DETERMINATION OF CISPLATIN IN HUMAN BLOOD PLASMA AND URINE USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY FOR ONCOLOGICAL PATIENTS WITH A VARIETY OF FATTY TISSUE MASS FOR PREDICTION OF TOXICITY

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Aim: The research was aimed to analyze a level of triglycerides in blood serum as a possible new marker of toxicity, particularly in patients with excess body weight, receiving cisplatin. *Materials and Methods:* Study involved 20 oncological patients with stage III lung cancer, who received palliative treatment with cisplatin. High-performance liquid chromatography was used for quantitative determination of pure cisplatin in urine and blood samples. Cisplatin concentration of the test samples was determined based on the data obtained from the calibration graph. *Results:* Quantitative determination of pure cisplatin is quite complicated. The elimination half-time for one of the groups was observed higher almost by half than for other patients. Higher dose of cisplatin showed a significant association with increase in triglyceride levels. We found a close correlation between body mass index and triglyceride changes during chemotherapy ($p = 0.001$; $r = 0.67$). The results indicate that a higher body mass index gives higher fluctuations of triglyceride levels in blood serum. Analyses of correlation between level of triglycerides and elimination half-time show that by an increase in the level of triglycerides in the blood serum cisplatin elimination half-time is prolonged (R^2 Linear = 0.596). Cisplatin concentration in urine is higher and elimination takes longer time at elevated levels of triglycerides, where close correlation between fraction of excreted substance in urine and concentration parameters was seen ($p < 0.01$). Also good correlation for body mass index with fraction of excreted substance in urine and concentration parameters was observed ($p < 0.05$). *Conclusion:* Clearance of cisplatin, which was determined by the chromatographic method, is reduced in individuals with increased adipose tissue mass. Research data suggest that overweight affects cisplatin elimination from the body. The greater body fat mass can contribute to a greater rise of triglyceride level in blood serum. Triglycerides in blood plasma may serve as an additional indicator of higher cisplatin toxicity as a cardiotoxicity marker.
Key Words: cancer, chemotherapy, cisplatin, triglycerides, chromatographic method.

Cancer is a major burden of disease worldwide. Each year, tens of millions of people are diagnosed with cancer around the world, and more than half of the patients eventually die from it. In many countries, cancer ranks the second most common cause of death following cardiovascular diseases [1].

Increase in cancer incidence is due to the aging of the population (in most cases, the tumors are diagnosed in older people), as well as the high prevalence of risk factors. The risk factors that have a significant impact on mortality from malignant tumors are: an in-

creased body mass index (BMI), inadequate fruit and vegetable consumption, low physical activity, tobacco and alcohol consumption, air pollution, unsafe sex, various manipulations made in inappropriate environment and circumstances (preventive and therapeutic injections, tattooing, etc.). World Health Organization (WHO) data show that 35% of cancer deaths are caused by these risk factors [2].

Survival rates of cancer patients are affected by various factors, such as tumor localization, clinically morphological risk factors (patient age, tumor morphological variant, tumor differentiation grade, stage of disease, reproductive factors, etc.), the patient's general health, co-existing conditions, specific anti-cancer treatment options.

Chemotherapy is one of cancer treatment options. In chemotherapy, anticancer agents that kill the cancer cells in the body are used. Even if the cancer is removed with a surgery, cancer cells may be located in nearby tissue or elsewhere in the body. The aim of chemotherapy is to treat cancer in several ways: either to reduce tumor volume before surgery or irradiation, or eradicate cancer cells that may be dispersed in the surrounding tissues or organs after tumor surgery (i.e. preventive chemotherapy), or relieve cancer symptoms (such as pain), or to con-

trol the tumor growth if the tumor has already spread (palliative therapy).

Each chemotherapy drug has its own internationally defined dose of administration, which is calculated by taking into account body square meters. Dose of drug is calculated individually for each patient depending on body weight and height. The patient's general condition and co-morbidity is also taken into account. Individuals respond differently to received chemotherapy and this response may have significant clinical importance. Calculation of a definite dose of chemotherapy reduces the possible toxic reactions of drugs [3]. There are 5 basic conditions that determine treatment tactics, i.e., the right medication, right patient, right dosage, right administration route and right timing [4]. This helps a physician to make therapy more rational and avoid therapeutic errors, as too small doses of medication have no therapeutic effect and that affects overall survival and disease-free period, while too high dose increases the toxic effects in the body for up to a possible death.

High-performance liquid chromatography (HPLC) is irreplaceable modern analytical method for the separation and identification of different chemical compounds in both simple and in highly complex test samples. Its main advantage is speed and accuracy. HPLC is currently the most popular method in biochemistry, in particular for pharmacokinetic and pharmacodynamic studies. When the smaller particle size of separation column is used (ultra high-performance liquid chromatography — UHPLC), separation method goes more effective and more sensitive. UHPLC, with its shorter analysis time and quicker column equilibration, is ideally suited to rapid method development.

MATERIALS AND METHODS

Patients. Patient group with 20 cancer patients was established for determination of cisplatin in biological liquids — blood and urine. The stage III lung cancer patients who received palliative treatment with cisplatin were selected for this study. Based on BMI patients were divided into two groups. One group were patients where the BMI < 29.0 kg/m^2, another of BMI > 29.0 kg/m^2. Patients received a dose of cisplatin calculated at 75 mg/m^2. The patient blood serum triglyceride levels additional was to evaluated the pharmacokinetics of cisplatin relationship with body fat mass.

The *serum material* for determination of cisplatin was collected as follows — blood plasma (2 ml, purple tube), 0 (prior to treatment), 20, 40, 60, 120 and 180 min, 24, 48 and 72 h after the end of the infusion (duration of cisplatin infusion 1.00 h). A sample of blood allowed clotting for 30 min at the room temperature, then centrifuged for 10 min × 2500 rpm, then frozen at −20 °C till further analyses.

Cisplatin analysis by high-performance liquid chromatography-mass spectrometry (HPLC-MS). HPLC combined with mass spectrometry (MS) was used to determine the presence of cisplatin in blood and urine samples. All solvents used were of analytical grade. Acetonitrile and formic acid were purchased from Sigma-Aldrich (St. Louis, USA). The used deionized water (18.2 MΩ) was prepared by a Milli-Q water purification system from Millipore (Billerica, Massachusetts, USA). Chromatographic analyses performed on a modular UHPLC system, Agilent *1290 Infinity* series (Agilent Technologies). Liquid chromatography (LC) separations achieved by using an Extend-C18 (Agilent) column 2.1 × 15 mm, 1.8 µm. Elution solvents consist of 0.1% formic acid in acetonitrile and 0.1% formic acid in water in gradient mode at flow rate 250 µl·min^{-1}. The injection volume was 1.0 µl. The high-resolution mass spectra (HRMS) were taken, respectively, on an Agilent 6230 TOF LC/MS (Agilent Technologies, Germany) with electrospray ionization. MS operating conditions were as following: positive ionization mode, gas temperature of 325 °C, nitrogen flow rate of 10 l/min, nebulizer pressure 40 psi, capillary voltage 3500 V and applied fragmentor was 100 V. Internal reference mass 121.050873 m/z and 922.009798 m/z (G1969-85001 ES-TOF Reference Mass Solution Kit, Agilent Technologies & Supelco) for all sample analyses were used. One full mass spectrum was acquired in profile mode, with mass range from *m/z* 50 to 1000.

Direct infusion of the cisplatin showed that there are no detectable ions found for cisplatin. Peak detection and spectrum extraction were performed with MassHunter 5.00 Software (Agilent).

Sample and standard preparation. Frozen cisplatin-plasma samples were thawed, 5% diethyldithiocarbamate (DDTC) 0.1N NaOH solution was prepared. Cisplatin derivatives were prepared as follows — to 500 µl of plasma were added 100 µl of 5% DDTC solution. The sample was homogenized by vortexing approximately 15–20 s and incubated for 15 min at 45 °C. After 15 min 1400 µl of 70% acetonitrile were added to the sample. The sample was homogenized by vortexing about 15–20 s, and then placed into a centrifuge for 15 min at 10,000 rpm. A 1.0 µl resulting upper layer aliquot was injected into the liquid chromatography-mass spectrometry (LC-MS) system. The standard solutions of cisplatin derivative were prepared in the following concentrations: 0.5; 1.0; 5.0; 10.0; 25.0; 50.0 and 100.0 µg/ml in acetonitrile. Solutions of each concentration were injected into LC-MS system. All experiments were performed in triplicate. Calibration curves of standard solutions were constructed by plotting the average peak area against concentration, and a regression equation was computed. A mixture of standard solutions was injected three times and the corresponding peak areas were recorded. The relative standard deviation was determined to be less than 1%. The obtained calibration curve showed linearity of correlation coefficient (R^2) in the concentration range 0.99994.

Urine sample was collected for 6 h, 12 h, 24 h (Day 1), 48 h, 72 h (Day 3) and 96 h (Day 5) after received cisplatin infusion. Collected urine for ice — 20 °C.

Ethical aspects. Positive opinion of the trial preparation and development given the Latvian University of Research Institute of Cardiology clinical-physiological study of medicinal and pharmaceutical products of clinical research ethics committee. Before the study, the patients were informed about the details of the study and approved the voluntary consent to participate in it. The study respondents of maintained confidentiality and anonymity.

Statistical analysis. Patient data were analysed using descriptive statistical methods, making a comparative analysis (*t*-test, *chi*-square (χ^2) test by correlation analysis (Pearson correlation coefficient)) using Microsoft Excel 2007 and SPSS software.

The aim of statistical analysis of research data was to evaluate the validity and theoretical probability distributions of the resulting measurements (distance, thickness, circumference and weight), as well as follow up on the statistical hypotheses. Therefore, the common (popular) descriptive statistical methods were very widely used in this study.

The variables, measured in relation scale and normally distributed (by Gaussian normal distribution) were analysed using parametric statistics methods. In other cases, non-parametric statistical methods were used. Hypotheses about the adequacy of the data to the normal probability distribution were mainly tested by Kolmogorov — Smirnov test. The Student's *t*-test was used to test uniformity of two arithmetic mean groups, for three or more groups of arithmetic mean the analysis of variance (ANOVA) was used. For comparison of the number of cases different statistical methods were used, such as χ^2-test and Fisher's exact test. The correlation and linear regression methods were used to predict the interaction between different variables and event analysis.

For statistical data processing first a database was created in software MS Excel, and then the data were converted for professional research data statistical processing program SPSS (Statistical Pacade for Social Sciences) version 16.0 for Windows. All hypothesis tests used a duplex (2-tailed) statistical hypotheses and hypothesis was rejected if the probability (significance level) was < 5% or $p < 0.05$.

The statistical software Statistica 6.0 was also used for processing of the data. In cases where data did not correspond to the normal distribution, a data transformation was performed, but after statistical analyses — opposite transformation. Non-parametric Spearman rank correlation was done in case of qualitative data. Differences between groups, correlations and regressions were considered significant, if $p < 0.05$.

RESULTS

HPLC-MS is an analytic chemical method, which combines physical separation of a blend by LC and mass analysis of a substance by MS. LC-MS analytical method is characterized by very high sensitivity and specificity. By using time of flight (TOF) detector, it is possible to determine compounds of highly complex samples according to the mole mass, avoiding matrix effect interference.

Quantitative determination of pure cisplatin is quite complicated. Cisplatin as a chemical compound has not typical ultraviolet absorption therefore derivatisation should be performed by sodium DDTC in order to determine cisplatin by HPLC. This obtained compound absorbs ultraviolet at 254 nm and is determined using LC.

Direct infusion of the cisplatin showed that there are no detectable ions found for cisplatin. The direct infusion into MS system of cisplatin DDTC derivative generate full scan spectra with a predominant ion at m/z 492 and m/z 640 correspond to $[Pt(DDTC)_2]^+$ and $[Pt(DDTC)_3]^+$ ions respectively (Fig. 1). This suggests that after DDTC derivatization, cisplatin is converted to Pt-$(DDTC)_2$ and Pt-$(DDTC)_3$.
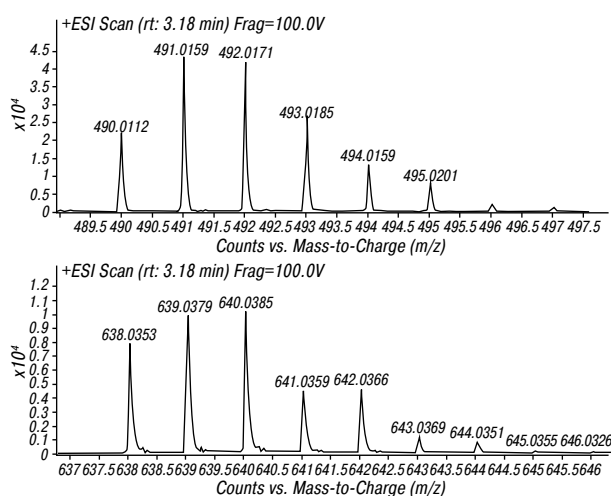

Fig. 1. High-resolution mass spectrum of cisplatin DDTC derivative from the standard solution, obtained by Agilent 6230 TOF LC/MS

Fig. 1 shows the magnified mass spectrum of a DDTC derivative of cisplatin from the standard solution, which is very close to spectrum from study by Yaroshenko *et al*. [5]. Other signals in mass spectra correspond to the signals formed by ions of different Pt isotopes.

In our further experiments cisplatin DDTC derivate which refers to formation of $[Pt(DDTC)_3]^+$ ion was used because of its higher intensity.

The base peak chromatogram in Fig. 2 shows the signal of peak from the most intense mass in mass spectrum of m/z 640, plotted versus time.
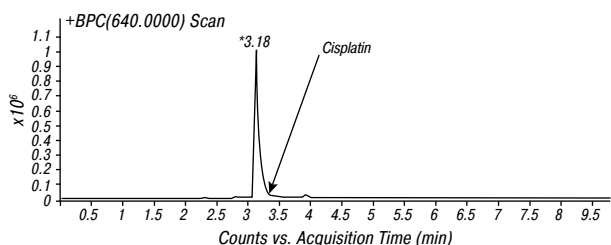

Fig. 2. Base peak chromatogram at m/z 640 of cisplatin DDTC derivative Pt-$(DDTC)_3$ in patient's P01 urine after 12 h

Pharmacokinetic measurements of cisplatin in patients' blood serum. The calibration curve

was designed from standard solutions of cisplatin derivative and cisplatin concentrations in µg/ml were determined from the standard curve for each sample of patient serum. Concentrations were converted into mg/l for calculations of pharmacokinetic data of cisplatin. From the chromatograms of test samples during analyses it was concluded that the selected method can be used in quantitative determination of cisplatin in human blood serum. Separated peaks were symmetrical and did not overlap with other compounds existing in the matrix.

Changes of cisplatin concentration in blood serum at different time intervals were identified, where the key was to find the maximum concentration of cisplatin in certain period of time (C_{max}). In this study C_{max} of cisplatin was reached 2 h after cisplatin injection.

Small differences were observed in the elimination half-times ($t_{1/2}$) for patients involved in this study. The $t_{1/2}$ for one of the groups (P13; P14; P15 and P16) was observed higher almost by half than for other patients. This could be explained by patients being overweight as the average weight for this group was 97.21 kg. Data on the $t_{1/2}$ for other patients were not significantly different, they were calculated as average values and applied to the calculations of following pharmacokinetic parameters for all patients (Table 1).

From the equations that were calculated and obtained using Excel 2013 software, individual elimination constants (k_{el}) were calculated and shown as the degree in equation (see Table 1). They are presented with a negative (−) sign, because it is a process of elimination, and k_{el} indicates the percentage (%) of cisplatin eliminated per hour.

Table 1. Pharmacokinetic data of cisplatin. Calculated individual k_{el} presented as the degree in equation with a negative (−) sign, because it is a process of elimination and k_{el} indicates the percentage (%) of cisplatin eliminated per hour

Patients	Equation	C(0), mg/l	$t_{1/2}$ of I phase, h	AUC, mg·h/l	CL, l/h	$t_{1/2}$, h
P01	$y = 1.17e^{-0.035x}$	1.17	16.90	33.43	3.74	19.80
P02	$y = 1.40e^{-0.025x}$	1.40	22.35	56.00	2.23	27.72
P03	$y = 0.73e^{-0.026x}$	0.73	11.95	28.08	4.45	26.65
P04	$y = 0.85e^{-0.024x}$	0.85	18.24	35.42	3.53	28.88
P05	$y = 0.91e^{-0.036x}$	0.91	12.60	25.28	5.93	19.25
P06	$y = 0.74e^{-0.022x}$	0.74	16.12	33.64	4.46	31.50
P07	$y = 1.58e^{-0.038x}$	1.58	18.24	41.58	3.61	20.38
P08	$y = 0.76e^{-0.023x}$	0.76	15.40	33.04	4.54	30.13
P09	$y = 1.72e^{-0.021x}$	1.72	15.07	81.90	1.59	33.00
P10	$y = 2.22e^{-0.028x}$	2.22	16.90	79.29	1.64	24.75
P11	$y = 1.92e^{-0.023x}$	1.92	27.72	83.48	1.56	30.13
P12	$y = 2.24e^{-0.027x}$	2.24	23.90	82.96	1.57	25.67
P13	$y = 0.45e^{-0.011x}$	0.45	8.06	40.91	3.18	63.00
P14	$y = 0.69e^{-0.014x}$	0.69	9.76	49.29	2.64	49.50
P15	$y = 0.62e^{-0.015x}$	0.62	9.36	41.33	3.15	46.20
P16	$y = 0.74e^{-0.019x}$	0.74	9.76	38.95	3.34	36.47
P17	$y = 0.98e^{-0.018x}$	0.98	38.50	54.44	2.39	38.50
P18	$y = 1.07e^{-0.022x}$	1.07	31.50	48.64	2.67	43.31
P19	$y = 0.97e^{-0.019x}$	0.97	36.47	51.05	2.55	46.20
P20	$y = 1.17e^{-0.022x}$	1.17	31.50	53.18	2.44	38.50

Note: C – concentration; $t_{1/2}$ – elimination half-time; AUC – area under the concentration curve; CL – clearance.

The elimination rate constant k_{el} was expressed from the established equation and then used to calculate the total area under the concentration curve (AUC) and the clearance (discharge of blood plasma volumes from the amount of substance per unit of time). Within

the framework of our work, the AUC results ranged from 25.28 to 83.48 mg·h/l (see Table 1). The mean AUC result for all oncological patients, receiving cisplatin at a dose of 125–150 mg, was 49.59 mg·h/l. Clearance estimates were summarized in Table 1. The results show that the clearance during treatment ranged 1.56–4.54 l/h. These results were very similar to the literature data, leading to the conclusion that results of our pharmacokinetic data are true and can be used for further research.

One of the pharmacokinetic parameters is the mean substance resistance time in the body (MRT — *mean residence time*). MRT = $1/k_{el}$, but since k_{el} = $0.693/t_{1/2}$, then the formula is = 1.44 x $t_{1/2}$ (one divided by 0.693 equals 1.44). The mean $t_{1/2}$ in elimination phase is 33.98 h, the average MRT was 48.93 h.

Determination of cisplatin in urine. Using the resulting calibration graph, amount of cisplatin in urine was calculated for all patients. Based on the concentration in the urine, which was determined for 20 patients, concentration curves were obtained. To show how visually the curves look for patients depending on the dose, they were divided into groups of patients, depending on the received chemotherapy dose. Accordingly, three groups of patients were acquired.

Pharmacokinetic parameters such as renal clearance, fraction of excreted substance in percentage and creatinine clearance were defined. It was observed that the AUC was higher for patients on the first day compared to the last day. Respectively, for those patients who received 130 mg or greater dose of cisplatin, the AUC lowered slowly in last two days of the study days. There are no data available to explain it. Perhaps this is related to the significant untapped biochemical processes, with cisplatin binding to molecules that hold cisplatin and do not let it to be removed gradually, as well as with cisplatin metabolism, which still has not been fully understandable. At the cisplatin dose of 130 mg and above, there was a change in the fraction of excreted substance (%) by increase. Literature states that cisplatin excretes with urine very long time after the end of therapy, such as for patients receiving chemotherapy for a long time and who were followed for more than 10 years, in the urine were excreted 1.26 ± 0.50 41 µg Pt after 24 h [6]. It would be useful to determine cisplatin urinary excretion for patients involved in the study after a long period of time, in parallel to determination of renal functions. This would allow the assessment of renal status, if it is not affected by the long cisplatin elimination after completion of therapy, or to determine the renal regeneration.

Changes of triglyceride levels in blood serum in patients receiving cisplatin therapy. Higher dose of cisplatin showed a significant association with increase in triglyceride levels. Also higher body fat mass showed a relationship with increase in triglyceride levels. According to clinical tests, triglyceride levels in blood serum changed 24 h after received cisplatin therapy. The highest triglyceride levels were reached 48–72 h after treatment.

It was observed that triglycerides have a correlation with thickness of fatty tissue folds. The closest correlation was with the thickness of fat tissue fold under the shoulder blade ($p < 0.001$; $r = 0.56$). We found a close correlation between BMI and triglyceride changes during chemotherapy ($p = 0.001$; $r = 0.67$). The results indicate that a higher BMI gives higher fluctuations of triglyceride levels in blood serum.

Levels of triglycerides in the blood serum increased more at higher doses of cisplatin (Table 2). Higher triglyceride changes were observed at higher doses of cisplatin or greater dose differences. Increase of triglycerides in blood serum correlated with the person's weight, especially the adipose tissue mass, greater changes were with increased weight. In general, gender has not such a big role in the cisplatin dose application, but fat tissue mass is essential. Amount of adipose tissue mass contributes to an increase in serum triglycerides. In conclusion, the greater body fat mass can contribute to a greater rise of triglyceride level in blood serum ($p < 0.001$).

Separately were analysed the data of 20 oncological patients, splitting them into two groups depending on the BMI $\geqslant 29$ kg/m^2 (see Table 2).

It was observed by Pearson correlation that the level of triglycerides in the blood affects the $t_{1/2}$ of cisplatin ($p < 0.01$). It is likely that cisplatin is eliminated more slowly from the blood serum at higher levels of triglycerides. Analyses of correlation between level of triglycerides and $t_{1/2}$ show that by an increase in the level of triglycerides in the blood serum cisplatin $t_{1/2}$ is prolonged (R^2 Linear = 0.596) (Fig. 3).
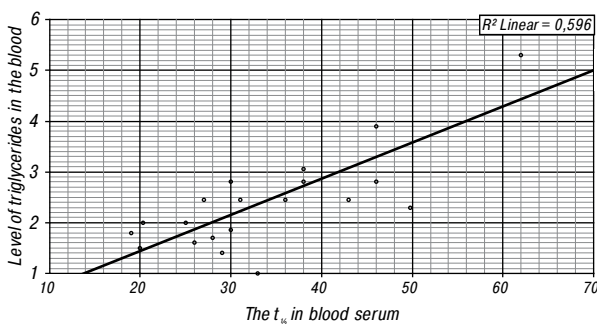

Fig. 3. Correlation between the levels of triglycerides and the $t_{1/2}$ of cisplatin

Also cisplatin concentration in urine is higher and elimination takes longer time at elevated levels of triglycerides (see Table 2), where close correlation between fe (%) and concentration parameters was seen ($p < 0.01$). Also good correlation for BMI with f_e (%) and concentration parameters was observed ($p < 0.05$), suggesting that overweight affects cisplatin elimination from the body. It is concluded that cisplatin from the serum and urine is excreted more slowly in patients with obesity and also maintains a lasting increase of triglycerides in the blood. There are no research data in the literature at the moment for comparison.

Table 2. Analysis of triglycerides, pharmacokinetic and anthropometric data of patients receiving cisplatin therapy

		BMI, kg/m^2	Dose of cisplatin 75 mg/m^2	Trigly-cerides in blood serum 1	Trigly-cerides in blood serum 2	$t_{1/2}$, h	CL, l/h	C, mg/l	f_e, %
BMI, kg/m^2	Pearson correlation	1	−0.008	0.060	0.284	0.255	0.227	**0.507***	**0.502***
	Sig. (2-tailed)		0.973	0.802	0.225	0.278	0.337	0.023	0.024
	N	20	20	20	20	20	20	20	20
Dose of cisplatin 75 mg/m^2	Pearson correlation	−0.008	1	0.105	0.016	−0.275	0.589**	0.381	0.389
	Sig. (2-tailed)	0.973		0.659	0.947	0.240	0.006	0.098	0.090
	N	20	20	20	20	20	20	20	20
Triglycerides in blood serum 1	Pearson correlation	0.060	0.105	1	0.798**	**0.731****	0.073	0.077	0.446*
	Sig. (2-tailed)	0.802	0.659		0.000	0.000	0.759	0.746	0.049
	N	20	20	20	20	20	20	20	20
Triglycerides in blood serum 2	Pearson correlation	0.284	0.016	0.798**	1	**0.772****	0.093	0.166	0.472*
	Sig. (2-tailed)	0.225	0.947	0.000		0.000	0.698	0.483	0.036
	N	20	20	20	20	20	20	20	20
$t_{1/2}$, h	Pearson correlation	0.255	−0.275	**0.731****	**0.772****	1	−0.239	−0.059	0.215
	Sig. (2-tailed)	0.278	0.240	0.000	0.000		00.311	0.804	0.362
	N	20	20	20	20	20	20	20	20
CL, l/h	Pearson correlation	0.227	0.589**	0.073	0.093	−0.239	1	**0.777****	**0.685****
	Sig. (2-tailed)	0.337	0.006	0.759	0.698	0.311		0.000	0.001
	N	20	20	20	20	20	20	20	20
C, mg/l	Pearson correlation	**0.507***	0.381	0.077	0.166	−0.059	**0.777****	1	0.823**
	Sig. (2-tailed)	0.023	0.098	0.746	0.483	0.804	0.000		0.000
	N	20	20	20	20	20	20	20	20
f_e, %	Pearson correlation	**0.502***	0.389	0.446*	0.472*	0.215	0.685**	**0.823****	1
	Sig. (2-tailed)	0.024	0.090	0.049	0.036	0.362	0.001	0.000	
	N	20	20	20	20	20	20	20	20

Note: *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). $t_{1/2}$ — elimination half-time — time in which the concentration of pharmaceutical agent in the blood (plasma, serum) reduces by half; CL, l/h — the liberation amount of plasma volume from the substance per unit of time; C — concentration of cisplatin in urine, mg/l; f_e, % — fraction of excreted substance in urine, which is usually expressed as percentage.

DISCUSSION

It is important to determine precisely a dose of chemotherapy agent as inadequate high dose of medication can cause serious side effects and even death, but too low dose reduces the effectiveness of treatment and overall survival. Accurate determination of the dose is still debatable both through already mentioned calculation formulas and by taking into account described toxicity of chemotherapy drugs, when the patient receives inadequate dose. Described studies on patients with obesity have trend not to prescribe the calculated dose, due to fear of side effects, but that is not always justified. It affirms the need for new and accurate methods of calculation for doses of chemotherapy drugs.

A LC-MS method was selected to prove that the body frame has a vital role in chemotherapy dosage, which would allow better prediction of the therapeutic toxicity. A new, innovative method was developed for derivatisation of cisplatin and following determination by MS with UHPLC-TOF [7], in cooperation with the Faculty of Chemistry at the University of Latvia.

Differences in the elimination of cisplatin were observed depending on the content of muscles or fat tissues in the body mass. It was observed that cisplatin is eliminated more slowly in patients with overweight (obesity) than in patients with normal body weight. Comparing results of the obtained total AUC with the scientific literature, we can conclude that they have a slight difference. In the scientific literature, which studied several groups of patients with different tumor types, including lung cancer, the AUC was calculated as the average of all the groups, which was 23.19 ± 2.052 mg·h/l [8, 9], but in our study the AUC results ranged $25.28–83.48$ mg·h/l. The mean AUC for all in our study involved oncological patients was 49.59 mg·h/l. The difference in results can be explained by the different doses. The peak concentration increases with increasing dose of cisplatin and therefore the total AUC is bigger. In research study, described in the literature, doses of cisplatin received ranged from 50 mg to 100 mg [8], but in this work have been studied and analysed the patients who received cisplatin at a doses of $125–150$ mg. Comparison of the cisplatin dose to AUC in both studies, a large difference between the results was not observed. In the literature reviewed study clearance calculations were carried out that during treatment ranged from 2.30 l/h up to 7.98 l/h, but in our calculations they were between 1.56 l/h and 4.54 l/h. These results were very similar to those in the described study, which suggests that pharmacokinetic data of our study are true and can be used for further research.

HPLC-MS is used for detection of a variety of substances and medicines in biological solutions. It was used in our study to determine the concentration of cisplatin from the constituent Cisplatin in the blood serum and urine of oncological patients. MS spectra with a signal at m/z 640, which corresponds to $[Pt(DDTC)_3]^+$ ions, were obtained during analysis of samples. Acquired bands were symmetrical and did not overlap with other existing matrix compounds.

A new, innovative method was developed for derivatisation of cisplatin and following determination by MS with UHPLC-TOF. Pharmacokinetic parameters of cisplatin were determined for twenty patients with lung cancer diagnosed in stage III. Cisplatin was measured by standard curve concentrations in mg/ml. Approximately at the average dose of 135 mg, the mean C_{max} in twenty patients was 2487.03 mg/ml, which was very close to the literature data. C_{max} for all patients ranged from 795.7 mg/ml up to 6429.1 mg/ml, this could be explained by a variety of doses administered individually to each patient. Elderly patients often have impaired renal function, which does not allow 100% cisplatin dose application in order to avoid serious adverse reactions. The $t_{1/2}$ was observed almost 50% higher for patient group (P13, P14, P15, and P16). That could be explained by the increase in body weight for this group (an average of 97.21 kg), so that proves the hypothesis that cisplatin is eliminated more slowly in patients with a higher body weight (obesity) than in patients with normal body weight. This confirmed one of our working hypotheses. Clearance during treatment ranged from 1.56 l/h to 4.54 l/h. These results were very similar to the literature data and suggest that resulting pharmacokinetic data in our study are true and can be used for further research.

Despite the fact that there are many studies on pharmacokinetics of cisplatin, many of these studies did not specify the pharmacokinetic data or often mentioned that the data are consistent with the norm. It was concluded from the review of literature data on the pharmacokinetics of cisplatin that there are no certain limits and averages, and the mechanism of action of cisplatin is not clear. There are no whole some data on the pharmacokinetics of cisplatin, which can be influenced by various factors such as the patient's overweight, age, gender, co-morbidities, drug storage and administration, etc.

It was concluded from results of our study that there are two significant factors that affected the level of triglycerides in the blood. One of them is the higher dose of cisplatin, the other is the larger body fat mass. Since patients received multiple courses of chemotherapy, then a gradual increase in triglycerides was observed with each subsequent dose of cisplatin, especially in patients with obesity. Positive correlation (Pearson) was found for triglyceride level changes, depending on the cisplatin dose, between the initial triglyceride levels and body fat, muscle and bone masses ($p < 0.001$). Increased dose of cisplatin showed a significant association with increased levels of triglycerides. Also, a higher body fat mass showed association with an increased level of triglycerides. Correlation was found between level of triglycerides and fat fold thickness. The closest correlation was with fat fold thickness under the shoulder blade ($p < 0.001$; r = 0.56). A close correlation was found between BMI and triglyceride changes during chemotherapy ($p = 0.001$; r = 0.67). Results indicate that higher BMI gives greater fluctuations of triglyceride levels in blood serum. The adipose tissue mass became more important for cisplatin dose determination in women ($p < 0.05$), although women were not the

biggest of statistical groups. Both the adipose tissue mass ($p < 0.001$) and muscle mass ($p < 0.05$) were essential in dose determination, especially at the dose differences ($p < 0.01$). Overall, the gender has not such a big role in the cisplatin dose application, essential is the fat tissue mass. Adipose tissue mass contributes to an increase in serum triglycerides.

Cisplatin is characterized by cumulation (accumulation) of dose, when part of the medication remains from the previous administration, particularly at doses higher than 100 mg/m^2 [10]. It should further be studied the relationship between cisplatin dose cumulation and an increase in triglyceride levels in the blood, and obesity. Associated between increased triglyceride levels and cisplatin treatment is unclear. It is important to mention that cancer patients have abnormal metabolic processes in the body. Cancer patients with weight loss have increased triglyceride and fatty acid metabolism in comparison with patients without weight loss [11]. An increased triglyceride concentration in blood plasma was observed in weight losing cancer patients compared to stable weight cancer patients, indicating on enhanced lipolysis [12]. This makes it difficult to precisely analyse changes in triglyceride levels in the blood serum.

Results of our study showed a significant relationship between the dose of cisplatin and the level of triglycerides in the blood serum. Also the study, carried out with stem cell tumors, mentioned that the level of triglycerides in the blood serum tended to increase in the group of cisplatin therapy patients, but this trend was not statistically significant [13]. However, in another study by a 5-year follow-up after cisplatin therapy of testicle tumor, there were no significant differences in plasma triglyceride levels among patients with cisplatin therapy and without it [14]. In the longer term cisplatin may have no effect on triglycerides in the blood plasma, as it has been observed in animal studies that a few days after cisplatin therapy there were elevated triglyceride levels in blood plasma and also in proximal renal tubules, thus causing cisplatin toxicity in the kidneys [15, 16]. This is an important factor for patients, especially in women with a high-carbohydrate diet, because additional accumulation of triglycerides as a result of overproduction of insulin may increase cisplatin nephrotoxicity [17, 18]. Additional research study is necessary to carry out on relationship of cisplatin pharmacokinetics with obesity and levels of triglycerides in the blood. Higher dose of cisplatin and increased body fat mass are important risk factors for elevated triglyceride levels in the blood serum. Determination of the level of triglycerides in blood plasma may serve as an additional indicator and toxicity marker for higher toxicity of cisplatin.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, *et al.* Global cancer statistics, 2002. CA Cancer J Clin 2005; **55**: 74–108.

2. World health report 2009. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: World Health Organization, 2009: 9.

3. Favier M, de Cazanove F, Saint-Martin F, *et al.* Preventing medication errors in antineoplastic therapy. Am J Hosp Pharm 1994; **51**: 832–3.

4. Hilan-Dandan R, Brunton LL, Goodman LS. Goodman and Gilman's Manual of Pharmacology and Therapeutics. McGraw-Hill, New York, 2014.

5. Yaroshenko DV, Grigoriev AV, Sidorova AA, *et al.* Chromatographic determination of sildenafil in blood plasma using spectrophotometric and mass-spectrometric detection. J Anal Chem 2013; **68**: 886–94.

6. Kawai Y, Taniuchi S, Okahara S, *et al.* Relationship between cisplatin or nedaplatin-induced nephrotoxicity and renal accumulation. Biol Pharm Bull 2005; **28**: 1385–8.

7. Dass C. Fundamentals of contemporary mass spectrometry. New Jersey: John Wiley & Sons, 2007.

8. de Jongh FE, Verweij J, Loos WJ, *et al.* Body-surface area-based dosing does not increase accuracy of predicting cisplatin exposure. J Clin Oncol 2001; **19**: 3733–9.

9. Cattel L, De Simone M, Passera R, *et al.* Pharmacokinetics of cisplatin in semi-closed hyperthermic peritoneal perfusion (HPP) for treatment of peritoneal carcinomatosis. Anticancer Res 2004; **24**: 2041–5.

10. Polovich M, White JM, Kelleher LO. Chemotherapy and biotherapy guidelines and recommendations for practice, 2nd ed. Pittsburg: Oncology Nursing Society, 2005.

11. Shaw JH, Wolfe RR. An integrated analysis of glucose, fat, and protein metabolism in severely traumatized patients. Studies in the basal state and the response to total parenteral nutrition. Ann Surg 1989; **209**: 63–72.

12. Drott C, Persson H, Lundholm K. Cardiovascular and metabolic response to adrenaline infusion in weight-losing patients with and without cancer. Clin Physiol 1989; **9**: 427–39.

13. Ellis PA, Fitzharris BM, George PM, *et al.* Fasting plasma lipid measurements following cisplatin chemotherapy in patients with germ cell tumors. J Clin Oncol 1992; **10**: 1609–14.

14. Koc G, Divrik TR, Unlu N, *et al.* Does cisplatin-based chemotherapy effect on blood lipid levels of patients with germ cell testicular tumor in long-term follow-up. Int Urol Nephrol 2011; **43**: 1095–100.

15. Johnson AC, Stahl A, Zager RA. Triglyceride accumulation in injured renal tubular cells: alterations in both synthetic and catabolic pathways. Kidney Int 2005; **67**: 2196–209.

16. Portilla D, Li S, Nagothu KK, *et al.* Metabolomic study of cisplatin-induced nephrotoxicity. Kidney Int 2006; **69**: 2194–204.

17. Ginsberg HN, Zhang YL, Hernandez-Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. Arch Med Res 2005; **36**: 232–40.

18. Dong MW, Zhang K. Ultra-high-pressure liquid chromatography (UHPLC) in method development. TrAC 2014; **63**: 21–30.