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# Heterogeneity of the Mac-1 expression on peripheral blood neutrophils in patients with different types of epithelial ovarian cancer

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ABSTRACT

The expression level of Mac-1 on the surface of neutrophils is an important indicator of neutrophil activation. Under pathological conditions, Mac-1 is believed a key adhesion molecule that facilitates cancer progression and mediates the adhesion of tumour cells to the endothelium of blood vessels. Our previous findings indicated that circulating peripheral blood neutrophils in patients with advanced epithelial ovarian cancer (EOC) expressed enhanced levels of Mac-1, which was functionally associated with an increased adhesive function of neutrophils. The objective of the current study was to analyse whether the value of individual components of the differential white cell count, including the neutrophil and lymphocyte ratios, which are markers of blood neutrophil activation, might be associated with certain types of ovarian cancer. We showed the increase in Mac-1 expression along with a parallel decrease of L-selectin and PSGL-1 on peripheral blood neutrophils of patients with EOC of early and advanced FIGO stages, which indicates an activated state of neutrophils in comparison to neutrophils of individuals without cancer. Despite a significant difference between Mac-1 expression in patients with and without cancer, a dramatic increase in Mac-1 expression was observed in the blood of patients with undifferentiated carcinomas compared with patients with other histological types of EOC. Moreover, the expression level of Mac-1 correlated with the number of neutrophils in patients with serous, endometrioid and undifferentiated EOC. The results of an ROC analysis demonstrated that the patients with the undifferentiated type of EOC form a distinct group with regard to Mac-1 expression on blood neutrophils. The results suggested a diverse biological cadre of immune cells in patients with undifferentiated ovarian carcinomas compared with patients with other histological types of EOC.



## 1. Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death from gynaecologic malignancies. The high mortality rate and poor overall prognosis of patients are usually ascribed to a late diagnosis (approximately 80% are diagnosed at an advanced stage). However, the high mortality rate and poor prognosis are also dependent on the histopathology and grade of the tumour, the type of ovarian cancer and the residual disease that remains after primary surgery (Heintz et al., 2006; Kurman and Shih, 2011).

It has long been known that inflammatory processes accompany the development of cancer. The close association among the numbers of neutrophils, the numbers of lymphocytes or inflammatory markers and the progression of ovarian cancer has been shown (Nowak et al., 2010; Watanabe et al., 2014; Williams et al., 2014). Specifically, Williams et al. (2014) revealed that the ratio of neu-

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trophil to lymphocyte number in the peripheral blood of patients with EOC correlated positively with disease progression and that it is enhanced in invasive *versus* borderline tumours. Despite that the main role of the immune system is to eliminate cancer cells, the role of neutrophils in the mediation of cancer progression is now being discussed. In pathological conditions, neutrophils can facilitate the attachment of tumour cells (both in the circulation and at the site of the tumour) to endothelial cells and enhance their metastatic potential or invasiveness (Starkey et al., 1984; Welch et al., 1989; Wu et al., 2001). In physiological conditions, adhesion molecules such as L-selectin, PSGL-1, and the integrins Mac-1 (CD11b/CD18), and LFA-1 (leukocyte function associated antigen-1, CD11a/CD18) that are expressed on the surface of neutrophils, are involved in transendothelial migration, which is a crucial step in the response of neutrophils to inflammation (Steeber et al., 1997; Williams et al., 2011). It is believed that tumour cells that circulate in the blood utilize the neutrophil adhesion machinery, including Mac-1, to facilitate their ability to metastasize (Spicer et al., 2012). Nevertheless, the relationship between the expression level of neutrophil adhesion molecules and the invasiveness of different types of ovarian cancer is not yet understood. The designation "epithelial ovarian cancer" includes a heterogeneous group of epithelial malignant tumours of the ovary, peritoneum and fallopian tube, which exhibit different characteristics, origins, phenotypes and invasiveness (Kurman and Shih, 2011; Prat, 2014; Shih and Kurman, 2004). However, the relationship between the neutrophil activation state and the clinicopathological features of different epithelial ovarian carcinomas has not been considered and discussed thus far.

The primary marker that is commonly assessed as an important indicator of neutrophil activation is the integrin Mac-1, which is involved in neutrophil adherence, migration, phagocytosis and bacterial killing (Schymeinsky et al., 2007). In physiological conditions, the level of Mac-1 expression on neutrophils in the peripheral blood is low, and it increases dramatically under the influence of pro-inflammatory factors. In pathological conditions, it is believed that the Mac-1 integrin is a key molecule on neutrophils that supports cancer metastasis. Our previous findings indicated that circulating peripheral blood neutrophils in patients with invasive EOC expressed enormously high levels of Mac-1 adhesive proteins, which are functionally associated with increased adhesive ability of these cells (Klink et al., 2008).

This study investigates whether Mac-1 expression on neutrophils circulating in the blood of patients with EOC is associated with cancer histology, FIGO stage (clinical stage of the disease according to the International Federation of Gynaecology and Obstetrics), grading and type (I or II) of cancer. The expression of Mac-1 on neutrophils in patients from different EOC groups is discussed in the context of the expression level of other adhesion molecules that are expressed on neutrophils (i.e., integrin LFA-1, L-selectin and PSGL-1). In addition, we investigated whether varied expression levels of Mac-1 were accompanied by changes in the number of neutrophils and lymphocytes in the blood.

## 2. Materials and methods

### 2.1. Chemical reagents and antibodies

Histopaque 1077, Hanks' balanced salt solution (HBSS), bovine serum albumin (BSA), formyl-methionyl-leucyl-phenylalanine (fMLP), propidium iodide (PI) and luminol were obtained from Sigma–Aldrich (USA). The BD CellFix and BD FACS Lysing Solution was from BD Biosciences (Franklin Lakes, New Jersey, USA). Dextran T500 was from Pharmacosmos A/S (Holbaek, Denmark). Fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (D-PBS) and Hanks' balanced salt solution (HBSS), were purchased from Life Technologies (Carlsbad, California, USA). Mouse anti-human CD15 FITC-conjugated monoclonal antibody (clone C3D-1) was purchased from DAKO A/S (Glostrup, Denmark). Phycoerythrin (PE)-conjugated mouse anti-human CD11b antibody (clone D12), PerCy5-conjugated mouse anti-human CD62L antibody (L-selectin, clone DREG-56), PE-conjugated PSGL-1 mouse anti-human CD162 antibody, mouse anti-human CD11a primary antibody were obtained from BD Biosciences (Franklin Lakes, New Jersey, USA).

### 2.2. Patients

Our study was approved by the local Ethical Committee and was conducted in the Gynaecologic Departments of the Polish Mother's Memorial Hospital—Research Institute, Lodz, Poland. We confirmed that the patients, who were admitted to the gynaecologic departments due to a pelvic mass, were actually diagnosed with an ovarian tumour. Patients with a history of any previous malignant neoplasia or gynaecologic operation, transplantation, autoimmune disease, diabetes, thyroid problems or any signs of infection were excluded from the study. After operative treatment and a histopathologic examination, we finally included 81 patients with epithelial ovarian cancer in our analysis.

The age of the patients ranged from 24 to 88 years, and the ovaries or fallopian tubes were the primary sites of malignancy for all women. The clinical stage of ovarian cancer was established after laparotomy and pathologic examination and was based on the International Federation of Gynaecology and Obstetrics (FIGO) classification system and guidelines (Benedet et al., 2000; Prat, 2014). All tissues removed during surgery were examined by a pathologist and were assessed for the following: tumour grade, histological type and the presence of metastases, which were determined according to FIGO and WHO classifications.

We also allocated all cases to two broad categories of ovarian cancer (types I and II) according to the clinicopathologic- and molecular/genetic-based classification system proposed by Shih and Kurman (2004). Type I tumours included low-grade serous, mucinous, low-grade endometrioid and clear-cell cancers. Type II included high-grade serous, high-grade endometrioid and undifferentiated carcinomas. The clinicopathological characteristics of the ovarian cancer patients in our study are presented in Table 1.

The majority of patients were in an advanced stage of the disease (III–IV FIGO stage—81 %). Serous (40%) and undifferentiated (23%) carcinomas were the predominant type; 67% of the tumours were grade 3, and type II ovarian cancer was established in 63% of the cases (Table 1). The control group (specified as the "non-cancer" group) consisted of 50 age-matched generally healthy women who were admitted to the gynaecologic department because of static disorders of the vagina. There were no history or signs of endometriosis in the control group.

### 2.3. Blood sample collection

A complete blood profile was determined using an automatic blood cell counter (Vitros 5.1/Vitros 350, Ortho Clinical Diagnostics, Inc., (Raritan, New Jersey, USA) in the Diagnostic Laboratory at the Polish Mother's Memorial Hospital Research Institute, Lodz, Poland one day prior to surgery. In regards to the *in vitro* experiment, the 9-ml aliquots of peripheral venous blood were obtained from patients in the morning prior to surgery. A total of 10 U/ml of heparin was used as an anticoagulant.