imuninės sistemos slopinimą lemia operacija ar operacijos ir KV sąveika. Chirurginis gydymas tebėra KV gydymo pagrindas [12], o KV sergančios pacientės neišvengiamai patiria pooperacinius imuninės sistemos pokyčius.

IŠVADOS

Atlikus PKVL analizę, išsiaiškinta, kad KV sergančių pacienčių imuninė sistema reikšmingai slopinama ir vyksta pokyčiai, prisidedantys prie naviko augimą skatinančios aplinkos. Šie imuniniai pokyčiai išliko ankstyvuoju pooperaciniu laikotarpiu bei atsirado papildomų pokyčių, galinčių skatinti naviko augimą.

Atsižvelgiant į tyrimo uždavinius, padarytos šios išvados:

1. Lyginant su kontroline grupe, prieš operaciją KV sergančių pacienčių leukocitų, neutrofilų bei tombocitų kiekis buvo padidėjęs, o hemoglobino koncentracija sumažėjusi. Jų NLR ir PLR rodikliai buvo didesni.
2. Lyginant su kontroline grupe, prieš operaciją KV sergančioms pacientėms nustatyti mažesni santykiniai CD3+ T, CD8+ T ląstelių

bei neklasifikuojamų monocitų kiekiai ir mažesnis M1 / M2 monocitų santykis. Santykiniai CD19+ B ląstelių, M2 monocitų kiekiai ir CD4 / CD8 santykis buvo padidėjęs. Po operacijos dauguma prieš operaciją nustatytų pokyčių išliko ar dar labiau išryškėjo.

3. Prieš operaciją KV sergančių pacienčių PKVL metabolinis ir funkcinis aktyvumas nesiskyrė nuo kontrolinės grupės. Pirmą dieną po operacijos metabolinis ir funkcinis aktyvumas buvo sumažėjęs, o ROS gamyba padidėjusi.
4. Prieš operaciją KV grupėje baltymų iRNR raiška PKVL buvo susilpnėjusi. Ankstyvuoju pooperaciniu laikotarpiu daugumos baltymų iRNR raiška PKVL toliau silpnėjo. Dalies analizuotų citokinų raiška KV grupės pacienčių monocituose buvo pakitusi esant skirtingiems citokinų raiškos modeliams.
5. Iš analizuotų kraujo serumo baltymų reikšmingai pakitęs buvo tik vienas: pirmą dieną po operacijos KV grupėje smarkiai padidėjo IL-6 koncentracija.

PRAKTINĖS REKOMENDACIJOS

Remiantis šio tyrimo rezultatais, tiesioginių klinikinių rekomendacijų pateikti negalima. Mūsų tyrimo rezultatai rodo, kad būtini tolesni tyrimai. Pirma, reikia tyrimų, skirtų nustatyti ankstyvojo pooperacinio laikotarpio imuninių pokyčių klinikinę svarbą ir jų įtaką ligos eigai. Šių sąsajų nustatymas sudarytų pagrindą būsimiems klinikiniams tyrimams. Antra, tikslinga atlikti klinikinius tyrimus, skirtus įvertinti galimus gydymo būdus pooperaciniams imuninės sistemos pokyčiams koreguoti. Svarbu atkreipti dėmesį, kad galimų intervencijų laikotarpis gali apimti ne tik ankstyvąjį pooperacinį, bet ir intraoperacinį laikotarpį.

LITERATURE

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–263.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA: A Cancer Journal for Clinicians. 2022;72(1):7–33.
3. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: cancer today. Lyon, France: International Agency for Research on Cancer. 2024; Available at: https://gco.iarc.who.int/today. Accessed 03 Oct, 2024.
4. World Health Organization, International Agency for Research on Cancer. Cancer tomorrow. 2024; Available at: https://gco.iarc.who.int/tomorrow. Accessed Oct 01, 2024.
5. Akter S, Rahman MA, Hasan MN, Akhter H, Noor P, Islam R, et al. Recent advances in ovarian cancer: therapeutic strategies, potential biomarkers, and technological improvements. Cells (Basel, Switzerland). 2022;11(4):650.
6. Vergote I, Denys H, De Greve J, Gennigens C, Van De Vijver K, Kerger J, et al. Treatment algorithm in patients with ovarian cancer. Facts Views Vis Obgyn. 2020;12(3):227–239.
7. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018;68(4): 284–296.
8. Peres LC, Cushing-Haugen KL, Köbel M, Harris HR, Berchuck A, Rossing MA, et al. Invasive epithelial ovarian cancer survival by histotype and disease stage. J Natl Cancer Inst. 2019;111(1):60–68.
9. Li X, Li Z, Ma H, Li X, Zhai H, Li X, et al. Ovarian cancer: Diagnosis and treatment strategies (Review). Oncol Lett. 2024;28(3):441.
10. Luvero D, Angioli R, Celoro F, Plotti F, Terranova C, Guzzo F, et al. Tailored treatment strategies in first line therapy for ovarian cancer patients: a critical review of the literature. Pharmaceuticals (Basel). 2024;17(6):778.
11. Drakes ML, Stiff PJ. Ovarian Cancer: Therapeutic Strategies to Overcome Immune Suppression. Adv Exp Med Biol. 2021;1330:33–54.
12. Ledermann JA, Matias-Guiu X, Amant F, Concin N, Davidson B, Fotopoulou C, et al. ESGO-ESMO-ESP consensus conference recommendations on ovarian cancer: pathology and molecular biology and early, advanced and recurrent disease. Ann Oncol. 2024;35(3):248–266.
13. Bryant A, Johnson E, Grayling M, Hiu S, Elattar A, Gajjar K, et al. Residual disease threshold after primary surgical treatment for advanced epithelial ovarian cancer, Part 1: a systematic review and network meta-analysis. Am J Ther. 2022;30(1):e36–e55.
14. Zhang L, Hou L, Wu J, Li C, Hu T, Zhu C, et al. Peripheral blood mononuclear cells (PBMCs), an ideal liquid biopsy approach to evaluate systematic immunity and predict response of neoadjuvant chemo-immunotherapy in resectable NSCLC. JCO. 2022;40(16):e20618.

15. Salas-Benito D, Vercher E, Conde E, Glez-Vaz J, Tamayo I, Hervas-Stubbs S. Inflammation and immunity in ovarian cancer. EJC Suppl. 2020;15:56–66.
16. Yang Y, Yang Y, Yang J, Zhao X, Wei X. Tumor microenvironment in ovarian cancer: function and therapeutic strategy. Front Cell Dev Biol. 2020;8:758.
17. Ye S, Chen W, Zheng Y, Wu Y, Xiang L, Li T, et al. Peripheral lymphocyte populations in ovarian cancer patients and correlations with clinicopathological features. J Ovarian Res. 2022;15(1):43.
18. Napoletano C, Bellati F, Landi R, Pauselli S, Marchetti C, Visconti V, et al. Ovarian cancer cytoreduction induces changes in T cell population subsets reducing immunosuppression. J Cell Mol Med. 2010;14(12):2748–2759.
19. De Bruyn C, Ceusters J, Landolfo C, Baert T, Thirion G, Claes S, et al. Neo-adjuvant chemotherapy reduces, and surgery increases immunosuppression in first-line treatment for ovarian cancer. Cancers (Basel). 2021;13(23):5899.
20. Kovács AR, Lukács L, Pál L, Szűcs S, Kovács KS, 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). 2023;59(9):1602.
21. Mielczarek-Palacz A, Sikora J, Kondera-Anasz Z, Mickiewicz P, Mickiewicz A. Effect of Th1/Th2 cytokine administration on proinflammatory SKOV-3 cell activation. Arch Med Sci. 2016;12(6):1337–1347.
22. Habel A, Weili X, Hadj Ahmed M, Stayoussef M, Bouaziz H, Ayadi M, et al. Immune checkpoints as potential theragnostic biomarkers for epithelial ovarian cancer. Int J Biol Markers. 2023:3936155231186163.
23. Dumitru A, Dobrica E, Croitoru A, Cretoiu SM, Gaspar BS. Focus on PD-1/PD-L1 as a Therapeutic Target in Ovarian Cancer. Int J Mol Sci. 2022;23(20):12067.
24. Micheli DC, Jammal MP, Martins-Filho A, Côrtes JRXdM, Souza CNd, Nomelini RS, et al. Serum cytokines and CXCR2: potential tumour markers in ovarian neoplasms. Biomarkers. 2020;25(6):474–482.
25. Tang F, Tie Y, Tu C, Wei X. Surgical trauma-induced immunosuppression in cancer: Recent advances and the potential therapies. Clin Transl Med. 2020;10(1):199–223.
26. Amodeo G, Bugada D, Franchi S, Moschetti G, Grimaldi S, Panerai A, et al. Immune function after major surgical interventions: the effect of postoperative pain treatment. J Pain Res. 2018;11:1297–1305.
27. Yang F, Hua Q, Zhu X, Xu P. Surgical stress induced tumor immune suppressive environment. Carcinogenesis. 2024;45(4):185–198.
28. Ramirez MF, Ai D, Bauer M, Vauthey J, Gottumukkala V, Kee S, et al. Innate immune function after breast, lung, and colorectal cancer surgery. J Surg Res. 2015;194(1):185–193.
29. Angka L, Martel AB, Kilgour M, Jeong A, Sadiq M, de Souza CT, et al. Natural killer cell IFNγ secretion is profoundly suppressed following colorectal cancer surgery. Ann Surg Oncol. 2018;25(12):3747–3754.
30. Ng CSH, Lau KKW. Surgical trauma and immune functional changes following major lung resection. Indian J Surg. 2015;77(1):49–54.
31. Cabasag CJ, Fagan PJ, Ferlay J, Vignat J, Laversanne M, Liu L, et al. Ovarian cancer today and tomorrow: a global assessment by world region and human development index using GLOBOCAN 2020. Int J Cancer. 2022;151(9):1535–1541.

32. Zhang S, Cheng C, Lin Z, Xiao L, Su X, Zheng L, et al. The global burden and associated factors of ovarian cancer in 1990-2019: findings from the global burden of disease study 2019. BMC Public Health. 2022;22(1):1455.
33. Cabasag CJ, Butler J, Arnold M, Rutherford M, Bardot A, Ferlay J, et al. Exploring variations in ovarian cancer survival by age and stage (ICBP SurvMark-2): A population-based study. Gynecol Oncol. 2020;157(1):234–244.
34. Azangou-Khyavy M, Ghasemi E, Rezaei N, Khanali J, Kolahi A, Malekpour M, et al. Global, regional, and national quality of care index of cervical and ovarian cancer: a systematic analysis for the global burden of disease study 1990–2019. BMC Womens Health. 2024;24(1):69.
35. Huang J, Chan WC, Ngai CH, Lok V, Zhang L, Lucero-Prisno DE, et al. Worldwide burden, risk factors, and temporal trends of ovarian cancer: a global study. Cancers (Basel). 2022;14(9):2230.
36. Board E. Female genital tumours. WHO Classification of Tumours (International Agency for Research on Cancer). 2020.
37. Wang M, Bi Y, Jin Y, Zheng Z. Global incidence of ovarian cancer according to histologic subtype: a population-based cancer registry study. JCO Glob Oncol. 2024; 10(10):e2300393.
38. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017;14(1):9–32.
39. Prat J, D'Angelo E, Espinosa I. Ovarian carcinomas: at least five different diseases with distinct histological features and molecular genetics. Hum Pathol. 2018; 80:11–27.
40. De Leo A, Santini D, Ceccarelli C, Santandrea G, Palicelli A, Acquaviva G, et al. What is new on ovarian carcinoma: integrated morphologic and molecular analysis following the new 2020 World Health Organization classification of female genital tumors. Diagnostics. 2021;11(4):697.
41. Zhang X, Wu Y, Xu T, Cui M. CAR-T cells in the treatment of ovarian cancer: a promising cell therapy. Biomolecules. 2023;13(3):465.
42. Marques C, Ferreira da Silva F, Sousa I, Nave M. Chemotherapy-free treatment of recurrent advanced ovarian cancer: myth or reality? Int J Gynecol Cancer. 2023;33(4): 607–618.
43. Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Barroilhet L, Behbakht K, Berchuck A, et al. Ovarian cancer, version 2.2020, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2021;19(2):191–226.
44. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. Int J Gynaecol Obstet. 2021;155(S1):61–85.
45. Tzanis AA, Iavazzo C, Hadjivasilis A, Tsouvali H, Antoniou GA, Antoniou SA. Neoadjuvant chemotherapy versus primary debulking surgery in FIGO stage III and IV epithelial ovarian, tubal or peritoneal cancer: a systematic review and meta-analysis. Oncol Rev. 2022;16:10605.
46. Miras I, Estévez-García P, Muñoz-Galván S. Clinical and molecular features of platinum resistance in ovarian cancer. Crit Rev Oncol Hematol. 2024;201:104434.
47. O'Malley DM, Krivak TC, Kabil N, Munley J, Moore KN. PARP inhibitors in ovarian cancer: a review. Target Oncol. 2023;18(4):471–503.

48. Peng Z, Li M, Li H, Gao Q. PD-1/PD-L1 immune checkpoint blockade in ovarian cancer: Dilemmas and opportunities. Drug Discov Today. 2023;28(8):103666.
49. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer. 2023;22(1):138.
50. Hu Q, Bian Q, Rong D, Wang L, Song J, Huang H, et al. JAK/STAT pathway: Extracellular signals, diseases, immunity, and therapeutic regimens. Front Bioeng Biotechnol. 2023;11:1110765.
51. Rinne N, Christie EL, Ardasheva A, Chun HK, Demchenko N, Low C, et al. Targeting the PI3K/AKT/mTOR pathway in epithelial ovarian cancer, therapeutic treatment options for platinum-resistant ovarian cancer. Cancer Drug Resist. 2021;4(3):573–595.
52. Hudry D, Le Guellec S, Meignan S, Bécourt S, Pasquesoone C, El Hajj H, et al. Tumor-infiltrating lymphocytes (TILs) in epithelial ovarian cancer: heterogeneity, prognostic impact, and relationship with immune checkpoints. Cancers (Basel). 2022;14(21):5332.
53. Huffman OG, Chau DB, Dinicu AI, DeBernardo R, Reizes O. Mechanistic insights on hyperthermic intraperitoneal chemotherapy in ovarian cancer. Cancers (Basel). 2023;15(5):1402.
54. Aronson SL, Lopez-Yurda M, Koole SN, Schagen van Leeuwen JH, Schreuder HWR, Hermans RHM, et al. Cytoreductive surgery with or without hyperthermic intraperitoneal chemotherapy in patients with advanced ovarian cancer (OVHIPEC-1): final survival analysis of a randomised, controlled, phase 3 trial. Lancet Oncol. 2023;24(10):1109–1118.
55. van Driel WJ, Koole SN, Sikorska K, Schagen van Leeuwen JH, Schreuder HWR, Hermans RHM, et al. Hyperthermic intraperitoneal chemotherapy in ovarian cancer. N Engl J Med. 2018;378(3):230–240.
56. Graham R, MacDonald ND, Mould TA, Kotsopoulos IC. Hyperthermic intraperitoneal chemotherapy (HIPEC) in the management of ovarian cancer. Obstet Gynecol. 2024;26(2):76–83.
57. Cheng X, Li P, Jiang R, Meng E, Wu H. ADC: a deadly killer of platinum resistant ovarian cancer. J Ovarian Res. 2024;17(1):196.
58. Blanc-Durand F, Pautier P, Michels J, Leary A. Targeting the immune microenvironment in ovarian cancer therapy – mission impossible? ESMO Open. 2024;9(3).
59. Siminiak N, Czepczyński R, Zaborowski MP, Iżycki D. Immunotherapy in Ovarian Cancer. Arch Immunol Ther Exp (Warsz). 2022;70(1):19.
60. Wilczyński M, Wilczyński J, Nowak M. MiRNAs as regulators of immune cells in the tumor microenvironment of ovarian cancer. Cells. 2024;13(16):1343.
61. Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal. 2020;18(1):59–59.
62. Blanc-Durand F, Clemence Wei Xian L, Tan DSP. Targeting the immune microenvironment for ovarian cancer therapy. Front Immunol. 2023;14:1328651.
63. Pankowska KA, Będkowska GE, Chociej-Stypułkowska J, Rusak M, Dąbrowska M, Osada J. Crosstalk of immune cells and platelets in an ovarian cancer microenvironment and their prognostic significance. Int J Mol Sci. 2023;24(11):9279.

64. Charbonneau B, Goode EL, Kalli KR, Knutson KL, DeRycke MS. The immune system in the pathogenesis of ovarian cancer. Crit Rev Immunol. 2013;33(2):137–164.
65. Erdogan B, Webb DJ. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem Soc Trans. 2017;45(1):229–236.
66. Givel A, Kieffer Y, Scholer-Dahirel A, Sirven P, Cardon M, Pelon F, et al. miR200-regulated CXCL12β promotes fibroblast heterogeneity and immunosuppression in ovarian cancers. Nat Commun. 2018;9(1):1056.
67. Sjöberg E, Augsten M, Bergh J, Jirström K, Östman A. Expression of the chemokine CXCL14 in the tumour stroma is an independent marker of survival in breast cancer. Br J Cancer. 2016;114(10):1117–1124.
68. Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. Nat Rev Immunol. 2011;11(10):702–711.
69. Yin M, Shen J, Yu S, Fei J, Zhu X, Zhao J, et al. Tumor-associated macrophages (TAMs): a critical activator in ovarian cancer metastasis. Onco Targets Ther. 2019; 12:8687–8699.
70. El-Arabey AA, Denizli M, Kanlikilicer P, Bayraktar R, Ivan C, Rashed M, et al. GATA3 as a master regulator for interactions of tumor-associated macrophages with high-grade serous ovarian carcinoma. Cell Signal. 2020;68:109539.
71. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, et al. The cellular and molecular origin of tumor-associated macrophages. Science. 2014;344(6186):921–925.
72. Cheng H, Wang Z, Fu L, Xu T. Macrophage polarization in the development and progression of ovarian cancers: an overview. Front Oncol. 2019;9:421.
73. Colvin EK. Tumor-associated macrophages contribute to tumor progression in ovarian cancer. Front Oncol. 2014;4:137.
74. Larionova I, Tuguzbaeva G, Ponomaryova A, Stakheyeva M, Cherdyntseva N, Pavlov V, et al. Tumor-associated macrophages in human breast, colorectal, lung, ovarian and prostate cancers. Front Oncol. 2020;10:566511.
75. Yuan X, Zhang J, Li D, Mao Y, Mo F, Du W, et al. Prognostic significance of tumor-associated macrophages in ovarian cancer: a meta-analysis. Gynecol Oncol. 2017; 147(1):181–187.
76. Macciò A, Gramignano G, Cherchi MC, Tanca L, Melis L, Madeddu C. Role of M1-polarized tumor-associated macrophages in the prognosis of advanced ovarian cancer patients. Sci Rep. 2020;10(1):6096.
77. Goswami KK, Ghosh T, Ghosh S, Sarkar M, Bose A, Baral R. Tumor promoting role of anti-tumor macrophages in tumor microenvironment. Cell Immunol. 2017;316:1–10.
78. Schweer D, McAtee A, Neupane K, Richards C, Ueland F, Kolesar J. Tumor-associated macrophages and ovarian cancer: implications for therapy. Cancers (Basel). 2022;14(9):2220.
79. Nowak M, Klink M. The role of tumor-associated macrophages in the progression and chemoresistance of ovarian cancer. Cells. 2020;9(5):1299.
80. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203–213.

81. Fanale D, Dimino A, Pedone E, Brando C, Corsini LR, Filorizzo C, et al. Prognostic and predictive role of tumor-infiltrating lymphocytes (TILs) in ovarian cancer. Cancers (Basel). 2022;14(18):4344.
82. Sadelain M, Rivière I, Riddell S. Therapeutic T cell engineering. Nature. 2017; 545(7655):423–431.
83. Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. Annu Rev Immunol. 2009;27:591–619.
84. Pearce OMT, Delaine-Smith RM, Maniati E, Nichols S, Wang J, Böhm S, et al. Deconstruction of a metastatic tumor microenvironment reveals a common matrix response in human cancers. Cancer Discov. 2018;8(3):304–319.
85. Pinto MP, Balmaceda C, Bravo ML, Kato S, Villarroel A, Owen GI, et al. Patient inflammatory status and CD4+/CD8+ intraepithelial tumor lymphocyte infiltration are predictors of outcomes in high-grade serous ovarian cancer. Gynecol Oncol. 2018;151(1):10–17.
86. Goode EL, Block MS, Kalli KR, Vierkant RA, Chen W, Fogarty ZC, et al. Dose-response relationship of CD8+ tumor infiltrating lymphocytes and survival time in high-grade serous ovarian cancer. JAMA Oncol. 2017;3(12):e173290.
87. Hwang W, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol. 2012;124(2):192–198.
88. Yakubovich E, Cook DP, Rodriguez GM, Vanderhyden BC. Mesenchymal ovarian cancer cells promote CD8+ T cell exhaustion through the LGALS3-LAG3 axis. NPJ Syst Biol Appl. 2023;9(1):61.
89. Miyara M, Sakaguchi S. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. Immunol Cell Biol. 2011;89(3):346–351.
90. Cassar E, Kartikasari AER, Plebanski M. Regulatory T cells in ovarian carcinogenesis and future therapeutic opportunities. Cancers (Basel). 2022;14(22):5488.
91. Liu Y, Shan F, Sun Y, Kai H, Cao Y, Huang M, et al. Prognostic and immunotherapeutic potential of regulatory T cell-associated signature in ovarian cancer. J Cell Mol Med. 2024;28(8):e18248.
92. Groves CJ, Carrell J, Grady R, Rajan B, Morehouse CA, Halpin R, et al. CD19-positive antibody-secreting cells provide immune memory. Blood Adv. 2018;2(22):3163–3176.
93. Lundgren S, Berntsson J, Nodin B, Micke P, Jirström K. Prognostic impact of tumour-associated B cells and plasma cells in epithelial ovarian cancer. J Ovarian Res. 2016;9:21.
94. Kroeger DR, Milne K, Nelson BH. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. Clin Cancer Res. 2016;22(12):3005–3015.
95. Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, et al. CD20+ tumor-infiltrating lymphocytes have an atypical CD27– memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. Clin Cancer Res. 2012;18(12):3281–3292.
96. Yang C, Lee H, Jove V, Deng J, Zhang W, Liu X, et al. Prognostic significance of B-cells and pSTAT3 in patients with ovarian cancer. PLoS One. 2013;8(1):e54029.
97. Dong HP, Elstrand MB, Holth A, Silins I, Berner A, Trope CG, et al. NK- and B-cell infiltration correlates with worse outcome in metastatic ovarian carcinoma. Am J Clin Pathol. 2006;125(3):451–458.

98. Hoogstad-van Evert JS, Maas RJ, van der Meer J, Cany J, van der Steen S, Jansen JH, et al. Peritoneal NK cells are responsive to IL-15 and percentages are correlated with outcome in advanced ovarian cancer patients. Oncotarget. 2018;9(78):34810–34820.
99. Webb JR, Milne K, Watson P, Deleeuw RJ, Nelson BH. Tumor-infiltrating lymphocytes expressing the tissue resident memory marker CD103 are associated with increased survival in high-grade serous ovarian cancer. Clin Cancer Res. 2014; 20(2): 434–444.
100. Henriksen JR, Nederby L, Donskov F, Waldstrøm M, Adimi P, Jakobsen A, et al. Blood natural killer cells during treatment in recurrent ovarian cancer. Acta Oncol. 2020;59(11):1365–1373.
101. Shaul ME, Levy L, Sun J, Mishalian I, Singhal S, Kapoor V, et al. Tumor-associated neutrophils display a distinct N1 profile following TGFβ modulation: A transcriptomics analysis of pro- vs. antitumor TANs. Oncoimmunology. 2016;5(11):e1232221.
102. Lee W, Ko SY, Mohamed MS, Kenny HA, Lengyel E, Naora H. Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum. J Exp Med. 2019; 216(1):176–194.
103. Singel KL, Emmons TR, Khan ANH, Mayor PC, Shen S, Wong JT, et al. Mature neutrophils suppress T cell immunity in ovarian cancer microenvironment. JCI Insight. 2019;4(5):e122311, 122311.
104. Zhang C, Jiang X, Li Y, Pan X, Gao M, Chen Y, et al. Independent predictive value of blood inflammatory composite markers in ovarian cancer: recent clinical evidence and perspective focusing on NLR and PLR. J Ovarian Res. 2023;16:36.
105. Song L, Dong G, Guo L, Graves DT. The function of dendritic cells in modulating the host response. Mol Oral Microbiol. 2018;33(1):13–21.
106. Scarlett UK, Rutkowski MR, Rauwerdink AM, Fields J, Escovar-Fadul X, Baird J, et al. Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. J Exp Med. 2012;209(3):495–506.
107. Labidi-Galy SI, Treilleux I, Goddard-Leon S, Combes J, Blay J, Ray-Coquard I, et al. Plasmacytoid dendritic cells infiltrating ovarian cancer are associated with poor prognosis. Oncoimmunology. 2012;1(3):380–382.
108. Truxova I, Kasikova L, Hensler M, Skapa P, Laco J, Pecen L, et al. Mature dendritic cells correlate with favorable immune infiltrate and improved prognosis in ovarian carcinoma patients. J Immunother Cancer. 2018;6(1):139.
109. Bartůňková J. Dendritic Cell Immunotherapy for ovarian cancer: an overview of our achievements. Onco. 2024;4(1):55.
110. Nepal MR, Shah S, Kang K. Dual roles of myeloid-derived suppressor cells in various diseases: a review. Arch Pharm Res. 2024;47(7):597–616.
111. Okła K. Myeloid-derived suppressor cells (MDSCs) in ovarian cancer – looking back and forward. Cells. 2023;12(14):1912.
112. Mabuchi S, Sasano T, Komura N. Targeting myeloid-derived suppressor cells in ovarian cancer. Cells. 2021;10(2):329.
113. Meyer N, Hinz N, Schumacher A, Weißenborn C, Fink B, Bauer M, et al. Mast cells retard tumor growth in ovarian cancer: insights from a mouse model. Cancers (Basel). 2023;15(17):4278.
114. Valmiki S, Aid MA, Chaitou AR, Zahid M, Valmiki M, Fawzy P, et al. Extracellular matrix: a treasure trove in ovarian cancer dissemination and chemotherapeutic resistance. Curēus (Palo Alto, CA). 2021;13(3):e13864.

115. Alshehri S, Pavlovič T, Farsinejad S, Behboodi P, Quan L, Centeno D, et al. Extracellular matrix modulates outgrowth dynamics in ovarian cancer. Adv Biol (Weinh). 2022;6(12):e2200197.
116. Cho A, Howell VM, Colvin EK. The extracellular matrix in epithelial ovarian cancer – a piece of a puzzle. Front Oncol. 2015;5:245.
117. Gertych A, Walts AE, Cheng K, Liu M, John J, Lester J, et al. Dynamic changes in the extracellular matrix in primary, metastatic, and recurrent ovarian cancers. Cells. 2022;11(23):3769.
118. Puttock EH, Tyler EJ, Manni M, Maniati E, Butterworth C, Burger Ramos M, et al. Extracellular matrix educates an immunoregulatory tumor macrophage phenotype found in ovarian cancer metastasis. Nat Commun. 2023;14:2514.
119. Brown Y, Hua S, Tanwar PS. Extracellular matrix in high-grade serous ovarian cancer: Advances in understanding of carcinogenesis and cancer biology. Matrix Biol. 2023;118:16–46.
120. Ning F, Cole CB, Annunziata CM. Driving immune responses in the ovarian tumor microenvironment. Front Oncol. 2020;10:604084.
121. Pawłowska A, Rekowska A, Kuryło W, Pańczyszyn A, Kotarski J, Wertel I. Current understanding on why ovarian cancer is resistant to immune checkpoint inhibitors. Int J Mol Sci. 2023;24(13):10859.
122. Riely GJ, Wood DE, Ettinger DS, Aisner DL, Akerley W, Bauman JR, et al. Non-small cell lung cancer, version 4.2024, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2024;22(4):249–274.
123. Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23 Suppl 7:vii86–vii91.
124. Hastings WD, Anderson DE, Kassam N, Koguchi K, Greenfield EA, Kent SC, et al. TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. Eur J Immunol. 2009;39(9):2492–2501.
125. Sakuishi K, Ngiow SF, Sullivan JM, Teng MWL, Kuchroo VK, Smyth MJ, et al. TIM3+FOXP3+ regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. Oncoimmunology. 2013;2(4):e23849.
126. Browning L, Patel MR, Horvath EB, Tawara K, Jorcyk CL. IL-6 and ovarian cancer: inflammatory cytokines in promotion of metastasis. Cancer Manag Res. 2018;10: 6685–6693.
127. Litmanovich A, Khazim K, Cohen I. The role of interleukin-1 in the pathogenesis of cancer and its potential as a therapeutic target in clinical practice. Oncol Ther. 2018;6(2):109–127.
128. Schauer IG, Zhang J, Xing Z, Guo X, Mercado-Uribe I, Sood AK, et al. Interleukin-1β promotes ovarian tumorigenesis through a p53/NF-κB-Mediated inflammatory response in stromal fibroblasts. Neoplasia. 2013;15(4):409–420.
129. Josiany Carlos dS, Roberto Silva RJ, Tatiana MP, Bárbara da SM, Leticia Batista AR. The role of pro-inflammatory components, carcinoma-associated fibroblasts, and tumor-associated macrophages in ovarian cancer progression and metastasis. J Cancer Metastasis Treat. 2023;9(1):3.
130. van Horssen R, Ten Hagen TLM, Eggermont AMM. TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility. Oncologist. 2006;11(4): 397–408.

131. Gupta M, Babic A, Beck AH, Terry K. TNF-α expression, risk factors, and inflammatory exposures in ovarian cancer: evidence for an inflammatory pathway of ovarian carcinogenesis? Hum Pathol. 2016;54:82–91.
132. Wang X, Yang C, Körner H, Ge C. Tumor necrosis factor: What is in a name? Cancers (Basel). 2022;14(21):5270.
133. Charles KA, Kulbe H, Soper R, Escorcio-Correia M, Lawrence T, Schultheis A, et al. The tumor-promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. J Clin Invest. 2009;119(10):3011–3023.
134. Li Z, Chen L, Qin Z. Paradoxical roles of IL-4 in tumor immunity. Cell Mol Immunol. 2009;6(6):415–422.
135. Wilke CM, Wei S, Wang L, Kryczek I, Kao J, Zou W. Dual biological effects of the cytokines interleukin-10 and interferon-γ. Cancer Immunol Immunother. 2011; 60(11):1529–1541.
136. Uhlen M, Karlsson MJ, Zhong W, Tebani A, Pou C, Mikes J, et al. A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. Science. 2019;366(6472):eaax9198.
137. Keegan AD, Leonard WJ, Zhu J. Recent advances in understanding the role of IL-4 signaling. Fac Rev. 2021;10:71.
138. Carlini V, Noonan DM, Abdalalem E, Goletti D, Sansone C, Calabrone L, et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Front Immunol. 2023; 14:1161067.
139. Tait Wojno ED, Hunter CA, Stumhofer JS. The immunobiology of the interleukin-12 family: room for discovery. Immunity. 2019;50(4):851–870.
140. Kleiveland CR. Peripheral blood mononuclear cells. Cham (CH): Springer; 2015. p. 161–167.
141. Isolation of mononuclear cells. Available at: https://cdn.cytivalifesciences.com/api/public/content/digi-16156-pdf. Accessed Sep 26, 2023.
142. Rajtak A, Ostrowska-Leśko M, Żak K, Tarkowski R, Kotarski J, Okła K. Integration of local and systemic immunity in ovarian cancer: implications for immunotherapy. Front Immunol. 2022;13:1018256.
143. Santin AD, Hermonat PL, Ravaggi A, Bellone S, Roman JJ, Smith CV, et al. Phenotypic and functional analysis of tumor-infiltrating lymphocytes compared with tumor-associated lymphocytes from ascitic fluid and peripheral blood lymphocytes in patients with advanced ovarian cancer. Gynecol Obstet Invest. 2001;51(4):254–261.
144. Bamias A, Tsiatas ML, Kafantari E, Liakou C, Rodolakis A, Voulgaris Z, et al. Significant differences of lymphocytes isolated from ascites of patients with ovarian cancer compared to blood and tumor lymphocytes. Association of CD3+CD56+ cells with platinum resistance. Gynecol Oncol. 2007;106(1):75–81.
145. Kovacsovics-Bankowski M, Chisholm L, Vercellini J, Tucker CG, Montler R, Haley D, et al. Detailed characterization of tumor infiltrating lymphocytes in two distinct human solid malignancies show phenotypic similarities. J Immunother Cancer. 2014; 2(1):38.
146. Zhang W, Ling Y, Li Z, Peng X, Ren Y. Peripheral and tumor-infiltrating immune cells are correlated with patient outcomes in ovarian cancer. Cancer Med. 2023;12(8): 10045.

147. Preston CC, Maurer MJ, Oberg AL, Visscher DW, Kalli KR, Hartmann LC, et al. The ratios of CD8+ T cells to CD4+CD25+ FOXP3+ and FOXP3- T cells correlate with poor clinical outcome in human serous ovarian cancer. PLoS One. 2013;8(11): e80063.
148. Gong J, Jiang H, Shu C, Hu M, Huang Y, Liu Q, et al. Prognostic value of lymphocyte-to-monocyte ratio in ovarian cancer: a meta-analysis. J Ovarian Res. 2019; 12(1):51.
149. Rogado J, Pozo F, Troule K, Sánchez-Torres JM, Romero-Laorden N, Mondejar R, et al. Peripheral blood mononuclear cells predict therapeutic efficacy of immunotherapy in NSCLC. Cancers. 2022;14(12):2898.
150. Stout A, Facey N, Bhatnagar A, Rice K, Berditchevski F, Kearns D, et al. Profiling of tumour-infiltrating lymphocytes and tumour-associated macrophages in ovarian epithelial cancer-relation to tumour characteristics and impact on prognosis. Int J Mol Sci. 2024;25(8):4524.
151. Lee YJ, Chung YS, Lee J, Nam EJ, Kim SW, Kim S, et al. Pretreatment lymphocytopenia is an adverse prognostic biomarker in advanced-stage ovarian cancer. Cancer Med. 2019;8(2):564–571.
152. Hudry D, Le Guellec S, Meignan S, Bécourt S, Pasquesoone C, El Hajj H, et al. Tumor-infiltrating lymphocytes (TILs) in epithelial ovarian cancer: heterogeneity, prognostic impact, and relationship with immune checkpoints. Cancers. 2022;14(21).
153. Hao Su, Yueqi Jin, Changyu Tao, Hua Yang, Ence Yang, Wei-guang Zhang, et al. Th2 cells infiltrating high-grade serous ovarian cancer: a feature that may account for the poor prognosis. J Gynecol Oncol. 2023;34(4):1.
154. Horowitz M, Neeman E, Sharon E, Ben-Eliyahu S. Exploiting the critical perioperative period to improve long-term cancer outcomes. Nat Rev Clin Oncol. 2015; 12(4):213–226.
155. Shen H, Pang Q, Gao Y, Liu H. Effects of epidural anesthesia on the prognosis of ovarian cancer – a systematic review and meta-analysis. BMC Anesthesiol. 2023; 23:390.
156. Tohme S, Simmons RL, Tsung A. Surgery for cancer: a trigger for metastases. Cancer Res. 2017;77(7):1548–1552.
157. Jansen L, Dubois BFH, Hollmann MW. The effect of propofol versus inhalation anesthetics on survival after oncological surgery. J Clin Med. 2022;11(22):6741.
158. Kovács AR, Pál L, Szűcs S, Lukács L, Póka R, Lampé R. Phagocytic function of monocytes and neutrophil granulocytes in ovarian cancer. Orv Hetil. 2018;159(33): 1353–1359.
159. Brøchner AC, Mikkelsen S, Hegelund I, Hokland M, Mogensen O, Toft P. The immune response is affected for at least three weeks after extensive surgery for ovarian cancer. Dan Med J. 2016;63(6).
160. Wu M, Chen X, Lou J, Zhang S, Zhang X, Huang L, et al. Changes in regulatory T cells in patients with ovarian cancer undergoing surgery: preliminary results. Int Immunopharmacol. 2017;47:244–250.
161. Cheng X, Zhang H, Hamad A, Huang H, Tsung A. Surgery-mediated tumor-promoting effects on the immune microenvironment. Semin Cancer Biol. 2022;86(Pt 3): 408–419.
162. Alieva M, van Rheenen J, Broekman MLD. Potential impact of invasive surgical procedures on primary tumor growth and metastasis. Clin Exp Metastasis. 2018;35(4): 319–331.

163. Nowak M, Głowacka E, Lewkowicz P, Banasik M, Szyłło K, Zimna K, et al. Sub-optimal primary surgery leads to unfavorable immunological changes in ovarian cancer patients. Immunobiology. 2018;223(1):1–7.
164. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495–499.
165. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677–686.
166. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat Rev Immunol. 2013;13(5):349–361.
167. Bogdan C. Nitric oxide synthase in innate and adaptive immunity: an update. Trends Immunol. 2015;36(3):161–178.
168. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol. 2005;5(8):641–654.
169. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001;25(4):402–408.
170. Hoaglin DC, Iglewicz B. Fine-tuning some resistant rules for outlier labeling. J Am Stat Assoc. 1987;82(400):1147–1149.
171. Isingizwe ZR, Meelheim BA, Benbrook DM. Elevated platelet aggregation in patients with ovarian cancer: more than just increased platelet count. Cancers. 2024;16(21).
172. Feng Y, Wang Z, Cui R, Xiao M, Gao H, Bai H, et al. Clinical analysis and artificial intelligence survival prediction of serous ovarian cancer based on preoperative circulating leukocytes. J Ovarian Res. 2022;15(1):64.
173. Giannakeas V, Kotsopoulos J, Cheung MC, Rosella L, Brooks JD, Lipscombe L, et al. Analysis of Platelet Count and New Cancer Diagnosis Over a 10-Year Period. JAMA Netw Open. 2022;5(1):e2141633.
174. Gao Y, Liu CJ, Li HY, Xiong XM, Li GL, et al. Platelet RNA enables accurate detection of ovarian cancer: an intercontinental, biomarker identification study. Protein Cell. 2023 Jun 7;14(6):579–590.
175. Ceran MU, Tasdemir U, Colak E, Güngör T. Can complete blood count inflammatory parameters in epithelial ovarian cancer contribute to prognosis? – a survival analysis. J Ovarian Res. 2019;12(1):16.
176. Milne K, Alexander C, Webb JR, Sun W, Dillon K, Kalloger SE, et al. Absolute lymphocyte count is associated with survival in ovarian cancer independent of tumor-infiltrating lymphocytes. J Transl Med. 2012;10:33.
177. Park A, Govindaraj C, Xiang SD, Halo J, Quinn M, Scalzo-Inguanti K, et al. Substantially modified ratios of effector to regulatory T cells during chemotherapy in ovarian cancer patients return to pre-treatment levels at completion: implications for immunotherapy. Cancers. 2012;4(2):581–600.
178. Hu Q, Shen G, Li Y, Xie Y, Ma X, Jiang L, et al. Lymphocyte-to-monocyte ratio after primary surgery is an independent prognostic factor for patients with epithelial ovarian cancer: a propensity score matching analysis. Front Oncol. 2023;13:1139929.
179. Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E. Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. Am J Respir Cell Mol Biol. 2015;53(5):676–688.

180. Wiehagen KR, Girgis NM, Yamada DH, Smith AA, Chan SR, Grewal IS, et al. Combination of CD40 agonism and CSF-1R blockade reconditions tumor-associated macrophages and drives potent antitumor immunity. Cancer Immunol Res. 2017; 5(12):1109–1121.
181. Shibutani M, Maeda K, Nagahara H, Fukuoka T, Nakao S, Matsutani S, et al. The peripheral monocyte count is associated with the density of tumor-associated macrophages in the tumor microenvironment of colorectal cancer: a retrospective study. BMC Cancer. 2017;17(1):404.
182. Fadhil M, Abdul-Rasheed O, Al-Naqqash M. Clinical value of peripheral blood M2/M1 like monocyte ratio in the diagnosis of breast cancer and the differentiation between benign and malignant breast tumors. Acta Biochim Pol. 2019;66(4):437–443.
183. Patysheva M, Frolova A, Larionova I, Afanas'ev S, Tarasova A, Cherdyntseva N, et al. Monocyte programming by cancer therapy. Front Immunol. 2022;13: 994319.
184. Freedman RS, Ma Q, Wang E, Gallardo ST, Gordon IO, Shin JW, et al. Migration deficit in monocyte-macrophages in human ovarian cancer. Cancer Immunol Immunother. 2008;57(5):635–645.
185. Zaborowski MP, Stefens-Stawna P, Osztynowicz K, Piorunek T, Batura-Gabryel H, Dyzmann-Sroka A, et al. Granzyme B in peripheral blood mononuclear cells as a measure of cell-mediated immune response in paraneoplastic neurological syndromes and malignancy. Cancer Immunol Immunother. 2021;70(5):1277–1289.
186. Ingersoll SB, Stoltzfus GP, Merchant MH, Ahmad S, Edwards CR, Ahmed A, et al. Comparison of the cytotoxic response against ovarian cancer by immune effector cells isolated and expanded from normal donors and ovarian cancer patients. Cytotherapy. 2012;14(6):716–723.
187. AlamarBlue® Assay protocol. Available at: https://tools.thermofisher.com/content/sfs/manuals/PI-DAL1025-1100_TI%20alamarBlue%20Rev%201.1.pdf. Accessed Now 17, 2024.
188. Ding D, Xie L, Shen Y, Li J, Guo Y, Fu Y, et al. Insights into the role of oxidative stress in ovarian cancer. Oxid Med Cell Longev. 2021;2021:8388258.
189. Snezhkina AV, Kudryavtseva AV, Kardymon OL, Savvateeva MV, Melnikova NV, Krasnov GS, et al. ROS generation and antioxidant defense systems in normal and malignant cells. Oxid Med Cell Longev. 2019;2019:6175804.
190. Kalb DM, Adikari SH, Hong-Geller E, Werner JH. Single-cell correlations of mRNA and protein content in a human monocytic cell line after LPS stimulation. PLoS One. 2019;14(4):e0215602.
191. Prabahar A, Zamora R, Barclay D, Yin J, Ramamoorthy M, Bagheri A, et al. Unraveling the complex relationship between mRNA and protein abundances: a machine learning-based approach for imputing protein levels from RNA-seq data. NAR Genom Bioinform. 2024;6(1):lqae019.
192. Israelsson P, Labani Motlagh A, Nagaev I, Dehlin E, Nagaeva O, Lundin E, et al. Assessment of cytokine mRNA expression profiles in tumor microenvironment and peripheral blood mononuclear cells of patients with high-grade serous carcinoma of the ovary. J Cancer Sci Ther. 2017;9:422–429.
193. Fahmi MN, Pradjatmo H, Astuti I, Nindrea RD. Cytokines as prognostic biomarkers of Epithelial Ovarian Cancer (EOC): a systematic review and meta-analysis. Asian Pac J Cancer Prev. 2021;22(2):315–323.

194. Kumar P, Ranmale S, Mehta S, Tongaonkar H, Patel V, Singh AK, et al. Immune profile of primary and recurrent epithelial ovarian cancer cases indicates immune suppression, a major cause of progression and relapse of ovarian cancer. J Ovarian Res. 2023;16(1):114.
195. Indra B, Lipoeto N, Hon Tjong D, Rahman S. Alteration of interleukin-4, interleukin-6 levels, and post-operative pain intensity. Open Access Maced J Med Sci [Internet]. 2023;11(A):1–7.
196. Wang L. Prognostic effect of programmed death-ligand 1 (PD-L1) in ovarian cancer: a systematic review, meta-analysis and bioinformatics study. J Ovarian Res. 2019; 12(1):37.
197. Fanale D, Brando C, Corsini LR, Cutaia S, Di Donna MC, Randazzo U, et al. Low plasma PD-L1 levels, early tumor onset and absence of peritoneal carcinomatosis improve prognosis of women with advanced high-grade serous ovarian cancer. BMC cancer. 2023;23(1):437.

LIST OF PUBLICATIONS

Research articles, directly related to the topic of doctoral dissertation, published in journals with a citation index in Clarivate Analytics Web of Science platform:

1. **Ulevičius Jonas**, Jasukaitienė Aldona, Bartkevičiene Arenida, Žilvinas Dambrauskas, Antanas Gulbinas, Daiva Urbonienė, Saulius Paškauskas. *Dysregulation of Peripheral Blood Mononuclear Cells and Immune-Related Proteins during the Early Post-Operative Immune Response in Ovarian Cancer Patients*. Cancers (Basel). 2023 Dec 30;16(1):190. doi:10.3390/cancers16010190. **Impact factor: 5.2**
2. **Ulevičius Jonas**, Jasukaitienė Aldona, Bartkevičiene Arenida, Žilvinas Dambrauskas, Antanas Gulbinas, Daiva Urbonienė, Saulius Paškauskas. *Preoperative Immune Cell Dysregulation Accompanies Ovarian Cancer Patients into the Postoperative Period*. International Journal of Molecular Sciences. 2024 Jun 28;25(13):7087. doi:10.3390/ijms25137087. **Impact factor: 5.6**

LIST OF PRESENTATIONS AT SCIENTIFIC CONFERENCES

Presentations directly related to the topic of doctoral dissertation:

1. **Ulevičius Jonas**, Jasukaitienė Aldona, Žilvinas Dambrauskas, Antanas Gulbinas, Saulius Paškauskas, Artūras Sukovas. *Influence of cancer and surgery to immunosuppressive and proinflammatory factors in ovarian cancer patients' PBMCs.* International Journal of Gynecological Cancer: ESGO 2022 - European Congress on Gynaecological Oncology: meeting abstracts / European Society of Gynaecological Oncology (BMJ, 2022, vol. 32, suppl. 2) 27–30 October, 2022, Berlin, Germany.
2. **Ulevičius Jonas**, Jasukaitienė Aldona, Bartkevičiene Arenida, Žilvinas Dambrauskas, Antanas Gulbinas, Saulius Paškauskas. *Association between heme oxygenase 1 serum levels, expression in peripheral blood mononuclear cells, and advanced ovarian cancer stages*. In: International Health Sciences Conference for All (IHSC for All) "Precision Medicine": Abstract book 2024 : [March 25–26, 2024, Kaunas].

COPIES OF THE MANUSCRIPTS

cancers

MDPI

Article

Dysregulation of Peripheral Blood Mononuclear Cells and Immune-Related Proteins during the Early Post-Operative Immune Response in Ovarian Cancer Patients

Jonas Ulevicius [1,*], Aldona Jasukaitiene [1], Arenida Bartkeviciene [1], Zilvinas Dambrauskas [1], Antanas Gulbinas [1], Daiva Urboniene [2] and Saulius Paskauskas [3]

[1] Laboratory of Surgical Gastroenterology, Institute for Digestive Research, Medical Academy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania; aldona.jasukaitiene@lsmu.lt (A.J.); arenida.bartkeviciene@lsmu.lt (A.B.); zilvinas.dambrauskas@lsmu.lt (Z.D.); antanas.gulbinas@lsmu.lt (A.G.)

[2] Department of Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania; daiva.urboniene@lsmu.lt

[3] Department of Obstetrics and Gynecology, Medical Academy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania; saulius.paskauskas@lsmu.lt

* Correspondence: jonas.ulevicius@lsmu.lt; Tel.: +370-61587731

Simple Summary: This study investigated the immune response in ovarian cancer (OC) patients before and after surgery. The aim was to explore how the immune system is affected by surgery, with a specific focus on peripheral blood mononuclear cells (PBMCs). An analysis of blood samples revealed pre-operative immune imbalances in OC patients that were further exacerbated post-operatively. The study results suggest that OC patients experience a degree of immune suppression, particularly during the early post-operative period. This indicates a potential window of vulnerability that could facilitate cancer progression. Our findings may contribute to the development of better treatment strategies and improved outcomes for OC patients.

Abstract: Surgical treatment is a cornerstone of ovarian cancer (OC) therapy and exerts a substantial influence on the immune system. Immune responses also play a pivotal and intricate role in OC progression. The aim of this study was to investigate the dynamics of immune-related protein expression and the activity of peripheral blood mononuclear cells (PBMCs) in OC patients, both before surgery and during the early postoperative phase. The study cohort comprised 23 OC patients and 20 non-cancer controls. A comprehensive analysis of PBMCs revealed significant pre-operative downregulation in the mRNA expression of multiple immune-related proteins, including interleukins, PD-1, PD-L1, and HO-1. This was followed by further dysregulation during the first 5 post-operative days. Although most serum interleukin concentrations showed only minor changes, a distinct increase in IL-6 and HO-1 levels was observed post-operatively. Reduced metabolic and phagocytic activity and increased production of reactive oxygen species (ROS) were observed on day 1 post-surgery. These findings suggest a shift towards immune tolerance during the early post-operative phase of OC, potentially creating a window for treatment. Further research into post-operative PBMC activity could lead to the development of new or improved treatment strategies for OC.

Keywords: ovarian cancer; surgery; PBMC; IL-1β; IL-4; IL-6; IL-10; PD-1; PD-L1; HO-1

Citation: Ulevicius, J.; Jasukaitiene, A.; Bartkeviciene, A.; Dambrauskas, Z.; Gulbinas, A.; Urboniene, D.; Paskauskas, S. Dysregulation of Peripheral Blood Mononuclear Cells and Immune-Related Proteins during the Early Post-Operative Immune Response in Ovarian Cancer Patients. *Cancers* **2024**, *16*, 190. https://doi.org/10.3390/cancers16010190

Academic Editors: Ugo Cavallaro and Edward J. Pavlik

Received: 5 December 2023
Revised: 27 December 2023
Accepted: 29 December 2023
Published: 30 December 2023

1. Introduction

Ovarian cancer (OC) is the eighth most common cancer type in women worldwide, resulting in over 200,000 deaths annually. Most OC patients are diagnosed with advanced-stage disease, making it more difficult to treat [1,2]. Surgical removal of visible cancer tissue and chemotherapy are the most commonly employed treatment methods for OC.

When combined appropriately, these two treatment approaches can significantly increase the rates of progression-free survival and overall survival [3]. Chemotherapy is rarely administered as a stand-alone treatment in OC, with surgery being a crucial independent prognostic factor [3–5]. Nevertheless, even with the most effective surgical strategies and chemotherapy protocols, disease relapse is frequent, and long-term remission remains a challenge [6]. Consequently, both established and novel approaches are continually being investigated in the quest to improve treatment strategies [7,8].

Despite its benefits for the treatment of OC, surgery can cause tissue trauma and exert systemic effects on the patient [9,10]. It significantly influences the immune response through various mechanisms, including the release of damage-associated molecular patterns, generation of neutrophil extracellular traps, and the activation of myeloid-derived suppressor cells, regulatory T cells, and programmed death ligand-1 [9]. Considering the well-documented interplay between the immune system, the tumour microenvironment, and the progression of OC [6], it is reasonable to hypothesise that surgery could also impact the progression of OC. Although the precise effect on patients is not yet fully understood, surgical trauma has been shown to disrupt immune function, thereby facilitating the formation of metastases [9,11,12]. This phenomenon has been demonstrated in patients undergoing surgical treatment for colorectal, pancreatic, breast, and lung cancers, as well as various other cancer types [13–15]. However, specific data for OC patients are currently lacking.

Peripheral blood mononuclear cells (PBMCs) constitute a cohort of immune cells that are pivotal for the generation of anticancer immunity. After settling in the tumour tissue, PBMCs interact with cancer cells and affect cancer progression [6,16,17]. Despite extensive data on the tumour microenvironment [6,18] and the serum cytokine profile of OC patients [19,20], little is known regarding cytokine production by PBMCs [21,22]. Furthermore, there have been few investigations of post-surgical immune responses in OC patients [23–25]. Consequently, there are almost no published data on the activity of PBMCs in OC patients following surgical intervention.

To gain a better understanding of the relationship between OC progression and surgical treatment, it is crucial to investigate how PBMCs and immune-related proteins [6,26–28] respond to surgical interventions in OC patients. Investigating interleukins IL-1β, IL-4, IL-6, and IL-10 is fundamental for comprehending the immune intricacies in anticancer immunity. These interleukins play crucial roles in regulating inflammatory responses (IL-1β and IL-6), modulating immune cell function and differentiation (IL-4 and IL-10), and maintaining the delicate balance between pro- and anti-inflammatory signals [6,27]. The interaction between immune cells and cancer extends beyond cytokine production. Immune checkpoints like programmed cell death protein 1 (PD-1) and programmed cell death protein ligand 1 (PD-L1) promote immune tolerance and, when activated, can facilitate tumour progression [29,30]. Heme oxygenase 1 (HO-1), a well-known enzyme involved in eliminating oxidative stress, predominantly exhibits anti-inflammatory and immunosuppressive properties when expressed by tumour microenvironment cells [26]. While data exist showing the profiles of PD-1/PD-L1 and HO-1 in the serum and tumour microenvironment [29,31], we lack information on their expression in PBMCs post-operatively in OC patients.

We hypothesised that in the early post-operative period, OC patients experience an imbalance in their immune system that is closely associated with the function of PBMCs. The aim of the current study was to examine potential alterations in PBMC activity, concentration of interleukins, and other immune-related proteins in surgically treated OC patients, with a particular focus on the early post-operative period. By drawing parallels with findings from other cancer types, this work could potentially improve the treatment strategies for OC.

2. Materials and Methods

2.1. Study Design and Patient Selection

This prospective study involved a cohort of 23 patients diagnosed with epithelial OC and surgically treated between January 2021 and April 2023 at the Lithuanian University of Health Sciences Hospital, Kaunas clinics. Laparotomy and cytoreduction were performed for OC patients who had not undergone prior chemotherapy. The selection criteria for the study group were patients diagnosed with primary OC and FIGO stage III and IV [32]. The exclusion criteria included individuals with autoimmune disease, other confirmed or suspected cancer, and patients who had undergone surgery or received blood transfusions within the past month. Histopathological examination confirmed the diagnosis of OC. The control group consisted of 20 women who were age- and body mass index (BMI)-matched with the OC patients and were cancer-free. The same exclusion criteria used for the study group were applied to the controls. All participants gave informed consent before inclusion in the study. This research study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-16).

2.2. Blood Collection

Samples of peripheral venous blood were collected from OC patients and healthy controls. For OC patients, the blood samples were obtained prior to surgery, and at 1, 3, and 5 days after surgery. After reviewing the pilot results, we observed a consistent shift in the data between days 1 and 5 after the surgery. Consequently, certain assays were excluded from day 3 protocol. Blood sampling and the assay schedule for OC patients are outlined in Figure 1. Blood sampling from healthy controls was a one-time procedure for each participant and followed the same assay protocol used for the preoperative assessment of OC patients. Each blood collection consisted of three 10 mL vacutainers containing EDTA K2 (BD, Plymouth, UK), and two 3.5 mL vacutainers with serum clot activator (SST) from Weihai Hongyu (Weihai, China).

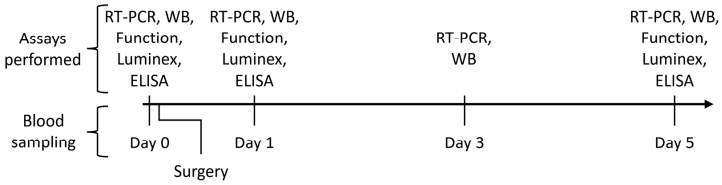


Figure 1. Blood sampling and assay schedule for the ovarian cancer (OC) patients. The lower section of the timeline shows the four specific time points for blood collection. The upper section of the figure outlines the assays conducted at each blood sampling: RT-PCR, real-time polymerase chain reaction; WB, Western blot assay; function, assessment of peripheral blood mononuclear cells (PBMC) metabolic and functional activity; Luminex assay; ELISA assay.

2.3. Isolation of PBMCs and Serum Preparation

Following collection in vacutainers with EDTA K2, blood samples were centrifuged for 10 min at 2470× *g* for plasma separation. PBMCs were isolated by gradient centrifugation with Ficoll-Paque PREMIUM medium (Cytiva, Uppsala, Sweden), according to the manufacturer's protocol [33]. They were then carefully collected by pipetting, and washed twice with PBS (Sigma-Aldrich, Taufkirchen, Germany) at 250× *g* for 5 min each. For downstream experiments, separate aliquots of the PBMCs were used for cell culture (PBMC functional evaluation by fluorometric or spectrophotometric assays) and Western blotting (WB). An aliquot was also mixed with RNAlater® (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −20 °C or −80 °C for use in subsequent assays.

Blood samples collected in vacutainers with SST were allowed to sit for an hour to facilitate clot formation. Vacutainers were then centrifugated at 2500× *g* for 20 min at 4 °C. The resultant serum, situated above the gel layer, was meticulously retrieved and transferred into cryogenic tubes for storage at −80 °C until analysis.

2.4. RNA Extraction and Real-Time Polymerase Chain Reaction (RT-PCR)

Total RNA extraction from pre-prepared PBMC samples was performed using an RNA extraction kit (Abbexa, Cambridge, UK), as per the manufacturer's instructions [34]. The amount of purified RNA was quantified, and its purity was assessed with UV spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesised from 2 µg of RNA using the High/Medium-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) and the 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). Amplification of specific RNA was performed in a 20 µL reaction mixture containing 2 µL of template cDNA, 1 µL of primers, 10 µL of TaqMan™ Universal PCR Master Mix (Applied Biosystems, Waltham, MA, USA), and 7 µL of nuclease-free water. The following primers (Applied Biosystems, Waltham, MA, USA) were used: IL-1β (Hs001555410), IL-4 (Hs00174122), IL-6 (Hs00174131), IL-10 (Hs00961619), HO-1 (Hs0111025), PD-1 (Hs05043241), PD-L1 (Hs00204257), and GAPDH (Hs02786624). Primer efficacy and melting curve analyses were performed to ensure specific amplification. All experiments were conducted in triplicate and repeated as needed for result validation.

2.5. Western Blot Analysis

Proteins were extracted from PBMCs using RIPA lysis buffer (Abcam, Cambridge, MA, USA) supplemented with protease-phosphatase inhibitors (Roche, Basel, Switzerland). After centrifugation at 10,000× *g* for 10 min, the supernatants were collected and stored at −80 °C. The BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure protein concentration. Protein samples (45 µg) were mixed with Bolt MES SDS loading buffer (Invitrogen, Carlsbad, CA, USA) and heated at 97 °C for 5 min. The mixture was then loaded onto a 4–12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and subsequently transferred onto poly-vinylidene fluoride (PVDF) membranes for 40 min at 30 V. The membranes were then exposed to a 5% blocking buffer (Invitrogen, Waltham, MA, USA) for 40 min at room temperature. They were subsequently incubated with primary antibodies either overnight at 4 °C or for 1 h at room temperature. The following primary antibodies were used: rabbit polyclonal anti-HO-1 (ab137749, Abcam, Cambridge, MA, USA) at a 1:1000 dilution, and mouse monoclonal anti-GAPDH (AM4300, Invitrogen, Waltham, MA, USA) at a 1:3000 dilution. Following incubation with a primary antibody, the membranes were washed and then incubated with the appropriate horseradish-peroxidase-conjugated (HRP) or alkaline phosphatase-conjugated (AP) secondary antibody (HRP) from Invitrogen (Carlsbad, CA, USA). Incubation times were either 1 h or 30 min at 37 °C. Subsequent rounds of washing were performed before the membranes were exposed for 5 min to chemiluminescence substrates (Invitrogen, Carlsbad, CA, USA) or West Pico Stable peroxidase buffer with luminol enhancer (Thermo Scientific, Waltham, MA, USA). The protein bands were visualised with a ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). Subsequent quantitative analysis was performed using ImageJ software version 1.53a (National Institutes of Health, Bethesda, MD, USA).

2.6. PBMC Culture and Assessment of Activity

After isolation from patients and healthy participants, PBMCs were resuspended at a concentration of 1 million cells per mL for cell activity analysis. The samples were grown in an RPMI 1640 medium (Gibco Life Technologies Limited, Paisley, UK) without phenol red and supplemented with 10% foetal bovine serum (FBS) (Gibco Life Technologies Limited, Paisley, UK) and 1% penicillin/streptomycin (Gibco Life Technologies Limited, Paisley, UK).

After incubating the samples for 30 min at 37 °C, they were distributed into dark and clear 96-well plates, with each well containing 90,000 cells suspended in the medium. Following 3 h of activation with various effectors, the dark well plates were used in fluorometric or spectrophotometric assays to evaluate the functional activity of PBMCs. Specifically, these were phagocytosis and the production of reactive oxygen species (ROS) and nitrous oxide (NO). Simultaneously with functional activity tests, PBMC viability was determined through resazurin metabolism evaluation. Clear well plates were used to assess metabolic activity with the AlamarBlue® assay (Thermo Fisher Scientific, Waltham, MA, USA). Similar to the procedure for the functional assays (described below), three categories of wells were prepared: wells containing the specific activator, negative controls, or media controls. After cell activation, the AlamarBlue® assay was performed 4 hours later in accordance with the manufacturer's instructions. This was followed by spectrophotometric measurements (The Sunrise, Tecan, Grodig, Austria) [35]. For baseline viability assessment, resazurin metabolism was concurrently measured in wells containing PBMCs without the activators. Resazurin metabolism was subsequently evaluated through fluorometric analysis.

2.7. Assessment of Phagocytosis

Phagocytosis, the process by which cells engulf and eliminate foreign particles or pathogens, is a critical mechanism used by immune cells, particularly macrophages and dendritic cells. Assessing phagocytic activity serves as a valuable marker for immune cell function in the context of anticancer immunity due to its role in clearing cancer cells, debris, and promoting an anti-tumour immune response [30,36]. Lipopolysaccharide (LPS) was used to induce phagocytosis in PBMCs. LPS (Sigma-Aldrich, Rehovot, Israel) was introduced into three wells of a 96-well dark plate, with each well receiving 10 µL of a 5 µg/mL solution. Negative controls were established in another three wells using 10 µL of LPS diluent. The final three wells acted as media controls and contained only RPMI and 10 µL of 5 µg/mL LPS without cells. After 2 h of incubation and activation of phagocytic function, the dark plate was subjected to centrifugation and the supernatant was then removed. Subsequently, 100 µL of pHrodo Green Zymosan Bioparticles (Invitrogen, Eugene, OR, USA) [37] was added to each well, followed by incubation at 37 °C for one hour. Fluorescence measurements were performed using a fluorimeter (Fluoroskan Ascent, Thermo Fisher Scientific, Waltham, MA, USA), with excitation set at 510 nm and detection at 538 nm.

2.8. Assessment of ROS Production

ROS production is significant for evaluating immune cell function, given its role in diverse immunological processes such as signalling, inflammation, and pathogen defence. Particularly in anticancer immunity, ROS acts as a marker due to its involvement in mediating cytotoxic effects against tumours [30,38]. The production of ROS was evaluated using tert-butyl hydroperoxide (TBHP) as an organic peroxide and oxidative-stress-inducing agent. PBMCs were stained with DCFDA (Cellular ROS Assay Kit, Abcam, Cambridge, UK) and then treated with 10 µL of 2.5 mM TBHP (Abcam, Cambridge, UK), according to the manufacturer's protocol [39]. TBHP was administered to three wells, while another set of three wells were designated as negative controls with 10 µL of TBHP diluent. Three wells received only RPMI with 10 µL of 2.5 mM TBHP, thus serving as media controls without cells. Following a 3 h incubation at 37 °C, fluorescence measurements were conducted using a fluorimeter (Fluoroskan Ascent, Thermo Fisher Scientific, Waltham, MA, USA) with excitation set at 485 nm and detection at 538 nm.

2.9. Assessment of NO Production

NO has a dual role in oxidative stress regulation, acting both as a free radical and in balancing oxidative stress levels within cells. It plays a significant role in immune responses against cancer cells, contributing to tumour cell destruction and regulating anti-tumour immune responses [30,40,41]. Nitrite production in PBMCs was induced using

LPS and L-arginine [42]. NO production was assessed in clear 96-well plates after 3 h of treatment with 10 µL of LPS (Sigma-Aldrich, Rehovot, Israel) at a concentration of 5 µg/mL in conjunction with 100 mM of L-arginine (Sigma-Aldrich, Tokyo, Japan). LPS and L-arginine were introduced into three wells, while an additional three wells were designated as negative controls and contained 10 µL of LPS and L-arginine diluent. In three additional wells, RPMI with 10 µL of 5 µg/mL LPS and 100 mM of L-arginine was added without cells as a medium control. After 2.5 h of activation at 37 °C, the samples were combined with 100 µL of Griess reagent (Invitrogen, Eugene, OR, USA) [43] and incubated at room temperature for 30 min. This was followed by spectrophotometric measurement (The Sunrise, Tecan, Grodig, Austria) at 550 nm absorption.

2.10. Analysis of Serum Cytokines Using Luminex

Serum levels of free IL-1β, IL-4, IL-6, and IL-10 were measured using magnetic bead-based multiplex assays (Human Cytokine Premixed Multi-Analyte Kit from R&D) and a Luminex® 100 analyser (Luminex Corporation, Austin, TX, USA). Frozen serum samples were thawed and then centrifuged for 4 min at 4 °C and 16,000× *g* to eliminate any debris or precipitates. The subsequent steps followed the manufacturer's protocol. Analyte-specific antibodies were pre-coated onto magnetic microparticles containing embedded fluorophores, each set at specific ratios for individual microparticle regions. Microparticles, standards, and samples were dispensed into wells, leading to immobilisation of the antibodies on target substances. After washing to remove unbound components, the samples were exposed to a mixture of biotinylated detection antibodies and a streptavidin–phycoerythrin (SAPE) reporter. Further washes eliminated unbound SAPE, and the microparticles were then resuspended in a buffer for analysis with the Luminex® 100 instrument. The Luminex instrument uses lasers to excite the beads, thereby identifying the bead region and its corresponding assigned analyte. The intensity of the PE-derived signal, which is directly proportional to the amount of bound analyte, was measured by another laser. Multiple measurements of the mean fluorescence intensity (MFI) were taken at each bead region to ensure robust detection. Cytokine concentrations were determined relative to a standard curve that plotted MFI against protein concentration.

2.11. Analysis of Serum Proteins Using ELISA

PD-1, PD-L1, and HO-1 concentrations in the serum were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, MA, USA) for PD-1 (ab252360), PD-L1 (ab277712) and HO-1 (ab229429), as recommended by the manufacturer [44–46]. The serum concentrations of PD-1, PD-L1, and HO-1 were derived by evaluating the optical density of samples, followed by interpolation with standard curves.

2.12. Statistical Analysis

Statistical analyses were performed using SPSS (version 22.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism software (version 9.5.1; GraphPad Software Inc., La Jolla, CA, USA). Since most variables were not normally distributed, the Mann–Whitney and Wilcoxon tests were applied for comparison of independent and dependent variables, respectively. All quantitative results were presented as the median with interquartile range, unless stated otherwise. Relative differences in the transcription level of the aforementioned proteins were determined by normalising to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta CT}$ method [47]. The activity of PBMCs was assessed by computing the ratio between samples in wells with activators and the negative controls, employing either fluorometric or spectrophotometric assays. Resazurin metabolism in inactivated PBMCs was assessed via a fluorometric assay and subsequently normalised against the control group. The level of significance was assumed to be $p < 0.05$.

3. Results

3.1. Participant Characteristics

The median age of participants was 58 (14) years. The OC patients and controls did not differ in terms of age and BMI. The distribution of OC histological types was consistent with that observed in the general population [48]. Clinicopathological data for the OC patients and controls are shown in Table 1.

Table 1. Clinicopathological characteristics of the ovarian cancer (OC) and control groups.

Characteristic	OC Group (n = 23)	Control Group (n = 20)	p-Value
Age (years) *	58 (14)	60.5 (10)	0.48
Body mass index (kg/m^2) *	24 (7.4)	24.7 (6.6)	0.97
Stage of OC **			
IIIA	4 (17.4)	NA	NA
IIIB	4 (17.4)	NA	NA
IIIC	7 (30.4)	NA	NA
IVA	1 (4.4)	NA	NA
IVB	7 (30.4)	NA	NA
Histological type of OC **			
Low-grade serous carcinoma	1 (4.4)	NA	NA
High-grade serous carcinoma	15 (65.1)	NA	NA
Endometrioid carcinoma	2 (8.7)	NA	NA
Clear cell carcinoma	1 (4.4)	NA	NA
Mucinous carcinoma	1 (4.4)	NA	NA
Serous endometrioid carcinoma	3 (13)	NA	NA

* median with interquartile range; ** number of cases with percentage. The stage of OC is presented according to FIGO [32].

3.2. Immune-Related Protein Expression Is Downregulated in the PBMCs of OC Patients and Further Dysregulated Postoperatively

We conducted a comprehensive analysis of the expression of cytokines and immune-regulating proteins in PBMCs, focusing primarily on the mRNA level. As shown in Figure 2, significant decreases in the expression of almost all of the studied proteins were observed in the OC group prior to surgery compared to healthy controls, with the most pronounced being IL-6. The expression of IL-10 was not significantly different.

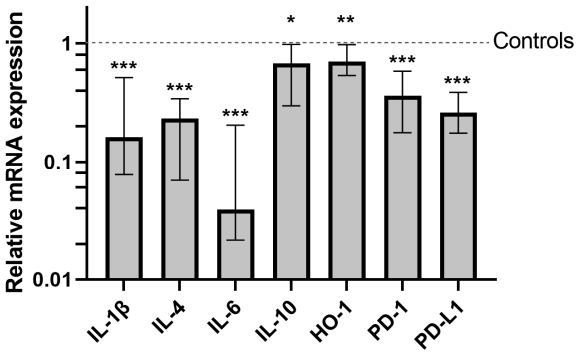


Figure 2. The mRNA expression of interleukins, HO-1, PD-1, and PD-L1 was significantly downregulated in the PBMCs of OC patients before surgery, with the exception of IL-10. The histogram shows the relative expression of mRNA compared to healthy controls, with the values normalised to one. * $p > 0.05$; ** $p = 0.02$; *** $p \leq 0.001$.

The OC group also showed changes in the interleukin expression in PBMCs during the early post-surgical period (Figure 3). Changes in interleukin mRNA expression were particularly pronounced on days 1 and 3 after surgery, mainly showing downregulation. Further to its unaltered expression in OC patients compared to healthy controls, IL-10 was the only interleukin that showed increased mRNA expression on the first day after surgical treatment. However, the expression of all interleukins in PBMCs showed a tendency to return to their pre-surgical state by day 5.

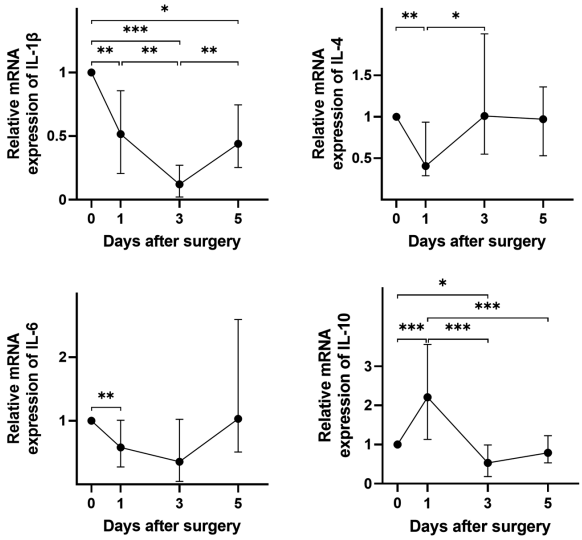


Figure 3. Surgical treatment alters the expression of interleukin mRNAs in the PBMCs of OC patients. The relative expression levels of different interleukin mRNAs are shown at days 1, 3, and 5 post-surgery compared to the pre-surgical baseline. All *p*-values comparing the different days were >0.05 unless otherwise specified. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

The changes in mRNA expression of other immune-related proteins in PBMCs varied. The decreased expression of PD-L1 was most pronounced on day 1 post-surgery. The expression of HO-1 and PD-1 decreased consistently throughout the observation period, reaching their lowest level on day 5 post-surgery. However, the decrease was only significant for HO-1 (Figure 4A). In parallel, the protein level of HO-1 in PBMCs peaked on day 1 after surgical treatment, followed by a return to the presurgical state (Figure 4B). All original immunoblots are included in Supplementary File S1.

3.3. *Surgical Treatment of OC Patients Affects PBMC Activity in the Early Post-Operative Period*

No significant differences were detected in the metabolic and functional activities of PBMCs between OC patients before surgery and healthy participants. Nevertheless, a slight decline in PBMC activity could be seen in most of the observations (Figure 5). A significant decrease in metabolic activity was observed after surgery, yet this change was not evident in activated PBMCs. Additionally, trends for increased activity on post-operative day 5 were observed. A marked shift in the functional activity was also observed, especially on day 1 post-surgery. This was characterised by a significant increase in relative ROS production and a significant reduction in the relative phagocytic activity. However, by day 5 post-surgery, these changes had reverted to preoperative levels.

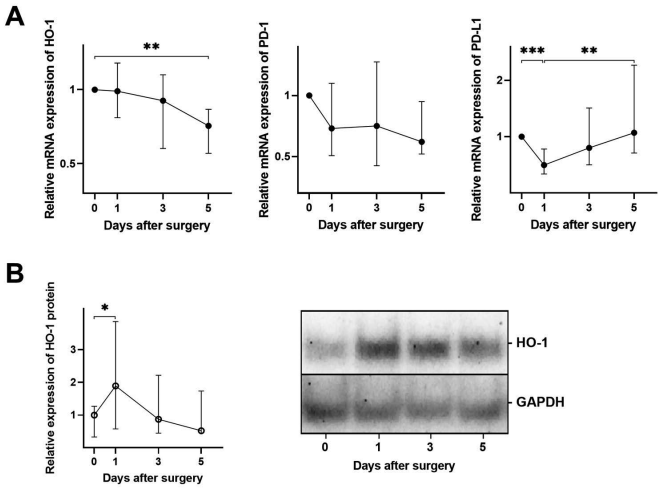


Figure 4. Surgical treatment causes dysregulation of HO1, PD-1, and PD-L1 expression in PBMCs from OC patients. (**A**) Relative expression of HO-1, PD-1, and PD-L1 mRNAs at days 1, 3, and 5 after surgical treatment compared to the pre-surgical baseline. (**B**) Changes in the HO-1 protein level in PBMCs (left), as determined by densitometric scanning of immunoblots and normalised to GAPDH (representative immunoblot on the right). All *p*-values comparing samples from different days were >0.05 unless otherwise specified. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

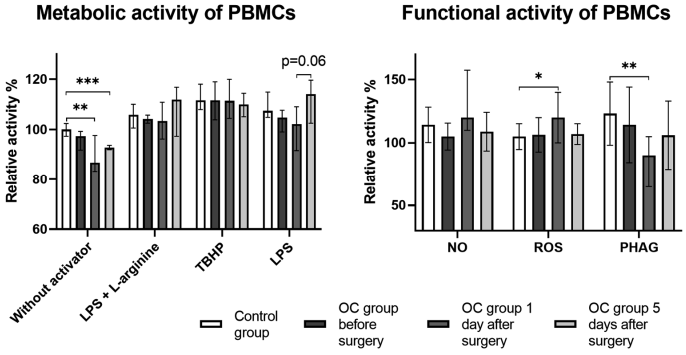


Figure 5. PBMC activity in healthy controls and OC patients before and after surgical treatment. Functional activity (**right panel**) encompassed the evaluation of nitric oxide (NO) and reactive oxygen species (ROS) production, alongside measuring phagocytic activity (PHAG). Metabolic activity (**left panel**) represents PBMCs viability assessed via the resazurin metabolism assay (AlamarBlue) without an activator, and subsequently repeated with the same activators used in the functional activity assays (LPS, lipopolysaccharide; L-arginine; TBHP, tert-butyl hydroperoxide). The relative activity shown was computed by comparing fluorometric or spectrophotometric assay results between observations with and without activators. Resazurin metabolism without activators was assessed using a fluorometric assay and then normalised to the control group. All *p*-values between groups are >0.05 unless stated otherwise. * $p = 0.02$; ** $p \leq 0.01$; *** $p = 0.001$.

3.4. Surgical Treatment Alters the Serum Concentrations of Immune-Related Proteins in OC Patients

Finally, we evaluated the concentrations of immune-related proteins in the serum of participants. No significant differences in interleukin concentrations were observed between the controls and OC patients, even after the surgical treatment of OC patients (Figure 6A). The only exception was IL-6, which showed a marked increase on the first day after surgery, but then returned to baseline levels at day 5 post-surgery. Additionally, OC patients showed a trend for lower serum levels of the free PD-1 receptor compared to controls (Figure 6B). The HO-1 concentration was comparable between OC patients and controls. However, a consistent increase in the HO-1 concentration was observed during the post-operative period in OC patients, reaching a peak (possibly not final) on day 5 (Figure 6B).

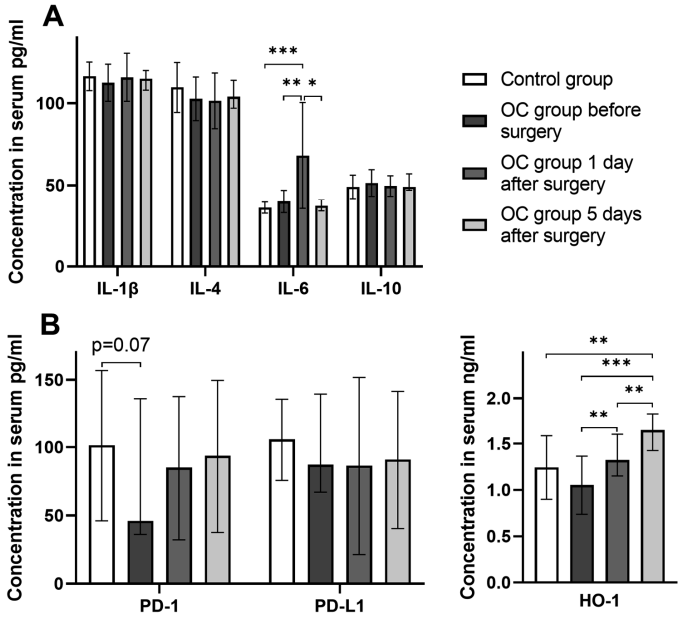


Figure 6. Changes in serum concentrations of interleukins, HO-1, PD-1, and PD-L1 in pre- and post-operative OC patients. (**A**) Serum levels of interleukins evaluated using Luminex assay. (**B**) Serum levels of PD-1, PD-L1, and HO-1 evaluated by the ELISA method. All *p*-values between groups are >0.05 unless stated otherwise. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

4. Discussion

4.1. Suppression of PBMCs in OC Patients Compared to Healthy Controls

OC is not immunogenic in origin. However, the immune response is a crucial factor in OC progression and exerts a significant local impact [6,18], as well as affecting the systemic immune response in OC patients [18,49]. PBMCs are a source of anticancer immunity [6,16] and are recognised for their significance in cancer formation and personalised treatment [17,28]. The current study provides compelling evidence for a distortion in the immune status of OC patients in the early post-operative period. Notably, our findings indicate that the mRNA expression of interleukins and various immune-related proteins is downregulated in the PBMCs of OC patients before surgery. This phenomenon was

consistent across the investigated cytokines and other immune-related proteins, irrespective of their immunosuppressive or immunostimulatory nature [27]. Although our results on differences in the metabolic and functional activity of PBMCs between OC and control groups did not reach statistical significance, previous research has shown reduced PBMC activity in OC patients [50–52]. In conjunction with results from the literature, our findings suggest that PBMCs are suppressed in OC patients.

The current study also investigated the serum levels of serum interleukins and immune-related proteins in OC patients. Although these can originate from various sources, including PBMCs [27], serum levels provide a more comprehensive view of the overall state of the body's immune system. The concentration of serum cytokines in OC patients before surgery was not significantly different to that of the controls. However, a trend for lower IL-1β and IL-4 levels was observed, together with higher IL-6 and IL-10 levels. Some of these findings concur with the findings of other investigators [19,20,53], although the results from different studies vary. This may be explained by the fact that many factors influence cytokine concentrations, including the stage and histology of OC [54].

4.2. Post-Operative Changes in Interleukin Levels in the Serum and PBMCs of OC Patients

In a novel approach, we investigated PBMCs in the context of surgical trauma in OC. To comprehensively analyse the immune response in our study, we selected two proinflammatory cytokines (IL-1β and IL-6) and two anti-inflammatory cytokines (IL-4 and IL-10). These all play pivotal roles in OC progression [20,27,55–57]. Despite their known stimulatory or suppressive functions in the immune response, the properties of these cytokines may impact tumour progression. Notably, there is more compelling evidence for a pro-tumourigenic role of IL-6 [55,58,59] compared to IL-1β, IL-4, and IL-10, for which conflicting data exist [6,57,60]. Our results indicate that during the early post-operative period, the mRNA expression of IL-1β, IL-4, and IL-6 in PBMCs was significantly downregulated, while that of IL-10 was upregulated. Aside from IL-6, we observed consistent serum interleukin levels throughout the study period. Moreover, no significant correlations were found between serum interleukin levels and their mRNA expression level in PBMCs. Given the current ambiguity concerning the effect of these interleukins on cancer progression, it is difficult to establish a direct link between the observed changes in interleukin levels and their potential effects on OC. It is perhaps more prudent to speculate that PBMCs undergo some degree of overall suppression during the early post-operative period. However, we did observe a distinct increase in the serum IL-6 level during the early post-operative phase. This was expected, given the patients had undergone major surgery [61,62]. According to the available data, this surge in the IL-6 concentration creates favourable conditions for cancer progression [55,58,59].

4.3. Post-Operative Changes in PBMC and Serum Levels of HO-1, PD-1, and PD-L1 in OC Patients

Many mechanisms are known to be involved in tumour progression and in the development of chemoresistance [63]. PD-1/PD-L1 is a well-known immune checkpoint that participates in the mechanism that allows tumours to evade the immune system [29]. The expression of PD-1/PD-L1 on tumour and immune cells has been shown to have prognostic significance [28,64]. Furthermore, researchers are actively exploring PD-1/PD-L1 as a target for immune checkpoint blockade in the treatment of OC. Despite some promising results, these treatment methods have so far shown only limited efficacy [65,66]. This limitation can be attributed to a poor understanding of the mechanisms of these therapeutic agents, as well as to challenges in selecting responsive patients [29,65]. Efforts are underway to collect more data and overcome these obstacles [67], but additional fundamental knowledge regarding this immune checkpoint is still needed, especially for post-operative patients. In the current study, we observed a post-operative decrease in PD-L1 mRNA expression and a trend for increased PD-1 serum concentration after the low preoperative levels compared

to the controls. The former may indicate that PBMCs shift towards increased immune tolerance during the early post-operative stage.

The heat shock protein HO-1 is an intracellular enzyme with antioxidant, anti-apoptotic, and cytoprotective properties. HO-1 has emerged as a critical factor associated with tumour progression in OC and other cancer types. It has been identified as a promoter of cancer cell proliferation, invasion, and migration, potentially fostering metastasis, although its precise mechanism of action remains uncertain [26,31]. HO-1 expression has been associated with cancer aggressiveness and poor clinical outcomes, making it a potential prognostic marker for OC patients [68]. Our findings suggest that during the early post-operative phase, OC patients may experience additional favourable conditions for tumour progression due to elevated post-operative serum levels of HO-1. This is despite a concurrent decrease in HO-1 mRNA expression within PBMCs.

4.4. Surgery Reduces the Activity of PBMCs in OC Patients

The current findings did not demonstrate a statistically significant reduction in the cytotoxic activity of immune cells in pre-operative OC patients. Nevertheless, previous studies support the notion of immune cell dysfunction in OC patients [51,52]. A particularly noteworthy result from the current work was the significant decline in PBMC phagocytic activity on day 1 after surgery in OC patients. Moreover, there was a notable decrease in baseline PBMC viability post-surgery, potentially impacting the results of PBMC function assays. However, following PBMC activation, metabolic activity rebounded. This suggests an independent decrease in PBMC functional activity post-surgery, despite the compromised viability. Increased ROS production was also observed during the same period. Although increased ROS production suggests heightened PBMC activity [69], high ROS levels can potentially promote OC progression [70,71], making this change less advantageous. A trend for the reversal of the day 1 post-surgical decrease in phagocytic activity was observed on day 5, indicating that the major changes in PBMC activity are likely to occur during the initial post-operative phase. It has also been reported that following optimal cytoreduction, peripheral immune cell activity in OC patients continues to normalise, eventually equalling that of healthy patients [72].

While there are additional data demonstrating immune suppression in OC patients post-surgery [73,74], it is essential to acknowledge that the observed changes might not be specific to the selected population. The existing data indicate that immune suppression following surgery extends beyond ovarian cancer, affecting various cancer types and even non-cancer populations [15,75]. Moreover, the extent of surgery and the type of anaesthesia employed significantly impact these outcomes [15,74]. Considering the extensive surgical interventions required for OC patients, investigating the post-surgery immune response becomes even more imperative and relevant.

4.5. Final Considerations Regarding the Post-Operative Immune Response in OC Patients

Given the significance of the immune response in OC progression [6], fluctuations in immune response dynamics could be critical for the disease course. Radical surgical treatment of OC results in positive impacts on the subsequent immune response [25] and greatly improves patient prognosis [4]. However, it may also have negative effects on metastasis formation [11]. Direct evidence linking the distortion of post-operative immune responses to metastasis formation in OC patients is lacking. By extrapolating results from other cancer types with regard to post-operative metastasis formation [9,11,12], as well as the evidence for immune suppression after surgery in OC patients [23–25], the current findings lead us to speculate that post-operative metastasis formation in OC patients could be a real phenomenon.

Our data revealed a significant suppression of the PBMC function in OC patients during the initial post-operative days, as well as dysregulated expression of immune-related proteins. We consider this imbalance in PBMC activity as a potential vulnerability that could facilitate the progression of OC. Therefore, further investigation of the post-operative

immune response is crucial, with a specific focus on PBMCs. Given the challenges in OC treatment, such as chemoresistance [63] and frequent disease relapse [6], we propose that the early post-operative period presents a valuable opportunity to enhance concurrent treatment strategies. These could include systemic intraoperative chemotherapy or hyperthermic intraperitoneal chemotherapy [7,76]. The establishment of patient selection criteria based on PBMC activity in the early post-operative phase could also play a pivotal role in developing new treatments and refining existing treatment modalities for OC patients.

5. Conclusions

In conclusion, this study has provided further insights into early post-operative immune changes in OC patients, revealing significant dysregulation in PBMC activity and immune-related protein expression. Surgical treatment exacerbates the pre-operative suppression of PBMC activity and causes changes in the mRNA expression levels of IL-1β, IL-4, IL-6, IL-10, HO-1, PD-1, and PD-L1 in PBMCs. While the interpretation of this dysregulation can be complex, notable fluctuations in serum IL-6 and HO-1 levels, together with suppressed phagocytosis by PBMCs, indicate a shift towards immune tolerance post-surgery. Although not all findings reached statistical significance, the overall trend indicates compromised PBMC activity in OC patients during the early post-operative period, with the most significant changes seen on the first day after surgery. These findings emphasise the need to assess the impact of surgery on the immune response during the early post-operative phase, as this may create a vulnerable window for OC to progress. Further refinement of patient selection strategies based on the post-operative activity of PBMCs could optimise treatment approaches and improve the long-term prognosis of OC patients.

6. Study Limitations

While this study provides valuable insights, it has some limitations. The relatively small sample size may limit the generalisability of the findings. However, given the novelty and scale of experiments conducted on post-surgical OC patients, sample size calculations were challenging. Additionally, although we evaluated changes in PBMC activity and immune-related protein expression, our study did not assess their impact on disease progression and clinical outcomes. Moreover, we did not include a control group consisting of individuals with a benign gynaecological pathology to examine the specific impact of OC to our results. Considering the extensive nature of surgery for OC patients, selecting suitable patients for an adequate control group is challenging.

7. Practical Recommendations

Future research with larger cohorts is necessary to validate the current findings and gain a better understanding of the clinical implications of immune dysregulation in OC patients.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cancers16010190/s1. File S1: Western blot images.

Author Contributions: Conceptualisation, S.P., Z.D. and A.G.; Methodology, J.U., Z.D., A.G., A.J. and S.P.; Formal Analysis, J.U. and A.J.; Investigation, J.U., S.P., A.B. and A.J.; Data curation, J.U., A.B., D.U. and A.J.; Writing—Original Draft Preparation, J.U.; Writing—Review and Editing, S.P., Z.D., A.G. and A.J.; Visualisation, J.U. and A.J.; Supervision, S.P. and Z.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Kaunas Regional Biomedical Research Ethics Committee was obtained (No. BE-2-16; date of approval 5 February 2020).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to acknowledge the support of Laboratory of Surgical Gastroenterology (Institute for Digestive Research, Medical Academy, Lithuanian University of Health Sciences) for providing laboratory facilities for this research.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Torre, L.A.; Trabert, B.; DeSantis, C.E.; Miller, K.D.; Samimi, G.; Runowicz, C.D.; Gaudet, M.M.; Jemal, A.; Siegel, R.L. Ovarian cancer statistics, 2018. *CA Cancer J. Clin.* **2018**, *68*, 284–296. [CrossRef] [PubMed]
2. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
3. Vergote, I.; Denys, H.; De Greve, J.; Gennigens, C.; Van De Vijver, K.; Kerger, J.; Vuylsteke, P.; Baurain, J.F. Treatment algorithm in patients with ovarian cancer. *Facts Views Vis. Obgyn.* **2020**, *12*, 227–239. [PubMed]
4. Colombo, N.; Sessa, C.; Bois, A.d.; Ledermann, J.; McCluggage, W.G.; McNeish, I.; Morice, P.; Pignata, S.; Ray-Coquard, I.; Vergote, I.; et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: Pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Int. J. Gynecol. Cancer* **2019**, *29*, 728–760. [CrossRef] [PubMed]
5. Bryant, A.; Johnson, E.; Grayling, M.; Hiu, S.; Elattar, A.; Gajjar, K.; Craig, D.; Vale, L.; Naik, R. Residual Disease Threshold After Primary Surgical Treatment for Advanced Epithelial Ovarian Cancer, Part 1: A Systematic Review and Network Meta-Analysis. *Am. J. Ther.* **2022**, *30*, e36–e55. [CrossRef] [PubMed]
6. Yang, Y.; Yang, Y.; Yang, J.; Zhao, X.; Wei, X. Tumor Microenvironment in Ovarian Cancer: Function and Therapeutic Strategy. *Front. Cell. Dev. Biol.* **2020**, *8*, 758. [CrossRef] [PubMed]
7. Huffman, O.G.; Chau, D.B.; Dinicu, A.I.; DeBernardo, R.; Reizes, O. Mechanistic Insights on Hyperthermic Intraperitoneal Chemotherapy in Ovarian Cancer. *Cancers* **2023**, *15*, 1402. [CrossRef]
8. Akter, S.; Rahman, M.A.; Hasan, M.N.; Akhter, H.; Noor, P.; Islam, R.; Shin, Y.; Rahman, M.H.; Gazi, M.S.; Huda, M.N.; et al. Recent Advances in Ovarian Cancer: Therapeutic Strategies, Potential Biomarkers, and Technological Improvements. *Cells* **2022**, *11*, 650. [CrossRef]
9. Tang, F.; Tie, Y.; Tu, C.; Wei, X. Surgical trauma-induced immunosuppression in cancer: Recent advances and the potential therapies. *Clin. Transl. Med.* **2020**, *10*, 199–223. [CrossRef]
10. Amodeo, G.; Bugada, D.; Franchi, S.; Moschetti, G.; Grimaldi, S.; Panerai, A.; Allegri, M.; Sacerdote, P. Immune function after major surgical interventions: The effect of postoperative pain treatment. *J. Pain Res.* **2018**, *11*, 1297–1305. [CrossRef]
11. Alieva, M.; van Rheenen, J.; Broekman, M.L.D. Potential impact of invasive surgical procedures on primary tumor growth and metastasis. *Clin. Exp. Metastasis* **2018**, *35*, 319–331. [CrossRef] [PubMed]
12. Tohme, S.; Simmons, R.L.; Tsung, A. Surgery for Cancer: A Trigger for Metastases. *Cancer Res.* **2017**, *77*, 1548–1552. [CrossRef] [PubMed]
13. Ramirez, M.F.; Ai, D.; Bauer, M.; Vauthey, J.; Gottumukkala, V.; Kee, S.; Shon, D.; Truty, M.; Kuerer, H.M.; Kurz, A.; et al. Innate immune function after breast, lung, and colorectal cancer surgery. *J. Surg. Res.* **2015**, *194*, 185–193. [CrossRef] [PubMed]
14. Angka, L.; Martel, A.B.; Kilgour, M.; Jeong, A.; Sadiq, M.; de Souza, C.T.; Baker, L.; Kennedy, M.A.; Kekre, N.; Auer, R.C. Natural Killer Cell IFNγ Secretion is Profoundly Suppressed Following Colorectal Cancer Surgery. *Ann. Surg. Oncol.* **2018**, *25*, 3747–3754. [CrossRef]
15. Ng, C.S.H.; Lau, K.K.W. Surgical trauma and immune functional changes following major lung resection. *Indian J. Surg.* **2015**, *77*, 49–54. [CrossRef]
16. Kleiveland, C.R. *Peripheral Blood Mononuclear Cells*; Springer: Cham, Switzerland, 2015; Chapter 15.
17. Zhang, L.; Hou, L.; Wu, J.; Li, C.; Hu, T.; Zhu, C.; Wu, C.; Chen, C. Peripheral blood mononuclear cells (PBMCs), an ideal liquid biopsy approach to evaluate systematic immunity and predict response of neoadjuvant chemo-immunotherapy in resectable NSCLC. *J. Clin. Oncol.* **2022**, *40*, e20618. [CrossRef]
18. Salas-Benito, D.; Vercher, E.; Conde, E.; Glez-Vaz, J.; Tamayo, I.; Hervas-Stubbs, S. Inflammation and immunity in ovarian cancer. *EJC Suppl.* **2020**, *15*, 56–66. [CrossRef]
19. Micheli, D.C.; Jammal, M.P.; Martins-Filho, A.; Côrtes, J.R.X.d.M.; Souza, C.N.d.; Nomelini, R.S.; Murta, E.F.C.; Tavares-Murta, B.M. Serum cytokines and CXCR2: Potential tumour markers in ovarian neoplasms. *Biomarkers* **2020**, *25*, 474–482. [CrossRef]
20. Kampan, N.C.; Madondo, M.T.; Reynolds, J.; Hallo, J.; McNally, O.M.; Jobling, T.W.; Stephens, A.N.; Quinn, M.A.; Plebanski, M. Pre-operative sera interleukin-6 in the diagnosis of high-grade serous ovarian cancer. *Sci. Rep.* **2020**, *10*, 2213. [CrossRef]
21. Nowak, M.; Klink, M.; Glowacka, E.; Sulowska, Z.; Kulig, A.; Szpakowski, M.; Szyllo, K.; Tchorzewski, H. Production of cytokines during interaction of peripheral blood mononuclear cells with autologous ovarian cancer cells or benign ovarian tumour cells. *Scand. J. Immunol.* **2010**, *71*, 91–98. [CrossRef]

22. Zhang, X.; Xu, J.; Ke, X.; Zhang, S.; Huang, P.; Xu, T.; Huang, L.; Lou, J.; Shi, X.; Sun, R.; et al. Expression and function of Toll-like receptors in peripheral blood mononuclear cells from patients with ovarian cancer. *Cancer Immunol. Immunother.* **2015**, *64*, 275–286. [CrossRef] [PubMed]
23. Drakes, M.L.; Stiff, P.J. Ovarian Cancer: Therapeutic Strategies to Overcome Immune Suppression. *Adv. Exp. Med. Biol.* **2021**, *1330*, 33–54. [CrossRef] [PubMed]
24. De Bruyn, C.; Ceusters, J.; Landolfo, C.; Baert, T.; Thirion, G.; Claes, S.; Vankerckhoven, A.; Wouters, R.; Schols, D.; Timmerman, D.; et al. Neo-Adjuvant Chemotherapy Reduces, and Surgery Increases Immunosuppression in First-Line Treatment for Ovarian Cancer. *Cancers* **2021**, *13*, 5899. [CrossRef] [PubMed]
25. Napoletano, C.; Bellati, F.; Landi, R.; Pauselli, S.; Marchetti, C.; Visconti, V.; Sale, P.; Liberati, M.; Rughetti, A.; Frati, L.; et al. Ovarian cancer cytoreduction induces changes in T cell population subsets reducing immunosuppression. *J. Cell. Mol. Med.* **2010**, *14*, 2748–2759. [CrossRef]
26. Luu Hoang, K.N.; Anstee, J.E.; Arnold, J.N. The Diverse Roles of Heme Oxygenase-1 in Tumor Progression. *Front. Immunol.* **2021**, *12*, 658315. [CrossRef]
27. Mielczarek-Palacz, A.; Sikora, J.; Kondera-Anasz, Z.; Mickiewicz, P.; Mickiewicz, A. Effect of Th1/Th2 cytokine administration on proinflammatory SKOV-3 cell activation. *Arch. Med. Sci.* **2016**, *12*, 1337–1347. [CrossRef] [PubMed]
28. Habel, A.; Weili, X.; Hadj Ahmed, M.; Stayoussef, M.; Bouaziz, H.; Ayadi, M.; Mezlini, A.; Larbi, A.; Yaacoubi-Loueslati, B. Immune checkpoints as potential theragnostic biomarkers for epithelial ovarian cancer. *Int. J. Biol. Markers* **2023**, *38*, 203–213. [CrossRef] [PubMed]
29. Dumitru, A.; Dobrica, E.; Croitoru, A.; Cretoiu, S.M.; Gaspar, B.S. Focus on PD-1/PD-L1 as a Therapeutic Target in Ovarian Cancer. *Int. J. Mol. Sci.* **2022**, *23*, 12067. [CrossRef]
30. Gordon, S.R.; Maute, R.L.; Dulken, B.W.; Hutter, G.; George, B.M.; McCracken, M.N.; Gupta, R.; Tsai, J.M.; Sinha, R.; Corey, D.; et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* **2017**, *545*, 495–499. [CrossRef]
31. Lu, J.; Abudukeyoumu, A.; Zhang, X.; Liu, L.; Li, M.; Xie, F. Heme oxygenase 1: A novel oncogene in multiple gynecological cancers. *Int. J. Biol. Sci.* **2021**, *17*, 2252–2261. [CrossRef]
32. Berek, J.S.; Renz, M.; Kehoe, S.; Kumar, L.; Friedlander, M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. *Int. J. Gynaecol. Obstet.* **2021**, *155* (Suppl. 1), 61–85. [CrossRef]
33. Isolation of Mononuclear Cells. Available online: https://cdn.cytivalifesciences.com/api/public/content/digi-16156-pdf (accessed on 26 September 2023).
34. RNA Extraction Kit (abx098089). Available online: https://www.abbexa.com/documents/manual/abx098089_ifu.pdf (accessed on 7 October 2023).
35. AlamarBlue®Assay Protocol. Available online: https://tools.thermofisher.com/content/sfs/manuals/PI-DAL1025-1100_TI%20alamarBlue%20Rev%201.1.pdf (accessed on 27 September 2023).
36. Mantovani, A.; Sica, A.; Sozzani, S.; Allavena, P.; Vecchi, A.; Locati, M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **2004**, *25*, 677–686. [CrossRef] [PubMed]
37. pHrodoTM Red, Deep Red, and Green BioParticlesTM Conjugates for Phagocytosis. Available online: https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp35361.pdf (accessed on 28 September 2023).
38. Nathan, C.; Cunningham-Bussel, A. Beyond oxidative stress: An immunologist's guide to reactive oxygen species. *Nat. Rev. Immunol.* **2013**, *13*, 349–361. [CrossRef] [PubMed]
39. DCFDA/H2DCFDA—Cellular ROS Assay Kit (ab113851). Available online: https://www.abcam.com/products/assay-kits/dcfda--h2dcfda-cellular-ros-assay-kit-ab113851.html (accessed on 28 September 2023).
40. Bogdan, C. Nitric oxide synthase in innate and adaptive immunity: An update. *Trends Immunol.* **2015**, *36*, 161–178. [CrossRef] [PubMed]
41. Bronte, V.; Zanovello, P. Regulation of immune responses by L-arginine metabolism. *Nat. Rev. Immunol.* **2005**, *5*, 641–654. [CrossRef] [PubMed]
42. Qiu, Y.; Yang, X.; Wang, L.; Gao, K.; Jiang, Z. L-Arginine Inhibited Inflammatory Response and Oxidative Stress Induced by Lipopolysaccharide via Arginase-1 Signaling in IPEC-J2 Cells. *Int. J. Mol. Sci.* **2019**, *20*, 1800. [CrossRef] [PubMed]
43. Griess Reagent Kit for Nitrite Determination (G-7921). Available online: https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp07921.pdf (accessed on 28 September 2023).
44. Human Heme Oxygenase 1 (HO-1) CatchPoint SimpleStep ELISA Kit (ab229429). Available online: https://www.abcam.com/ps/products/229/ab229429/documents/Human-Heme-Oxygenase-1-ELISA-Kit-protocol-book-v4-ab229429%20(website).pdf (accessed on 29 September 2023).
45. Human PD-L1 SimpleStep ELISA Kit (ab277712). Available online: https://www.abcam.com/ps/products/277/ab277712/documents/Human-PD-L1-ELISA-Kit-[28-8]-protocol-book-v2-ab277712%20(website).pdf (accessed on 29 September 2023).
46. Human PD-1 SimpleStep ELISA Kit (ab252360). Available online: https://www.abcam.com/ps/products/252/ab252360/documents/Human-PD-1-ELISA-Kit-protocol-book-v2-ab252360%20(website).pdf (accessed on 29 September 2023).

47. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
48. Reid, B.M.; Permuth, J.B.; Sellers, T.A. Epidemiology of ovarian cancer: A review. *Cancer Biol. Med.* **2017**, *14*, 9–32. [CrossRef]
49. Nelson, B.H. The impact of T-cell immunity on ovarian cancer outcomes. *Immunol. Rev.* **2008**, *222*, 101–116. [CrossRef]
50. Freedman, R.S.; Ma, Q.; Wang, E.; Gallardo, S.T.; Gordon, I.O.; Shin, J.W.; Jin, P.; Stroncek, D.; Marincola, F.M. Migration deficit in monocyte-macrophages in human ovarian cancer. *Cancer Immunol. Immunother.* **2008**, *57*, 635–645. [CrossRef]
51. Ingersoll, S.B.; Stoltzfus, G.P.; Merchant, M.H.; Ahmad, S.; Edwards, C.R.; Ahmed, A.; Oyer, J.L.; Finkler, N.J.; Holloway, R.W.; Edwards, J.R. Comparison of the cytotoxic response against ovarian cancer by immune effector cells isolated and expanded from normal donors and ovarian cancer patients. *Cytotherapy* **2012**, *14*, 716–723. [CrossRef]
52. Zaborowski, M.P.; Stefens-Stawna, P.; Osztynowicz, K.; Piorunek, T.; Batura-Gabryel, H.; Dyzmann-Sroka, A.; Kozubski, W.; Nowak-Markwitz, E.; Michalak, S. Granzyme B in peripheral blood mononuclear cells as a measure of cell-mediated immune response in paraneoplastic neurological syndromes and malignancy. *Cancer Immunol. Immunother.* **2021**, *70*, 1277–1289. [CrossRef] [PubMed]
53. Kampan, N.C.; Kartikasari, A.E.R.; Deceneux, C.; Madondo, M.T.; McNally, O.M.; Flanagan, K.L.; Aziz, N.A.; Stephens, A.N.; Reynolds, J.; Quinn, M.A.; et al. Combining TNFR2-Expressing Tregs and IL-6 as Superior Diagnostic Biomarkers for High-Grade Serous Ovarian Cancer Masses. *Cancers* **2023**, *15*, 667. [CrossRef] [PubMed]
54. Yabuno, A.; Matsushita, H.; Hamano, T.; Tan, T.Z.; Shintani, D.; Fujieda, N.; Tan, D.S.P.; Huang, R.Y.; Fujiwara, K.; Kakimi, K.; et al. Identification of serum cytokine clusters associated with outcomes in ovarian clear cell carcinoma. *Sci. Rep.* **2020**, *10*, 18503. [CrossRef] [PubMed]
55. Browning, L.; Patel, M.R.; Horvath, E.B.; Tawara, K.; Jorcyk, C.L. IL-6 and ovarian cancer: Inflammatory cytokines in promotion of metastasis. *Cancer Manag. Res.* **2018**, *10*, 6685–6693. [CrossRef]
56. Kartikasari, A.E.R.; Huertas, C.S.; Mitchell, A.; Plebanski, M. Tumor-Induced Inflammatory Cytokines and the Emerging Diagnostic Devices for Cancer Detection and Prognosis. *Front. Oncol.* **2021**, *11*, 692142. [CrossRef]
57. Li, Z.; Chen, L.; Qin, Z. Paradoxical Roles of IL-4 in Tumor Immunity. *Cell. Mol. Immunol.* **2009**, *6*, 415–422. [CrossRef]
58. Souza, J.C.; Ribeiro, R.S.; Pimenta, T.M.; Martins, B.S.; Rangel, L.B.A. The role of pro-inflammatory components, carcinoma-associated fibroblasts, and tumor-associated macrophages in ovarian cancer progression and metastasis. *J. Cancer Metastasis Treat.* **2023**, *9*, 3. [CrossRef]
59. Lan, T.; Chen, L.; Wei, X. Inflammatory Cytokines in Cancer: Comprehensive Understanding and Clinical Progress in Gene Therapy. *Cells* **2021**, *10*, 100. [CrossRef]
60. Wilke, C.M.; Wei, S.; Wang, L.; Kryczek, I.; Kao, J.; Zou, W. Dual biological effects of the cytokines interleukin-10 and interferon-γ. *Cancer Immunol. Immunother.* **2011**, *60*, 1529–1541. [CrossRef]
61. Indra, B.; Lipoeto, N.; Hon Tjong, D.; Rahman, S. Alteration of Interleukin-4, Interleukin-6 Levels, and Post-operative Pain Intensity. *Open Access Maced. J. Med. Sci.* **2023**, *11*, 1–7. [CrossRef]
62. Hsu, T.; Lin, C.; Sun, F.; Chen, M. Postoperative Serum Levels of Interleukin-6 are Affected by Age in Patients with Colorectal Cancer. *Int. J. Gerontol.* **2017**, *11*, 75–79. [CrossRef]
63. Yeldag, G.; Rice, A.; Del Río Hernández, A. Chemoresistance and the Self-Maintaining Tumor Microenvironment. *Cancers* **2018**, *10*, 471. [CrossRef] [PubMed]
64. Wang, L. Prognostic effect of programmed death-ligand 1 (PD-L1) in ovarian cancer: A systematic review, meta-analysis and bioinformatics study. *J. Ovarian Res.* **2019**, *12*, 37. [CrossRef] [PubMed]
65. Zhu, J.; Yan, L.; Wang, Q. Efficacy of PD-1/PD-L1 inhibitors in ovarian cancer: A single-arm meta-analysis. *J. Ovarian Res.* **2021**, *14*, 112. [CrossRef]
66. Świderska, J.; Kozłowski, M.; Kwiatkowski, S.; Cymbaluk-Płoska, A. Immunotherapy of Ovarian Cancer with Particular Emphasis on the PD-1/PDL-1 as Target Points. *Cancers* **2021**, *13*, 6063. [CrossRef] [PubMed]
67. Bacot, S.M.; Harper, T.A.; Matthews, R.L.; Fennell, C.J.; Akue, A.; KuKuruga, M.A.; Lee, S.; Wang, T.; Feldman, G.M. Exploring the Potential Use of a PBMC-Based Functional Assay to Identify Predictive Biomarkers for Anti-PD-1 Immunotherapy. *Int. J. Mol. Sci.* **2020**, *21*, 9023. [CrossRef]
68. Zhao, Z.; Xu, Y.; Lu, J.; Xue, J.; Liu, P. High expression of HO-1 predicts poor prognosis of ovarian cancer patients and promotes proliferation and aggressiveness of ovarian cancer cells. *Clin. Transl. Oncol.* **2018**, *20*, 491–499. [CrossRef]
69. Piktel, E.; Ościłowska, I.; Suprewicz, Ł.; Depciuch, J.; Marcińczyk, N.; Chabielska, E.; Wolak, P.; Wollny, T.; Janion, M.; Parlinska-Wojtan, M.; et al. ROS-Mediated Apoptosis and Autophagy in Ovarian Cancer Cells Treated with Peanut-Shaped Gold Nanoparticles. *Int. J. Nanomed.* **2021**, *16*, 1993–2011. [CrossRef]
70. Snezhkina, A.V.; Kudryavtseva, A.V.; Kardymon, O.L.; Savvateeva, M.V.; Melnikova, N.V.; Krasnov, G.S.; Dmitriev, A.A. ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 6175804. [CrossRef]
71. Ding, D.; Xie, L.; Shen, Y.; Li, J.; Guo, Y.; Fu, Y.; Liu, F.; Han, F. Insights into the Role of Oxidative Stress in Ovarian Cancer. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 8388258. [CrossRef] [PubMed]
72. Kovács, A.R.; Pál, L.; Szűcs, S.; Lukács, L.; Póka, R.; Lampé, R. Phagocytic function of monocytes and neutrophil granulocytes in ovarian cancer. *Orvosi Hetil.* **2018**, *159*, 1353–1359. [CrossRef] [PubMed]

73. Wu, M.; Chen, X.; Lou, J.; Zhang, S.; Zhang, X.; Huang, L.; Sun, R.; Huang, P.; Pan, S.; Wang, F. Changes in regulatory T cells in patients with ovarian cancer undergoing surgery: Preliminary results. *Int. Immunopharmacol.* **2017**, *47*, 244–250. [CrossRef] [PubMed]
74. Dong, H.; Zhang, Y.; Xi, H. The effects of epidural anaesthesia and analgesia on natural killer cell cytotoxicity and cytokine response in patients with epithelial ovarian cancer undergoing radical resection. *J. Int. Med. Res.* **2012**, *40*, 1822–1829. [CrossRef] [PubMed]
75. Hogan, B.V.; Peter, M.B.; Shenoy, H.G.; Horgan, K.; Hughes, T.A. Surgery induced immunosuppression. *Surgeon* **2011**, *9*, 38–43. [CrossRef]
76. Lim, M.C.; Chang, S.; Park, B.; Yoo, H.J.; Yoo, C.W.; Nam, B.H.; Park, S. Survival After Hyperthermic Intraperitoneal Chemotherapy and Primary or Interval Cytoreductive Surgery in Ovarian Cancer: A Randomized Clinical Trial. *JAMA Surg.* **2022**, *157*, 374–383. [CrossRef]


International Journal of
Molecular Sciences

MDPI

Article

Preoperative Immune Cell Dysregulation Accompanies Ovarian Cancer Patients into the Postoperative Period

Jonas Ulevicius [1,*], Aldona Jasukaitiene [1], Arenida Bartkeviciene [1], Zilvinas Dambrauskas [1], Antanas Gulbinas [1], Daiva Urboniene [2] and Saulius Paskauskas [3,*]

[1] Laboratory of Surgical Gastroenterology, Institute for Digestive Research, Medical Academy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania; aldona.jasukaitiene@lsmu.lt (A.J.); arenida.bartkeviciene@lsmu.lt (A.B.); zilvinas.dambrauskas@lsmu.lt (Z.D.); antanas.gulbinas@lsmu.lt (A.G.)
[2] Department of Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania; daiva.urboniene@lsmu.lt
[3] Department of Obstetrics and Gynecology, Medical Academy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania
* Correspondence: jonas.ulevicius@lsmu.lt (J.U.); saulius.paskauskas@lsmu.lt (S.P.); Tel.: +370-61587731 (J.U.)

Abstract: Ovarian cancer (OC) poses a significant global health challenge with high mortality rates, emphasizing the need for improved treatment strategies. The immune system's role in OC progression and treatment response is increasingly recognized, particularly regarding peripheral blood mononuclear cells (PBMCs) and cytokine production. This study aimed to investigate PBMC subpopulations (T and B lymphocytes, natural killer cells, monocytes) and cytokine production, specifically interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), and tumor necrosis factor alpha (TNFα), in monocytes of OC patients both preoperatively and during the early postoperative period. Thirteen OC patients and 23 controls were enrolled. Preoperatively, OC patients exhibited changes in PBMC subpopulations, including decreased cytotoxic T cells, increased M2 monocytes, and the disbalance of monocyte cytokine production. These alterations persisted after surgery with subtle additional changes observed in PBMC subpopulations and cytokine expression in monocytes. Considering the pivotal role of these altered cells and cytokines in OC progression, our findings suggest that OC patients experience an enhanced pro-tumorigenic environment, which persists into the early postoperative period. These findings highlight the impact of surgery on the complex interaction between the immune system and OC progression. Further investigation is needed to clarify the underlying mechanisms during this early postoperative period, which may hold potential for interventions aimed at improving OC management.

Keywords: ovarian cancer; surgery; peripheral blood mononuclear cells (PBMC); interleukin-1 beta (IL-1β); interleukin-4 (IL-4); interleukin-6 (IL-6); interleukin-10 (IL-10); interleukin-12 (IL-12); tumor necrosis factor alpha (TNFα)

Citation: Ulevicius, J.; Jasukaitiene, A.; Bartkeviciene, A.; Dambrauskas, Z.; Gulbinas, A.; Urboniene, D.; Paskauskas, S. Preoperative Immune Cell Dysregulation Accompanies Ovarian Cancer Patients into the Postoperative Period. *Int. J. Mol. Sci.* **2024**, *25*, 7087. https://doi.org/10.3390/ijms25137087

Academic Editors: Kuo-Hu Chen and Giovanni Tossetta

Received: 21 May 2024
Revised: 18 June 2024
Accepted: 26 June 2024
Published: 28 June 2024

1. Introduction

Ovarian cancer (OC) is a significant health concern worldwide, with high morbidity and mortality rates. It is recognized as the most fatal gynecologic malignancy, accounting for more than two thirds of mortality within this category [1]. This is largely attributed to the prevalence of advanced-stage ovarian cancer diagnoses in most patients [1,2]. Current treatment modalities, primarily comprising surgery and chemotherapy, notably enhance rates of progression-free survival (PFS) and overall survival (OS), although the long-term prognosis remains unsatisfactory [3]. Researchers continually strive to develop improved treatment strategies, yet the optimal approach for ovarian cancer treatment remains elusive [4,5]. There is a growing recognition of the importance of the immune system in

ovarian cancer progression and treatment response with accumulating evidence suggesting that alterations in immune cell populations and cytokine production play crucial roles in the tumor microenvironment and its progression [6–9].

Peripheral blood mononuclear cells (PBMCs) are a heterogeneous population of immune cells comprising lymphocytes, monocytes, and dendritic cells. PBMCs are pivotal in modulating inflammation and regulating immune responses against tumors [10–12]. In OC patients, alterations in PBMC subpopulations and cytokine production have been observed, reflecting the intricate interplay between the immune system and tumor development [12,13]. PBMCs exhibit multifaceted immune responses against cancer through cytotoxicity, cytokine secretion, antigen presentation, and regulatory functions [11]. Functionally diverse mature T lymphocytes (CD3+ T cells) are mainly categorized into CD3+/CD4+ T lymphocytes (CD4+ T cells) and CD3+/CD8+ T lymphocytes (CD8+ T cells), each contributing significantly to antitumor immunity. CD4+ T cells, also known as T helper lymphocytes, modulate immune responses through cytokine secretion, influencing inflammation and tumor growth as well as assisting in the activation of other immune cells [14,15]. Conversely, CD8+ T cells, termed cytotoxic T lymphocytes, eliminate infected or malignant cells via cytokine release and cytolytic molecules [15,16]. B lymphocytes (CD19+ B cells) regulate humoral immunity, influence CD4+ T cell function, and may impact tumor progression, although their specific effect on ovarian cancer remains incompletely understood [17,18]. Innate immune cells, such as CD3−/CD16 + 56+ lymphocytes, also referred to as natural killer (NK) cells, primarily exhibit anticancer activity but constitute a minor fraction of the tumor microenvironment [12,19,20]. Conversely, macrophages, derived from CD14+ cells (monocytes), comprise a substantial portion of the tumor microenvironment and wield significant influence over tumor progression [11,12,21]. The impact of monocytes varies depending on subtype, with differentiation into M1 macrophages possessing tumor-inhibiting properties, while M2 macrophages are characterized by tumor-promoting effects [22]. While these immune cells have defined roles, it is essential to recognize that all PBMCs can be modulated by various factors, potentially altering their effect on cancer progression [11,12]. Furthermore, considering the immune system's involvement in OC progression, various ratios involving PBMCs and other immune cells, such as the neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR), CD4+ T cells to CD8+ T cells (CD4/CD8) ratio, and M1 to M2 monocytes (M1/M2) ratio, are currently under investigation for their prognostic utility in OC patients [23–25].

For a more comprehensive understanding of the immune response in OC patients undergoing surgical treatment, it is essential to explore cytokines and their response to surgery. Several crucial cytokines, including but not limited to interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), and tumor necrosis factor alpha (TNFα), are pivotal for understanding the complexities of the immune system's response to cancer [11,26,27]. These cytokines play critical roles in regulating inflammatory reactions, primarily with IL-1β, IL-6, and TNFα being involved. Additionally, they modulate the function and differentiation of immune cells through their immune suppressive effects (IL-4 and IL-10) and immune stimulation effects (IL-12) [11,26].

Cytoreductive surgery is a cornerstone of OC treatment, which is often performed together with chemotherapy. The removal of tumor tissue has long-term benefits by decreasing disease progression, yet short-term tissue damage might counteract these gains. While radical surgery is recognized as an independent positive prognostic factor [28], the subsequent tissue trauma has a significant impact on the immune response through multiple mechanisms, leading to immune dysfunction [29,30], and potentially promoting metastatic progression [31,32]. Despite being substantiated in various cancer types, this phenomenon has not been validated in OC [33,34]. Furthermore, extensive research has investigated alterations in PBMC subpopulations and cell ratios in OC patients, aiming at patient stratification and prognosis assessment [13,23,25,35,36]. However, data regarding surgery's impact on PBMC subpopulations in OC patients are limited [37–39] with scant information available on postoperative cytokine production in PBMCs [40].

We postulated that during the initial postoperative period, OC patients may encounter dysregulation in their peripheral PBMC subpopulations. Our aim was to examine PBMC subpopulations and cytokine production within monocytes following surgery, focusing on the early postoperative period. These results may highlight immune dysregulation during early postoperative recovery in OC patients, contributing to the improvement of OC management strategies.

2. Results

2.1. Participant Characteristics

The median age of participants in the study group, 58 (49–67) years, was similar to that of the controls. Body mass index (BMI) did not differ significantly between OC patients and controls. The histologic types observed in OC patients reflected those seen in the general population [41]. Further clinicopathologic characteristics of both controls and OC patients are outlined in Table 1.

Table 1. Clinicopathological characteristics of the studied participants.

Characteristic	OC Group (n = 13)	Control Group (n = 23)	p-Value
Age (years), median (IQR)	58 (49–67)	59 (49–61)	0.75
Body mass index (kg/m^2), median (IQR)	22.9 (22.1–28.8)	23.7 (21.9–28.7)	0.85
Stage of OC, n (%)			
IIIA	3 (23.1)	NA	NA
IIIB	3 (23.1)	NA	NA
IIIC	4 (30.7)	NA	NA
IVB	3 (23.1)	NA	NA
Histologic type of OC, n (%)			
Low-grade serous carcinoma	1 (7.7)	NA	NA
High-grade serous carcinoma	9 (69.2)	NA	NA
Mucinous carcinoma	1 (7.7)	NA	NA
Serous endometrioid carcinoma	2 (15.4)	NA	NA

OC, ovarian cancer; IQR, interquartile range; n, number of cases; NA, not applicable. The stages of OC are presented according to the 2021 FIGO (The International Federation of Gynecology and Obstetrics) staging system [42].

2.2. Alterations in Peripheral Blood Cell Counts, Ratios, and Monocyte Cytokine Expression in Ovarian Cancer Patients

Complete blood count (CBC) analysis was conducted for both controls and OC patients prior to surgery. Our findings indicated a tendency for OC patients to have higher white blood cell (WBC) counts compared to controls, which was primarily attributable to the heightened neutrophil levels in OC patients. Furthermore, OC patients exhibited elevated platelet (Plt) counts alongside a trend suggesting diminished red blood cell (RBC) counts. Analysis of hemoglobin (Hb) concentration indicated that OC patients showed lower levels, measuring 124 (115–130) g/L, compared to controls, which exhibited levels of 135 (126–140) g/L (p = 0.01). Detailed data from the CBC analysis are presented in Table 2.

After PBMC isolation, samples underwent analysis to identify alterations in PBMC subpopulations in OC patients. Preoperatively, OC patients exhibited elevated proportions of CD19+ B cells and decreased levels of CD3+ T cells and CD8+ T cell subsets (Figure 1A). Monocyte subpopulation analysis revealed equal proportions of M1 monocytes between controls and OC patients preoperatively. However, M2 monocytes exhibited approximately 2.5 times higher proportions in OC patients preoperatively, while non-classified monocytes demonstrated lower proportions (Figure 1B).

Table 2. Comparison of preoperative peripheral blood cell counts between the control group and OC patients.

Cell Type	OC Group (n = 13)	Control Group (n = 23)	p-Value
WBCs	6.8 (4.9–7.9)	5.6 (4.4–6.4)	0.09
Neutrophils	4.5 (3.3–5.3)	3.1 (2.3–3.5)	0.01
Eosinophils	0.1 (0.04–0.2)	0.1 (0.1–0.1)	0.36
Basophils	0.03 (0.03–0.06)	0.04 (0.02–0.04)	0.8
Lymphocytes	1.3 (1.1–1.8)	1.9 (1.3–2.3)	0.13
Monocytes	0.5 (0.2–1)	0.4 (0.4–0.5)	0.72
RBCs	4.2 (4–4.4)	4.5 (4.4–4.7)	0.06
Plts	372 (322–400)	267.5 (234–289)	<0.001

WBCs, white blood cells; RBCs, red blood cells; Plts, platelets. RBC counts are expressed in units of $\times 10^{12}$/L; other cell counts are expressed in units of $\times 10^{9}$/L. Cell counts are displayed as median values with interquartile ranges.

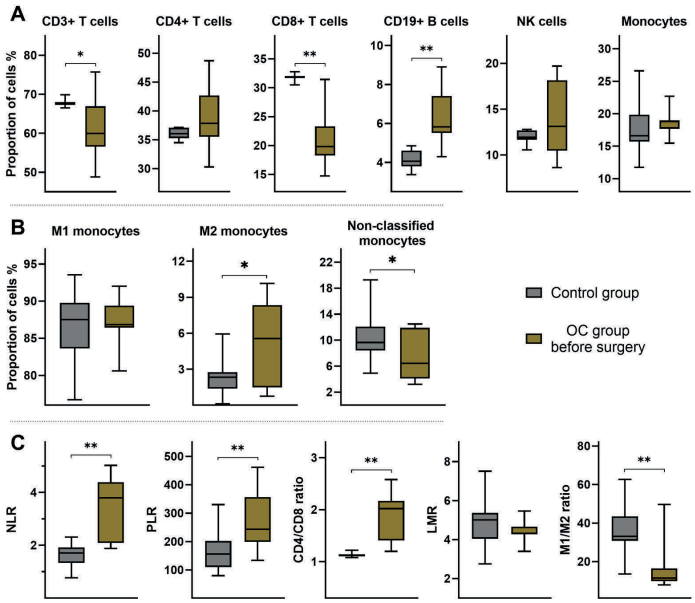


Figure 1. Changes in peripheral blood mononuclear cell (PBMC) subpopulations and ratios in preoperative OC patients. (**A**) The upper graphs illustrate the median proportions of T lymphocytes (CD3+ T cells), T helper lymphocytes (CD4+ T cells), cytotoxic T lymphocytes (CD8+ T cells), B lymphocytes (CD19+ B cells), natural killer cells (NK cells), and monocytes within all PBMCs. (**B**) The middle graphs illustrate the median proportions of monocyte subsets within all monocytes, including M1 (CD45+/CD80+/CD86+), M2 (CD45+/CD163+/CD206+), and non-classified monocytes (monocytes without a distinct phenotype). (**C**) The lower graphs depict median values of the neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), CD4+ T cells to CD8+ T cells ratio (CD4/CD8 ratio), lymphocyte to monocyte ratio (LMR), and the M1 to M2 monocytes ratio (M1/M2 ratio). They represent data from the control group and OC patients before surgery. The boxes in the graphs represent the IQR, with the median indicated by a line inside the box. Error bars extend from the box to the minimum and maximum values of the dataset. All p-values between groups are >0.05 unless stated otherwise. * $p < 0.05$; ** $p \leq 0.001$.

Further analysis of CBC results revealed higher NLR and PLR in the OC group before surgery. Additionally, the analysis of PBMCs ratios showed a significantly higher CD4/CD8 ratio and markedly decreased M1/M2 ratio in OC patients preoperatively. However, the LMR between the investigated groups remained unchanged (Figure 1C). To enhance understanding of the interrelationships among the investigated ratios and all peripheral blood cells, we conducted a detailed correlation analysis. The results of the correlation analysis in preoperative OC patients are illustrated in Figure 2, and those of the control group are presented in Figure S1.

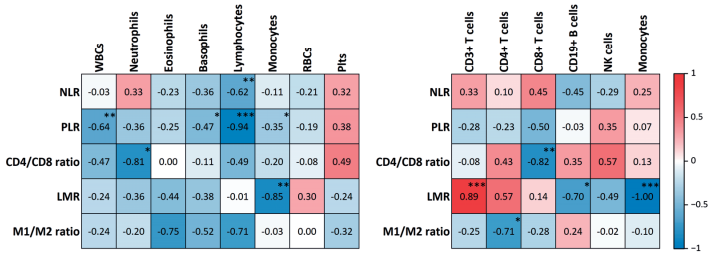


Figure 2. Correlation between peripheral blood cell ratios and peripheral blood cells in preoperative OC patients. The left graph presents the correlation between the ratios and quantitative values of blood cell counts. The right graph illustrates the correlation between the ratios and proportional values of PBMCs among all PBMCs. The listed values indicate Spearman's correlation coefficient (rs). Colors in the graphs range from blue to red, indicating rs from −1 to 1, respectively. All *p*-values of correlations are >0.05 unless otherwise stated. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Cytokine expression analysis in the two main subtypes of monocytes revealed that the majority of the analyzed cytokine expressions were comparable between the control group and OC patients with several notable differences observed in M2 monocytes. Specifically, TNFα expression exhibited a tendency to be less expressed, and IL-10 expression was significantly lower in OC patients. Conversely, IL-6 expression in M2 monocytes was higher in OC patients before surgery (Figure 3).

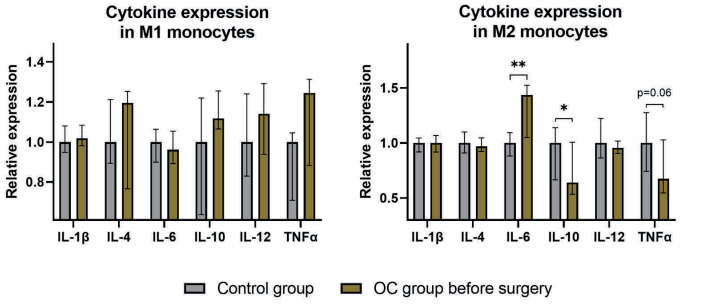


Figure 3. Alterations in cytokine expression in monocytes among preoperative OC patients. The graphs depict the fold change in cytokine expression, including interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), and tumor necrosis factor alpha (TNFα), in M1 monocytes (left graph) and M2 monocytes (right graph). Columns represent median fluorescence intensity (MFI) values in OC patients before surgery, normalized to the control group, with error bars indicating the IQR. All *p*-values between groups are >0.05 unless stated otherwise. * $p < 0.05$; ** $p \leq 0.001$.

2.3. Alterations in Peripheral Blood Cell Counts, Ratios, and Monocyte Cytokine Expression in Ovarian Cancer Patients Postoperatively

The analysis of postoperative changes in PBMC subpopulations in OC patients revealed a spectrum of fluctuations, although the majority of these alterations did not reach statistical significance compared to the preoperative baseline (Figure 4A,B). However, significant differences emerged when postoperative OC patients were compared to controls. One day following surgery, there was a statistically significant increase in CD4+ T cell counts, with values of 36.1 (35.3–37.1)% vs. 40.9 (37.7–46.8)%, respectively ($p < 0.001$). Additionally, the proportion of CD19+ B cells reached its peak 1 day postoperatively, with values of 4.1 (3.8–4.6)% vs. 6.7 (5.7–7.9)%, respectively ($p < 0.001$). Similarly, M2 monocytes exhibited a more than threefold rise in proportion compared to controls with values of 2.3 (1.4–2.8)% vs. 7.5 (6.7–7.8)%, respectively ($p < 0.001$). Conversely, analysis of non-classified monocytes showed opposing results, with levels further decreased 1 day postoperatively at 4.4 (4.1–4.8)% compared to control levels of 9.6 (8.4–12.1)% ($p < 0.001$). The analysis of postoperative PBMC ratios did not reveal significant alterations in OC patients compared to their preoperative state (Figure 4C).

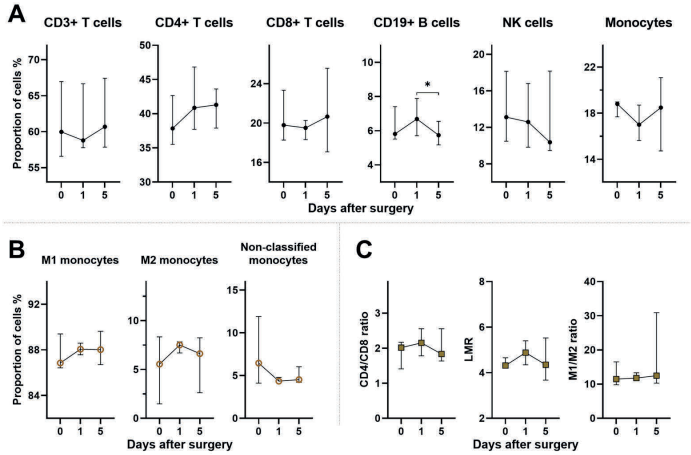


Figure 4. Changes in PBMC subpopulations and ratios in pre- and postoperative OC patients. (**A**) The upper graphs illustrate the median proportions of CD3+ T cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, NK cells, and monocytes within all PBMCs. (**B**) The lower left graphs illustrate the median proportions of monocyte subsets within all monocytes, including M1, M2, and non-classified monocytes. (**C**) The lower right graphs depict median values of the CD4/CD8 ratio, LMR, and the M1/M2 ratio. The data represent OC patients before surgery (0) and on postoperative days 1 and 5. Graphs show median values with error bars indicating the IQR. All *p*-values between groups are >0.05 unless stated otherwise. * $p < 0.05$.

The majority of analyzed cytokine expressions exhibited comparability before and after surgery in OC patients (Figure 5). However, notable differences were observed in comparison to cytokine expression in controls. Specifically, TNFα expression in M1 monocytes reached its peak 1 day postoperatively, being 1.24 times higher compared to controls, with median fluorescence intensity (MFI) values of 634 (449–663) vs. 789 (633–845), respectively ($p = 0.02$). Additionally, following a trend of decreased TNFα expression in M2 monocytes preoperatively, its expression equilibrated to control levels 1 day postoperatively (MFI values 535 (398–683) vs. 487 (298–621), respectively, $p = 0.64$). Similar fluctuations were observed in IL-10 expression in M2 monocytes, with its expression equalizing to controls

on day 5 postoperatively (MFI values 640 (426–729) vs. 645 (363–701), respectively, $p = 0.53$). The most pronounced fluctuations were noted in IL-6 expression in M2 monocytes, which peaked on day 1 postoperatively, being 1.5 times higher in OC patients compared to controls (MFI values 2602 (2296–2850) vs. 3894 (3196–4028), respectively, $p < 0.001$). Furthermore, it exhibited a tendency to decline toward control levels on day 5 postoperatively.

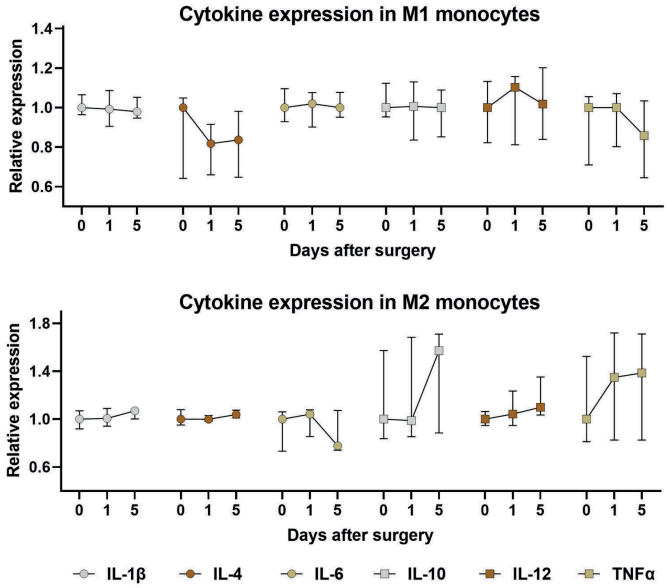


Figure 5. Alterations in cytokine expression in monocytes among pre- and postoperative OC patients. The graphs depict the fold change in cytokine expression (IL-1β, IL-4, IL-6, IL-10, IL-12, TNFα) in M1 monocytes (upper graph) and M2 monocytes (lower graph). Columns represent median MFI values in OC patients on days 1 and 5 after surgery, normalized to OC patients preoperatively (0), with error bars indicating the IQR. All *p*-values between groups are >0.05.

3. Discussion

3.1. Peripheral Blood Cell Counts, Ratios and Monocyte Cytokine Expression Are Altered in Preoperative OC Patients

OC significantly affects the tumor microenvironment, thereby impacting all cells within the tumor tissue, including immune cells [11,12]. Furthermore, the influence of OC extends beyond the tumor microenvironment to include various peripheral blood constituents, such as RBCs, WBCs, and Plts [43,44]. In our study, we observed higher concentrations of neutrophils and markedly elevated Plt counts in preoperative OC patients. These observed changes, when considering the inclusion and exclusion criteria, are likely attributable to the malignancy rather than infectious processes. Interestingly, Plts have gained significant attention in cancer progression investigations. Notably, higher Plt counts have been associated with a worse prognosis [45]. Conversely, elevated Plt counts have been linked to increased cancer incidences, including OC [46]. Studies on tumor-educated platelets (TEPs) also demonstrate promising results, suggesting their potential as a novel OC biomarker [47]. Subsequent investigation into PBMC subpopulation changes in preoperative OC patients revealed significant alterations, particularly in the proportions of CD8+ T cells and CD19+ B cells. Although CD19+ B cells are recognized for their regu-

latory role in tumors, with data supporting their prognostic value [13], their impact on anticancer immunity remains diverse and not fully understood [17,18]. In contrast, CD8+ T cells clearly contribute to anticancer immunity with their role in eliminating cancer cells established [11,48]. Therefore, a decreased proportion of CD8+ T cells in our study suggests impaired anticancer immunity. In the context of CD3+ T cells, which demonstrated an 8% decrease in OC patients, interpretation can be challenging. Firstly, existing literature predominantly emphasizes the significance of OC progression in relation to tumor-infiltrating CD3+ T cells [48,49]. Nevertheless, peripheral lymphocytes have also been shown to correlate with disease progression, with lower counts of peripheral lymphocytes being disadvantageous [50,51], independent of tumor-infiltrating lymphocytes [51]. Furthermore, the CD3+ T cell population is highly heterogeneous, with its significance lying in subsequent subsets. In addition to the aforementioned CD8+ T cells, other smaller subsets, such as tumor-promoting T regulatory lymphocytes and T helper 2 (Th2) lymphocytes, as well as tumor-inhibiting T helper 1 (Th1) lymphocytes, have a clearer impact on anticancer immunity [11,12]. Table 3 presents a brief overview of the findings within our study along with their potential implications on OC progression.

Table 3. Effects of PBMCs and cytokines on OC progression and main findings in preoperative OC patients.

Immune System Component	Effect on OC Progression	Current Study Findings in Preoperative OC Patients
CD3+ T cells	Complex	↓
CD4+ T cells	Complex	↔
CD8+ T cells	Tumor-inhibiting	↓
CD19+ B cells	Complex	↑
NK cells	Tumor-inhibiting	↔
M1 monocytes	Tumor-inhibiting	↔
M2 monocytes	Tumor-promoting	↑
IL-1β	Tumor-promoting	↔
IL-4	Complex	↔
IL-6	Tumor-promoting	↑
IL-10	Complex	↓
IL-12	Tumor-inhibiting	↔
TNFα	Complex	Tendecy of ↓

↑ increased level compared to controls; ↔ equal level compared to controls; ↓ decreased level compared to controls.

While not all PBMCs are universally recognized to correlate with disease prognosis, certain cell ratios may serve this purpose more effectively [23,24]. Upon the evaluation of lymphocyte-related ratios, we observed increased NLR, PLR, and CD4/CD8 ratios. While these were not primary outcomes, their alignment with the existing literature validates our findings [13,23]. Such alterations are under investigation for their prognostic value in disease progression or treatment success. Specifically, a high NLR and PLR have been demonstrated to correlate to poor PFS, OS, worse surgical outcomes, or poor response to chemotherapy [13,52,53]. Although less investigated, the CD4/CD8 ratio has attracted attention, with evidence suggesting that patients with a lower CD4/CD8 ratio, and therefore a higher proportion of CD8+ T cells, demonstrate better clinical outcomes [24,54]. Notably, we did not observe any significant changes in LMR, contrary to reports indicating its decrease in OC, which correlates with poor clinical outcomes [35,55]. Furthermore, a detailed correlation analysis was conducted between the investigated ratios, absolute counts of peripheral blood cells, and proportional values of PBMCs in OC patients and the control group. While these specific data are not included in the primary focus of this manuscript, they offer valuable insights that may contribute to further research into the immune response mechanisms observed in OC patients.

The monocyte–macrophage cell lineage plays a pivotal role in the tumor microenvironment, representing key components of the innate immune response that are influenced

by the adaptive immune system [11,12]. In cancer tissue, monocytes differentiate into tumor-associated macrophages (TAMs) [21,56], predominantly sourced from peripheral blood monocytes [57]. TAMs exhibit two distinct phenotypes, M1 and M2, determined by their cytokine environment, which in turn influences their impact on tumor progression. M1 macrophages are proinflammatory and suppress tumor growth, while M2 macrophages are anti-inflammatory and promote tumor growth [21,22]. Our findings show that OC patients exhibit a higher proportion of M2 monocytes before surgery along with a significantly lower M1/M2 monocyte ratio compared to controls. These results imply a state of immune suppression in OC patients, considering the characteristics of M2 monocytes. Our findings align with previous research indicating decreased PBMCs activity in OC patients, although there has been less focus on peripheral monocytes specifically [58–60]. Importantly, the majority of studies examining the role of the M1/M2 macrophages ratio in OC management have focused on macrophages within cancer tissue, with data showing that a high M1/M2 macrophage ratio is related to better OS and PFS rates [25,61,62]. The examination of the M1/M2 monocyte ratio in peripheral blood underscores the novelty of our study, highlighting the potential utility of peripheral monocyte ratios in future research.

It is important to note that different histological types of OC exhibit distinct immune profiles in both tumor tissue and peripheral blood [11,12,25]. While the distribution of histological types of OC patients in our study was comparable to that of the general population [41], the majority of cases were high-grade serous carcinoma. Therefore, our findings may be specific to this histology. When comparing the subgroups of high-grade serous carcinoma with other OC histological types in our study, we did not find any significant differences. Given the small sample size, these findings are most likely underpowered.

We further directed our analysis toward monocyte cytokine production to assess their activity in OC patients, as the monocyte/macrophage cell line is the most predominant line in OC tissue [22,23]. Cytokines play a pivotal role in immune cell communication, facilitating our understanding of immune system functions, albeit their effects can be complex to interpret due to their multifaceted nature [11,26]. Among the cytokines we investigated, IL-6 and IL-1β emerged as the most pro-tumorogenic [63–65]. Other cytokines, regardless of their proinflammatory (TNFα) or anti-inflammatory effects (IL-4, IL-10), may have diverse effects on promoting tumor growth [11,26,65–69]. In contrast, IL-12 acts as a key regulator of the immune response against tumors, promoting M1 macrophage polarization, the Th1 lymphocytes response, the activation of CD8+ T cells and NK cells, along with other mechanisms inhibiting tumor growth and progression [11,26,65]. Regarding our investigated cytokines, we did not find any statistically significant changes in IL-1β, IL-4, and IL-12 expression in OC patients' monocytes. However, it is noteworthy that IL-4 is predominantly expressed by basophils, while IL-12 is mostly secreted by B lymphocytes and, to a lesser extent, by monocytes [70]. IL-10, known for its diverse actions but primarily recognized for its pro-tumorogenic potential [11,67], exhibited significant downregulation in M2 monocytes preoperatively. Additionally, TNFα displayed a tendency toward decreased expression in OC patients preoperatively. Despite TNFα's acknowledged antitumor properties [66], current evidence supports its indirect involvement in pro-tumorogenic pathways [69,71,72]. Therefore, alterations in IL-10 and TNFα expression levels indicate a potentially less pro-tumorogenic environment. Furthermore, a noteworthy overexpression of IL-6 was observed in M2 monocytes of OC patients preoperatively. Considering IL-6's established role in tumor progression [63–65], these variations suggest an intensified pro-tumorogenic environment in OC patients preoperatively. Summing up the cytokine expression data, it becomes evident that OC patients experience a disbalance in cytokine production. However, given the complex roles of the analyzed cytokines in inflammation and tumor progression, the final interpretation of our data remains ambiguous.

These findings from preoperative OC patients further highlight the systemic nature of OC, indicating that alterations in the immune system extend beyond the tumor microenvironment to involve the peripheral immune system. Despite the complexity in interpreting

cytokine expression changes, the observed shifts in PBMCs in OC patients provide insights into the immunosuppressive environment in OC.

3.2. Early Postoperative Period Is Related to Additional Subtle Alterations in Peripheral Blood Cell Counts, Ratios and Monocyte Cytokine Expression in OC Patients

Surgery has been demonstrated to exert a significant impact on the immune system, with evidence suggesting that surgical interventions can impair immune function, potentially creating a window for the development of metastases [29,31,32]. Considering the significance of surgery in cancer patients, we further aimed to clarify the alterations occurring in PBMC subpopulations during the early postoperative period. Despite some insignificant fluctuations, our results suggest that surgery did not induce a subsequent decrease in CD3+ T cell and CD8+ T cell levels. Other authors similarly have not observed significant changes in CD3+ T and CD8+ T cell populations postoperatively [37,39]. Compared to controls, CD4+ T cells showed a significant increase 1 day postoperatively with CD19+ B cells demonstrating the most pronounced elevation. However, owing to the heterogeneous nature of CD4+ T cells [14,15] and inconclusive evidence regarding the impact of CD19+ B cells on cancer progression [17,18], definitive conclusions cannot be drawn from these findings alone. Nevertheless, other authors have reported beneficial shifts in smaller subsets of CD4+ T cell, particularly T regulatory cells, as early as day 2 after surgery, which persisted and became more pronounced 1 month after surgery [38,39]. It is noteworthy that another study reported opposite results 7 days postoperatively [37]. The postoperative analysis of lymphocyte-related ratios revealed minimal alterations, highlighting the potential utility of these ratios for prognosis in postoperative patients. This complements the preoperative use of LMR [35,55] and, to a lesser extent, the CD4/CD8 ratio [24,54].

Postoperative analysis of monocyte subsets indicated that M2 monocytes were most pronounced on the 1st day after surgery, although the M1/M2 ratio remained unchanged. However, investigations into peripheral blood monocytes following surgery are currently lacking. While not directly comparable to our findings, other studies have shown altered monocyte function after surgery with impaired peripheral monocyte function observed before surgery and persisting up to the seventh day after surgery [73,74]. Additionally, another study demonstrated the suppressed function of all PBMCs in the early postoperative period [40]. Although our study did not directly analyze monocyte activity, considering the immunosuppressive characteristics of M2 monocytes/macrophages [22] and the role of TAMs, particularly M2, in the metastatic progression of OC [21], our findings could have significant implications. Another intriguing discovery was the similarity in levels of M1 monocytes between controls and pre- and postoperative OC patients. This suggests that the change in M2 monocyte levels occurred at the expense of unclassified monocytes, whose dynamics in OC patients showed opposite trends compared to M2 monocytes. These findings support the plastic polarization theory, wherein existing subtypes may not be final and can change in response to environmental stimuli [21,75]. Furthermore, there is a growing interest in the role of monocytes in OC treatment [56]. In animal models of diverse cancer types, it has been demonstrated that the repolarization of monocytes from an M2 to M1 phenotype via immunotherapy can extend survival [76]. Consistent with the existing literature, our findings confirm that subsets of peripheral monocytes are indeed a subject for further investigation in OC patients during the early postoperative period.

In terms of cytokine expression in monocytes postoperatively, we observed only minor changes. Although we did not find significant alterations in cytokine expression postoperatively compared to preoperative levels, there was an increase in TNFα and IL-6 levels 1 day after surgery compared to controls. Additionally, IL-10 levels returned to levels similar to controls after being reduced before surgery. Thus, in addition to the preoperative cytokine disbalance, further subtle dysregulation in cytokine expression was noted. Given the known impact of these cytokines on OC progression [63–65,69,71,72], it

could be speculated that the observed postoperative cytokine increase indicates a stronger immune suppression.

In summary, the postoperative analysis of PBMC subpopulations and monocyte cytokine expression revealed subtle changes. No strong correlations between OC surgical treatment and these changes were evident. While some fluctuations pose challenges in interpretation, others suggest that preoperative alterations in PBMCs and monocyte cytokine expression, indicative of immune suppression, persist after surgery and are not reversed during the early postoperative period. Conversely, considering the subtle postoperative changes, they may suggest an even more intensified pro-tumorigenic environment.

4. Materials and Methods

4.1. Study Design and Patient Selection

This prospective study enrolled 13 patients diagnosed with epithelial OC who underwent surgical treatment at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos between January 2021 and April 2023. Surgical procedures included laparotomy and cytoreduction for OC patients without prior chemotherapy. The study group comprised patients with primary OC at stages III and IV according to the 2021 FIGO (International Federation of Gynecology and Obstetrics) staging system [42], excluding those with autoimmune diseases, other confirmed or suspected cancers, recent surgeries or blood transfusions within the past month. OC diagnosis was confirmed through histopathologic examination. A control group of 23 women matched in age and body mass index (BMI) with OC patients and free of cancer was included, adhering to the same exclusion criteria as the study group. Certain occurring non-malignant comorbidities, such as primary arterial hypertension, heart rhythm disorders, hypercholesterolemia, and obesity, were allowed in both the OC and control groups. Informed consent was obtained from all participants prior to enrolment, and the study was conducted in accordance with the Declaration of Helsinki principles and approved by the Kaunas Regional Biomedical Research Ethics Committee (Approval No. BE-2-16).

4.2. Blood Collection

Peripheral venous blood specimens were gathered from both OC patients and healthy controls. OC patient blood samples were acquired preoperatively and at 1 and 5 days postoperatively. Blood collection from healthy controls was a single-event procedure. A CBC analysis was conducted for both the control group and OC patients before surgery. Subsequent analyses were carried out for both groups, including blood sampling after surgery (Figure 6). Blood draws were conducted using either one 4 mL and one 10 mL vacutainer or a single 10 mL vacutainer containing K2EDTA (BD, Plymouth, UK). After blood sampling, the tubes were gently mixed to prevent clotting and promptly analyzed to minimize sample degradation.

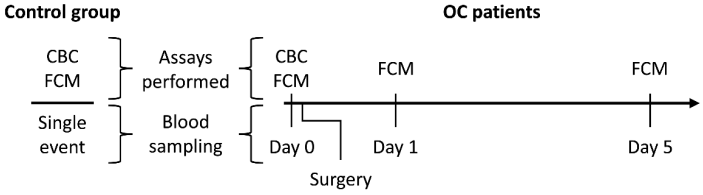


Figure 6. Blood sampling and assay schedule for study subjects. The lower section depicts blood collection events and their timing for the control group and OC patients. The upper section outlines the assays performed at each blood sampling: CBC, complete blood count; FCM, flow cytometry.

4.3. Complete Blood Count Analysis

Venous blood samples collected in 4 mL K2EDTA-containing tubes were used for CBC analysis. An automated hematology analyzer UniCellDxH 800 (Beckman Coulter, Brea, CA, USA) was employed following the manufacturer's instructions. The analyzer measured CBC parameters, including RBC count, Hb concentration, Plt count, WBC count, and differential leukocyte count.

4.4. Peripheral Blood Mononuclear Cells Isolation

Following blood collection in 10 mL K2EDTA-containing vacutainers, PBMCs were subsequently isolated using a Ficoll–Paque PREMIUM density gradient medium (Cytiva, Uppsala, Sweden) according to the manufacturer's protocol. The isolated PBMCs were then carefully collected and subjected to two washes with PBS (Sigma-Aldrich, Steinheim, Germany) at 250× *g* for 5 min each. Once isolated from both OC patients and healthy participants, the PBMCs were resuspended to a concentration of 1 million cells per mL and divided into 1 mL aliquots for further analysis.

4.5. Flow Cytometric Analysis of Lymphocyte and Monocyte Subsets

To perform flow cytometric lymphocyte immunophenotyping, we used the BD multitest 6-color TBNK kit (BD, Franklin Lakes, NJ, USA). The following subsets of lymphocytes were identified: CD3+ (T lymphocytes), CD3+/CD4+ (T helper lymphocytes), CD3+/CD8+ (cytotoxic T lymphocytes), CD19+ (B lymphocytes) and CD3−/CD16+56+ (NK cells). PBMCs aliquots containing 1 million cells each were incubated with a mixture of monoclonal antibodies (mAbs): CD3-FITC (Leu-4), CD4 PE-Cy™7 (Leu-3a), CD8 APC-Cy™7 (Leu-2a), CD16 PE (Leu-11c), CD19 APC (Leu-12), CD56 PE (Leu-19), and CD45 PerCP-Cy5.5 (2D1) according to manufacturer's protocol. Following staining, the cells underwent washing steps and were analyzed using a 10-color flow cytometer, FACSLyric (BD, San Jose, CA, USA). Data acquisition was set to record up to 10,000 events per sample. Lymphocyte populations were gated based on granularity/complexity (side scatter—SSC) and CD45 expression, with the percentage of cells expressing specific antigens within the lymphocyte gate determined.

For the discrimination of monocytes/macrophages and their subsets, PBMCs were phenotypically analyzed using a panel of mAbs, including CD3 PE (UCHT1), CD14 BV510 (MφP9), CD16 APC (B73.1), CD80 APC-H7 (L307.4), CD86 PE-Cy7 (2331 (FUN-1)), CD163 BV605 (GHI/61), CD206 FITC (19.2), HLA-DR PerCP (L243), CD19 PE (HIB19), CD56 PE (555516) and CD66b PE (G10F5).

4.6. Flow Cytometric Analysis of Monocyte Cytokine Expression

For the assessment of cytokine production within monocytes, mAbs targeting IL-1β (H1b-98) Pacific Blue, IL-4 (MP4-25D2), IL-6 (MQ2-13A5), IL-10 (JES3-19F1), IL12 (C8.6), and TNFα (MAb11) (all purchased from BD, San Jose, CA, USA) were utilized. Intracellular cytokine accumulation was facilitated using the BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit with BD GolgiStop™ protein transport inhibitor containing monensin (BD, San Jose, CA, USA). Multicolor staining for cell surface antigens and intracellular cytokines was performed according to the manufacturer's protocol provided with the kit. PMT voltage and compensation settings were adjusted using BD™ CompBeads Anti-Mouse Ig, κ/Negative Control Compensation Particles Set (BD, San Jose, CA, USA). A data acquisition goal of up to 30,000 total events per sample was set for monocyte/macrophage subsets and cytokine analysis. The M1 subset, characterized by CD45+/CD80+/CD86+ markers, and M2, identified by CD45+/CD163+/CD206+, were quantified as a percentage of cells within the lymphocyte gate. Cytokine expression levels were quantified as mean MFI values.

4.7. Statistical Analysis

Statistical analyses were conducted using SPSS (version 22.0; IBM Corp, Armonk, NY, USA) and GraphPad Prism software (version 9.5.1; GraphPad Software Inc., La Jolla, CA, USA). Given the sample size and non-normal distribution of most variables, we employed the Mann–Whitney test for independent variables and the Wilcoxon test for dependent variables comparison. Spearman's correlation coefficient (rs) was used to examine correlations between variables. Quantitative results were presented as median with interquartile range (IQR), defined as quartile 1 to quartile 3, unless otherwise specified. Relative changes of cytokines were displayed after normalization to controls or preoperative OC patients (set at 1). Statistical significance was defined as $p < 0.05$.

5. Conclusions

5.1. Summary of Findings

Our investigation examines the immune dynamics in OC patients with a particular focus on PBMCs and cytokine production in the early postoperative period. Preoperatively, OC patients showed changes in PBMC subpopulations, with a decrease in CD8+ T cells and an increase in M2 monocytes, indicating systemic immune suppression that persisted after surgery. Cytokine analysis in OC patients showed an imbalance, with higher levels of tumor-promoting cytokines, notably IL-6, both before and after surgery, and increased levels of IL-10 and TNFα postoperatively. These findings highlight the systemic impact of OC and the potential consequences of surgery, showing that an impaired immune system and a tumor-promoting environment are present before surgery and accompany OC patients into the early postoperative period. Further research into postoperative immune dynamics in OC patients is needed, as the early postoperative period may offer opportunities for developing more targeted treatment strategies to improve OC outcomes.

5.2. Study Limitations

While our study provides valuable insights, several limitations need to be acknowledged. Primarily, the relatively small sample size may limit the generalizability of our findings, potentially characterizing our investigation as a pilot study. However, the novelty of certain experiments involving postoperative OC patients made it difficult to calculate an appropriate sample size. Additionally, our inclusion criteria restricted enrolment to stage III and IV OC patients according to the 2021 FIGO classification, which further limits the comparability of findings across different disease stages, consequently impacting generalizability. Furthermore, while we assessed changes in PBMC subpopulations and cytokine production, we did not investigate their implications for disease progression and clinical outcomes. Moreover, the absence of a control group with individuals undergoing benign gynecologic surgeries limits our ability to see the specific impact of OC on our results. Given the extensive nature of OC surgery, selecting appropriate control patients is inherently challenging. Lastly, the observational design of our study does not allow us to establish cause-and-effect relationships, requiring further research to understand the underlying mechanisms of immune changes in OC patients undergoing surgical treatment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms25137087/s1.

Author Contributions: Conceptualization, S.P., Z.D. and A.G.; Methodology, J.U., Z.D., A.G., A.J. and S.P.; Formal Analysis, J.U., D.U. and A.B.; Investigation, J.U., S.P., A.B. and A.J.; Data Curation, J.U., A.B., D.U. and A.J.; Writing—Original Draft Preparation, J.U.; Writing—Review and Editing, S.P., Z.D., A.G., D.U. and A.J.; Visualization, J.U. and A.J.; Supervision, S.P. and Z.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-16; date of approval 5 February 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to acknowledge the support of Laboratory of Surgical Gastroenterology (Institute for Digestive Research, Medical Academy, Lithuanian University of Health Sciences) and The Department of Laboratory Medicine (Medical Academy, Lithuanian University of Health Sciences) for providing laboratory facilities for this research.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
2. Ali, A.T.; Al-Ani, O.; Al-Ani, F. Epidemiology and risk factors for ovarian cancer. *Prz. Menopauzalny* **2023**, *22*, 93–104. [CrossRef] [PubMed]
3. Fabbro, M.; Colombo, P.; Leaha, C.M.; Rouanet, P.; Carrère, S.; Quenet, F.; Gutowski, M.; Mourregot, A.; D'Hondt, V.; Coupier, I.; et al. Conditional Probability of Survival and Prognostic Factors in Long-Term Survivors of High-Grade Serous Ovarian Cancer. *Cancers* **2020**, *12*, 2184. [CrossRef] [PubMed]
4. Akter, S.; Rahman, M.A.; Hasan, M.N.; Akhter, H.; Noor, P.; Islam, R.; Shin, Y.; Rahman, M.D.H.; Gazi, M.S.; Huda, M.N.; et al. Recent Advances in Ovarian Cancer: Therapeutic Strategies, Potential Biomarkers, and Technological Improvements. *Cells* **2022**, *11*, 650. [CrossRef] [PubMed]
5. Drakes, M.L.; Stiff, P.J. Ovarian Cancer: Therapeutic Strategies to Overcome Immune Suppression. *Adv. Exp. Med. Biol.* **2021**, *1330*, 33–54. [CrossRef] [PubMed]
6. Habel, A.; Weili, X.; Hadj Ahmed, M.; Stayoussef, M.; Bouaziz, H.; Ayadi, M.; Mezlini, A.; Larbi, A.; Yaacoubi-Loueslati, B. Immune checkpoints as potential theragnostic biomarkers for epithelial ovarian cancer. *Int. J. Biol. Markers* **2023**, *38*, 3936155231186163. [CrossRef] [PubMed]
7. Zhang, X.; Wu, Y.; Xu, T.; Cui, M. CAR-T Cells in the Treatment of Ovarian Cancer: A Promising Cell Therapy. *Biomolecules* **2023**, *13*, 465. [CrossRef]
8. Marques, C.; Ferreira da Silva, F.; Sousa, I.; Nave, M. Chemotherapy-free treatment of recurrent advanced ovarian cancer: Myth or reality? *Int. J. Gynecol. Cancer* **2023**, *33*, 607–618. [CrossRef]
9. Zhang, J.; Wang, L. The Emerging World of TCR-T Cell Trials Against Cancer: A Systematic Review. *Technol. Cancer Res. Treat.* **2019**, *18*, 1533033819831068. [CrossRef]
10. Zhang, L.; Hou, L.; Wu, J.; Li, C.; Hu, T.; Zhu, C.; Wu, C.; Chen, C. Peripheral blood mononuclear cells (PBMCs), an ideal liquid biopsy approach to evaluate systematic immunity and predict response of neoadjuvant chemo-immunotherapy in resectable NSCLC. *J. Clin. Oncol.* **2022**, *40*, e20618. [CrossRef]
11. Yang, Y.; Yang, Y.; Yang, J.; Zhao, X.; Wei, X. Tumor Microenvironment in Ovarian Cancer: Function and Therapeutic Strategy. *Front. Cell Dev. Biol.* **2020**, *8*, 758. [CrossRef]
12. Salas-Benito, D.; Vercher, E.; Conde, E.; Glez-Vaz, J.; Tamayo, I.; Hervas-Stubbs, S. Inflammation and immunity in ovarian cancer. *EJC Suppl.* **2020**, *15*, 56–66. [CrossRef] [PubMed]
13. Ye, S.; Chen, W.; Zheng, Y.; Wu, Y.; Xiang, L.; Li, T.; Ping, B.; Zhang, X.; Yang, H. Peripheral lymphocyte populations in ovarian cancer patients and correlations with clinicopathological features. *J. Ovarian Res.* **2022**, *15*, 43. [CrossRef] [PubMed]
14. Ziegler, S.F. Division of labour by CD4(+) T helper cells. *Nat. Rev. Immunol.* **2016**, *16*, 403. [CrossRef] [PubMed]
15. Kishton, R.J.; Sukumar, M.; Restifo, N.P. Metabolic regulation of T cell longevity and function in tumor immunotherapy. *Cell Metab.* **2017**, *26*, 94–109. [CrossRef] [PubMed]
16. Zhang, N.; Bevan, M.J. CD8(+) T cells: Foot soldiers of the immune system. *Immunity* **2011**, *35*, 161–168. [CrossRef] [PubMed]
17. Quail, D.F.; Joyce, J.A. Microenvironmental regulation of tumor progression and metastasis. *Nat. Med.* **2013**, *19*, 1423–1437. [CrossRef] [PubMed]
18. Gupta, P.; Chen, C.; Chaluvally-Raghavan, P.; Pradeep, S. B Cells as an Immune-Regulatory Signature in Ovarian Cancer. *Cancers* **2019**, *11*, 894. [CrossRef] [PubMed]
19. Pandey, V.; Oyer, J.L.; Igarashi, R.Y.; Gitto, S.B.; Copik, A.J.; Altomare, D.A. Anti-ovarian tumor response of donor peripheral blood mononuclear cells is due to infiltrating cytotoxic NK cells. *Oncotarget* **2016**, *7*, 7318–7328. [CrossRef]
20. Sun, Y.; Yao, Z.; Zhao, Z.; Xiao, H.; Xia, M.; Zhu, X.; Jiang, X.; Sun, C. Natural killer cells inhibit metastasis of ovarian carcinoma cells and show therapeutic effects in a murine model of ovarian cancer. *Exp. Ther. Med.* **2018**, *16*, 1071–1078. [CrossRef]

21. Yin, M.; Shen, J.; Yu, S.; Fei, J.; Zhu, X.; Zhao, J.; Zhai, L.; Sadhukhan, A.; Zhou, J. Tumor-Associated Macrophages (TAMs): A Critical Activator in Ovarian Cancer Metastasis. *Onco. Targets Ther.* **2019**, *12*, 8687–8699. [CrossRef] [PubMed]
22. Cheng, H.; Wang, Z.; Fu, L.; Xu, T. Macrophage Polarization in the Development and Progression of Ovarian Cancers: An Overview. *Front. Oncol.* **2019**, *9*, 421. [CrossRef] [PubMed]
23. Zhang, C.; Jiang, X.; Li, Y.; Pan, X.; Gao, M.; Chen, Y.; Pang, B. Independent predictive value of blood inflammatory composite markers in ovarian cancer: Recent clinical evidence and perspective focusing on NLR and PLR. *J. Ovarian Res.* **2023**, *16*, 36. [CrossRef]
24. Preston, C.C.; Maurer, M.J.; Oberg, A.L.; Visscher, D.W.; Kalli, K.R.; Hartmann, L.C.; Goode, E.L.; Knutson, K.L. The ratios of CD8+ T cells to CD4+CD25+ FOXP3+ and FOXP3- T cells correlate with poor clinical outcome in human serous ovarian cancer. *PLoS ONE* **2013**, *8*, e80063. [CrossRef]
25. Yuan, X.; Zhang, J.; Li, D.; Mao, Y.; Mo, F.; Du, W.; Ma, X. Prognostic significance of tumor-associated macrophages in ovarian cancer: A meta-analysis. *Gynecol. Oncol.* **2017**, *147*, 181–187. [CrossRef] [PubMed]
26. Kartikasari, A.E.R.; Huertas, C.S.; Mitchell, A.; Plebanski, M. Tumor-Induced Inflammatory Cytokines and the Emerging Diagnostic Devices for Cancer Detection and Prognosis. *Front. Oncol.* **2021**, *11*, 692142. [CrossRef]
27. Mielczarek-Palacz, A.; Sikora, J.; Kondera-Anasz, Z.; Mickiewicz, P.; Mickiewicz, A. Effect of Th1/Th2 cytokine administration on proinflammatory SKOV-3 cell activation. *Arch. Med. Sci.* **2016**, *12*, 1337–1347. [CrossRef]
28. Colombo, N.; Sessa, C.; Bois, A.d.; Ledermann, J.; McCluggage, W.G.; McNeish, I.; Morice, P.; Pignata, S.; Ray-Coquard, I.; Vergote, I.; et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: Pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Int. J. Gynecol. Cancer* **2019**, *29*, 728–760. [CrossRef]
29. Tang, F.; Tie, Y.; Tu, C.; Wei, X. Surgical trauma-induced immunosuppression in cancer: Recent advances and the potential therapies. *Clin. Transl. Med.* **2020**, *10*, 199–223. [CrossRef]
30. Hogan, B.V.; Peter, M.B.; Shenoy, H.G.; Horgan, K.; Hughes, T.A. Surgery induced immunosuppression. *Surgeon* **2011**, *9*, 38–43. [CrossRef]
31. Alieva, M.; van Rheenen, J.; Broekman, M.L.D. Potential impact of invasive surgical procedures on primary tumor growth and metastasis. *Clin. Exp. Metastasis* **2018**, *35*, 319–331. [CrossRef] [PubMed]
32. Tohme, S.; Simmons, R.L.; Tsung, A. Surgery for Cancer: A Trigger for Metastases. *Cancer Res.* **2017**, *77*, 1548–1552. [CrossRef] [PubMed]
33. Ramirez, M.F.; Ai, D.; Bauer, M.; Vauthey, J.; Gottumukkala, V.; Kee, S.; Shon, D.; Truty, M.; Kuerer, H.M.; Kurz, A.; et al. Innate immune function after breast, lung, and colorectal cancer surgery. *J. Surg. Res.* **2015**, *194*, 185–193. [CrossRef] [PubMed]
34. Ng, C.S.H.; Lau, K.K.W. Surgical trauma and immune functional changes following major lung resection. *Indian J. Surg.* **2015**, *77*, 49–54. [CrossRef] [PubMed]
35. Gong, J.; Jiang, H.; Shu, C.; Hu, M.; Huang, Y.; Liu, Q.; Li, R. Prognostic value of lymphocyte-to-monocyte ratio in ovarian cancer: A meta-analysis. *J. Ovarian Res.* **2019**, *12*, 51. [CrossRef] [PubMed]
36. Paik, E.S.; Sohn, I.; Baek, S.; Shim, M.; Choi, H.J.; Kim, T.; Choi, C.H.; Lee, J.; Kim, B.; Lee, Y.; et al. Nomograms Predicting Platinum Sensitivity, Progression-Free Survival, and Overall Survival Using Pretreatment Complete Blood Cell Counts in Epithelial Ovarian Cancer. *Cancer Res. Treat.* **2017**, *49*, 635–642. [CrossRef] [PubMed]
37. Nowak, M.; Głowacka, E.; Lewkowicz, P.; Banasik, M.; Szyłło, K.; Zimna, K.; Bednarska, K.; Klink, M. Sub-optimal primary surgery leads to unfavorable immunological changes in ovarian cancer patients. *Immunobiology* **2018**, *223*, 1–7. [CrossRef] [PubMed]
38. Wu, M.; Chen, X.; Lou, J.; Zhang, S.; Zhang, X.; Huang, L.; Sun, R.; Huang, P.; Pan, S.; Wang, F. Changes in regulatory T cells in patients with ovarian cancer undergoing surgery: Preliminary results. *Int. Immunopharmacol.* **2017**, *47*, 244–250. [CrossRef] [PubMed]
39. Napoletano, C.; Bellati, F.; Landi, R.; Pauselli, S.; Marchetti, C.; Visconti, V.; Sale, P.; Liberati, M.; Rughetti, A.; Frati, L.; et al. Ovarian cancer cytoreduction induces changes in T cell population subsets reducing immunosuppression. *J. Cell. Mol. Med.* **2010**, *14*, 2748–2759. [CrossRef]
40. Ulevicius, J.; Jasukaitiene, A.; Bartkeviciene, A.; Dambrauskas, Z.; Gulbinas, A.; Urboniene, D.; Paskauskas, S. Dysregulation of Peripheral Blood Mononuclear Cells and Immune-Related Proteins during the Early Post-Operative Immune Response in Ovarian Cancer Patients. *Cancers* **2023**, *16*, 190. [CrossRef]
41. Reid, B.M.; Permuth, J.B.; Sellers, T.A. Epidemiology of ovarian cancer: A review. *Cancer Biol. Med.* **2017**, *14*, 9–32. [CrossRef] [PubMed]
42. Berek, J.S.; Renz, M.; Kehoe, S.; Kumar, L.; Friedlander, M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. *Int. J. Gynaecol. Obstet.* **2021**, *155* (Suppl. S1), 61–85. [CrossRef] [PubMed]
43. Li, Z.; Hong, N.; Robertson, M.; Wang, C.; Jiang, G. Preoperative red cell distribution width and neutrophil-to-lymphocyte ratio predict survival in patients with epithelial ovarian cancer. *Sci. Rep.* **2017**, *7*, 43001. [CrossRef] [PubMed]
44. Ceran, M.U.; Tasdemir, U.; Colak, E.; Güngör, T. Can complete blood count inflammatory parameters in epithelial ovarian cancer contribute to prognosis?—A survival analysis. *J. Ovarian Res.* **2019**, *12*, 16. [CrossRef] [PubMed]
45. Hufnagel, D.H.; Cozzi, G.D.; Crispens, M.A.; Beeghly-Fadiel, A. Platelets, Thrombocytosis, and Ovarian Cancer Prognosis: Surveying the Landscape of the Literature. *Int. J. Mol. Sci.* **2020**, *21*, 8169. [CrossRef]

46. Giannakeas, V.; Kotsopoulos, J.; Cheung, M.C.; Rosella, L.; Brooks, J.D.; Lipscombe, L.; Akbari, M.R.; Austin, P.C.; Narod, S.A. Analysis of Platelet Count and New Cancer Diagnosis Over a 10-Year Period. *JAMA Netw. Open* **2022**, *5*, e2141633. [CrossRef] [PubMed]
47. Gao, Y.; Liu, C.J.; Li, H.Y.; Xiong, X.M.; Li, G.L.; In 't Veld, S.G.; Cai, G.Y.; Xie, G.Y.; Zeng, S.Q.; Wu, Y.; et al. Platelet RNA enables accurate detection of ovarian cancer: An intercontinental, biomarker identification study. *Protein Cell* **2023**, *14*, 579–590. [CrossRef] [PubMed]
48. Wang, W.; Zou, W.; Liu, J.R. Tumor-infiltrating T cells in epithelial ovarian cancer: Predictors of prognosis and biological basis of immunotherapy. *Gynecol. Oncol.* **2018**, *151*, 1–3. [CrossRef]
49. Santoiemma, P.P.; Powell, D.J. Tumor infiltrating lymphocytes in ovarian cancer. *Cancer Biol. Ther.* **2015**, *16*, 807–820. [CrossRef]
50. Lee, Y.J.; Chung, Y.S.; Lee, J.; Nam, E.J.; Kim, S.W.; Kim, S.; Kim, Y.T. Pretreatment lymphocytopenia is an adverse prognostic biomarker in advanced-stage ovarian cancer. *Cancer Med.* **2019**, *8*, 564–571. [CrossRef]
51. Milne, K.; Alexander, C.; Webb, J.R.; Sun, W.; Dillon, K.; Kalloger, S.E.; Gilks, C.B.; Clarke, B.; Köbel, M.; Nelson, B.H. Absolute lymphocyte count is associated with survival in ovarian cancer independent of tumor-infiltrating lymphocytes. *J. Transl. Med.* **2012**, *10*, 33. [CrossRef] [PubMed]
52. Huang, Q.; Zhou, L.; Zeng, W.; Ma, Q.; Wang, W.; Zhong, M.; Yu, Y. Prognostic Significance of Neutrophil-to-Lymphocyte Ratio in Ovarian Cancer: A Systematic Review and Meta-Analysis of Observational Studies. *Cell. Physiol. Biochem.* **2017**, *41*, 2411–2418. [CrossRef] [PubMed]
53. Tian, C.; Song, W.; Tian, X.; Sun, Y. Prognostic significance of platelet-to-lymphocyte ratio in patients with ovarian cancer: A meta-analysis. *Eur. J. Clin. Investig.* **2018**, *48*, e12917. [CrossRef] [PubMed]
54. Waki, K.; Kawano, K.; Tsuda, N.; Komatsu, N.; Yamada, A. CD4/CD8 ratio is a prognostic factor in IgG nonresponders among peptide vaccine-treated ovarian cancer patients. *Cancer Sci.* **2020**, *111*, 1124–1131. [CrossRef] [PubMed]
55. Cai, L.; Song, Y.; Zhao, X. Prognostic significance of lymphocyte monocyte ratio in patients with ovarian cancer. *Medicine* **2020**, *99*, e19638. [CrossRef]
56. Schweer, D.; McAtee, A.; Neupane, K.; Richards, C.; Ueland, F.; Kolesar, J. Tumor-Associated Macrophages and Ovarian Cancer: Implications for Therapy. *Cancers* **2022**, *14*, 2220. [CrossRef] [PubMed]
57. Franklin, R.A.; Liao, W.; Sarkar, A.; Kim, M.V.; Bivona, M.R.; Liu, K.; Pamer, E.G.; Li, M.O. The Cellular and Molecular Origin of Tumor-associated Macrophages. *Science* **2014**, *344*, 921–925. [CrossRef] [PubMed]
58. Freedman, R.S.; Ma, Q.; Wang, E.; Gallardo, S.T.; Gordon, I.O.; Shin, J.W.; Jin, P.; Stroncek, D.; Marincola, F.M. Migration deficit in monocyte-macrophages in human ovarian cancer. *Cancer Immunol. Immunother.* **2008**, *57*, 635–645. [CrossRef] [PubMed]
59. Ingersoll, S.B.; Stoltzfus, G.P.; Merchant, M.H.; Ahmad, S.; Edwards, C.R.; Ahmed, A.; Oyer, J.L.; Finkler, N.J.; Holloway, R.W.; Edwards, J.R. Comparison of the cytotoxic response against ovarian cancer by immune effector cells isolated and expanded from normal donors and ovarian cancer patients. *Cytotherapy* **2012**, *14*, 716–723. [CrossRef]
60. Zaborowski, M.P.; Stefens-Stawna, P.; Osztynowicz, K.; Piorunek, T.; Batura-Gabryel, H.; Dyzmann-Sroka, A.; Kozubski, W.; Nowak-Markwitz, E.; Michalak, S. Granzyme B in peripheral blood mononuclear cells as a measure of cell-mediated immune response in paraneoplastic neurological syndromes and malignancy. *Cancer Immunol. Immunother.* **2021**, *70*, 1277–1289. [CrossRef]
61. Zhang, M.; He, Y.; Sun, X.; Li, Q.; Wang, W.; Zhao, A.; Di, W. A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. *J. Ovarian Res.* **2014**, *7*, 19. [CrossRef] [PubMed]
62. Macciò, A.; Gramignano, G.; Cherchi, M.C.; Tanca, L.; Melis, L.; Madeddu, C. Role of M1-polarized tumor-associated macrophages in the prognosis of advanced ovarian cancer patients. *Sci. Rep.* **2020**, *10*, 6096. [CrossRef] [PubMed]
63. Browning, L.; Patel, M.R.; Horvath, E.B.; Tawara, K.; Jorcyk, C.L. IL-6 and ovarian cancer: Inflammatory cytokines in promotion of metastasis. *Cancer Manag. Res.* **2018**, *10*, 6685–6693. [CrossRef] [PubMed]
64. Souza, J.C.; Ribeiro, R.S.; Pimenta, T.M.; Martins, B.S.; Rangel, L.B.A. The role of pro-inflammatory components, carcinoma-associated fibroblasts, and tumor-associated macrophages in ovarian cancer progression and metastasis. *J. Cancer Metastasis Treat.* **2023**, *9*, 3. [CrossRef]
65. Lan, T.; Chen, L.; Wei, X. Inflammatory Cytokines in Cancer: Comprehensive Understanding and Clinical Progress in Gene Therapy. *Cells* **2021**, *10*, 100. [CrossRef]
66. van Horssen, R.; Ten Hagen, T.L.M.; Eggermont, A.M.M. TNF-alpha in cancer treatment: Molecular insights, antitumor effects, and clinical utility. *Oncologist* **2006**, *11*, 397–408. [CrossRef] [PubMed]
67. Wilke, C.M.; Wei, S.; Wang, L.; Kryczek, I.; Kao, J.; Zou, W. Dual biological effects of the cytokines interleukin-10 and interferon-γ. *Cancer Immunol. Immunother.* **2011**, *60*, 1529–1541. [CrossRef]
68. Li, Z.; Chen, L.; Qin, Z. Paradoxical Roles of IL-4 in Tumor Immunity. *Cell. Mol. Immunol.* **2009**, *6*, 415–422. [CrossRef] [PubMed]
69. Wang, X.; Yang, C.; Körner, H.; Ge, C. Tumor Necrosis Factor: What Is in a Name? *Cancers* **2022**, *14*, 5270. [CrossRef]
70. Monaco, G.; Lee, B.; Xu, W.; Mustafah, S.; Hwang, Y.Y.; Carré, C.; Burdin, N.; Visan, L.; Ceccarelli, M.; Poidinger, M.; et al. RNA-Seq Signatures Normalized by mRNA Abundance Allow Absolute Deconvolution of Human Immune Cell Types. *Cell Rep.* **2019**, *26*, 1627–1640.e7. [CrossRef]
71. Gupta, M.; Babic, A.; Beck, A.H.; Terry, K. TNF-α expression, risk factors, and inflammatory exposures in ovarian cancer: Evidence for an inflammatory pathway of ovarian carcinogenesis? *Hum. Pathol.* **2016**, *54*, 82–91. [CrossRef]

72. Charles, K.A.; Kulbe, H.; Soper, R.; Escorcio-Correia, M.; Lawrence, T.; Schultheis, A.; Chakravarty, P.; Thompson, R.G.; Kollias, G.; Smyth, J.F.; et al. The tumor-promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. *J. Clin. Investig.* **2009**, *119*, 3011–3023. [CrossRef] [PubMed]
73. Kovács, A.R.; Pál, L.; Szűcs, S.; Lukács, L.; Póka, R.; Lampé, R. Phagocytic function of monocytes and neutrophil granulocytes in ovarian cancer. *Orv. Hetil.* **2018**, *159*, 1353–1359. [CrossRef] [PubMed]
74. 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* **2023**, *59*, 1602. [CrossRef] [PubMed]
75. Tarique, A.A.; Logan, J.; Thomas, E.; Holt, P.G.; Sly, P.D.; Fantino, E. Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. *Am. J. Respir. Cell Mol. Biol.* **2015**, *53*, 676–688. [CrossRef]
76. Wiehagen, K.R.; Girgis, N.M.; Yamada, D.H.; Smith, A.A.; Chan, S.R.; Grewal, I.S.; Quigley, M.; Verona, R.I. Combination of CD40 Agonism and CSF-1R Blockade Reconditions Tumor-Associated Macrophages and Drives Potent Antitumor Immunity. *Cancer. Immunol. Res.* **2017**, *5*, 1109–1121. [CrossRef]

CURRICULUM VITAE

Name, Surname:	**Jonas Ulevičius**
Address:	Obstetrician–gynaecologist, MD, at the Hospital of Lithuanian University of Health Sciences, Kaunas Clinics, Department of Obstetrics and Gynaecology, Eivenių 2, LT-50161 Kaunas, Lithuania.
E-mail:	jonas.ulevicius@lsmu.lt

Education:

2007–2013	Master's degree in Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania.
2013–2017	Medical Residency in Obstetrics and Gynaecology, Lithuanian University of Health Sciences, Department of Obstetrics and Gynaecology, Kaunas, Lithuania.
2019–2024	Doctoral (PhD) Postgraduate Studies, Lithuanian University of Health Sciences, Kaunas, Lithuania.

Work experience:

2017–present	Obstetrician–gynaecologist, MD, at the Hospital of Lithuanian University of Health Sciences, Kaunas Clinics, Department of Obstetrics and Gynaecology, Kaunas, Lithuania.
2017–present	Lecturer at Lithuanian University of Health Sciences, Department of Obstetrics and Gynaecology, Kaunas, Lithuania.
2017–2021	Obstetrician–gynaecologist, MD, Vilkaviškis Hospital, Vilkaviškis, Lithuania.

PADĖKA

Nuoširdžiai dėkoju visiems, kurie prisidėjo prie šios disertacijos rengimo ir palaikė mane šiame kelyje.

Esu labai dėkingas savo mokslinio darbo vadovui prof. dr. Sauliui Paškauskui – už neįkainojamą patirtį, ramybę, gebėjimą visada išlaikyti „šviesą tunelio gale“ ir visapusišką pagalbą rengiant šią disertaciją.

Ypatingą padėką skiriu prof. dr. Žilvinui Dambrauskui – už nepakeičiamus, konstruktyvius patarimus ir nuolatinį palaikymą viso disertacijos rengimo metu.

Nuoširdžiai dėkoju prof. dr. Antanui Gulbinui už suteiktą galimybę būti Chirurginės gastroenterologijos laboratorijos doktorantu, vertingus patarimus ir dalijimąsi savo žiniomis.

Su pagarba ir dėkingumu prisimenu šviesaus atminimo prof. dr. Rūtą Jolantą Nadišauskienę ir dėkoju už suteiktą galimybę tobulėti.

Labai ačiū prof. dr. Mindaugui Kliučinskui už palaikymą, sudarytas sąlygas ruošti disertaciją ir pagalbą organizuojant disertacijos gynimą.

Esu dėkingas ir didelę pagarbą reiškiu prof. dr. Kristinai Jarienei už pradžią, norą mokyti ir vertingą bendrą patirtį.

Dėkoju prof. dr. Kristinai Berškienei už mokymą ir patarimus ieškant geriausių statistinių sprendimų.

Esu labai dėkingas visiems Chirurginės gastroenterologijos laboratorijos darbuotojams – už nuoširdžią paramą žodžiais ir darbais, už kantrybę mokant, skirtą laiką ir didelį indėlį apdorojant pacienčių mėginius.

Labai dėkoju visam Akušerijos ir ginekologijos klinikos kolektyvui už įvairiapusę paramą bei pagalbą renkant tyrimo duomenis.

Nuoširdžiai dėkoju savo artimiesiems ir draugams – už palaikymą, išklausymą ir buvimą kartu.

Didžiausią dėkingumą jaučiu savo šeimai. Nuoširdus ačiū mylimai žmonai Giedrei ir nuostabioms dukroms Agotai bei Barborai už begalinę kantrybę, palaikymą, meilę, prasmę ir brangų laiką kartu. Ačiū!