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CLINICAL-LABORATORY EVALUATION OF COAGULATION AND FIBRINOLYSIS IN PATIENTS WITH MALIGNANT DISEASES

ABSTRACT

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CONTENT

Abbreviations 4
I. Introduction 5
II. Aim and tasks 7
III. Materials and methods 8
1. Clinical material 8
2. Biological material 9
3. Methods 9
IV. Results and discussion 14
1. Breast cancer 14
2. Lung cancer 23
3. Non-Hodgkin's lymphoma (NHL) 32
4. Comparative analysis of markers of coagulation and fibrinolysis between patient groups. 41
5. Discussion 43
V. Conclusions 48
VI. Contributions 49
VII. Publications and patricipation in scientific forums 50

ABBREVIATIONS:

aPTT – activated partial thromboplastin time
AT III – Antithrombin III
DVT – deep venous thrombosis
FDP – fibrin degradated products
Fib – fibrinogen
F1+2 – prothrombin fragment 1+2
INR – International normalized ration
NHL – Non-Hodgkin's lymphoma
PT – prothrombin time
PTE – pulmonary thromboembolism
TAT – thrombin-antithrombin complex
TF – Tissue factor
t-PA – tissue plasminogen activator
TT – thrombin time
VTE – venous thromboembolism

I. INTRODUCTION

Malignant diseases are a large part in human pathology. According to the World Health Organization, they are the second cause of death in developed countries, giving way to cardiovascular diseases. A common complication in patients with malignant pathology is thrombosis. Already 130 years ago, the link between carcinogenesis and blood coagulation disorders was established. Approximately 50% of patients with malignant disease and over 90% of those with metastatic lesions show evidence of some abnormality in clotting and/or fibrinolysis, with thrombosis being the second most common cause of death in these patients. Clinically proven coagulation disorders are very often the first sign of malignancy. Changes in laboratory parameters that indicate activation of coagulation and fibrinolysis in carcinoma are a result of tumor growth, neoangiogenesis and impaired function of the affected organ. This applies mainly to the procoagulant activity of tumor cells, endothelial and inflammatory cells. In addition, there are abnormalities in the body's normal defense mechanisms - vascular-endothelial dysfunction, decreased circulating inhibitors, cell-associated anticoagulants and fibrinolytic activators. On the other hand, the use of chemotherapeutic drugs can also contribute to haemocoagulation disorders. Thus, coagulopathy and angiogenesis in the presence of carcinoma actually appear to be anatomically and functionally related. They predispose cancer patients to an increased risk of thrombotic events or bleeding complications.

In our country, there are still no systematic studies that focus on the clinical-laboratory evaluation of changes in the hemostasis system in malignant diseases. The present research is a modern and up-to-date attempt to contribute in this direction, to describe and follow up the changes in the laboratory markers of activation of coagulation and fibrinolysis - fibrinogen, thrombin-antithrombin complex (TAT), tissue factor (TF), prothrombin fragment 1+2 (F1+2), Antithrombin III (AT III), D-dimer and tissue plasminogen activator (t-PA) in patients with malignant diseases, to evaluate the diagnostic reliability of the investigated markers.

Complementing the informative value and providing the physicians the results from the investigated markers of coagulation and fibrinolysis is an important and responsible clinical-laboratory task. Its implementation would help not only for assessment of the thrombogenic risk, but also for diagnose, treatmeant and prevention of thrombotic complications.

The study of markers of coagulation activation and fibrinolysis together with routine hemostasis tests in clinically healthy individuals and in patients

with malignant pathology would allow finding a marker or a complex of markers that best reflects changes in the hemostatic system and the risk of thrombotic events. The question whether markers of coagulation activation and fibrinolysis can be a promising prognostic and/or diagnostic marker in predicting the risk of thrombotic complications in patients with malignant diseases still does not have a clear and definitive answer, which requires further scientific studies in this area. These considerations motivated us to conduct the present research and determined its aim and tasks.

II. AIM AND TASKS OF THE RESEARCH

AIM

To investigate the changes in laboratory parameters of coagulation and fibrinolysis - fibrinogen (Fib), thrombin-antithrombin complex (TAT), tissue factor (TF), prothrombin fragment 1+2 (F1+2), Antithrombin III (AT III), D - dimer and tissue plasminogen activator (t-PA) in patients with malignant diseases on systemic and/or radiotherapy, to evaluate their diagnostic reliability in the specific diseases.

TASKS

To achieve the set aim, it was necessary to complete the following tasks:

1. To determine the levels of fibrinogen, D-dimer, F1+2, TAT, AT III, TF and t-PA in patients with breast cancer, lung cancer and non-Hodgkin's lymphoma during treatment.
- baseline, before therapy (Visit 1)
- monitoring response to therapy (Visit 2)
- after completion of therapy (Visit 3)
2. To follow up the dynamics of the laboratory parameters, reflecting the changes in the coagulation and fibrinolysis systems.
3. To assess the correlations between the investigated markers and the changes in the subsystems of hemostasis.
4. To assess the diagnostic reliability of the investigated markers
5. To compare the changes in the levels of the investigated markers of coagulation and fibrinolysis between the patient groups.

III. MATERIALS AND METHODS

1. CLINICAL MATERIAL

1.1. Type of the Research

The research is single-center, controlled and prospective.

1.2. Number and distribution of patients and healthy subjects

185 individuals aged between 18-70 of Bulgarian nationality were included in the study. 120 patients from the Clinic of Medical Oncology and Clinic of Radiation Oncology at UMHAT "Sv. Georgi" – Plovdiv, divided into 3 patient groups: breast cancer (n=38), lung cancer (n=42) and non-Hodgkin's lymphoma (NHL) (n=40) were investigated between September 2017 and May 2019. For the same period of time, we studied a control group of 65 clinically healthy volunteers, 30 women and 35 men. The study was approved by the Committee on Scientific Ethics at Medical University of Plovdiv with decision No. R-1722/12.03.2018. The clinical- laboratory tests were carried out in the Clinical Laboratory of UMHAT "Sv.Georgi" - Plovdiv. The following inclusion and exclusion criteria were used to select the target patient group:

- **Inclusion criteria:** patients ≥ 18 years; histologically verified disease; newly diagnosed; localized or metastatic stage; patients on systemic and/or radiotherapy.
- **Exclusion criteria**: history of arterial and/or venous thrombosis (in the last three months before the examination); history of significant cardiovascular disease: NYHA grade III/IV heart failure; atrial fibrillation; arterial bypass; angioplasty and vascular stenting; valvular prosthesis; ECOG/Performance status ≥4; diabetes; more than two of the specified factors: smoking, hypercholesterolemia, antihypertensive therapy, oral contraceptives; other concomitant malignant disease; acute viral or bacterial infection in the last two weeks before the examination; exacerbated chronic inflammatory process in the last two weeks before the examination; kidney and liver failure; treatment with vit. K antagonists, new oral direct anticoagulants, direct parenteral anticoagulants in the last three months; treatment with vit. K in the last one month.

1.3. Design of the reasearch

For the aim of the study, the target group of patients was followed prospectively within three planned visits, which were defined as:

- **Visit 1:** baseline, before starting therapy
- **Visit 2:** monitoring response to therapy

- **Visit 3:** after completion of therapy

For patients with solid tumors, visit 2 is after the third course of chemotherapy, and visit 3 after the sixth course. In patients with non-Hodgkin's lymphoma, visit 2 is after the fourth course, and visit 3 after the eighth course of chemotherapy. In healthy controls, markers of coagulation and fibrinolysis were examined once.

1.4. Documentation and information sources.

Survey cards were used for all selected participants. Written informed consent was obtained from all participants enrolled in the study. The sociodemographic and health data of each of the individuals are included in an individual patient record. A history of illness from hospitalization in the Clinic of Medical Oncology and Clinic of Radiation Oncology, UMHAT "Sv. Georgi" - Plovdiv was used. A written information form about the nature, risks and expected benefits of the study for the patient/healthy volunteer was prepared.

2. BIOLOGICAL MATERIAL

The biological material from all participants was taken according to the standard requirements for patient preparation and venous blood collection for clinical-laboratory tests. The procedure was carried out in compliance with all the regulated conditions and requirements regarding the collection of biological material, ensuring complete safety at work. The following closed venous blood collection systems were used: For CBC and DBC - Monovette Sarstedt EDTA, 2.7 ml; ESR - Monovette Sarstedt Sodium Citrate, 3.5 ml; Clinical-chemical analyses, tumor markers - Monovette Sarstedt, Serum, 2.6 ml; parameters of coagulation and fibrinolysis Monovette Sarstedt Sodium Citrate, 2.9 ml.

3. METHODS

The following methods were used for collection the scientific information:

1. Survey method for registering the subjects included in the study and the received data from the individual survey cards

2. **Clinical-diagnostic methods**

a) primary physical examination

b) clinical follow-up - history and physical status

3. Instrumental methods - radiography, computer tomography

4. Clinical-laboratory tests - aPTT, PT, TT, fibrinogen, D-dimer, AT III, TF F1+2, TAT, t-PA, CBC, DBC, ESR, glucose, urea, creatinine, uric acid, total

protein, albumin, AST, ALT, CRP, sodium, potassium, chlorine, CA 15-3, CEA, β2-microglobulin

CLINICAL-LABORATORY METHODS

Determination of plasma concentration of D-dimer

Principle of the method

An automated, immunoturbidimetric method for quantitative determination of D-dimer. The test is based on a reaction between polystyrene particles loaded with monoclonal antibodies and D-dimer in the plasma. The change in absorbance measured at 540 nm was reported. Determination of plasma D-dimer levels was performed on a coagulation system Sysmex CS 2000i with an immunological channel for D-dimer reading.

Analytical reliability

Intra-assay imprecision: CV 4–6%, inter-assay imprecision: CV 6–8%, inaccuracy – d% from -3.50 to + 3.20%

Determination of ATIII activity

Quantitative assessment of the functional activity of AT III in plasma was performed by the chromogenic substrate method.

Principle of the method

A chromogenic method based on automatic measurement in a two-step test

$$\text{AT III sample} + \text{Thrombin}_{\text{excess}} \xrightarrow{\text{Heparin}} [\text{AT III - Thrombin}] + \text{Thrombin}_{\text{residual}}$$

Tos-Gly-Pro-Arg-ANBA-IPA + остатъчен тромбин → Tos-Gly-Pro-Arg-OH + ANBA+ IPA

The plasma is incubated with the thrombin reagent in the presence of heparin. The residual activity of thrombin upon its interaction with a chromogenic substrate is then determined at 405 nm, which is inversely proportional to the activity of AT III in the patient sample. The analysis is performed on a coagulation system Sysmex CS 2000i with photo-optical reading, immunological and chromogenic channel.

Analytical reliability

Intra-assay imprecision: CV 2.3-4.2%, inter-assay imprecision: CV 4.6-5.8%, inaccuracy - d % from -4.20 to + 3.50 %

Determination of plasma concentration of fibrinogen

Principle of the method

Chronometric method von Klaus – it measures the time for clot formation in plasma, diluted with a buffer 1:10 after the addition of thrombin in a high concentration. Plasma fibrinogen concentration was determined on coagulation system Sysmex CS 2000i with photo-optical reading. Clotting time is inversely proportional to plasma fibrinogen concentration.

Analytical reliability

Intra-assay imprecision: CV - 2.3–3.8%, inter-assay imprecision: CV 3.05–4.5%, inaccuracy – d% from -2.76 to + 2.83%

Determination of plasma concentration of tissue factor

Plasma concentration of tissue factor was determined by enzyme-linked immunosorbent assay ELISA

Principle – indirect immunoenzymatic method. ELISA kit was used, with cat. No. ab108903, Abcam, UK. A specific antibody against tissue factor is attached to the solid phase of the plate wells. The standards, controls, and patient samples are added to each well, respectively. Incubate and then wash. A specific biotinylated anti-tissue factor antibody was added to each well. Incubation and washing follow again. Streptavidin-peroxidase conjugate is added, incubated, and unbound conjugate is washed away. A chromogenic substrate tetramethylbenzidine (TMB) was added to each well and used to visualize the streptavidin-peroxidase reaction. It gives a blue colour, which changes to yellow after adding an acid stop solution. The concentration of tissue factor is directly proportional to the intensity of the color. Absorbance was measured on an ELISA Reader at 450 nm and 570 nm.

Analytical reliability

Intra-assay imprecision: CV 3.9%, inter-assay imprecision: CV 9.9%

Determination of plasma concentration of prothrombin fragment 1+2 (F1+2)

Plasma concentration of prothrombin fragment 1+2 (F1+2) was determined by enzyme-linked immunosorbent assay ELISA.

Principle - immunoenzymatic sandwich method. ELISA kit with cat. No. ABIN612785, Abcam, UK was used. The wells of the plate are loaded with a specific antibody against F1. The standards, controls, and patient samples are added to each well, respectively. A specific biotinylated antibody against F2 was

then added. Avidin-HRP conjugate was added to each well and incubated. After addition of TMB substrate, only those wells that contain F 1+2 bound to the biotinylated antibody and enzyme-bound avidin show a colour change. The enzyme-substrate reaction was stopped by adding sulfuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of F 1+2 in the samples is then determined by comparing the optical density of the samples on the standard curve.

Analytical reliability

Intra-assay imprecision: CV<10%, inter-assay imprecision: CV<12%

Determination of thrombin-antithrombin complex – TAT

Plasma concentration of TAT was determined by enzyme-linked immunosorbent assay ELISA.

Principle – sandwich immunoenzymatic assay. ELISA kit was used - No. ET1020-1, Abcam, UK. A monoclonal antibody specific for antithrombin is attached to the solid phase of the plate wells. Add the standards, controls, and samples to each well, incubate, then wash. The next step is the addition of the biotinylated polyclonal antibody specific for thrombin. Incubation and washing follow again. Streptavidin-peroxidase conjugate is added, incubated, unbound conjugate is washed off, and chromogenic substrate (TMB) is added. The reaction was stopped by adding sulfuric acid solution, the color changed from blue to yellow, and the color intensity was measured spectrophotometrically at 450 nm and at 570 nm.

Analytical reliability

Intra-assay imprecision: CV 4.8 %, inter-assay imprecision: CV 10 %

Determination of plasma concentration of t-PA

Plasma concentration of t-PA was determined by enzyme-linked immunosorbent assay ELISA

Principle – sandwich immunoenzymatic assay. ELISA kit with cat. No. BMS 258/2, Thermofisher Scientific, USA was used. A specific antibody against t-PA was attached to the solid phase of the plate wells. The standards, controls, and patient samples are added to each well, respectively. Incubate and then wash. HRP-conjugated polyclonal antibody against t-PA was added to each well. Incubate again and wash. Chromogenic substrate (TMB) was added to each well. After a short incubation, a blue color is obtained, which changes to yellow after the addition of an acid stop solution. The concentration of t-PA is

directly proportional to the intensity of the stain. Absorbance was measured at 450 nm.

Analytical reliability

Intra-assay imprecision: CV 3.6 %, inter-assay imprecision: CV 6.5 %

Clinical-chemical and hematological tests

Hematological tests (CBC, DBC) were performed on an hematology system Advia 2120 (Siemens, Germany), with original reagents of the manufacturer, ESR - manual measurement according to the Westergreen method. Clinical-chemical tests (glucose, urea, creatinine, uric acid, total serum protein, albumin, AST, ALT, CRP, sodium, potassium, chloride) were performed on a clinical-chemical system Olympus AU480 (Beckman Coulter, USA) according to original programs and with original reagents of the manufacturer. Tumor markers: CA 15-3, CEA - determination was performed on an Architect immunological system according to original programs and with original reagents of the manufacturer. β2-microglobulin – serum concentration was measured on a clinical chemical system Olympus AU480 (Beckman Coulter, USA) according to original programs and with original reagents of the manufacturer. Coagulation tests: aPTT, PT, TT, conventional methods with one- and two-step coagulation techniques with Dade Behring reagents on a coagulation system Sysmex CS 2000i (Siemens Diagnostica) were used.

STATISTICAL METHODS

Systematization, processing and analysis of data in the form of quantitative and qualitative variables were implemented with the statistical package of social science software IBM SPSS Statistics v. 26. A significance level of $\alpha<0.05$ was adopted for all tests.

The t-test, Mann-Whitney test, one-way ANOVA, ANOVA with repeated measurements, Shapiro-Wilk test, Spearman's rho-coefficient (ρ) for rank correlation, Kruskal-Wallis test, Friedman test, Wilcoxon signed ranks test and ROC curves were used for presenting the obtained results.

IV. RESULTS AND DISCUSSION

A total of 185 individuals participated in the study, of which 120 were patients and 65 were healthy controls. The patients were divided according to the type of diagnosis into three patient groups – breast cancer n=38 (31.7%); lung cancer n=42 (35%), non-Hodgkin's lymphoma (NHL) n=40 (33.3 %). Variables that have a normal distribution as assessed by the Shapiro-Wilks test ($p>0.05$) are presented as mean ($\bar{x}$) ± standard deviation (Sx). The parametric statistical methods t-test, one-way ANOVA and ANOVA with repeated measurements were applied to them. In cases where the requirement for normal distribution was not met ($p<0.05$), non-parametric tests were used - Mann-Whitney test, Friedman test, Wilcoxon test and Kruskal-Wallis test. Data are presented using positional means – median (Me) and 25th and 75th percentiles (range). Gender distribution is presented in numbers and percentages and compared between groups using the Chi-square test.

1. Breast cancer

Demographic and clinical data of the patient group and healthy subjects

38 women with breast cancer and a control group of 30 clinically healthy women were studied. The control and patient groups were comparable in terms of gender and age. The average age of the patient group was 56.47±9.64 years, and of the control group 59.20±11.74 years. (Table 1).

Table 1. Mean age of the patient group and healthy subjects

group	Number (N) women	age ($\bar{x}$ ± SD)*	p-value
Breast cancer	38	56.47±9.64	p=0.297
Controls	30	59.20±11.74	

* $\bar{x}$ – mean, SD – standard deviation

The distribution of women with breast cancer in a localized stage (I and II stage) is 60.5% (n=23), advanced stage (III and IV stage) – 39.5% (n=15), (Fig. 1).

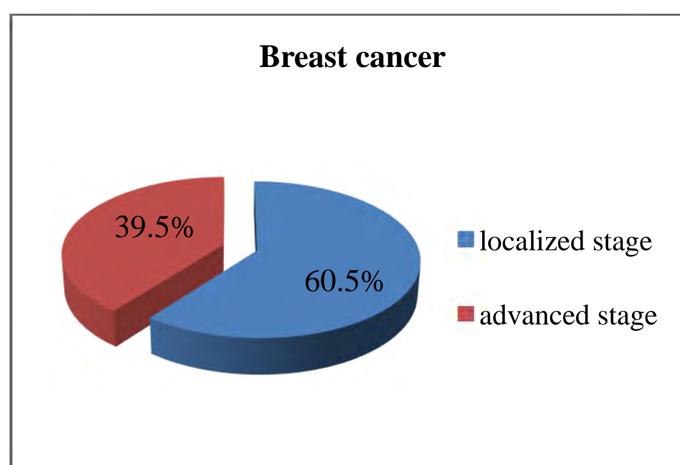

Figure 1. Pie chart of the distribution of breast carcer patients by stage

Clinical-laboratory parameters of coagulation and fibrinolysis in patients with breast carcer and controls

The clinical-laboratory parameters of coagulation and fibrinolysis in breast cancer group (n=38) and the control group of healthy subjects (n=30) are presented in Table. 2.

Table 2. Descriptive characteristics of parameters of coagulation and fibrinolysis in breast carcer patients and healthy controls

Parameter	Group	Mean	Standard deviation	Median	25 percentile	75 percentile
TF pg/ml – Visit 1	Breast cancer	196.17	92.08	198.15	112.15	263.68
	Controls	138.81	54.41	140.45	101.25	169.28
TF pg/ml – Visit 2	Breast cancer	167.44	73.75	182.85	111.68	224.70
TF pg/ml – Visit 3	Breast cancer	164.66	71.90	184.90	100.00	208.03
D-dimer mg/l – Visit 1	Breast cancer	1.13	0.91	0.87*	0.54	1.34
	Controls	0.35	0.14	0.33*	0.23	0.42
D-dimer mg/l – Visit 2	Breast cancer	0.85	0.56	0.73*	0.45	1.11
D-dimer mg/l – Visit 3	Breast cancer	0.75	0.61	0.56*	0.33	1.03
TAT ng/ml – Visit 1	Breast cancer	10.03	4.87	8.52*	6.28	12.64
	Controls	5.43	2.23	4.77*	3.81	6.74

TAT ng/ml – Visit 2	Breast cancer	8.95	4.41	7.83*	5.54	11.8
TAT ng/ml – Visit 3	Breast cancer	8.04	4.29	7.06*	5.12	9.26
F 1+2 ng/ml – Visit 1	Breast cancer	19.70	8.27	21.10	11.73	25.93
	Controls	9.97	4.94	10.60	5.33	12.45
F 1+2 ng/ml – Visit 2	Breast cancer	17.75	6.35	18.25	12.70	22.40
F 1+2 ng/ml – Visit 3	Breast cancer	15.80	6.48	13.95	11.20	19.60
Fib g/l – Visit 1	Breast cancer	3.61	1.09	3.23	2.85	4.37
	Controls	2.97	0.57	2.88	2.60	3.23
Fib g/l – Visit 2	Breast cancer	3.46	0.82	3.44	2.88	3.86
Fib g/l – Visit 3	Breast cancer	3.42	0.96	3.24	2.76	3.84
AT III % – Visit 1	Breast cancer	87.30	8.21	87.40	81.80	93.73
	Controls	93.65	6.93	93.85	89.00	98.53
AT III % – Visit 2	Breast cancer	86.72	7.13	86.50	80.28	92.48
AT III % – Visit 3	Breast cancer	88.15	6.99	88.85	82.20	92.95
t-PA pg/ml – Visit 1	Breast cancer	3688.61	1688.73	3560.00	2620.00	5257.50
	Controls	2337.03	1702.45	1980.50	1121.00	2575.00
t-PA pg/ml – Visit 2	Breast cancer	3367.50	1508.11	3437.50	2180.00	4275.00
t-PA pg/ml – Visit 3	Breast cancer	3335.11	1466.87	3335.00	2260.00	4420.00

* the variable is not with normal distribution

Comparative analysis of clinical laboratory parameters of coagulation and fibrinolysis in patients and healthy controls

We compared the clinical laboratory parameters of coagulation and fibrinolysis between the breast cancer group (n=38) and the control group (n=30) and the data are presented graphically and described (Fig. 2-8).

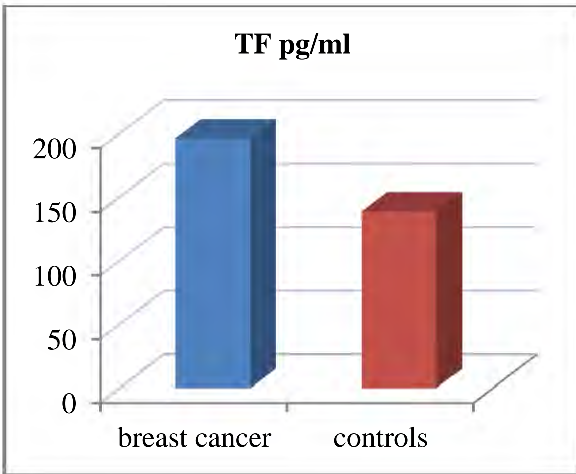

Figure 2. Mean levels of TF pg/ml in controls and patients

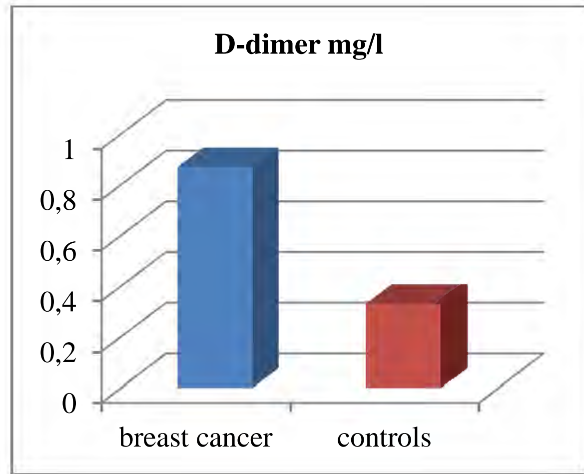

Figure 3. Mean levels of D-dimer mg/l in controls and patients

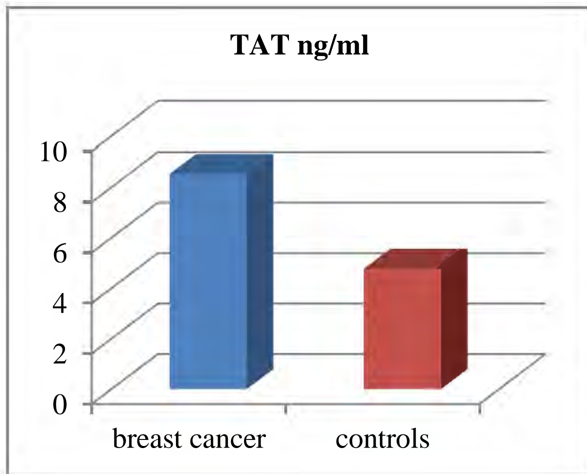

Figure 4. Mean levels of TAT ng/ml in controls and patients

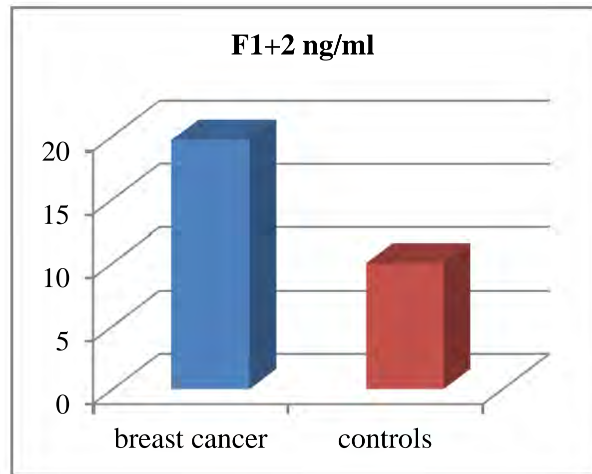

Figure 5. Mean levels of F1+2 ng/ml in controls and patients

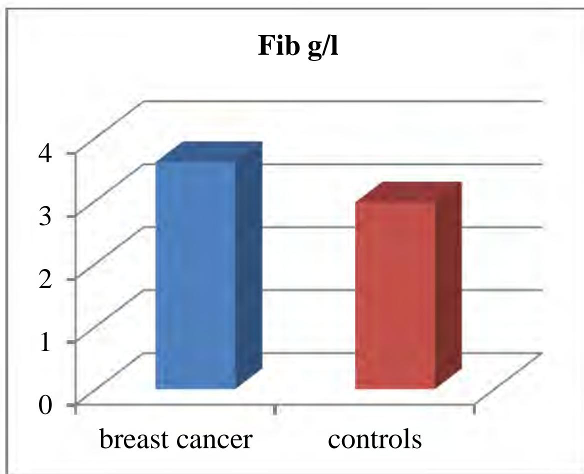

Figure 6. Mean levels of fibrinogen g/l in controls and patients

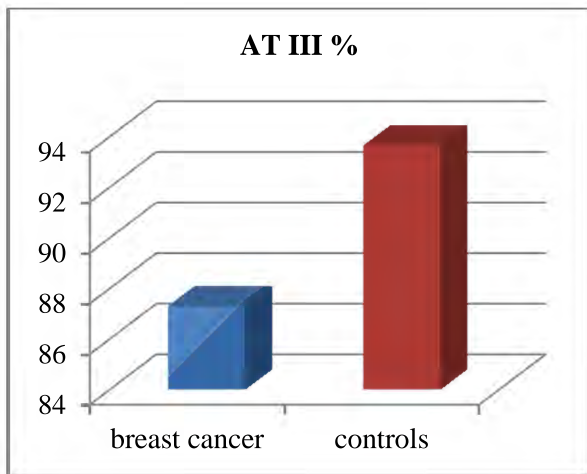

Figure 7. Mean levels of AT III % in controls and patients

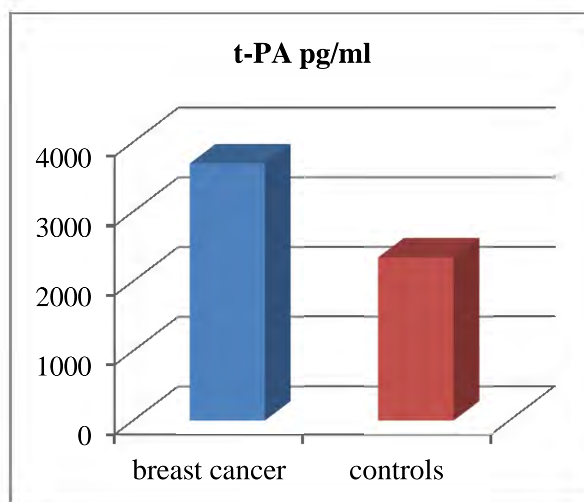

Figure 8. Mean levels of t-PA pg/ml in controls and patients

There was a statistically significant difference between the mean values of TF in the breast cancer group (196.17±92.08 pg/ml) and the control group (138.81±54.41 pg/ml) – t=3.20, p=0.002 (Fig. 2). Statistically significantly higher levels of D-dimer were found in the group of patients with breast cancer 0.87 mg/l (0.54 mg/l; 1.34 mg/l) compared to the control group 0.33 mg/l (0.23 mg/l; 0.42 mg /l), p=0.000 (Fig. 3). The mean TAT levels in the patient group of 8.52 ng/ml (6.28 ng/ml; 12.64 ng/ml) were statistically significantly higher than those in the control group 4.77 ng/ml (3.81 ng/ml; 6.74 ng /ml), p=0.000 (Fig. 4). A statistically significant difference was found between the mean values of F 1+2 in the group of patients with breast cancer (19.70±8.27 ng/ml) and the control group (9.97±4.94 ng/ml) – t=6.02, p=0.000 (Fig 5). Statistically significantly higher levels of fibrinogen were found in the patient group (3.61±1.09 g/l) compared to the control group (2.97±0.57 g/l) – t= 3.08, p=0.003 (Fig. 6). In breast cancer patients, a significantly lower activity of ATIII (87.30±8.21 %) was found compared to the control group (93.65±6.93 %) – t=-3.39, p=0.001 (Fig. 7). A statistically significant difference was demonstrated between the plasma levels of t-PA in the patient group (3688.61±1688.73 pg/ml) and the control group (2337.03±1702.45 pg/ml) – t=3.27, p=0.002 (Fig. 8).

Examination of screening coagulation tests showed significantly shortened aPTT and PT presented in sec, increased PT% and lower INR values in patients compared to controls. No statistically significant difference was found in thrombin time (sec) (Table 3). The values of the three parameters remain in the reference interval for both groups.

Table 3. Comparative analysis of screening coagulation tests in patients and healthy controls

Parameter	Group	N	Mean	Standard deviation	Standard error	P-value
PT sec	Controls	30	11.42	0.49	0.09	**p=0.036**
	Breast cancer	38	11.09	0.74	0.12	
PT INR	Controls	30	1.01	0.05	0.01	**p=0.034**
	Breast cancer	38	0.98	0.07	0.01	
PT %	Controls	30	87.98	9.64	1.76	**p=0.017**
	Breast cancer	38	95.14	13.44	2.18	
aPTT sec	Controls	30	27.81	2.55	0.47	**p=0.000**
	Breast cancer	38	24.34	1.63	0.26	
TT sec	Controls	30	16.85	0.65	0.12	p=0.143
	Breast cancer	38	16.58	0.81	0.13	

Dynamics of the markers of coagulation and fibrinolysis in patients with breast cancer during treatment

For variables with a normal distribution, the one-way ANOVA with repeated measurements and Greenhouse-Geisser correction was used to determine the presence of significant differences in the levels of the investigated markers during treatment. Values are presented as mean ± standard deviation. Plasma D-dimer and TAT levels did not meet the requirement for a Gaussian distribution, so that the non-parametric Friedman test and the Wilcoxon signed-rank test were used for them. Data are presented as median (Me) and 25th and 75th percentiles (range). It was found that factor “time” (visit) has a significant influence on the plasma levels of markers of coagulation and fibrinolysis. The obtained results are presented graphically and then described (Fig. 9-15).

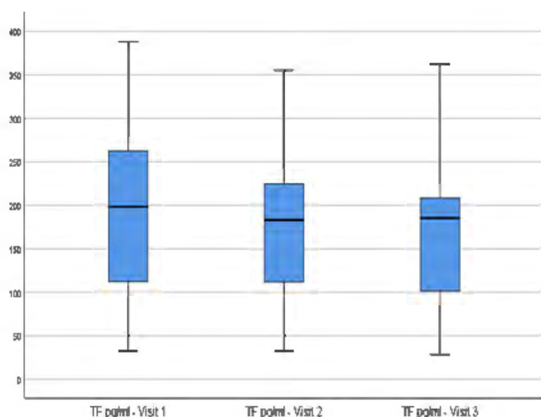

Figure 9. Dynamics of TF pg/ml in patients with breast cancer during treatment

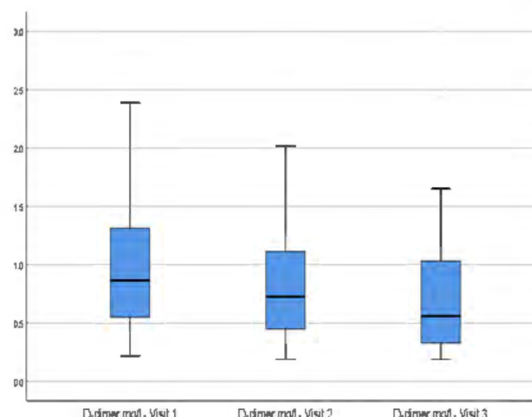

Figure 10. Dynamics of D-dimer mg/l in patients with breast cancer during treatment

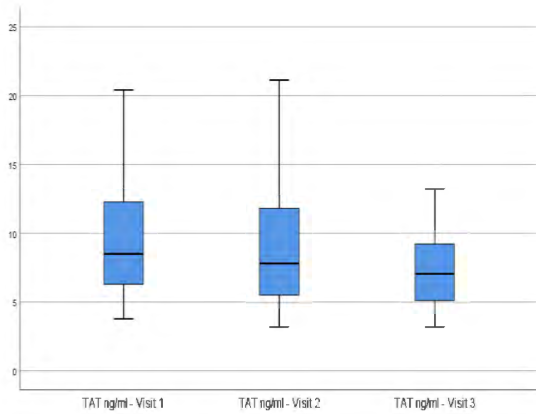

Figure 11. Dynamics of TAT ng/ml in patients with breast cancer during treatment

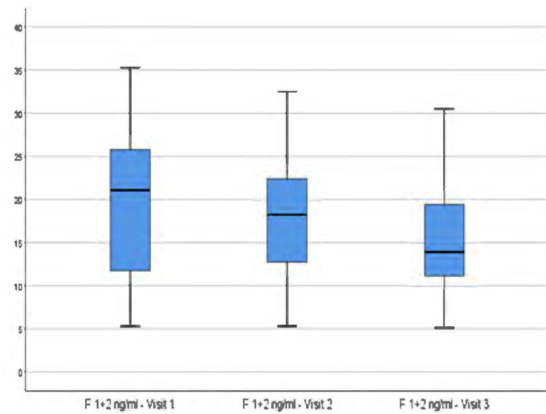

Figure 12. Dynamics of F1+2 ng/ml in patients with breast cancer during treatment

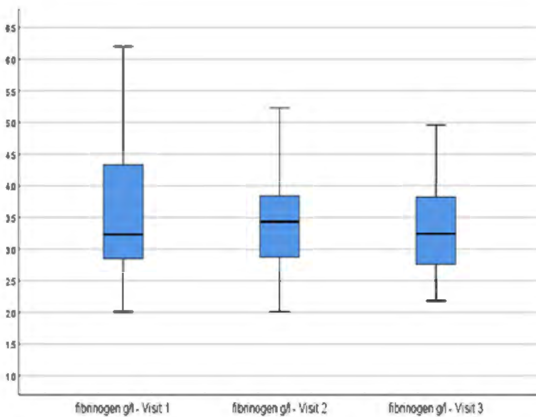

Figure 13. Dynamics of fibrinogen g/l in patients with breast cancer during treatment

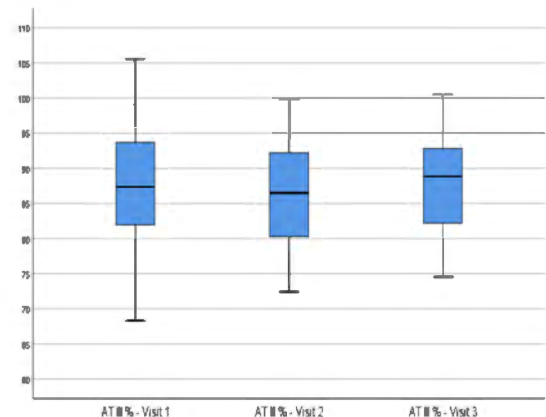

Figure 14. Dynamics of ATIII % in patients with breast cancer during treatment

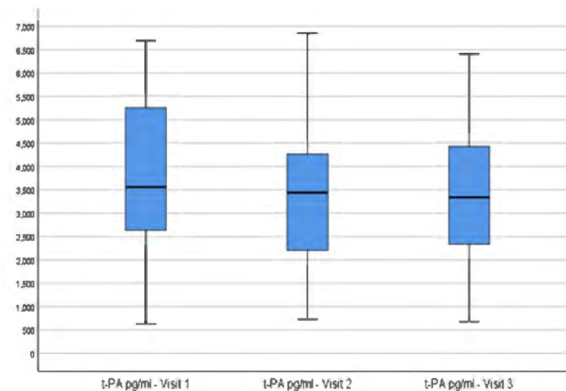

Figure 15. Dynamics of t-PA pg/ml in patients with breast cancer during treatment

Mean TF values reported in the group (n=38) of breast cancer patients differed statistically significantly between time points (F(1.092,40.39)=13.83, p=0.000). Post hoc tests using the Bonferroni correction reported statistically significantly lower TF levels at the second (167.44±73.75 pg/ml) and third visits

(164.66±71.90 pg/ml) compared to the first (196.17±92.08 pg/ml), respectively p=0.003, p=0.000 (Fig. 9). Statistically significantly lower levels of D-dimer were reported at the second 0.73 mg/l (0.45 mg/l; 1.11 mg/l) and third visit 0.56 mg/l (0.33 mg/l; 1.03 mg/l) compared to the first 0.87 mg/l (0.54 mg/l; 1.34 mg/l), respectively p=0.016, p=0.001 (Fig. 10). Plasma TAT levels also decreased during the applied treatment strategy, with a statistically significant difference at the second 7.83 mg/l (5.54 ng/ml; 11.8 ng/ml) and third visit 7.06 ng/ml (5.12 ng/ml; 9.26 ng/ml) versus the first 8.52 ng/ml (6.28 ng/ml; 12.64 ng/ml), respectively p=0.018, p=0.006 (Fig. 11). Lower levels of F 1+2 were reported in each subsequent visit – visit 1 (19.70±8.27 ng/ml), visit 2 (17.75±6.35 ng/ml) and visit 3 (15.80±6.48 ng/ml) with statistically significantly difference between time points (F(1.321,48.88)=11.59, p=0.000) (Fig. 12). No statistically significant difference was found in plasma levels of fibrinogen between time points (F(1.528,56.53)=0.76, p=0.438) (Fig.13). ATIII activity was statistically significantly higher at the third visit (88.15±6.99%) compared to the second (86.72±7.13%), p=0.023 (Fig. 14). t-PA values differed statistically significantly between time points (F(1.185, 43.85)=5.56, p=0.018). Plasma levels decreased in visits 2 and 3 compared to visit 1, but when comparing them in pairs, no statistically significant difference was reported (Fig. 15).

Correlations between the investigated markers of coagulation and fibrinolysis in patients with breast cancer and in healthy controls

We assessed the correlations between the investigated markers of coagulation and fibrinolysis in patients with breast cancer and in healthy controls

In women with breast cancer at visit 1, a positive, moderate correlation was found between TF and D-dimer (r=0.480, p=0.002), TF and TAT (r=0.508, p=0.001) and D-dimer and Fib (r=0.514, p=0.001). A positive, strong relationship was observed between F1+2 and TAT (r=0.782, p=0.000) and Fib and TAT (r=0.601, p=0.000). A very strong, positive association exists between TAT and D-dimer (r=0.811, p=0.000) and between F1+2 and D-dimer (r=0.825, p=0.000). A moderate, negative relationship in women with breast cancer at visit 1 was between Fib and AT III (r=-0.530, p=0.001), and a strong, negative relationship was observed between D-dimer and AT III (r=- 0.658, p=0.000), TAT and AT III (r=-0.651, p=0.000), F1+2 and AT III (r=-0.600, p=0.000).

In the control group of clinically healthy women (n=30), only a few correlations were found. A positive, moderate relationship was found between TF and fibrinogen (r=0.487, p=0.006) and between TAT and F1+2 (r=0.482,

p=0.007). A moderate, negative relationship was observed between fibrinogen and AT III (r=-0.434, p=0.017).

Diagnostic reliability of the investigated markers.

Receiver-operating characteristic (ROC) curves were described and sensitivity and specificity of TF, D-dimer, TAT and F1+2 were calculated. To compare the curves, the area under the curve (AUC) and 95% confidence interval (CI) were calculated. In addition, the optimal cut-off values for each of the markers were established. The level of significance (p-value) was set at 0.05. The cut-off values, sensitivity, specificity and AUC for each of the markers are presented in the corresponding tables below the ROC-curves. The area under the ROC-curve for TF (Fig. 16) is the smallest (AUC=0.675, 95% CI 0.545 – 0.804, p=0.014) (Table 4). The cut-off value is 194.45 pg/ml, 55.3% diagnostic sensitivity and 86.7% diagnostic specificity (Table 5).

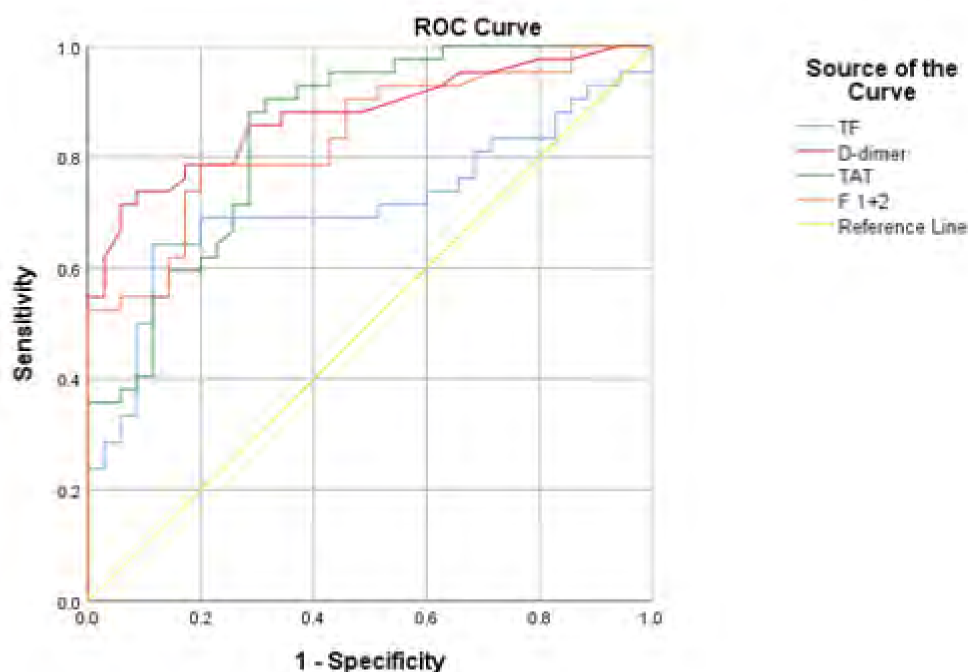

Figure 16. ROC curves: TF, D-dimer, TAT и F1+2 in breast cancer patients

Table 4. AUC of the investigated parameters

Parameter	AUC	P-value	95% CI	
TF	0.675	0.014	0.547	0.804
D-dimer	0.872	0.000	0.789	0.956
TAT	0.821	0.000	0.723	0.920
F1+2	0.823	0.000	0.727	0.919

Table 5. Accuracy indices of TF, D-dimer, TAT and F1+2 in breast cancer patients

Parameter	Cut-off value	Sensitivity	Specificity	Youden index
TF	194.45 pg/ml	55.3%	86.7%	0.420
D-dimer	0.49 mg/l	78.9%	86.7%	0.656
TAT	6.10 ng/ml	81.6%	70.0%	0.516
F1+2	13.75 ng/ml	63.2%	90.0%	0.532

The area under the ROC curve of D-dimer is over 0.80 (AUC = 0.872), which makes this parameter is a good marker for distinguishing patients at increased risk of thrombotic complications. The discriminant value was 0.49 mg/l, with 78.9% diagnostic sensitivity and 86.7% diagnostic specificity (Fig. 16, Table 5). The area under the ROC curve of thrombin-antithrombin complex was also above 0.80 (AUC=0.821) with diagnostic sensitivity 81.6% and specificity 70% (Fig. 16, Table 5). The cut-off value is 6.10 ng/ml, which allows a good differentiation of patients at a prothrombotic state. Diagnostic sensitivity of prothrombin fragment 1+2 was 63.2% and specificity 90% (Fig. 16, Table 5) at a cut-off value of 13.75 ng/ml. Although the diagnostic sensitivity is lower compared to that of D-dimer and thrombin-antithrombin complex, the area under the ROC curve is above 0.80 (AUC = 0.823), which allows a good differentiation of patients at increased risk of of thrombotic complications.

When comparing the areas under the ROC-curves of the investigated parameters (Fig. 16, Table 4), an overlap between the AUC at 95% CI for D-dimer, TAT and F1+2 is noticed, which indicates similarity in their diagnostic reliability. The high diagnostic reliability of the discriminant values for D-dimer, TAT and F1+2 would help distinguish patients at increased risk of developing thrombotic complications.

2. Lung cancer

Demographic and clinical data of the patient group and healthy subjects

42 men with lung cancer and a control group of 35 clinically healthy men were studied. The control and patient groups were comparable in terms of gender and age. The mean age of the patient group was 59.69±8.42 years, and of the control group 58.14±9.99 years (Table 6).

Table 6. Mean age of the patient group and healthy subjects

Group	Number (N) men	Age ($\bar{x}$ ± SD)*	P-value
Lung cancer	42	59.69±8.42	p=0.463
Controls	35	58.14±9.99	

* $\bar{x}$ – mean, SD – standard deviation

The distribution of men with lung cancer in a localized stage (stage I and II) is 38.0% (n=16), and in an advanced stage (stage III and IV) – 62.0% (n=26) (Fig. 17)

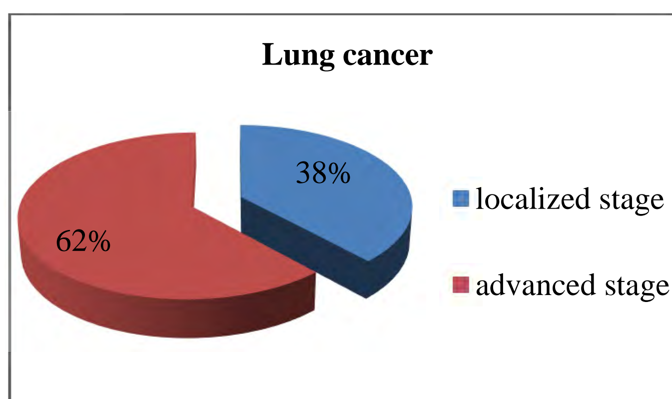

Figure 17. Pie chart of the distribution of lung cancer patients by stage

Clinical-laboratory parameters of coagulation and fibrinolysis in patients with lung cancer and healthy controls

The clinical-laboratory parameters of coagulation and fibrinolysis in the group of patients diagnosed with lung cancer (n=42) and the control group (n=35) are presented in Table. 7.

Table 7. Descriptive characteristics of parameters of coagulation and fibrinolysis in lung cancer patients and healthy controls

Parameter	Group	Mean	Standard deviation	Median	25 percentile	75 percentile
TF pg/ml – Visit 1	Lung cancer	210.35	92.26	202.3	122.93	290.58
	Controls	142.3	53.96	138.80	105.60	153.60
TF pg/ml – Visit 2	Lung cancer	181.99	73.68	185.70	122.85	243.20
TF pg/ml – Visit 3	Lung cancer	184.22	85.51	183.65	111.58	262.03
D-dimer mg/l – Visit 1	Lung cancer	1.67	1.60	1.08*	0.56	2.41
	Controls	0.38	0.17	0.33*	0.23	0.48
D-dimer mg/l – Visit 2	Lung cancer	1.34	1.33	0.86*	0.56	1.81
D-dimer mg/l – Visit 3	Lung cancer	1.14	1.12	0.80*	0.33	1.28
TAT ng/ml – Visit 1	Lung cancer	11.35	5.60	9.42*	6.89	16.55
	Controls	5.56	2.96	4.90*	3.70	7.72

TAT ng/ml – Visit 2	Lung cancer	10.03	4.80	9.29*	6.29	12.38
TAT ng/ml – Visit 3	Lung cancer	9.29	5.57	8.33*	4.17	12.54
F 1+2 ng/ml – Visit 1	Lung cancer	21.49	9.57	22.90	14.35	28.83
	Controls	10.10	5.33	9.80	5.40	13.30
F 1+2 ng/ml – Visit 2	Lung cancer	19.01	8.72	18.45	12.33	25.33
F 1+2 ng/ml – Visit 3	Lung cancer	18.54	9.58	16.30	11.18	27.40
Fib g/l – Visit 1	Lung cancer	4.30	1.39	4.26	2.90	5.31
	Controls	3.08	0.56	2.90	2.80	3.45
Fib g/l – Visit 2	Lung cancer	4.03	1.41	3.76	2.92	4.86
Fib g/l – Visit 3	Lung cancer	4.11	1.55	4.05	2.76	5.20
AT III % – Visit 1	Lung cancer	82.69	9.02	83.70	74.88	91.20
	Controls	91.68	6.85	93.00	85.80	96.90
AT III % – Visit 2	Lung cancer	83.87	8.66	85.15	76.73	92.13
AT III % – Visit 3	Lung cancer	84.28	9.20	84.75	76.63	92.23
t-PA pg/ml – Visit 1	Lung cancer	4067.57	1882.85	4255.00	2413.75	5722.50
	Controls	2406.17	1499.54	2018.00	1130.00	3200.00
t-PA pg/ml – Visit 2	Lung cancer	3864.98	1896.42	4040.00	1989.25	5070.00
t-PA pg/ml – Visit 3	Lung cancer	3942.38	1912.60	3825.00	2071.50	5546.00

* the variable is not with normal distribution

Comparative analysis of clinical-laboratory parameters of coagulation and fibrinolysis in patients and healthy controls

We compared the clinical-laboratory parameters of coagulation and fibrinolysis between the group of patients diagnosed with lung cancer (n=42) and the control group (n=35), the data are presented graphically and described (Figs. 18-24).

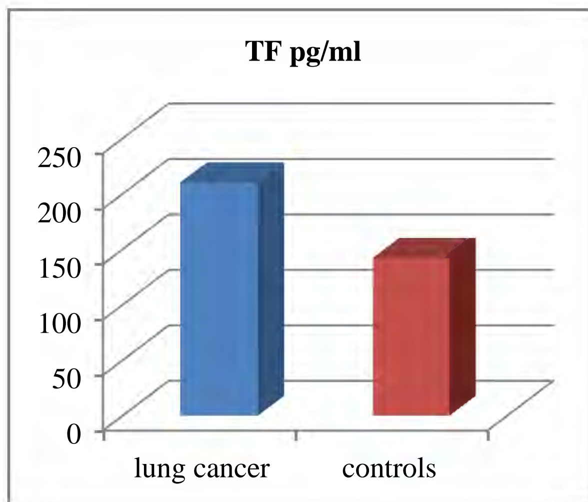

Figure 18. Mean levels of TF pg/ml in controls and patients

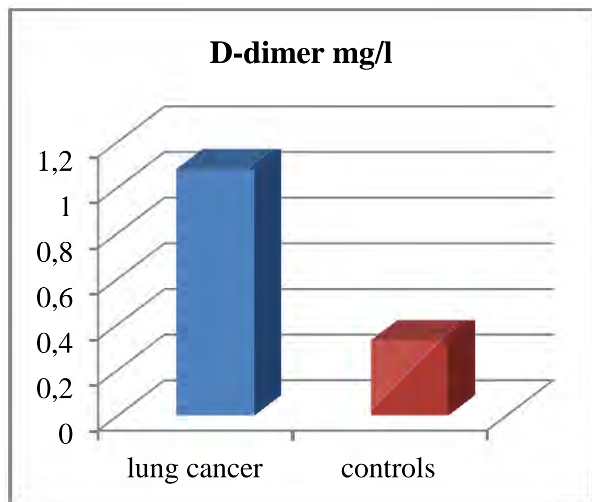

Figure 19. Mean levels of D-dimer mg/l in controls and patients

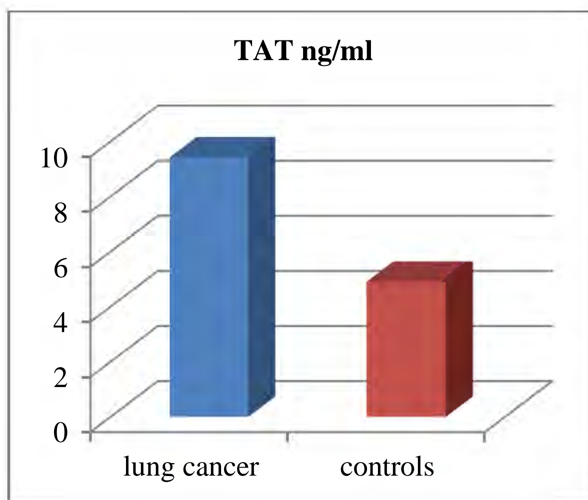

Figure 20. Mean levels of TAT ng/ml in controls and patients

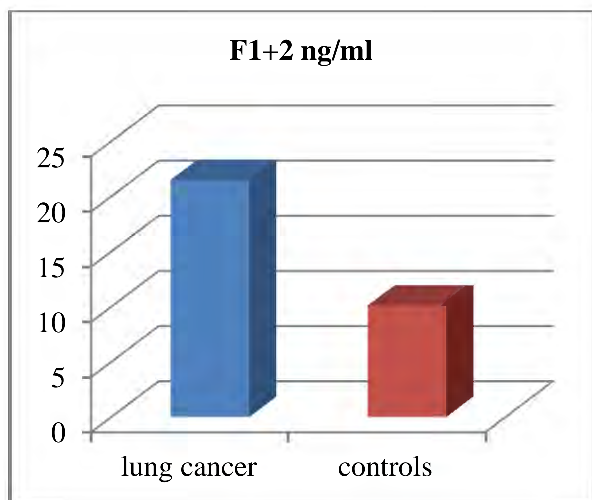

Figure 21. Mean levels of F1+2 ng/ml in controls and patients

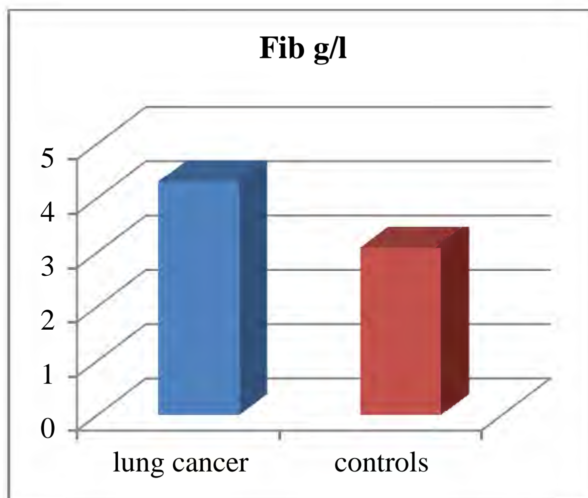

Figure 22. Mean levels of fibrinogen g/l in controls and patients

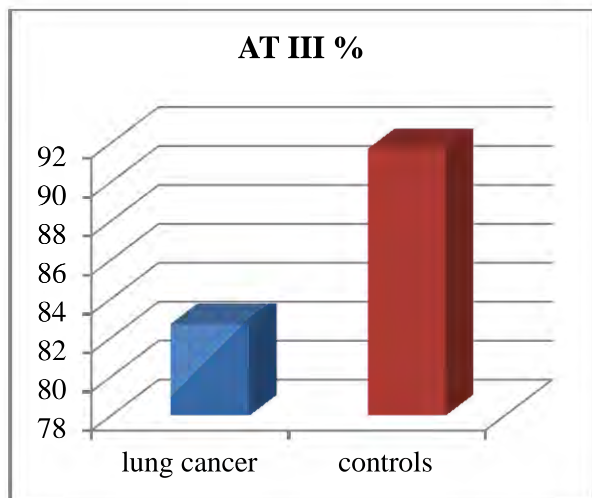

Figure 23. Mean levels of AT III % in controls and patients

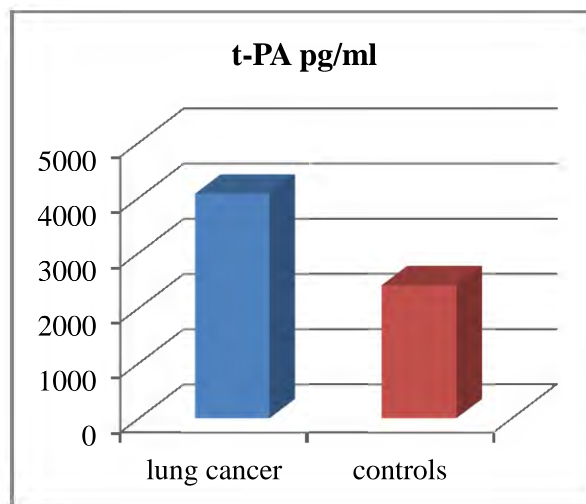

Figure 24. Mean levels of t-PA pg/ml in controls and patients

A statistically significant difference was demonstrated between the mean values of TF in the group of patients with lung cancer (210.35±92.26 pg/ml) and the control group (142.30±53.96 pg/ml) – t=4.03, p=0.000 (Fig. 18) . The mean values of D-dimer in the patient group 1.08 mg/l (0.56 mg/l; 2.41 mg/l) were statistically significantly higher than those in the control group 0.33 mg/l (0.23 mg/l; 0.48 mg/l), p=0.000 (Fig. 19). A statistically significant difference was demonstrated between the mean TAT levels in the lung cancer patients 9.42 ng/ml (6.89 ng/ml; 16.55 ng/ml) and the controls 4.90 ng/ml (3.70 ng/ml; 7.72 ng/ml), p=0.000, being twice as high in patients (Fig. 20). The mean values of F 1+2 in the group of patients with lung cancer (21.49±9.57 ng/ml) were statistically significantly higher than those in the control group (10.10±5.33 ng/ml) – t=6.58, p=0.000 (Fig. 21). A statistically significant difference was found between the levels of fibrinogen in the patient group (4.30±1.39 g/l) and the control group (3.08±0.56 g/l) – t= 5.23, p=0.003 (Fig. 22). The levels of ATIII% activity in the patients (82.69±9.02%) were statistically significantly lower than those in the control group (91.68±6.85%) – t=-4.97, p=0.000 (Fig. 23). A statistically significant difference was found between the mean values of t-PA in the group of patients with lung cancer (4067.57±1882.85 pg/ml) and the control group (2406.17±1499.54 pg/ml) – t=4.22, p=0.000 (Fig. 24).

Table 8. Comparative analysis of screening coagulation tests in patients and healthy controls

Parameter	Group	N	Mean	Standard deviation	Standard error	p-value
PT %	Controls	35	89.79	12.36	2.09	**p=0.026**
	Lung cancer	42	96.37	12.86	1.98	
PT sec	Controls	35	11.34	0.65	0.11	**p=0.027**
	Lung cancer	42	11.01	0.63	0.09	
PT INR	Controls	35	1.00	0.06	0.01	**p=0.035**
	Lung cancer	42	0.97	0.06	0.01	
aPTT sec	Controls	35	26.84	2.01	0.34	**p=0.000**
	Lung cancer	42	24.05	2.03	0.31	
TT sec	Controls	35	16.67	0.80	0.14	p=0.069
	Lung cancer	42	17.05	0.97	0.15	

Examination of screening coagulation tests revealed significantly shortened aPTT and PT presented in sec, increased PT% and lower INR values in patients compared to controls. No statistically significant difference was found in thrombin time (sec) between patients and controls (Table 8). The values of the three parameters remain in the reference interval for both groups. The results of routine hemostatic parameters in the lung cancer patients are similar to those of patients with breast cancer

Dynamics of the markers of coagulation and fibrinolysis in patients with lung cancer during treatment

We searched a presence of significant differences in the levels of the investigated markers between the visits during treatment. Values are presented as mean ±standard deviation for TF, F 1+2, fibrinogen, AT III and t-PA. As in breast cancer patients, plasma levels of D-dimer and TAT do not have a normal distribution and are presented as median (Me) and 25th and 75th percentiles (range). It was found that the factor “time” affects the studied markers. The data are presented graphically and then described (Figs. 25-31).

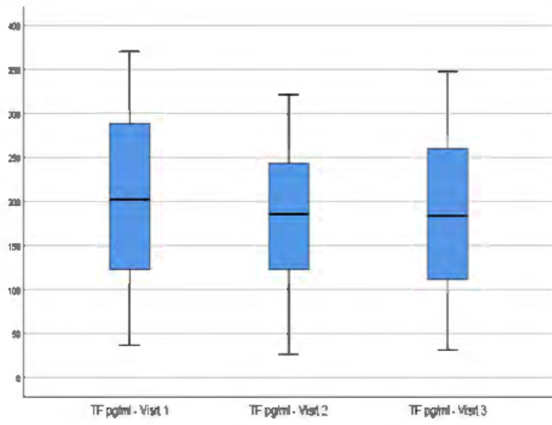

Figure 25. Dynamics of TF pg/ml in lung cancer patients during treatment

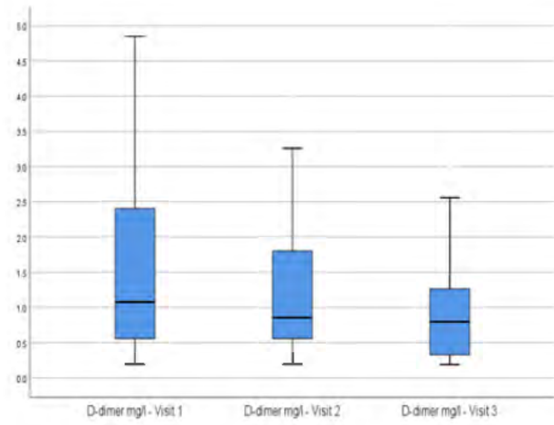

Figure 26. Dynamics of D-dimer mg/l in lung cancer patients during treatment

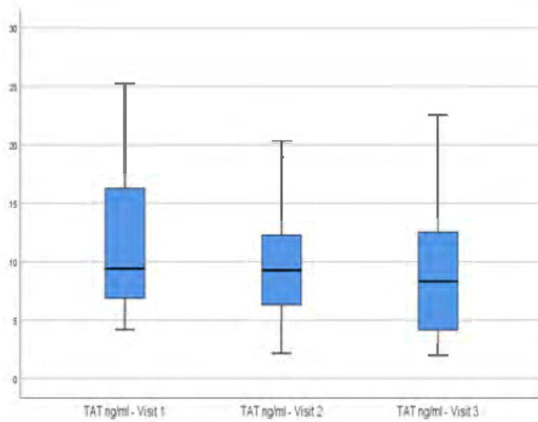

Figure 27. Dynamics of TAT ng/ml in lung cancer patients during treatment

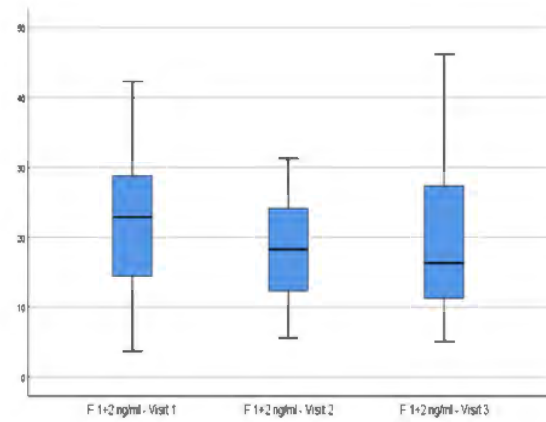

Figure 28. Dynamics of F1+2 ng/ml in lung cancer patients during treatment

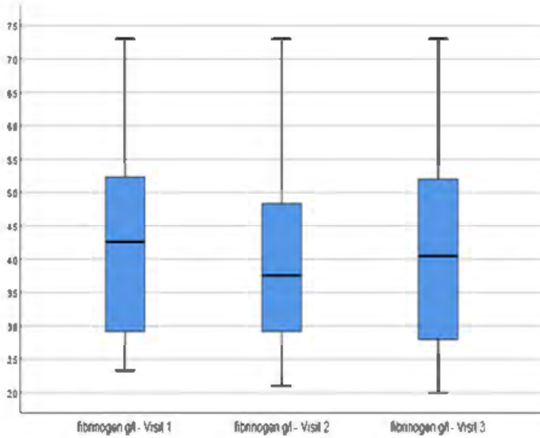

Figure 29. Dynamics of fibrinogen g/l in lung cancer patients during treatment

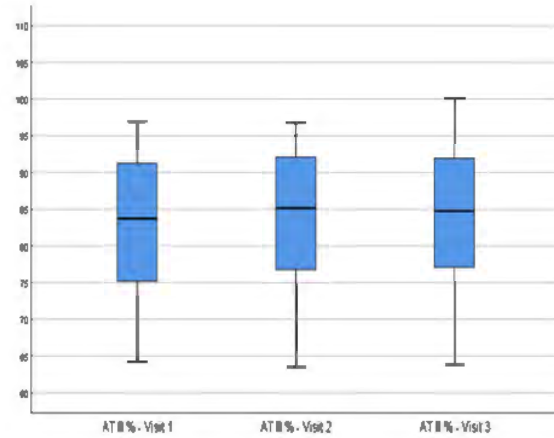

Figure 30. Dynamics of ATIII % in lung cancer patients during treatment

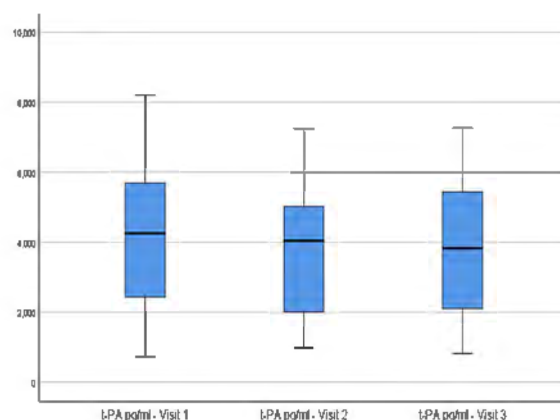

Figure 31. Dynamics of t-PA pg/ml in lung cancer patients during treatment

Mean TF values reported in the group (n=42) of lung cancer patients differed statistically significantly between time points (F(1.304, 53.47)=10.23, p=0.001). Post hoc tests using the Bonferroni correction reported statistically significantly lower TF levels at the second (181.99±73.68 pg/ml) and third visits (184.22±85.51 pg/ml) compared to the first (210.35±92.26 pg/ml), respectively p=0.000, p=0.020 (Fig. 25). Plasma levels of D-dimer at the second 0.86 mg/l (0.56 mg/l; 1.81 mg/l) and third visit 0.80 mg/l (0.33 mg/l; 1.28 mg/l) were also statistically significantly lower compared to the first 1.08 mg/l (0.56 mg/l; 2.41 mg/l), respectively p=0.011, p=0.007 (Fig. 26). TAT levels were also statistically significantly lower at the second 9.29 mg/l (6.29 ng/ml; 12.38 ng/ml) and third visit 8.33 ng/ml (4.17 ng/ml; 12.54 ng/ml) compared to the first 9.42 ng/ml ml (6.89 ng/ml; 16.55 ng/ml), respectively p=0.017, p=0.010 (Fig. 27). Mean F 1+2 values also differed statistically significantly between time points (F(1.150, 47.16)=6.26, p=0.013). Post hoc tests using the Bonferroni correction reported statistically significantly lower levels of F 1+2 at the second (19.01±8.72 ng/ml) and third visits (18.54±9.58 ng/ml) compared to the first (21.49±9.57 ng/ml ml), respectively p=0.019, p=0.017 (Fig. 28). There were no statistically significant differences in fibrinogen values at visits during treatment (F(1.402, 57.47)=2.39, p=0.117) (Fig. 29). The activity of AT increases during the systemic therapy, with statistically significantly higher levels of ATIII at the second (83.87±8.66 %) and third visit (84.29±9.20 %) compared to the first (82.69±9.02 %), respectively p =0.018, p=0.025 (Fig. 30). Similar to fibrinogen leves, no statistically significant difference was found in t-PA levels between the visits (F(1.631,66.86)=1.84, p=0.173) (Fig. 31).

Correlations between the investigated markers of coagulation and fibrinolysis in patients with lung cancer and in healthy controls

In men with lung cancer at visit 1, a positive, moderate correlation was found between TF and D-dimer (r=0.531, p=0.000), TF and AT III (r=-0.466,

p=0.002) and D-dimer and Fib (r=0.541, p=0.000). A positive, strong relationship was observed between F1+2 and TAT (r=0.635, p=0.000), D-dimer and TAT (r=0.626, p=0.000) and D-dimer and F1+2 (r=0.793, p=0.000). A moderate, negative relationship in men with lung cancer at visit 1 was between F1+2 and AT III (r=-0.562, p=0.000), and a strong, negative relationship was observed between D-dimer and AT III (r =-0.610, p=0.000).

In the control group of clinically healthy men (n=35), only one statistically significant negative, moderate relationship between TF and fibrinogen was found (r=-0.487, p=0.016).

Diagnostic reliability of the investigated markers.

The receiver-operating characteristic (ROC) curves were described and the sensitivity and specificity of the markers of coagulation and fibrinolysis - TF, D-dimer, TAT and F1+2 were calculated. The cut-off values, the area under the ROC-curve, the sensitivity, the specificity for each of the markers are presented in the corresponding tables below the ROC-curves.

Of the four investigated markers, the area under the ROC-curve for TF is the smallest (AUC=0.713) (Fig. 32, Table 9). The cut-off value was 189.95 pg/ml, with 64.3% diagnostic sensitivity and 88.6% diagnostic specificity (Table 10).

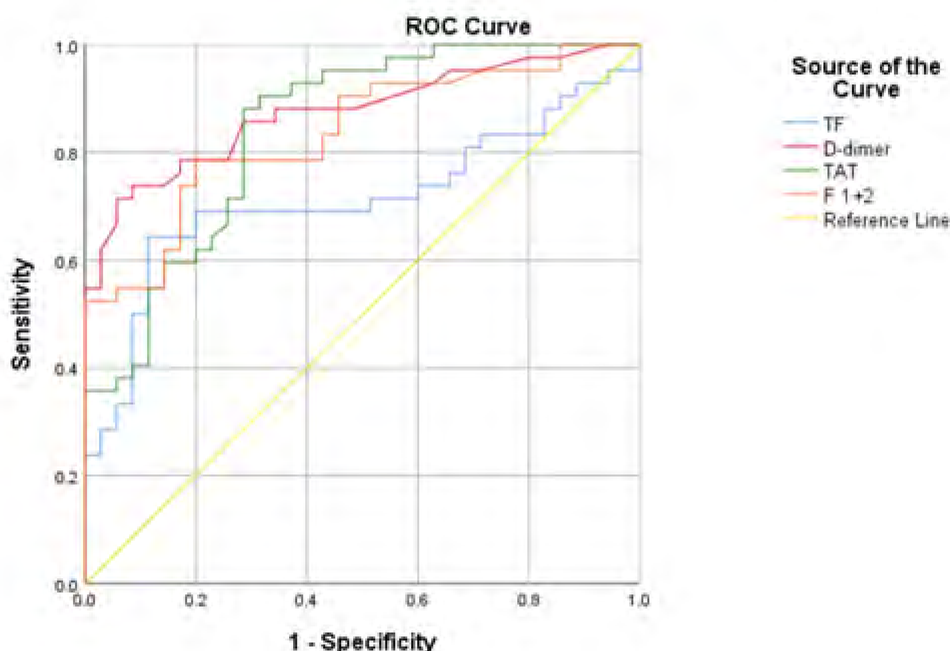

Figure 32. ROC curves: TF, D-dimer, TAT and F1+2 in lung cancer patients

Table 9. AUC fo investigated parameters

Parameter	AUC	p-value	95% Confidence Interval	
TF	0.713	0.001	0.594	0.832
D-dimer	0.874	0.000	0.796	0.953
TAT	0.843	0.000	0.756	0.930
F 1+2	0.836	0.000	0.748	0.924

Table 10. Accuracy indices of TF, D-dimer, TAT and F1+2 in lung cancer patients

Parameter	Cut-off value	Sensitivity	Scecificity	Youden index
TF	189.95 pg/ml	64.3%	88.6%	0.53
D-dimer	0.68 mg/l	71.4%	94.3%	0.657
TAT	5.7 ng/ml	88.1%	71.4%	0.595
F1+2	14.85 ng/ml	73.8%	82.9%	0.567

The area under the ROC curve of D-dimer is above 0.80 (AUC=0.874), which makes this parameter a good marker for distinguishing patients at an increased risk of thrombotic complications. The discriminant value is 0.68 mg/l, with 71.4% diagnostic sensitivity and 94.3% diagnostic specificity (Fig. 32, Table 10). The area under the ROC curve of TAT was also above 0.80 (AUC = 0.843), with a diagnostic sensitivity of 88.1% and a specificity of 71.4%. The cut-off value is 5.70 ng/ml, which allows a good differentiation of patients with a prothrombotic state (Fig. 32, Table 10). The area under the ROC curve of F 1+2 is above 0.80 (AUC= 0.836), with diagnostic sensitivity 73.8% and specificity 82.9% (Fig. 32, Table 10). The cut-off value of 14.85 ng/ml allows a good differentiation of patients at increased risk of thrombotic complications.

In Fig. 32, Tabl. 9, the areas under the ROC-curves of the investigated markers are compared. Overlap was observed between AUC of D-dimer, TAT, F1+2 at 95% CI, suggesting similarity in their diagnostic reliability, with D-dimer having the highest diagnostic sensitivity and specificity and the largest area under the ROC curve (AUC=0.874).

3. Non-Hodgkin's lymphoma (NHL)

Demographic and clinical data of the patient group and healthy controls

40 patients with NHL were studied - 17 of them women (42.5%) and 23 men (57.5%) and a control group of 65 clinically healthy volunteers, 30 women (46.2%) and 35 men (53.8%), (p=0.715). The mean age of the patient group (n=40) diagnosed with NHL was 60.33±8.81 years, and that of the control group

(n=65) was 58.63±10.76 years (p=0.404). The control and patient groups were comparable in terms of gender and age (Table 11).

Table 11. Demographic data of the patient group and healthy controls

Variables		NHL (N=40)	Controls (N=65)	P-value
age ($\bar{x}$ ± SD)*		60.33±8.81	58.63±10.76	p=0.404
gender (N; %)	men	23 (57.5%)	35 (53.8%)	p=0.715
	women	17 (42.5%)	30 (46.2%)	

* $\bar{x}$ – mean, SD – standard deviation

The distribution of patients with NHL in a localized stage (stage I and II) is 30% (n=12), in the advanced stage (stage III and IV) – 70.0% (n=28) (Fig. 33). According to the course of the disease, 62.5% (n=25) of the patients had aggressive NHL and 37.5% (n=15) had indolent NHL (Fig. 33).

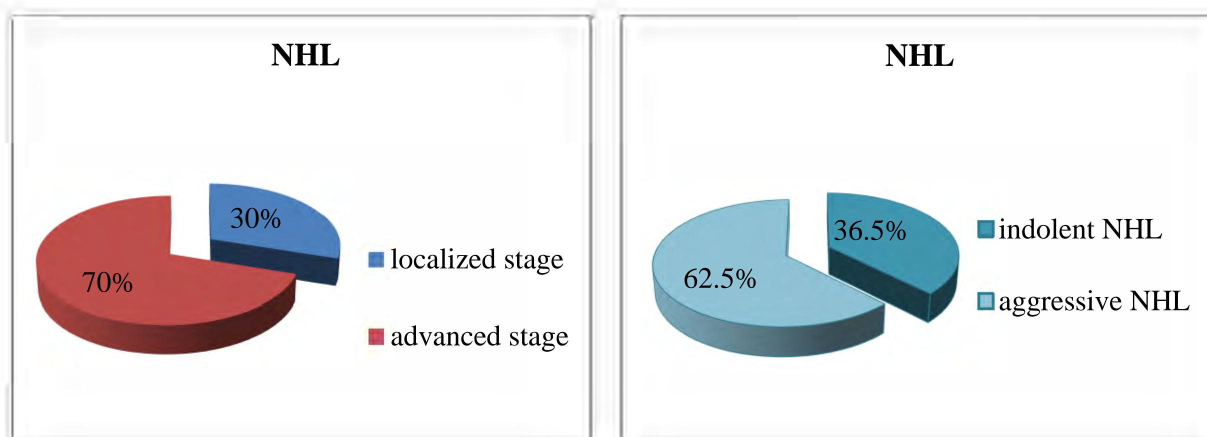

Figure 33. Pie chart of the distribution of NHL patients by stage and course

Clinical-laboratory parameters of coagulation and fibrinolysis in patients with NHL and healthy controls

The clinical-laboratory parameters of coagulation and fibrinolysis in the group of patients diagnosed with NHL (n=40) and the control group (n=65) are presented in Table. 12. TF, F1+2, fibrinogen, AT III, t-PA have a normal distribution both in patients with NHL and in the control group, while plasma levels of TAT and D-dimer do not met this requirement for normal distribution.

Table 12. Descriptive characteristics of parameters of coagulation and fibrinolysis in patients with NHL and healthy controls

Parameter	Group	Mean	Standard deviation	Median	25 percentile	75 percentile
TF pg/ml – Visit 1	NHL	201.12	85.25	203.35	122.75	269.03
	Controls	140.69	53.77	138.80	105.60	154.45
TF pg/ml – Visit 2	NHL	179.38	80.36	181.85	98.83	252.90
TF pg/ml – Visit 3	NHL	176.22	80.55	180.30	102.93	244.68
D-dimer mg/l – Visit 1	NHL	1.34	1.13	0.83*	0.50	2.25
	Controls	0.37	0.16	0.33*	0.23	0.45
D-dimer mg/l – Visit 2	NHL	0.91	0.13	0.58*	0.23	1.61
D-dimer mg/l – Visit 3	NHL	0.95	1.02	0.51*	0.20	1.30
TAT ng/ml – Visit 1	NHL	12.23	5.79	12.54*	6.90	16.80
	Controls	5.50	2.63	4.90*	3.79	7.23
TAT ng/ml – Visit 2	NHL	11.36	6.70	10.87*	5.21	17.08
TAT ng/ml – Visit 3	NHL	10.62	6.32	8.28*	4.51	17.73
F 1+2 ng/ml – Visit 1	NHL	21.59	9.39	21.30	12.65	28.40
	Controls	10.04	5.11	10.30	5.40	12.90
F 1+2 ng/ml – Visit 2	NHL	18.62	9.74	16.80	9.05	26.10
F 1+2 ng/ml – Visit 3	NHL	17.76	10.28	14.60	7.80	26.43
Fib g/l – Visit 1	NHL	3.95	1.18	3.66	2.93	4.47
	Controls	3.03	0.56	2.90	2.63	3.35
Fib g/l – Visit 2	NHL	3.82	1.35	3.50	2.83	4.29
Fib g/l – Visit 3	NHL	3.80	1.37	3.80	2.69	4.59
AT III % – Visit 1	NHL	83.92	8.30	83.10	78.23	93.08
	Controls	92.59	6.90	93.70	86.95	97.85
AT III % – Visit 2	NHL	85.32	8.15	84.60	78.23	93.28
AT III % – Visit 3	NHL	85.42	7.69	87.30	78.50	92.75

t-PA pg/ml – Visit 1	NHL	3617.15	1407.08	3701.00	2391.75	4620.00
	Controls	2374.26	1584.01	2005.00	1127.00	2956.00
t-PA pg/ml – Visit 2	NHL	3418.58	1412.91	3326.00	2278.50	4497.50
t-PA pg/ml – Visit 3	NHL	3387.80	1482.84	3222.00	2195.50	4693.00

* the variable is not with normal distribution

Comparative analysis of clinical-laboratory parameters of coagulation and fibrinolysis in patients with NHL and healthy controls

We compared the clinical-laboratory parameters of coagulation and fibrinolysis between the patient group, diagnosed with NHL (n=40), and the control group (n=65), the data are presented graphically and described (Figs. 34–40).

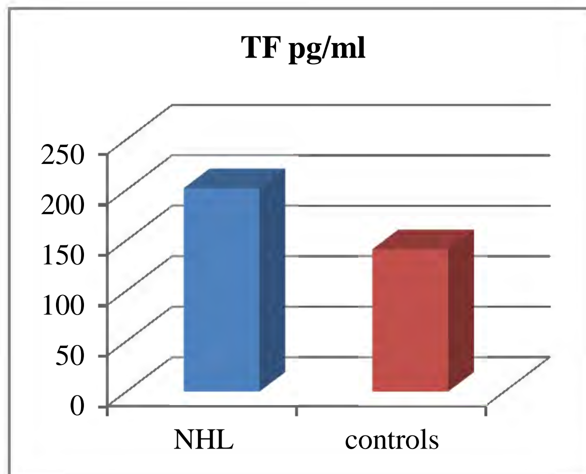

Figure 34. Mean levels of TF pg/ml in controls and patients

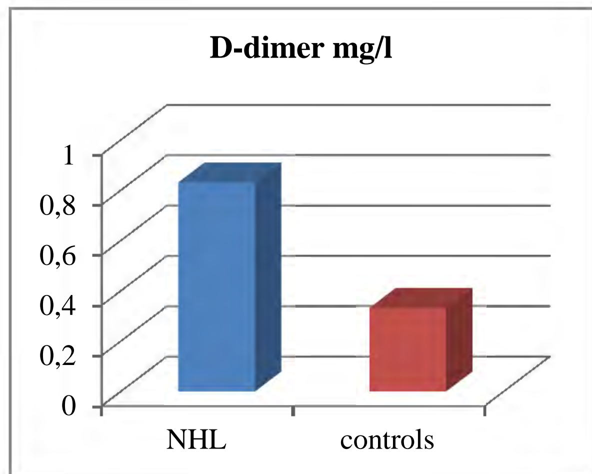

Figure 35. Mean levels of D-dimer mg/l in controls and patients

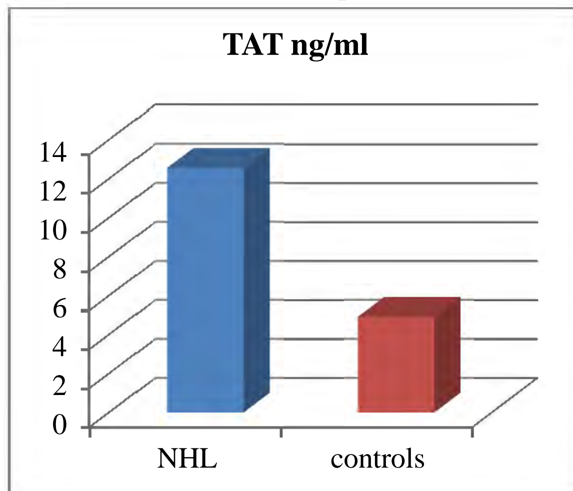

Figure 36. Mean levels of TAT ng/ml in controls and patients

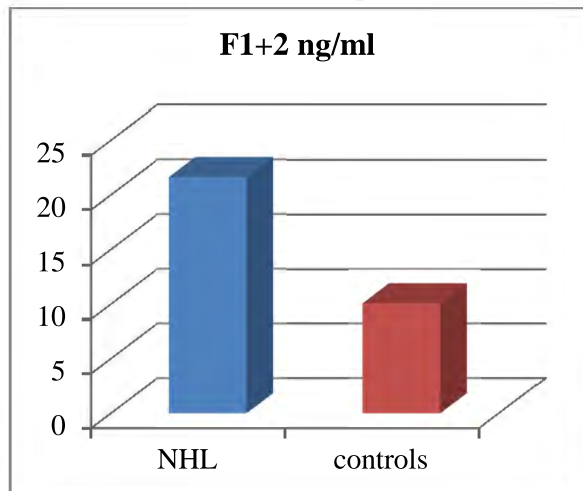

Figure 37. Mean levels of F1+2 ng/ml in controls and patients

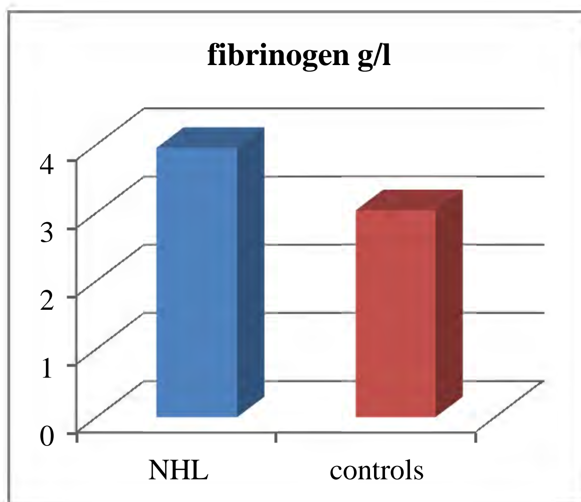

Figure 38. Mean levels of fibrinogen g/l in controls and patients

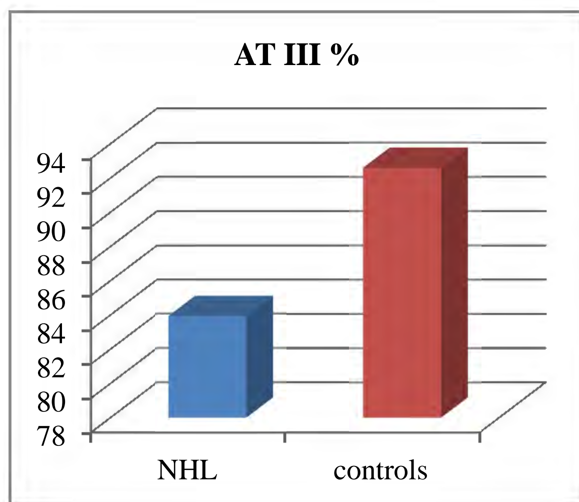

Figure 39. Mean levels of AT III% in controls and patients

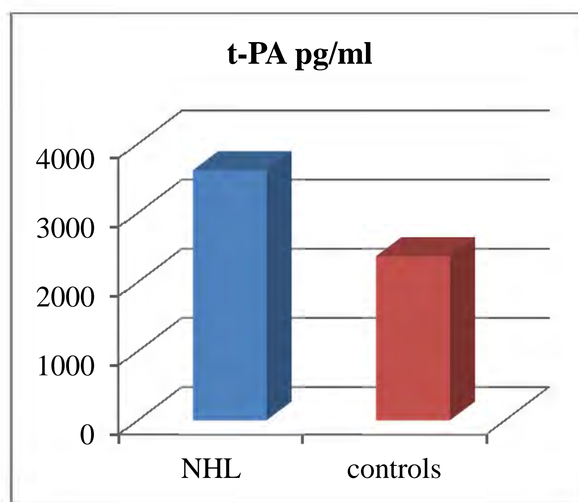

Figure 40. Mean levels of t-PA pg/ml in controls and patients

A statistically significant difference was demonstrated between the mean levels of TF in the group of patients with NHL (201.12±85.25 pg/ml) and the control group (140.69±53.77 pg/ml) – t=4.018, p=0.000 (Fig. 34). Mean D-dimer levels in the NHL patient group 0.83 mg/l (0.50 mg/l; 2.25 mg/l) were found to be statistically significantly higher than those in the control group 0.33 mg/l (0.23 mg/l ;0.45 mg/l), p=0.000 (Fig. 35). Statistically significantly higher plasma levels of TAT were demonstrated in the group of patients with NHL 12.54 ng/ml (6.90 ng/ml; 16.80 ng/ml) compared to the control group 4.90 ng/ml (3.79 ng/ml; 7.23 ng/ml), p=0.000 (Fig. 36). The mean levels of F 1+2 in the patient group (21.59±9.39 ng/ml) were twice higher than those in the control group (10.04±5.11 ng/ml) – t=7.151, p=0.000 (Fig. 37). A statistically significant difference was found between the plasma levels of fibrinogen in the group of patients with NHL (3.95±1.18 g/l) and the control group (3.03±0.56 g/l) – t= 4.623, p=0.000 (Fig. 38). The mean levels of ATIII activity in the group of patients with NHL (83.92±8.30 %) were lower than those in controls (92.59±6.90%), and the difference was

statistically significant - t=-5.785, p=0.000 (Fig. 39). A statistically significant difference was found between the plasma levels of t-PA in the patient group (3617.15±1407.08 pg/ml) and the control group (2374.26±1584.01 pg/ml) – t=4.070, p=0.000 (Fig. 40).

Examination of the screening coagulation tests revealed a significantly shortened aPTT and PT presented in sec, an increase in PT % and lower INR values in patients compared to controls. No statistically significant difference was found in thrombin time (sec) between patients and controls (Table 13). The values of the three parameters remain in the reference ranges for both groups.

Table 13. Comparative analysis of screening coagulation tests between patients and controls

Parameter	Group	N (number)	Mean	Standard deviation	Standard error	p-value
PT %	Controls	65	88.96	11.14	1.38	**p=0.016**
	NHL	40	94.77	12.85	2.03	
PT sec	Controls	65	11.38	0.58	0.07	**p=0.021**
	NHL	40	11.09	0.64	0.10	
PT INR	Controls	65	1.01	0.05	0.01	**p=0.020**
	NHL	40	0.98	0.06	0.01	
aPTT sec	Controls	65	27.29	2.31	0.29	**p=0.000**
	NHL	40	24.44	2.12	0.34	
TT sec	Controls	65	16.75	0.74	0.09	p=0.259
	NHL	40	16.92	0.71	0.11	

Dynamics of the investigated markers of coagulation and fibrinolysis in patients with non-Hodgkin's lymphoma during treatment

In patients with NHL it was also found that the “time” factor (visit) has a significant influence on the plasma levels of the markers of coagulation and fibrinolysis. The results are presented graphically and then described (Figs. 41-47).

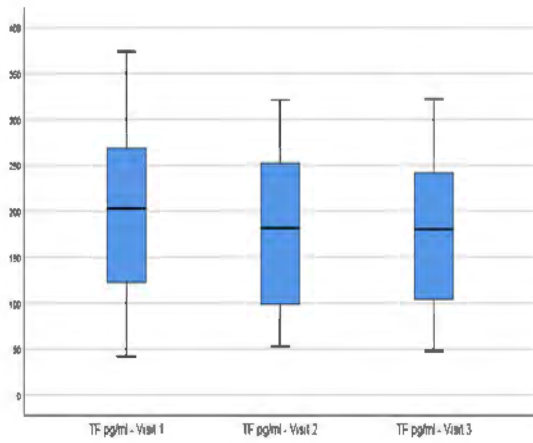

Figure 41. Dynamics of TF pg/ml in patients with NHL during treatment

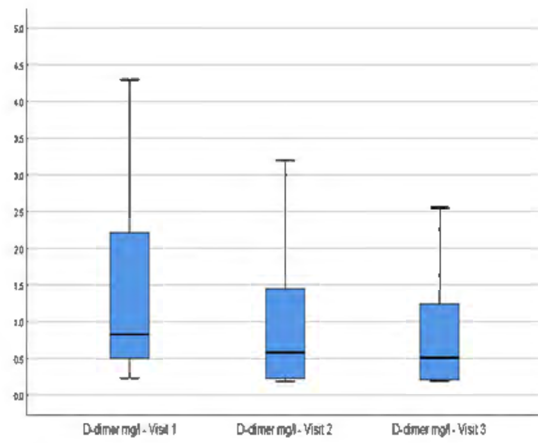

Figure 42. Dynamics of D-dimer mg/l in patients with NHL during treatment

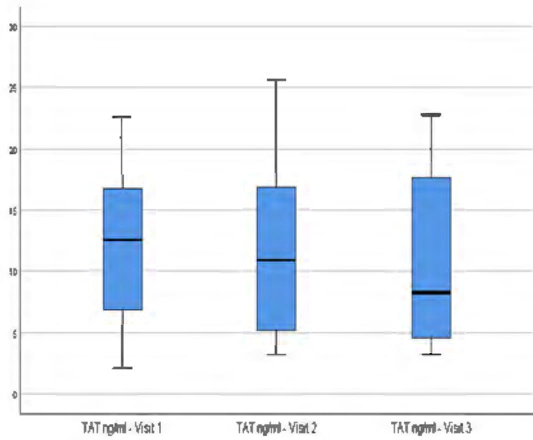

Figure 43. Dynamics of TAT ng/ml in patients with NHL during treatment

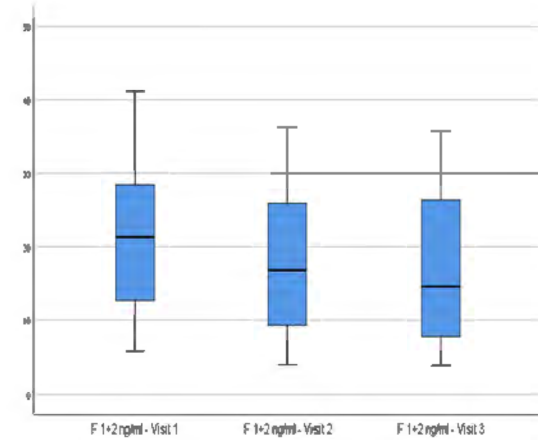

Figure 44. Dynamics of F1+2 ng/ml in patients with NHL during treatment

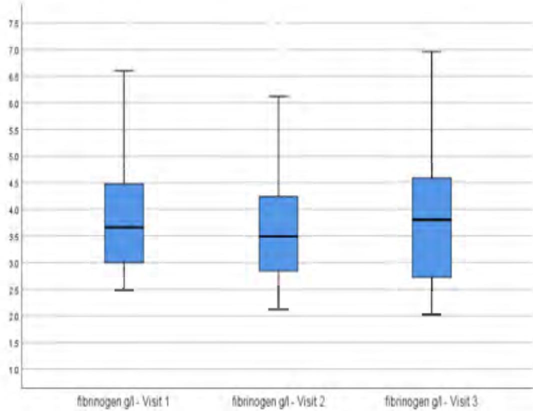

Figure 45. Dynamics of fibrinogen g/l in patients with NHL during treatment

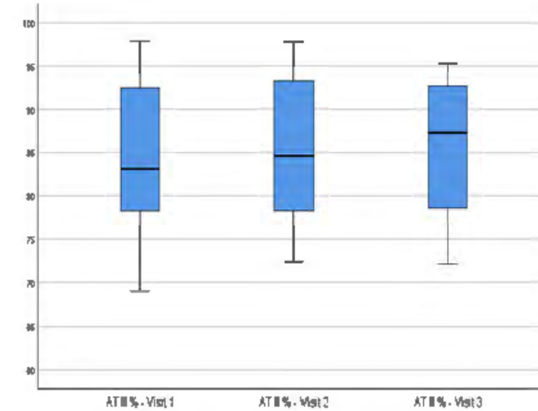

Figure 46. Dynamics of ATIII % in patients with NHL during treatment

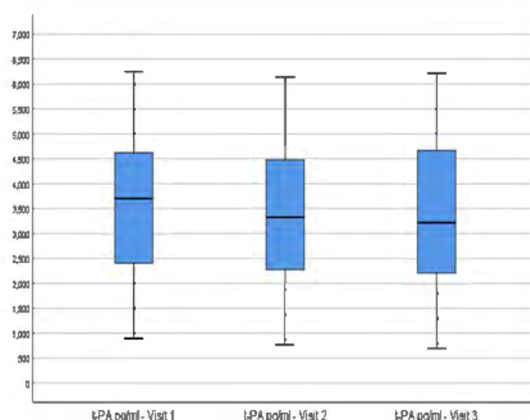

Figure 47. Dynamics of t-PA pg/ml in patients with NHL during treatment

Mean TF levels reported in the group (n=40) of NHL patients differed statistically significantly between time points (F(1.213,47.30)=9.84, p=0.002). Lower levels of TF were found at the second (179.38±80.36 pg/ml) and third visit (176.22±80.55 pg/ml) compared to the first (201.12±85.25 pg/ml), respectively p=0.009, p=0.006 (Fig. 41). Changes in plasma D-dimer levels were similar, with statistically significantly lower D-dimer levels reported at the second visit 0.58 mg/l (0.23 mg/l; 1.61 mg/l) and the third visit 0.51 mg/l (0.20 mg/l; 1.30 mg/l) compared to the first 0.83 mg/l (0.50 mg/l; 2.25 mg/l), respectively p=0.000, p=0.000) (Fig. 42). TAT levels were statistically significantly lower at the second 10.87 ng/ml (5.21 ng/ml; 17.08 ng/ml) and third visit 8.28 ng/ml (4.51 ng/ml; 17.73 ng/ml) compared to the first 12.54 ng/ml (6.90 ng/ml; 16.80 ng/ml), respectively p=0.020, p=0.005 (Fig. 43). Plasma levels of F 1+2 also decreased during the applied treatment strategy, with statistically significantly difference between time points (F(1.264,49.30)=32.59, p=0.000). We found lower levels of F 1+2 at the second (18.62±9.74 ng/ml) and third visit (17.76±10.28 ng/ml) compared to the first (21.59±9.39), respectively p=0.000 p=0.000 (Fig. 44). There was no statistically significant difference in fibrinogen levels between the visits (F(1.465,57.15)=1.45, p=0.241) (Fig. 45). The trend in ATIII activity is towards an increase, with statistically significantly higher levels of ATIII at the second (85.32±8.15%) and third visit (85.42±7.69%) compared to the first (83.92±8.30%), respectively, p=0.001, p=0.004 (Fig. 46). The mean levels of t-PA were statistically significantly lower at the second (3418.58±1412.90 pg/ml) and third visit (3387.80±1482.83 pg/ml) compared to the first (3617.15±1407.08 pg/ml), respectively, p=0.008, p =0.033 (Fig. 47).

Correlations between the investigated parameters of coagulation and fibrinolysis in patients with NHL and in healthy controls

In patients with NHL at visit 1, a positive, moderate correlation was found between TAT and fibrinogen (r=0.417, p=0.007), TF and TAT (r=0.505,

p=0.001) and D-dimer and Fib (r=0.466, p=0.002). A positive, strong relationship was observed between TAT and D-dimer (r=0.743, p=0.000), F1+2 and TAT (r=0.695, p=0.000) and between F1+2 and D-dimer (r=0.677, p=0.000). A moderate, negative relationship in patients with NHL at visit 1 is between TF and AT III (r=-0.571, p=0.000), F1+2 and AT III (r=-0.470, p=0.002), TAT and AT III (r=-0.535, p=0.000). In the control group, only weak associations were found that were not statistically significant.

Diagnostic reliability of the investigated parameters.

Of the four investigated parameters, the area under the ROC-curve for TF is the smallest (AUC=0.707) (Fig. 48, Table 14). The cut-off value is 197.55 pg/ml, with 55.5% diagnostic sensitivity and 87.7% diagnostic specificity (Table 15).

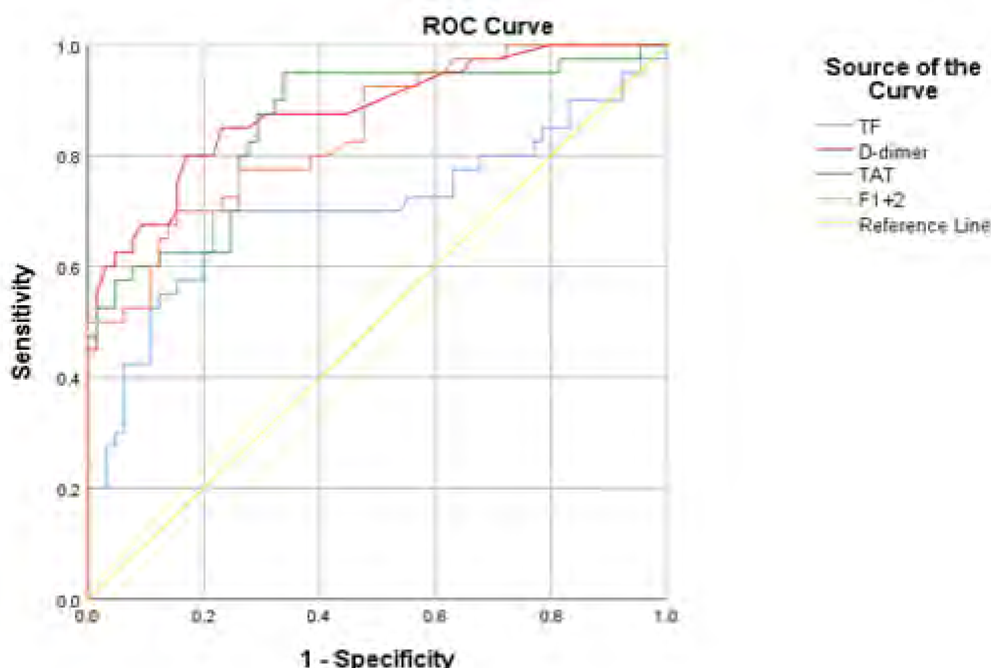

Figure 48. ROC curves: TF, D-dimer, TAT and F1+2 in NHL patients

Table 14. AUC of investigated parameters

Parameter	AUC	p-value	95% Confidence Interval	
TF	0.707	0.000	0.593	0.822
D-dimer	0.879	0.000	0.810	0.949
TAT	0.857	0.000	0.780	0.934
F1+2	0.842	0.000	0.765	0.919

Table 15. Accuracy indices of TF, D-dimer, TAT and F1+2 in NHL patients

Parameter	Cut-off value	Sensitivity	Specificity	Youden index
TF	197.55 pg/ml	55.5%	87.7%	0.427
D-dimer	0.495 mg/l	80.0%	83.1%	0.631
TAT	6.09 ng/ml	87.5%	70.8%	0.583
F1+2	16.65 ng/ml	65.0%	87.7%	0.527

The area under the ROC curve of D-dimer is over 0.80 (AUC = 0.879), which makes this parameter a good marker for distinguishing patients at increased risk of thrombotic complications. The discriminant value is 0.495 mg/l, with 80.0% diagnostic sensitivity and 83.1% diagnostic specificity (Fig. 48, Table 15). The area under the ROC curve of the thrombin-antithrombin complex was also above 0.80 (AUC = 0.857), with a diagnostic sensitivity of 87.5% and a specificity of 70.8%. The cut-off value is 6.09 ng/ml, which allows a good differentiation of patients with an increased risk of thrombotic complication (Fig. 48, Table 15). Diagnostic sensitivity of prothrombin fragment 1+2 was 65.0% and specificity 87.7% (Fig. 48, Table 15) at the cut-off value of 16.65 ng/ml. Despite the fact that the diagnostic sensitivity is lower than that of D-dimer and TAT, the area under the ROC curve for prothrombin fragment 1+2 is above 0.80 (AUC = 0.842), which allows a good differentiation of patients at prothrombotic state.

When comparing the areas under the ROC-curves of the investigated parameters, an overlap between the AUC of D-dimer, TAT and F1+2 at 95% CI is noted, which shows a similarity in their diagnostic reliability. These results are comparable to the data obtained in the other two patient groups.

4. Comparative analysis of markers of coagulation and fibrinolysis between patient groups.

We compared the mean levels of markers of coagulation activation and fibrinolysis between the three patient groups at baseline, before initiation of therapy. The only statistically significant difference was in plasma levels of fibrinogen, between the breast cancer and lung cancer groups (p=0.039), (Table 16 and Table 17).

Table 16. Comparative analysis of plasma level of D-dimer and TAT between patient groups

Parameter	Group	N	Mean	25 percetile	75 percetile	p - value
D-dimer mg/l Visit 1	Breast cancer	38	0.87	0.54	1.34	p=0.155
	Lung cancer	42	1.08	0.56	2.41	
	NHL	40	0.83	0.50	2.25	
TAT ng/ml Visit 1	Breast cancer	38	8.52	6.28	12.64	p=0.207
	Lung cancer	42	9.42	6.89	16.55	
	NHL	40	12.54	6.90	16.80	

Table 17. Comparative analysis of plasma level of TF, F1+2, AT III, fibrinogen, t-PA between patient groups

Parameter	Group	N	Mean	Standard	Средна грешка	p - value
TF pg/ml Visit 1	Breast cancer	38	196.17	92.08	14.94	p=0.773
	Lung cancer	42	210.35	92.26	14.24	
	NHL	40	201.12	85.25	13.48	
F 1+2 ng/ml Visit 1	Breast cancer	38	19.70	8.27	1.34	p=0.593
	Lung cancer	42	21.49	9.57	1.48	
	NHL	40	21.59	9.39	1.48	
Fib g/l Visit 1	**Breast cancer**	**38**	**3.61**	**1.09**	**0.18**	**p=0.039***
	Lung cancer	**42**	**4.30**	**1.39**	**0.21**	
	NHL	40	3.95	1.18	0.19	
AT III % Visit 1	Breast cancer	38	87.30	8.21	1.33	p=0.051
	Lung cancer	42	82.69	9.02	1.39	
	NHL	40	83.92	8.30	1.31	
t-PA pg/ml Visit 1	Breast cancer	38	3688.61	1688.73	273.95	p=0.426
	Lung cancer	42	4067.57	1882.85	290.53	
	NHL	40	3617.15	1407.08	222.48	

5. Discussion

We found significantly higher levels of TF in breast cancer, lung cancer and NHL patients compared to the control group, which is comparable to data of other authors (*Xia Q et al. 2020, Fernandez et al. PM 2002*). As a major initiator of the extrinsic coagulation activation pathway and its relationship with angiogenesis, which plays a major role in tumor pathology, our results support the hypothesis of an association between carcinogenesis and coagulation disorders. A number of studies have found that tissue overexpression of TF correlates with the presence of metastases, invasive potential, and aggressive tumor progression (*Bluff J.E et al. 2008, Han LY et al. 2006*). An increase in D-dimer is an indicator of fibrin formation and accumulation, which makes it a good indicator of the risk of thrombosis (thrombophlebitis, pulmonary thromboembolism). In patients with malignant diseases, the tumor tissue is covered with a fibrin mesh, which is a source of fibrin degradation products (FDP), in particular D-dimer. The finding of significantly higher D-dimer levels in our patient groups compared to the control group coincides with published results in other studies (*Jiang X et al. 2017, Mego M et al. 2015*). Pathologically increased thrombin formation and fibrin accumulation, which is characteristic of patients with malignant diseases, leads to increased formation of F1+2 and TAT. In our study, we found statistically significantly higher levels of F1+2, TAT in patients compared to healthy controls, which is similar to data published in other studies (*Lundbech M et al. 2020, Moik F et al. 2020*). Patients with malignancy may have decreased production of coagulation inhibitors and/or increased consumption. AT III regulates procoagulation activity and is an important natural thrombin inhibitor. It deactivates thrombin through an irreversible reaction, leading to the formation of thrombin-antithrombin complexes. Our data indicate that AT III activity is significantly lower in patient groups compared to healthy controls. This can be explained by a pathologically increased formation of thrombin in patients with tumor pathology and its increased consumption for the formation of TAT complexes. Other authors also demonstrated similar data (*Hong SK et al. 2010, Sun W et al. 2015*), although there were studies, that did not find a statistically significant difference between patients and the control group (*Di Micco P et al. 2002*). We found significantly higher levels of t-PA in all three patient groups compared to the control group, suggesting activation of fibrinolysis. Literature data on plasma t-PA levels in patients with malignant diseases are conflicting (*Borgfeldt C et al. 2003, Gronostaj K et al. 2016*). t-PA is secreted by endothelial and tumor cells, but the main regulator of its activity is actually PAI, which is also produced by them. Therefore, we hypothesize that a

probable imbalance in the PAI/t-PA system affected the activity of t-PA. In addition, endothelial damage characteristic of malignant diseases and influenced by chemotherapy most likely also contributes to changes in fibrinolytic factors. In breast cancer, lung cancer and NHL patients, we found statistically significantly higher levels of fibrinogen compared to healthy controls. In addition to the coagulation factor, fibrinogen is also an acute phase protein, facilitatating the adhesion of platelets to tumor cells, which leads to increased thrombin formation. All these changes contribute to hypercoagulability in patients with tumor pathology. The results obtained in our study are similar to the literature data published by other authors (*Mitsui S et al. 2022, Tian Y et al. 2017).*

Comparative analysis of screening hemostasis tests between patients and controls found significantly shortened aPTT and PT presented in sec, increased PT % and lower INR values in all three patient groups. No statistically significant difference was found in thrombin time (sec) between patients and controls. The values of the three parameters remain in the reference interval for both groups. Data in the literature regarding routine coagulation tests in patients with tumor diseases are heterogeneous. Mi XK et al reported a shortened PT and aPTT in breast cancer patients compared to controls (*Mi XK et al. 2017*). Di Micco P. et al. also found a shortened PT and aPTT in breast and gastric cancer patients compared to controls, but with no statistically significant difference between them (*Di Micco P et al. 2002*). There is also evidence of prolonged PT and aPTT in patients with tumors (*Tas F et al. 2013*). Conventional hemostasis tests do not have the necessary specificity and sensitivity to be used in the evaluation of thrombotic risk in this group of patients. Therefore, in our opinion, it is important to investigate specific hemostatic markers such as D-dimer, TAT, F1+2, which are indicative of increased thrombin accumulation. This would be useful for determining the risk of thrombotic complications and individualizing anticoagulant therapy.

We investigated changes in coagulation and fibrinolysis parameters in patients with lung cancer, breast cancer and non-Hodgkin's lymphoma during systemic therapy. In the longitudinal follow-up of the markers, it was found that the time factor (visit) has an influence. In breast cancer patients, a decrease in TF, TAT, F1+2, D-dimer levels was found at visit 2 (montoring response to therapy) and visit 3 (after completion of therapy) compared to visit 1 (baseline), as this difference is statistically significant. In parallel to this, the activity of AT III increased during treatment, the difference being statistically significant between visits 2 and 3. The plasma concentration of t-PA decreased in visits 2 and 3 compared to visit 1, but this difference was not statistically significant.

The results obtained in the group of patients with lung cancer were similar. In both patient groups, no statistically significant difference was found in the plasma levels of fibrinogen between visits.

In the group of patients with non-Hodgkin's lymphoma, the trend in the change of markers of coagulation and fibrinolysis is similar. There was a decrease in the levels of TF, thrombin-antithrombin complex (TAT), prothrombin fragments F1+2, D-dimers and t-PA in visit 2 and 3 compared to 1, and this difference was statistically significant. AT III activity increased during therapy, with the difference being statistically significant between visits 2 and 3.

Baseline increased levels of TF, D-dimer, F1+2, TAT were associated with lower AT III activity. The consumption of AT III, which is a natural inhibitor of coagulation, decreases - its activity after therapy increases or normalizes. In parallel, the levels of TF, D-dimer, F1+2, TAT as markers for activation of the coagulation cascade decrease. Judging by the results obtained by us, we can assume that the dynamics of the studied markers could be indicative for a suppressive effect of the systemic therapy on coagulation. We are inclined to assume that the characterized dynamic profile of a decrease in TF, TAT, F1+2, D-dimer and t-PA and an increase in AT III levels is informative for therapeutic effectiveness during treatment.

Despite the decrease in the markers of coagulation activation during follow-up, their levels remained higher after completion of therapy compared to the control group, from which it can be assumed that the prothrombotic state was present both before the initiation of therapy and during the treatment. The data obtained in our study correlate with results reported by other authors (*Inal T et al. 2014, Reitter EM et al. 2018).*

When comparing markers of coagulation and fibrinolysis between the three patient groups, the only statistically significant difference was in plasma fibrinogen levels at visit 1. Although no statistically significant difference was found in the other parameters, higher levels of TF, D-dimer , F 1+2, TAT in patients with lung cancer and NHL could be explained by the advanced stage of the disease at diagnosis in these patients.

In our study, we searched for a correlation between the parameters of coagulation and fibrinolysis in the patient groups and controls. Only a few correlations of moderate strength were found in the control groups, but strong, significant relationships appeared in the patient groups. Between TF, D-dimer, fibrinogen, F 1+2, TAT, the relationship is positive, while with AT III this relationship is negative. The increase in TF, D-dimer, fibrinogen, F1+2, TAT is associated with a decrease in the activity of AT - i.e. with its consumption. Positive significant correlation between D-dimer and F1+2 ($r=0.65$, $p<0.001$),

D-dimer and TAT (r=0.71, p<0.001), TAT and F1+2 (r=0.76, p<0.001) was demonstrated by other authors in studies of patients with suspected venous thromboembolism (*Ota S et al. 2008, Wexels F et al. 2016*). In the Vienna CATS study, a positive significant correlation was found between D-dimer and F1+2 (r=0.5, p<0.001) (*Ay C et al. 2009*). In our study, in addition to the reported quantitative changes, qualitative changes in the interaction between the investigated parameters were found in healthy controls and patients. These results demonstrate a coherent and complex alteration of interactions between hemostatic and fibrinolytic factors, most likely induced by the tumor, with tendency to hypercoagulable state.

When comparing the diagnostic reliability of markers of activation of coagulation and fibrinolysis in the three groups of malignant diseases, we found that the lowest was AUC in TF. The ROC curves of D-dimer, F1+2 and TAT have similar characteristics and their AUCs overlap (above 0.80). In all three patient groups, D-dimer showed the highest values for diagnostic sensitivity and specificity, followed by TAT and F1+2. These results confirm the fact that the three parameters are good markers of hypercoagulable state and for distinguish patients at risk of thrombotic complications. Our data are comparable to those of other authors. In a study of coagulation and fibrinolysis parameters in patients with gastric carcinoma, Fidan et al reported that the AUC for TAT was 0.874 (*Fidan E et al 2012*). Ota S. et al also reported similar data for the ROC curves of D-dimer, F1+2 and TAT (AUC >0.9) in the study of patients with different pathologies, the largest proportion of which were those with solid tumors (Ota S et et al. 2008). F1+2 and TAT are markers that directly reflect thrombin formation and coagulation activation. D-dimer is a marker for both coagulation activation and fibrin degradation and fibrinolysis activation. It also has a longer half-life than F1+2 and TAT. This could explain the higher values for diagnostic sensitivity and specificity of D-dimer compared to F1+2 and TAT in patients with malignant pathology at increased risk of thrombosis.

In conclusion, as a result of the data obtained in our study, it can be assumed that in newly diagnosed patients with malignant diseases, a procoagulant state is characteristic. It is due to specific procoagulant activity of tumor cells, change in coagulation and fibrinolysis systems, interaction of hemostasis factors with components of the inflammatory response. Subclinical abnormalities in coagulation status are characteristic in patients with malignant diseases. Although there is a tendency to decrease the markers of activation of coagulation and fibrinolysis and increase the activity of AT III during treatment, this condition persists.

Therapy for patients with malignant pathology depends on the type of tumor, its differentiation, stage and aggressiveness. VTE is a frequent complication in these patients, worsening the quality of life and the prognosis of the disease. The incidence of VTE also depends on the type of administered chemotherapy. This necessitates the need for an individual approach and assessment of the risk of thrombotic complications in patients with malignant diseases. This requires an individual plan for anticoagulant therapy in order to prevent and reduce the frequency of thrombosis and for treatment of associated pulmonary thromboembolism. The use of anticoagulants would reduce tumor growth, angiogenesis and metastasis. Most studies support the idea of the combined application of standard chemotherapeutics with anticoagulants, which represents an ideal opportunity to control tumor growth and reduce the risk of serious thrombotic complications.

V. CONCLUSIONS

1. Higher levels of TF, thrombin-antithrombin complex (TAT), prothrombin fragment 1+2, D-dimer, fibrinogen and lower AT III activity in patients with malignant diseases compared to healthy controls support our hypothesis of the relationship between carcinogenesis and blood coagulation disorders.
2. Plasma levels of t-PA in all three patient groups are also higher compared to healthy controls, but we assume that a possible imbalance in the PAI/t-PA system affected its activity.
3. Standard screening coagulation tests (aPTT, PT, TT) are not sufficiently informative about changes in the hemostasis system.
4. Systemic therapy significantly changes the dynamics of the investigated markers. In a longitudinal follow-up, the cytotoxic effect of therapy has a suppressive effect on the levels of TF, thrombin-antithrombin complexes (TAT), prothrombin fragment 1+2, D-dimer and t-PA.
5. The investigated markers decrease during treatment, but remain higher than those in the control group, which is indicative of the prothrombotic state in these patients.
6. The interaction between the markers of coagulation and fibrinolysis were also changed, with no correlations in healthy subjects, but statistically significant correlations in the patient groups. These results demonstrate a coherent and complex alteration of interaction between hemostatic and fibrinolytic factors, which is induced most likely by the tumor.
7. TAT, D-dimer and F1+2 have high values of accuracy indices assessed by ROC analysis and can be used as markers to differentiate patients at increased risk of thrombotic complications.

VI. CONTRIBUTIONS

1. The relationship between malignant diseases and blood coagulation disorders has been confirmed.

2. For the first time in the country, markers of coagulation activation thrombin-antithrombin complex (TAT) and prothrombin fragment 1+2 were investigated in patients with malignant diseases, in order to assess their diagnostic reliability.

3. For the first time in the country, t-PA was studied as a marker for activation of fibrinolysis in patients with malignant diseases.

4. For the first time in the country, changes in markers for activation of coagulation and fibrinolysis waere compared and evaluated - thrombin-antithrombin complex (TAT), prothrombin fragment 1+2, Antithrombin III (AT III), D-dimer and tissue plasminogen activator (t-PA) during therapy in patients with malignant diseases.

5. The investigation of markers of activation of coagulation and fibrinolysis in patients with malignant diseases proves their importance as elements of the multimarker approach discussed in the literature to assess the risk of thrombotic complications in these patients.

VII. PUBLICATIONS AND PARTICIPATION IN SCIETIFIC FORUMS

Publications in connection with the dissertation theme

1. S. Stoencheva, T. Deneva, Zh. Grudeva–Popova, E. Beleva, V. Popov. Determination of plasma tissue factor antigen and tissue factor-bearing microparticles activity in healthy bulgarian subjects, BJSTR**,** Vol. 19, Issue 3, pp. 14281-14286, July, 2019 ISSN: 2574 -1241
2. Stoencheva SS, Popov VG, Grudeva-Popova ZG, Deneva TI. Markers of activation of coagulation in cancer patients. Bratislava medical journal. 2023;124(1):29-35 doi: 10.4149/BLL_2023_004. PMID: 36519604. (IF_{2021}= 1.564)
3. Stoencheva S, Popov V, Grudeva-Popova Z, Deneva T. Alteration of coagulation and fibrinolysis in malicnancy, Knowledge – International Journal, Vol.56.3, 2023

Participation in scientific forums

1. S. Stoencheva, T. Deneva, D. Davcheva, B. Delev, E. Beleva "Hypercoagulable state in breast cancer patients" Clinical Chimica Acta 2019; Vol. 493, S391. 23rd IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine – Barcelona 19-23 May 2019
2. S. Stoencheva, E. Beleva, V. Popov, Zh. Grudeva-Popova, T. Deneva, Clinical - laboratory assessment of coagulation and fibrinolysis in patients with malignant diseases, Jubilee Scientific Conference, 75th. MU - Plovdiv, Medicine of the Future, Clinical intra-university project session 29-31.10.2020
3. S. Stoencheva, T. Deneva, E. Beleva, Zh. Grudeva - Popova, V. Popov Clinical – laboratory evaluation of coagulation and fibrinolysis in cancer patients, XXVIII BALKAN CLINICAL LABORATORY FEDERATION MEETING, XIII NATIONAL CONFERENCE OF CLINICAL LABORATORY, September 8-11, 2021, Sofia, Bulgaria – oral presentation

4. S. Stoencheva, Zh. Grudeva-Popova, T. Deneva, E. Beleva, V. Popov Changes in hemostasis parameters in patients with non-Hodgkin's lymphoma XIV NATIONAL CONFERENCE ON CLINICAL LABORATORY, 14-16.10.2022, Plovdiv – report
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Scientific projects

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