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The use of C_{trough} for the therapeutic drug monitoring of olaparib in patients with ovarian cancer

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Abstract. – OBJECTIVE: Olaparib is the poly-[Adenosine diphosphate ribose (ADP-ribose)] polymerase inhibitor (PARPI) used in maintenance therapy of patients with platinum-sensitive ovarian cancer with mutations in breast cancer genes 1/2 (BRCA1/2). Oncologists still do not have recommendations of treatment depending on efficient plasma concentrations of the PARP inhibitor. The aim of the study was the assessment of plasma trough concentrations of olaparib at steady state (C_{trough}) in ovarian cancer patients. The severity of olaparib adverse effects (AEs) was noted.

PATIENTS AND METHODS: The retrospective study involved 33 patients [mean standard deviation (SD)]; age 57.0 (8.4) years; weight 68.7 (13.7) kg; and body mass index (BMI) 26.4 (4.9) kg/m^2, with ovarian cancer treated with olaparib (tablets in dose 300 mg/12 h, 250 mg/12 h, 200 mg/12 h or capsules 400 mg/12 h, 200 mg/12 h, 100 mg/12 h). Plasma drug levels were measured by HPLC-UV method (λ = 254 nm; Symmetry C8 column; gradient flow). The severity of olaparib AEs was assessed by Common Terminology Criteria for Adverse Events (CTCAE) v5.0 scale. Drug interactions were analyzed.

RESULTS: In total, 130 measurements (n) of C_{trough} were determined in 33 patients (median sample frequency per patient was 4). The olaparib C_{trough} in patients with AEs was 87.840-7,213.262 ng/mL [coefficient of variation (CV) = 91%], in patients without AEs 48.021-7,073.350 ng/mL (CV = 88%). AEs were the following: fatigue (modest, n = 4, severe, n = 2), anemia (grade G1 n = 66, G2 n = 6, G3 n = 3), neutropenia (grade G1 n = 15, G2 n = 4), prediabetes (n = 1). There was a correlation between C_{trough} and olaparib-induced fatigue (p = 0.0015). The lower values of dose-adjusted olaparib concentrations (p = 0.0121) and dose/kg-adjusted olaparib concentrations (p = 0.0389) were correlated with higher grade of neutropenia.

CONCLUSIONS: There was a correlation between C_{trough}, expressed as ng/ml, ng/ml/mg or ng/ml/mg/kg, and fatigue degree, but not anemia. Patients with neutropenia had statistically significant lower plasma concentrations of olaparib.

Key Words:
Olaparib, Blood-level testing, C_{trough}, Adverse effects.

Introduction

Olaparib was the first poly-[Adenosine diphosphate ribose (ADP-ribose)] polymerase inhibitor (PARPI) approved in Europe in 2014 for the maintenance therapy of patients with platinum-sensitive serum ovarian cancer with mutations in BRCA1/2 genes who responded to platinum derivatives during the relapse treatment[1,2]. The results of the SOLO-1 trial (olarib *vs.* placebo) resulted in the approval of this drug for maintenance therapy after the first-line chemotherapy in patients with platinum-response ovarian cancer with mutations in *BRCA1/2* genes. Due to the promising results of clinical trials, olaparib can also be applied to patients with fallopian tube cancer and primary peritoneal cancer if they have responded (completely or partly) to chemotherapy based on platinum compounds, regardless of the *BRCA1/2* gene mutation status[2,3]. The initial positive results of the PROfound and POLO clinical trials led to the conclusion that olaparib may effectively treat prostate and pancreatic cancers[4,5].

The PARPIs act selectively on neoplastic cells. Therefore, they are a promising treatment strategy for oncological patients[6]. However, the

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olaparib therapy is usually discontinued or the dose of the drug is reduced due to adverse reactions such as: nausea (73%), fatigue (5%), vomiting (36%), diarrhea (30%), and anemia (26%)[1,2]. So far there have been no data on the correlation of the frequency of occurrence of these undesirable effects with the concentration of the drug in the blood plasma. However, it is known that therapeutic drug monitoring (TDM) based on measurements of the drug concentration in the blood is an important tool supporting safe pharmacotherapy. For some drugs it is enough to collect a sample and measure the maximum concentration ($C_{ss,max}$) and/or the trough concentration at steady state. Olaparib reaches the steady state after about 3-4 days and maximum concentration $C_{ss,max}$ within 1-3 hours after oral administration[7]. $C_{ss,max}$ is characterized by high inter-individual variability, therefore the determination of the minimum concentration before the administration of another dose of the drug may be of much greater clinical value. The aim of the study was the assessment of plasma trough concentrations of olaparib at steady state (C_{trough}) in ovarian cancer patients. Additionally, the severity of olaparib adverse effects (AEs) was noted[8].

Patients and Methods

Reagents

Olaparib was purchased from LGC Standards (Łomianki, Poland) and Internal Standard – acetaminophen from Sigma Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) mobile phase methanol and glacial acetic acid were from Merck. Water used in the mobile phase was deionized, distilled and filtered through a Millipore system Direct Q3® (Burlington, MA, USA) before use. Extractive mixture consists of ethyl acetate and chloroform from Merck.

The patients received Lynparza® (AstraZeneca Pharma Poland Sp. z o.o.) in tablets (batch: 70818, RN192) or in capsules (batch: RR950).

Subjects

The retrospective study was conducted at the Clinics of Gynecological Oncology Poznan University of Medical Sciences, and the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań, Poland with the approval from the Bioethics Committee, University of Medical Sciences, Poznań, Poland (697/20). The subjects of the study were patients with ovarian cancer who received olaparib between October 2020 and June 2021. The olaparib was taken in capsules of 50 mg. After stoppage of production by AstraZeneca the capsules were changed for tables a 100 mg and 150 mg, but any patient was switched from capsules to tablets. The patients were included in the study if they met the following criteria: treatment with olaparib above four days, age >18 years; no history of allergy to olaparib. The chief criteria for exclusion included allergy to olaparib, age under 18 years, status of the patient which did not allow the patient to continue the study. The baseline characteristics of all patients enrolled in the study are shown in Table I. All patients provided written consent to participate in the study.

Administration and Blood Sampling

The patients with ovarian cancer (n = 33) were treated with olaparib (tablets in dose 300 mg/12 h, 250 mg/12 h, 200 mg/12 h or capsules 400 mg/12 h, 200 mg/12 h, 100 mg/12 h). Blood samples (2 mL) were collected at steady state before morning drug administration. The blood samples were transferred into heparinized tubes and centrifuged at 2,880 g for 10 min at 4°C. Next the plasma was transferred to propylene tubes and stored at -20°C until analysis.

Assays

The concentrations of olaparib in plasma were assayed using the HPLC method with ultraviolet (UV) detection. Acetaminophen was used as the internal standard (IS). Samples were analyzed

Table I. Patients' characteristics.

Parameter	S ± SD (CV%)
Age [years]	57.00 ± 8.40 (15)
body weight [kg]	68.70 ± 13.70 (20)
BMI [kg/m^2]	26.40 ± 4.88 (18)
WBC [10^9/L]	5.26 ± 1.45 (28)
Neut [10^9/L]	3.13 ± 1.20 (38)
RBC [10^{12}/L]	3.50 ± 0.50 (14)
Hgb [mmol/L]	7.43 ± 0.90 (12)
HCT [L/L]	0.36 ± 0.04 (12)
ALT [U/L]	23.16 ± 9.10 (39)
AST [U/L]	22.70 ± 6.81 (30)
creatinine [mmol/L]	75.07 ± 16.95 (23)
Cl_{Cr} [mL/min]	76.64 ± 14.08 (18)

BMI – body mass index; WBC – white blood cells; neut – neutrophiles; RBC – red blood cells; HBG – hemoglobin; HCT – hematocrit; ALT – alanine transaminase; AST – aspartate transaminase; Cl_{Cr} – creatinine clearance. S – arithmetic mean; SD – standard deviation.

on Alliance 2695 HPLC UV-Vis system with Empower Software No. 1154 Waters Corporation (Milford, MA, USA). The separation was done using the gradient method. Phase A was 0.2% acetic acid in water, phase C was ultrapure water, phase D was methanol. The gradient started at 100% C and decreased linearly to 11% C, 1% A and increased 88% D in 17 min, then changed to 1% D, 98% C and on 25 min it returned to starting condition for column equilibration. The flow rate was 1 mL/min. Chromatography was run on Waters Symmetry C8, 5 µm, 4.6 mm x 250 mm analytical column. The column temperature was maintained at 25°C, the UV detection wavelength was set at 254 nm and the injection volume was 20 µL. The method was validated according to European Medicines Agency guidelines[9]. The method validation confirmed good precision [coefficient of variation (CV)% < 15%], accuracy (92.3-115.0%) and linearity (r = 0.9994) in the range of 100-4,000 ng/mL.

The severity of olaparib adverse effects was assessed by Common Terminology Criteria for Adverse Events (CTCAE) v5.0 scale. In the CTCAE scale the severity of adverse effects can be divided from mild (G1) to death (G5)[8]. The severity of neutropenia depends on number of neutrophils in blood samples (G1: < lower limits of normal (LLN)-1,500/µL; G2: 1,500-1,000 / µL; G3: 1,000-500/ µL; G4: < 500/ µL).

The severity of fatigue was assessed in dependency to rest impact and impact on activities of daily living (ADL) (G1: fatigue relieved by rest; G2: fatigue not relieved by rest, limiting instrumental ADL; G3: fatigue not relieved by rest, limiting self-care ADL). The severity of anaemia depends on haemoglobin level: G1: haemoglobin (Hgb) LLN - 10 g/dL (6.2 mmol/L); G2: Hgb 10.0 - 8.0 g/dL (6.2 - 4.9 mmol/L); G3: Hgb < 8.0 g/dL (< 4.9 mmol/L); transfusion indicated G4: life threatening consequences; urgent intervention indicated.

Statistical Analysis

The analyzed interval data did not follow the normal distribution (Shapiro-Wilk test) that is why the results were presented as medians and interquartile ranges Me [Q1-Q3]. In the case of comparing the data between the two groups, the Mann-Whitney test was used. When comparing more than two groups simultaneously, the Kruskal-Wallis' test was used together with Dunn's post-hoc test. All tests were analyzed at the significance level of $\alpha = 0.05$. Statistical analysis was performed using the statistical package TIBCO Software Inc. (2017, Palo Alto, CA, USA) and Statistica (data analysis software system), version 13 (available at: http://statistica.io).

Results

From October 2020 to June 2021, 33 patients were included in the study. The anthropometric and biochemical parameters of all the groups of patients are shown in Table I. All the data were expressed as the arithmetic mean and standard deviation (S±SD). The coefficient of variation (CV%) exceeding 30% indicates high inter subject variability. 11 patients (33.3%) were overweight, and 10 patients (30.3%) were obese. The patients were characterized by the normal hepatic function, except for 5 patients, whose alanine transaminase (ALT) (n = 3) and/or aspartate transaminase (AST) (n = 3) were above normal values (ALT < 45 U/L; AST < 35 U/L, respectively).

There were 25 patients (60.6%) with abnormal hemoglobin (Hgb) levels (norm: 7.45-10.00 mmol/L), 31 patients (93.9%) with abnormal red blood cell (RBC) levels (norm: $4\text{-}5\times10^{12}$/L), and 19 patients (57.6%) with abnormal hematocrit (HCT) levels (norm: 0.36-0.47 L/L). The Cl_{cr} values were estimated with the Cockroft-Gault formula. They were lower than normal in 31 patients (94%). There were only a few patients (2-6%) with $Cl_{cr} \geq 90$ (normal value), which most likely was caused by an earlier platinum-based chemotherapy. There were 25 patients (76%) with mild decreased Cl_{cr} levels, (Cl_{cr} 60-89 mL/min). The Cl_{cr} levels of the remaining 6 patients (18%) were characterized by moderate decrease (Cl_{cr} 30-59 mL/min), except for one case of a severe decrease (Cl_{cr} 15-29 mL/min).

The dosing schedule and olaparib C_{trough} values are shown in Table II. In the analyzed group 13 patients received olaparib tablets and 20 patients received capsules.

There were 25 patients (75.8%) with anemia observed in 75 concentration measurements (n = 75 samples), 4 patients (12.1%, n = 6 samples) with fatigue, 10 patients (30.3%, n = 19 samples) with neutropenia, 1 patient (3.0%, n = 1 sample) with prediabetes, and 1 patient (3.0%, n = 1 sample) with pulmonary fibrosis. Grade 1 (G1) anemia was recorded in 66 measurements of the olaparib concentration, G2 anemia in 6 measure-

Table II. C_{trough}, dose-adjusted olaparib concentrations and dose/kg-adjusted olaparib concentrations.

Parameter	Tablets			Capsules		
Dose	300 mg bid	250 mg bid	200 mg bid	400 mg bid	200 mg bid	100 mg bid
Number of patients	12	1	2	16	5	2
Drug level samples	57	6	3	44	15	5
C_{trough} [ng/mL] Me [Q1 – Q3]	1,209.07 [797.61-2,415.48]	974.68 [765.70-1,279.35]	504.60 [238.91-1,794.75]	1,209.07 [797.61-2,415.48]	974.68 [765.70-1,279.35]	504.60 [238.91-1,794.75]
Dose-adjusted olaparib concentrations [ng/mL/mg] S ± SD [min-max]	6.45 ± 1.30 [0.16-24.04]	4.60 ± 3.60 [0.66-17.42]	4.46 ± 4.35 [0.62-11.96]	6.45 ± 1.30 [0.16-24.04]	4.60 ± 3.60 [0.66-17.42]	4.46 ± 4.35 [0.62-11.96]
Dose/kg-adjusted olaparib concentrations [ng/ml/ mg/kg] S ± SD [min-max]	469.06 ± 62.88 [10.88-2047.89]	325.79 ± 265.95 [10.88-1,306.57]	268.16 ± 254.18 [37.49-717.54]	469.06 ± 62.88 [10.88-2,047.89]	325.79 ± 265.95 [10.88-1,306.57]	268.16 ± 254.18 [37.49-717.54]
Number of drug levels per patients median (range)		4 (1-9)			3 (1-6)	

bid – twice a day; S - arithmetic mean; SD – standard deviation; Me - median.

Table III. Adverse effects and C_{trough} of Olaparib.

CTCAE term	C_{trough} [ng/mL] S ± SD (CV%)				
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Anemia	1,590.03 ± 1,372.98 (86) n = 66	1,128.69 ± 561.22 (50) n = 6	1,155.15 ± 417.75 (36) n = 3	- n = 0	- n = 0
Neutropenia	1,079.44 ± 725.64 (67) n = 15	665.68 ± 749.05 (112) n = 4	- n = 0	- n = 0	NA -
Fatigue	– n = 0	2,965.07 ± 2,202.01 (74) n = 4	5,339.02 ± 2,452.71 (46) n = 2	NA -	NA -

n-drug level samples; NA - not applicable.

ments, and G3 anemia in 3 measurements. There were no cases of G4 or G5 anemia. Due to the specificity of the study, one patient could have developed G1, G2, and G3 anemia. Neutropenia was diagnosed in 10 patients (30.3%) in 19 concentration measurements, including G1 neutropenia in 15 measurements and G2 neutropenia in 4 measurements. Neither G3 nor G4 toxicity was observed.

There were 4 patients (12.1%) with fatigue diagnosed in 6 measurements, including 4 cases of light fatigue and 2 cases of moderate fatigue. Prediabetes was diagnosed in 1 patient.

The C_{trough} values of olaparib which resulted in the above mentioned adverse reactions are shown in Table III and Figure 1. There was no significant correlation between the olaparib C_{trough} value and respective dose – neither in the group on tablets (p = 0.6789) nor capsules (p = 0.1020). There was also calculation of dose-adjusted olaparib concentrations and dose/kg-adjusted olaparib concentrations. Dose-adjusted olaparib concentrations (ng/ml/mg) and dose/kg-adjusted olaparib concentrations (ng/ml/mg/kg) were calculated using the following equations: olaparib concentration (ng/ml)/olaparib daily dose (mg/day) and olaparib concentration (ng/ml)/olaparib daily dose per body mass kilogram (mg/kg/day), respectively.

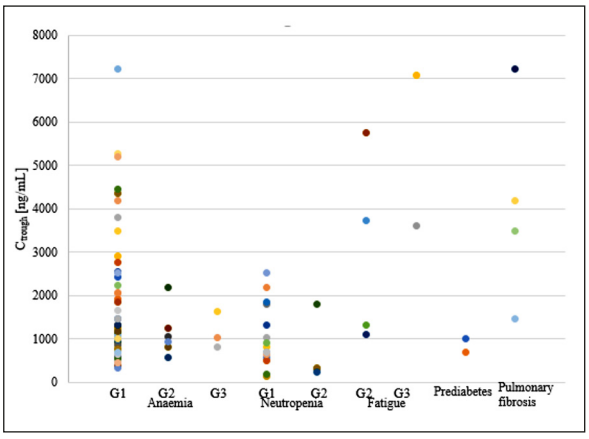

Figure 1. Olaparib C_{trough} plasma concentrations and respective adverse effects.

It was noticed that there is a statistical correlation between dose-adjusted olaparib concentrations (p = 0.0250) and dose/kg-adjusted olaparib concentrations (p = 0.0023) for the patient with fatigue. It should be marked that fatigue is correlated with the dose of olaparib and the fatigue should be decreased after the olaparib-dose reduction. The statistical correlation was also noticed for neutropenia. But conversely, the lower values of dose-adjusted olaparib concentrations (p = 0.0121) and dose/kg-adjusted olaparib concentrations (p = 0.0389) was correlated with higher grade of neutropenia.

The statistical correlation was not noticed for anemia and also for each of the grades of CTCAE separately.

Additionally, possible drug-drug interactions were considered. Drugs taken by the patients were analyzed to find inhibitors/inducers of cytochrome P450 3A4/3A5 (CYP3A4/5), P-glycoprotein (P-gp). The patients were taking the following drugs for comorbidities: ramipril (n = 1), pregabalin (n = 1), enoxaparin (n = 5), chloroquine (n = 1), betahistine (n = 1), atorvastatin (n = 2), levothyroxine (n = 3), tizanidine (n = 1), paroxetine (n = 1), pantoprazole (n = 2), bisoprolol (n = 5), rosuvastatin (n = 1), perindopril (n = 1), amlodipine (n = 1), citalopram (n = 1), venlafaxine (n = 1), furosemide (n = 1), spironolactone (n = 1), ferrous sulfate (n = 1), anastrozole (n = 1), indapamide (n = 1), sulpiride (n = 1), alprazolam (n = 1), nebivolol (n = 1), aspirin (n = 1). In the observed group of patients, 9 of them were diag-

nosed with hypertension, 9 had positive history of breast cancer, 5 Hashimoto disease, 2 hypercholesterolemia, 2 deep vein thrombosis in the past, 1 rheumatoid arthritis, 2 depression and 1 patient migraine.

Discussion

It is known that the olaparib therapy extended the time to progression (TTP) progression-free survival (PFS) and overall survival (OS). Clinical trials SOLO 1-3 confirmed the beneficial effect of olaparib on the PFS and its reduction of the risk of death. The olaparib monotherapy caused adverse drug reactions of mild or moderate severity (CTCAE Grade 1 or 2), which usually did not require the treatment to be discontinued. Various clinical trials involving Lynparza monotherapy showed that the most common adverse reactions (≥ 10%) observed in the patients receiving the drug were: nausea, vomiting, diarrhea, indigestion, fatigue, headache, dysgeusia, decreased appetite, dizziness, cough, dyspnea, anemia, neutropenia, thrombocytopenia, and leukopenia. Grade ≥ 3 adverse reactions observed in more than 2% of the patients included: anemia (16%), neutropenia (6%), fatigue/asthenia (5%), leukopenia (3%), thrombocytopenia (3%), and vomiting (2%). The most common adverse reactions which usually led to interruption of the therapy and/or dose reduction were: anemia (16.2%), vomiting (6.8%), nausea (6.2%), neutropenia (6.2%), and fatigue/asthenia (6.0%). The most common adverse reactions which usually led to discontinuation of the therapy were: anemia (1.8%), nausea (0.7%), fatigue/asthenia (0.7%), and thrombocytopenia (0.7%)[7]. Study19 showed that the incidence of adverse reactions to olaparib in patients after shorter and longer treatment periods was comparable. This may indicate that the duration of treatment does not reduce adverse drug reactions, but in some cases, they can only be reduced by decreasing the dose[2,10-12]. There is one study[13] which identified the correlation between olaparib plasma exposure and toxicity in patients treated for *BRCA1/2* mutated ovarian cancer. Increased predicted value of C_{trough} was significantly associated with a higher risk of severe adverse events (SAE) occurrence.

The patients in our study received olaparib tablets (2 x 300 mg) or capsules (2 x 400 mg). The efficacy and tolerability of olaparib tablets administered daily at a dose of 600 mg is comparable to capsules administered at a dose of 800 mg[14]. In the event of side effects, the dose was reduced, or the therapy was interrupted for not more than 28 days until the side effect abated. The drug concentrations measured in the patients who consented to participate in the study ranged widely (Table II). There were 130 C_{trough} measurements in total. The lowest concentration was 48.021 ng/mL, whereas the highest was 7,213.262 ng/mL.

The following adverse reactions were observed in the group of patients under analysis: 25 patients (75.8%) – anemia, 4 patients (18.2%) – fatigue, 10 patients (36.2%) – neutropenia, 1 patient (3%) – prediabetes, and 1 patient (3%) – pulmonary fibrosis.

Anemia

Anemia is one of the most common complications occurring during an olaparib therapy. In order to effectively control this side effect, it is advisable for patients to have a complete blood count test before they undergo maintenance therapy and then periodically during the olaparib therapy – before each subsequent course of the treatment (monthly) and in case of clinical indications. During the treatment, the severity of anemia is described according to the CTCAE v5.0 classification of adverse events[8]. If G1 or G2 anemia occurs, which was the most frequent incidence during registration trials, the therapy can be continued. However, G3 and G4 anemia is an indication for the transfusion of RBC concentrate, interruption of the treatment and reduction of the olaparib dose[2]. If G3 or G4 anemia occurs and it is necessary to transfuse RBCs, it is important to remember that the blood for the C_{trough} test should be collected before rather than after an RBC transfusion, because the mixing of the donor and recipient's blood may result in a measurement error. The analysis of the blood samples collected from the patients in our study showed that in most of the cases the values of the blood parameters (RBC [4-5 10^{12}/L]; Hgb [7.45-10.00 mmol/L]; HCT [0.36-0.47 L/L]) were under the limit. Symptomatic anemia was diagnosed in only 5 of 25 patients. It is necessary to reduce the olaparib dose administered to the patients whose hemoglobin level corresponds to G3 anemia (Hgb < 4.9 mmol/L) or who experience severe adverse reactions despite better results. This means that the patients who do not experience any negative effects of the therapy and have been diagnosed

with G1 or G2 anemia in a complete blood count test do not require medical intervention and reduction of the drug dose. Plasma C_{trough} for olaparib in 25 patients with anemia was in the range 331.57-7,213.26 ng/mL (Table III). There was not any correlation between C_{trough} (p = 0.2453), dose-adjusted olaparib concentrations (0.4259) and dose/kg-adjusted olaparib concentrations (0.2594) and anemia generally and depending on the degree of anemia.

Neutropenia

Neutropenia is a less common consequence of olaparib treatment than anemia. The following adverse reactions were classified as neutropenia during the research: agranulocytosis, febrile neutropenia, low granulocyte count, granulocytopenia, idiopathic neutropenia, neutropenia, neutropenic infection, neutropenic sepsis, and low neutrophil count. During registration trials these grade ≥ 3 adverse reactions were observed in less than 10% of the patients; respectively 5% had neutropenia and 3% had leukopenia. For this reason, the therapy was interrupted and/or the dose of the drug was reduced in 6% of the patients[7]. According to the CTCAE version 5.0, there are four grades of neutropenia[8]. Like in the case of anemia, olaparib treatment can be continued if G1 or G2 toxicity is diagnosed, but G3 and G4 toxicity is an indication to interrupt the olaparib therapy for up to 28 days and reduce the dose of the drug in further treatment. If symptomatic neutropenia is diagnosed, the administration of granulocyte colony stimulating factors (GCSF) should be taken into consideration[2]. The plasma C_{trough} for olaparib in 10 patients with neutropenia was in the range 124.96-2,525.99 ng/mL (Table III). There is a significant difference in C_{trough} between the patients with neutropenia Me-799.53 [Q1-576.37 – Q3-1,787.74] and without neutropenia Me-1,182.44 [Q1-812.16 – Q3-2,365.85] (p = 0.01) but there was no correlation between C_{trough} and neutropenia degree (p = 0.221). However, it was noticed that there is a statistical correlation between dose-adjusted olaparib concentrations (p = 0.0121) and dose/kg-adjusted olaparib concentrations (p = 0.0389) for the patients with or without neutropenia. The patients with neutropenia had statistically lower dose-adjusted olaparib concentrations and dose/kg-adjusted olaparib concentrations than the patients without neutropenia. All the patients who were classified to olaparib treatment had a normal neutrophils range.

Drug-Induced Fatigue

Cytotoxic-induced fatigue and cancer-related fatigue have been well described. Drug-induced fatigue deteriorates the quality of life and causes severe tiredness and depressed mood. The mechanism of this non-hematological adverse effect is still unknown. One of the highest drug-induced fatigue is connected with cyclophosphamide and gemcitabine[15]. It is also one of the most common class effects of adverse events, observed in 50-70% of patients on PARPIs[16]. Physical activity, like a 30-minute daily walk, may be recommended to patients to prevent this side effect, but the patient's condition should be considered, especially concurrent anemia or thrombocytopenia[15,17]. In our study olaparib-induced fatigue was observed in 6 patients (15.2%), in the grade G2-G3. The plasma C_{trough} for olaparib in these patients was in the range 1,090.63 to 7,073.35 ng/mL (Table III). There was correlation between C_{trough} and olaparib induced fatigue (p = 0.0015). Additionally, there is a statistical correlation between dose-adjusted olaparib concentrations (p = 0.0250) and dose/kg-adjusted olaparib concentrations (p = 0.0023) for the patient with or without fatigue. It should be marked that fatigue is correlated with the dose of olaparib and should decrease after the olaparib-dose reduction. It is important to notice that and to take the interview with patients carefully as it is one of the most neglected side effects of PARP-inhibitor treatment.

Pulmonary Fibrosis

Pulmonary fibrosis or pneumopathy is a rare adverse reaction to anticancer drugs such as: bleomycin, gemcitabine, docetaxel, paclitaxel[18]. The mechanism of olaparib-induced pneumopathy is still unclear. A few studies on the roles of PARPs involved in inflammation showed that they may be responsible for the development of pathological processes such as asthma, acute lung injury, acute inflammation and others (e.g., smoking) by increasing the production of pro-inflammatory cytokines [tumor necrosis factor alpha (TNFα), interleukin-5 (IL-5) and CXC motif chemokine ligand 2 (CXCL2)] and by activating the nuclear factor kappa B (NF-κB) pathway and upregulation of inducible nitric oxide synthase (iNOS)[19-20]. On the other hand, the deficiency of PARPs led to the expression of interleukin 6 (IL-6), CXC motif chemokine ligand 13 (CXCL13), and insulin-like growth factor binding protein-5 (IGFBP-5) cytokines found in the lung tissues affected by idiopathic pulmonary fibro-

sis[18]. The occurrence of pulmonary fibrosis in the patient was confirmed by computed tomography (CT). According to the recommendations of the maintenance treatment program, patients with platinum-sensitive ovarian cancer should be given a CT scan at least once in 12 weeks. The patient did not have typical symptoms of pulmonary fibrosis, such as dyspnea, reduced exercise tolerance, cough (which indicates G1 toxicity), so glucocorticosteroid therapy was not initiated, because it could reduce the effectiveness of anticancer treatment. Currently it is known that idiopathic pulmonary fibrosis may occur in patients infected with SARS-CoV-2, especially when they are treated in intensive care units[21]. As the study was conducted during the COVID-19 pandemic, the patient was tested to exclude a SARS-CoV-2 infection history. According to scientific publications, pneumonia, which may lead to pulmonary fibrosis, including fatal cases, has been reported in less than 1.0% of patients treated with olaparib in clinical trials[7]. The patient with pulmonary fibrosis was treated with olaparib capsules administered at a dose of 2 x 400 mg. Her C_{trough} levels were measured four times and ranged from 1,466.7 to as high as 7,213.3 ng/ml (Figure 1). When the adverse reaction was diagnosed, the dose of the drug was reduced to 2 x 200 mg.

Prediabetes

One patient without a diabetes history had an elevated glucose level when she received olaparib. Before the olaparib treatment her blood glucose level was c = 5.0 mmol/L (in the normal range). The patient had her fasting blood glucose level measured four times within 14 months of the olaparib treatment. The results were as follows: 5.15; 5.30; 5.30; and 6.00 mmol/L. The patient's increasing blood glucose levels during the treatment suggest a prediabetes condition, and more precisely – impaired fasting glucose (IFG). According to the American Diabetes Association (ADA), IFG is diagnosed at a fasting blood sugar level of 5.6-7.0 mmol/L. Hyperglycemia associated with targeted cancer therapy has been relatively well described as an adverse reaction to various drugs such as tyrosine kinase inhibitors (e.g., gefitinib, ceritinib, rociletinib), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), and mammalian target of rapamycin (mTOR) inhibitors (buparlisib, afuresertib, everolimus, respectively), programmed death-1 (PD-1) inhibitors (e.g., nivolumab)[22]. Olaparib-related hyperglycemia is a common metabolic side effect observed in 1-10% of patients[23]. Currently, there is no data explaining the effect of olaparib on carbohydrate metabolism. Xia et al[24] conducted an *in vitro* study on olaparib and observed that it significantly enhanced the secretion of GLP-1 (glucagon-like peptide 1). Moreover, it is postulated that on the one hand, PARP activation reduces the rate of glycolysis, but on the other hand, it may lead to acute endothelial dysfunction in the blood vessels of diabetic patients by oxidative/nitrosative stress – PARP pathway. The authors stress that the inhibition of PARP may have a protective effect on the microvessels of human diabetic subjects[25]. However, the mechanism of the potential hyperglycemic effect of olaparib is not clear. Three measurements of the C_{trough} of the patient with prediabetes showed values 493.77; 760.95; and 799.53 ng/mL (Figure 1).

In order to exclude the influence of the drugs taken by the patients with comorbidities on the concentration of olaparib, the patients' pharmacotherapy was also analyzed for drug interactions. Olaparib is metabolized by CYP3A4/5, and in vitro it is also a substrate for P-glycoprotein. Therefore, there is a high risk of drug interactions. If olaparib is taken with potent inhibitors, both drugs (e.g., itraconazole) and juices (e.g., grapefruit juice, Seville oranges), its level may increase and adverse reactions may occur, e.g. diarrhea, nausea, vomiting, dyspepsia, abdominal pain, anorexia, paleness, fatigue, dizziness, fainting, unusual bruising or bleeding, fever, chills, sore throat, muscle pain, blood in phlegm, shortness of breath, red or inflamed skin, body sores, and pain or burning during urination[23,26]. Patients should be aware of these symptoms. On the other hand, strong inducers of CYP3A4 (e.g., rifampin) may lead to reduced exposure to the drug and its faster elimination from the system, which will lower the efficacy of anticancer therapy[26]. There were no interactions between the analyzed drugs, but this does not necessarily mean they do not exist, because olaparib is a new drug and many interactions are still unknown[23]. One of the drugs taken by the patients was anastrozole, which is a CYP3A4 inhibitor, but no clinically significant interactions were observed. There was only a moderate interaction between olaparib and rosuvastatin. Olaparib may increase the concentration of statin, so patients should be alerted to potential adverse reactions related to myopathy (change

in the urine color, muscle pain, muscle weakness) or rhabdomyolysis (increase in creatine kinase)[23]. Interactions between olaparib and other co-administered drugs should be monitored due to the induction of cytochrome P450 1A2, 2B6, 3A4, 2C9, 2C19 (CYP1A2, 2B6, 3A4, 2C9, 2C19), and P-glycoprotein by olaparib[27]. The association between the C_{trough} and the drugs given before PARP-inhibitors was also verified. There is a study showing that pretreatment of carboplatin can induce elevation of PARP and lower plasma concentration of olaparib relative to monotherapy[28].

Conclusions

Nowadays, in the oncological guidelines it is important to individualize the treatment as much as possible to have the best oncological results with minimum side effects. Unfortunately, oncologists still do not have recommendations of treatment depending on efficient plasma concentrations of the PARP inhibitors. The study revealed big interpatient variability of olaparib C_{trough} in analyzed patients with ovarian cancer, no correlation with anemia degree, but there was correlation between C_{trough}, expressed as ng/ml, ng/ml/mg or ng/ml/mg/kg, and fatigue degree. Patients with neutropenia had statistically significant lower plasma concentrations of olaparib. All the results showed that C_{trough}, expressed as ng/ml, ng/ml/mg or ng/ml/mg/kg, is not worthy for anemia but it could be used as the prognostic factor for fatigue during olaparib treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

The retrospective study was conducted at the Gynecological Oncology Division of Poznan University of Medical Sciences, and the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań, Poland with the approval from the Bioethics Committee, University of Medical Sciences, Poznań, Poland (697/20).

Informed Consent

The informed consent was signed by each of the patients whose blood was taken during the study.

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Authors' Contribution

The study concept and design were prepared by Joanna Stanisławiak-Rudowicz, Edyta Szałek. Data acquisition was prepared by Joanna Stanisławiak-Rudowicz. Validation of analytical method and measurements of drug concentrations were done by Marta Grzebalska, Hanna Urjasz. Data analysis and interpretation were prepared by Joanna Stanisławiak-Rudowicz, Edyta Szałek, and Michał Michalak. Writing of manuscript were prepared by Joanna Stanisławiak-Rudowicz, Edyta Szałek and Marta Grzebalska Final corrections were done by Radosław Mądry and Edmund Grześkowiak.

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Data Availability

The data supporting this article are available from the corresponding author on reasonable request.

References

1) Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott C, Meier W, Shapira-Frommet R, Safra T, Matei D, Macpherson E, Watkins C, Carmichael J, Matulonis U. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med 2012; 366: 1382-1392.
2) Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, Matei D, Fielding A, Spencer S, Dougherty B, Orr M, Hodgson D, Barrett JC, Matulonis U. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014; 15: 852-861.
3) Basta A, Bidziński M, Bieńkiewicz A, Blecharz P, Bodnar L, Jach R, Knapp P, Kojs Z, Kotarski J, Markowska J, Misiek M, Sznurkowski JJ, Wicherek Ł, Sawicki W, Timorek-Lemieszczuk A, Bachrycz J, Mądry R. Zalecenia Polskiego Towarzystwa Ginekologii Onkologicznej dotyczące diagnostyki i leczenia raka jajnika. Curr Gynecol Oncol 2017; 15: 5-23.

4) de Bono J, Mateo J, Fizazi K, Saas F, Shore N Sandhu S, Chi KN, Santor O, Agwal N, Olmos D, Thiery-Vuillemin A, Twardowski P, Mehra N, Goessl C, Kang J, Burgents J, Wu W, Kohlmann A, Adelman CA, Hussain MA. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med 2020; 382: 2091-2102.

5) Golan T, Hammel P, Reni M, van Cutsem E, Macarulla T, Hall MJ, Park J-O, Hochhauser D, Arnold D, Oh D-Y, Reinacher-Schick A, Tortora G, Algul H, O'Relly EM, McGuinness D, Cui KY, Schlienger K, Locker GY, Kindler HL. Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer. N Engl J Med 2019; 381: 317-327.

6) Wiśnik E, Ryksa M, Koter-Michalak M. Inhibitory PARP 1: współczesne próby zastosowania w terapii przeciwnowotworowej i perspektywy na przyszłość. Postepy Hig Med Dosw (online) 2016; 70: 280-294.

7) Summary of Lynparza Product Characteristics. Available at: https://www.ema.europa.eu/en/documents/product-information/lynparza-epar-product-information_en.pdf.

8) National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). Available at: http://ctep.info.nih.gov.

9) Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009 Rev.1 Corr. 2**). London 2011; 7.

10) Ledermann JA, Harter P, Gourley C, Fiedlander M, Vergote I, Rustin G, Scott C, Meier W, Shapira-Frommer R, Safra T, Matei D, Fielding A, Spencer S, Rowe P, Lowe E, Hodgson D, Sovak MA, Matulonis U. Overall survival in patients with platinum-sensitive recurrent serous ovarian cancer receiving olaparib maintenance monotherapy: an updated analysis from a randomised, placebo-controlled, double-blind, phase 2 trial. Lancet Oncol 2016; 17: 1579-1589.

11) Penson RT, Valencia RV, Cibula D, Colombo N, Leath CA 3rd, Bidziński M, Kim JW, Nam JH, Madry R, Hernández C, Mora PAR, Ryu SY, Milenkova T, Lowe ES, Barker L, Scambia G. Olaparib Versus Nonplatinum Chemotherapy in Patients With Platinum-Sensitive Relapsed Ovarian Cancer and a Germline BRCA1/2 Mutation (SOLO3): A Randomized Phase III Trial. J Clin Oncol 2020; 38: 1164-1174.

12) Moore K, Colombo N, Scambia G, Kim B-G, Oaknin A Friedlander M, Lisyanskaya A, Floquet A, Leary A, Sonke GS, Gourley C, Banerjee S, Oza A, González-Martín A, Aghajanian C, Bradley W, Mathews C, Liu J, Lowe ES, Bloomfield R, DiSilvestro P. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med 2018; 379: 2495-2505.

13) Velev M, Puszkiel A, Blanchet B, de Percin S, Delanoy N, Medioni J, Gervais C, Balakirouchenane D, Khoudour N, Pautier P, Leary A, Ajgal Z, Hirsch L, Goldwasser, Alexandrere J, Beinse G. Association between Olaparib Exposure and Early Toxicity in BRCA-Mutated Ovarian Cancer Patients: Results from a Retrospective Multicenter Study. Pharmaceuticals (Basel) 2021; 14: 804-820.

14) Mateo J, Moreno V, Gupta A, Kaye SB, Dean E, Middleton MR, Freidlander M, Gourley C, Plummer R, Rustin G, Sessa C, Leunenn K, Ledermann J, Swaisland H, Figg A, Banister W, Nicum S, Molife LR. An Adaptive Study to Determine the Optimal Dose of the Tablet Formulation of the PARP Inhibitor Olaparib. Target Oncol 2016; 11: 401-415.

15) Hartvig P, Aulin J, Hugerth M, Wallenberg S, Wagenius G. Fatigue in cancer patients treated with cytotoxic drugs. J Oncol Pharm Pract 2006; 12: 155-164.

16) Madariaga A, Bowering V, Ahrari S, Oza AM, Lheureux S. Manage wisely: poly (ADP-ribose) polymerase inhibitor (PARPi) treatment and adverse events. Int J Gynecol Cancer 2020; 30: 903-915.

17) Hartvig P, Aulin J, Wallenberg S, Wagenius G. Physical exercise for cytotoxic drug-induced fatigue. J Oncol Pharm Pract 2006; 12: 183-191.

18) Grisay G, Percy C, Pierrard J, Seront E. A rare but life-threatening pneumopathy induced by Olaparib: From a clinical case to a review of literature. J Transl Sci 2019; 6: 1-3.

19) Ke Y, Zhang J, Lv X, Zeng X, Ba X. Novel insights into PARPs in gene expression: regulation of RNA metabolism. Cell Mol Life Sci 2019; 76: 3283-3299.

20) Kiefmann R, Heckel K, Doerger M, Schenkat S, Kupatt C, Stoeckelhuber M, Wesierska-Gadek J, Goetz AE. Role of PARP on iNOS pathway during endotoxin-induced acute lung injury. Intensive Care Med 2004; 30: 1421-1431.

21) Kumar Rai D, Sharma P, Kumar R. Post covid 19 pulmonary fibrosis. Is it real threat? Indian J Tuberc 2021; 68: 330-333.

22) Goldman JW, Mendenhall MA, Rettinger SR. Hyperglycemia Associated with Targeted Oncologic Treatment. Mechanisms and Management. Oncologist 2016; 21: 1326-1336.

23) Find Drugs & Conditions. Available at: www.drugs.com.

24) Xia Q, Lu S, Ostrovsky J, McCormack SE, Falk MJ, Grant SFA. PARP-1 Inhibition Rescues Short Lifespan in Hyperglycemic C. Elegans and Improves GLP-1 Secretion in Human Cells. Aging Dis 2018; 9: 17-30.

25) Pacher P, Szabo S. Role of Poly(ADP-Ribose) Polymerase-1 Activation in the Pathogenesis of Diabetic Complications: Endothelial Dysfunction, as a Common Underlying Theme. Antioxid Redox Signal 2005; 7: 1568-1580.

26) Dirix L, Swaisland H, Verheul H M V, Rottey S, Leunen K, Jerusalem G, Rolfo C, Nielsen D, Molife LR, Kristeleit R, de Vos-Geelen J, Mau-Sørensen M, Soetekouw P, van Herpen C, Fielding A, So K, Bannister W, Plummer R. Effect of Itraconazole and Rifampin on the Pharmacokinetics of Olaparib in Patients with Advanced Solid

Tumors: Results of Two Phase I Open-label Studies. Clin Ther 2016; 38: 2286-2299.

27) Valabrega G, Scotto G, Tuninetti V, Pani, Scaglione F. Differences in PARP Inhibitors for the Treatment of Ovarian Cancer: Mechanisms of Action, Pharmacology, Safety, and Efficacy. Int J Mol Sci 2021; 22: 4203-4219.

28) Peer CJ, Lee JM, Roth J, Rodgers L, Nguyen J, Annunziata CM, Minasian L, Kohn EC, Figg WD. Population pharmacokinetic analyses of the effect of carboplatin pretreatment on olaparib in recurrent or refractory women's cancers. Cancer Chemother Pharmacol 2017; 80: 165-175.