Original Article

Altered fibrin clot properties in advanced lung cancer: impact of chemotherapy

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Background: Faster formation of dense and poorly lyzable fibrin networks have been reported in patients at risk of thromboembolism, including cancer patients. We sought to investigate whether chemotherapy affects plasma fibrin clot properties and their determinants in lung cancer patients.

Methods: In this observational study we enrolled 83 consecutive patients with advanced inoperable lung cancer. Plasma fibrin clot permeability (K_s), turbidimetric analysis of clot formation, clot lysis time (CLT), microparticle-associated tissue factor (MP-TF) activity, and thrombin generation parameters were investigated at enrolment and 3–4 months after standard chemotherapy.

Results: Lung cancer patients after 4 (range, 4–5) cycles of chemotherapy had 35.6% higher D-dimer, 22.1% lower MP-TF activity, and unaltered fibrinogen compared with baseline. Chemotherapy resulted also in 7.5% increased K_s, 8.6% prolonged lag phase, and 5.4% shortened CLT, while thrombin generation was unchanged. Chemotherapy-related differences in clot structure were confirmed by scanning electron microscopy images. Fibrin clot properties after chemotherapy did not differ among histological types of lung cancer, cancer stages or chemotherapy regimens. Interestingly, never smoking (n=13, 16%) was associated with looser post-treatment fibrin structure as reflected by 12.3% higher K_s. Multiple linear regression showed that more advanced cancer stage, higher peak thrombin generation, and higher white blood cell count determined post-treatment change in K_s, while active smoking was associated with change in CLT.

Conclusions: Three-month chemotherapy in lung cancer patients improves clot properties despite unaffected thrombin generation, suggesting that anticancer treatment might quickly produce antithrombotic actions.

Keywords: Chemotherapy; clot lysis time (CLT); fibrin clot; lung cancer; thrombin generation

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Introduction

Lung cancer is the leading cause of cancer death among both men and women (1), however available treatment, including palliative strategies, significantly prolongs the life of patients with advanced lung cancer (2). Despite the introduction of new therapies such as chemotherapy and immunotherapy that in some cases make cancer a chronic disease, lung cancer remains the most prevalent fatal tumour (3). Approximately 3% of lung cancer patients develop venous thromboembolism (VTE) within 2 years since diagnosis (4) with a year cumulative incidence of 10.2% in patients with small cell lung carcinoma (SCLC) (5) and about 22% in those with non-small cell lung carcinoma (NSCLC), which accounts for more than 80% lung cancers (6,7). A hypercoagulable state in lung cancer involves tissue factor (TF), microparticles, anticoagulant and antifibrinolytic systems, platelets, and many other factors (8). The lung cancer chemotherapy is associated with three-fold increased risk for VTE (9). An imbalance between coagulation activation and fibrinolytic potential might contribute to thromboembolic complications during chemotherapy. Shortening of clotting time, increase in antithrombin levels and thrombin-antithrombin complexes, followed by decreased plasma D-dimer, plasmin-α_2-antiplasmin complex and fibrinogen levels have been observed in lung cancer patients during the first and the second cycle of standard anticancer therapy with a large variability of these parameters up to 3 weeks after treatment (10). Recent study has demonstrated that activated and apoptotic platelets, together with platelet-derived microparticles contribute to hypercoagulable state in patients with NSCLC after chemotherapy and can be closely related to thromboembolic complications during anticancer treatment (11).

Growing evidence indicates that the formation of clots composed of compact fibrin networks resistant to fibrinolysis, which is largely determined by environmental factors (12), predisposes to VTE (13). Cigarette smoking, a common risk factor for lung cancer, also negatively alters fibrin clot properties (14). Data on fibrin clot characteristics in cancer patients, however, are scarce. Less permeable fibrin clots relatively resistant to lysis have been observed in gastrointestinal cancer and multiple myeloma (15,16). Rheometry of blood clots in lung cancer patients has shown the formation of pathological clot microstructure with changes of physical properties, including higher fractal dimension in patients with extensive lung cancer compared with the localized disease (17).

To our knowledge, comprehensive analysis of the plasma clot formation, permeability, and lysis in patients with advanced lung cancer during chemotherapy has not been published so far. Therefore, we investigated plasma fibrin clot features and their determinants in lung cancer patients before and after chemotherapy.

Methods

Study population

From May to September 2014 in a prospective study we evaluated 83 white consecutive patients with advanced histologically or cytologically confirmed lung cancer who were recruited at the Department of Oncology, the John Paul II Hospital, Cracow, Poland. Patients were categorized into subjects with SCLC and those with NSCLC that included three main subtypes: adenocarcinoma, squamous cell cancer, and not otherwise specified (NOS) carcinoma, involving cases other than adenocarcinoma, squamous cell, large cell carcinoma or mixed/other histology.

The American Joint Committee on Cancer (AJCC) stages were determined according to the AJCC 7th edition staging system, using available clinical data. Initially all patients met all the following eligibility criteria for chemotherapy: an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; adequate organ function (leukocytes ≥3,000/μL and ≤12,000/μL; neutrophils ≥1,500/μL; platelets ≥100,000/μL; haemoglobin ≥9.0 g/dL; total bilirubin ≤1.5 mg/dL; aspartate aminotransferase and alanine aminotransferase ≤2.5× upper limit of normal; serum creatinine ≤1.5 mg/dL or creatinine clearance ≥60 mL/min; PaO_2 ≥60 mmHg).

The exclusion criteria were: any active infections, glomerular filtration rate <60 mL/min, hypo- or hyperthyroidism, any acute vascular events, and current anticoagulant therapy. Patients in whom prophylaxis with low-molecular-weight heparins (LMWH) was administered, were eligible.

Heart failure (HF) was defined as the presence of relevant symptoms and signs and left ventricular ejection fraction ≤45%. Arterial hypertension was diagnosed based on a history of hypertension (blood pressure >140/90 mmHg) or preadmission antihypertensive treatment. Diabetes mellitus was defined as fasting glucose ≥7.0 mM on two separate occasions or use of insulin or oral hypoglycaemic

agents. Coronary artery disease (CAD) was defined as hospitalization for angina, prior documented myocardial infarction or coronary revascularization. Ischemic stroke was diagnosed based on the World Health Organization criteria. Chronic obstructive pulmonary disease was defined by the presence of irreversible expiratory airflow limitation measured by spirometry. Current smoker was defined as a subject who has smoked 100 cigarettes and currently declares the use of one or more cigarettes per day, while former smoker as a person who has smoked at least 100 cigarettes in a lifetime, but currently (at least within the last month) does not smoke.

All patients commenced standard chemotherapy according to the histopathological type of cancer and co-morbidities (18). Patients on chemotherapy underwent standard chest computed tomography scans at the 9th or 12th week since its onset and were assessed according to the response evaluation criteria in solid tumours (RECIST 1.1) (19). The recommended thromboprophylaxis in high-risk patients was administered (20).

The Local Ethical Committee in Krakow approved the study (31/KBL/OIL/2013) and participants provided informed consent in accordance with the Declaration of Helsinki.

Laboratory investigations

Fasting blood samples were drawn from antecubital vein using minimal stasis. Blood samples were drawn at the time of enrolment before starting chemotherapy and before the third or fourth cycle of chemotherapy. Patients who received prophylactic LMWH were drawn at least 12 hours since the injection. Complete blood count, glucose and creatinine were assayed by routine laboratory techniques. Fibrinogen was determined using the Clauss method. Plasma D-dimer was measured with the Innovance D-dimer assay (Siemens, Marburg, Germany). Immunoenzymatic assay was used to determine the TF-bearing microparticles (MP-TF) activity (Hyphen BioMed, Neuville sur Oise, France).

Fibrin clot properties

In citrated plasma (vol/vol, 9:1 of 3.2% sodium citrate), the following variables describing a plasma clot formation, structure and lyzability were determined in duplicate by technicians blinded to the origin of the samples (intra-assay and inter-assay coefficients of variation, 5% to 7%).

Clot permeability

Permeation of plasma fibrin clots was determined as described (21). Briefly, 20 mM calcium chloride and 1 U/mL human thrombin (Sigma-Aldrich, St. Louis, MO, USA) were added to citrated plasma. Tubes containing the clots were connected to a reservoir of a Tris-buffered saline (TBS; 0.01 M Tris, 0.1 M NaCl, pH 7.4) and its volume flowing through the gels was measured. A permeation coefficient (K_s), which indicates the pore size, was calculated from the equation: $K_s = QxLx\eta/txAx\Delta p$, where Q is the flow rate in time t, L is the length of a fibrin gel, η is the viscosity of liquid (in poise), t is percolating time, A is the cross-sectional area (in cm^2) and Δp is a differential pressure (in dyne/cm^2).

After K_s measurement clots (n=6) were fixed using 2.5% glutaraldehyde, then removed from tubes, washed with distilled water, dehydrated in graded water-ethanol solutions, dried by the critical point procedure and sputter coated with gold. Samples were scanned in six different areas (microscope JEOL JCM-6000; JEOL Ltd., Tokyo, Japan).

Turbidity measurements

Plasma citrated samples were mixed 2:1 with a TBS containing 0.6 U/mL human thrombin (Sigma-Aldrich) and 50 mM $CaCl_2$ to initiate polymerization. Absorbance was read at 405 nm with a Perkin-Elmer Lambda 4B spectrophotometer (Molecular Devices). The lag phase of the turbidity curve, which reflects the time required for initial protofibril formation and maximum absorbance at the plateau phase (ΔAbs), indicating the number of protofibrils per fiber, were recorded (21).

Clot lysis assay

Clot lysis time (CLT) was measured as described previously (21). Briefly, to 75 µL of citrated plasma we added TF (dilution 105 times; Innovin, Dade Behring, Deerfield, IL, USA), $CaCl_2$ (final concentration, 17 mM), tissue plasminogen activator (tPA, final concentration, 30 U/mL; Boehringer Ingelheim, Ingelheim, Germany) and phospholipid vesicles (22) (final concentration, 10 mM). HEPES buffer was added to make a total volume of 150 µL.

Thrombin generation assay

To assess the thrombin generation profiles, we used the assay previously described (23,24). Briefly, corn trypsin inhibitor was added to citrated plasma (0.1 mg/mL, final concentration) and 80 µL of the sample was mixed with relipidated TF (5 pM, final concentration). Twenty µL of a

2.5 mM Z-GGR-AMC/90 mM $CaCl_2$ solution in HEPES was added. Twenty µL of a 120 µM phospholipid vesicles solution in HEPES was then added to plasma samples to achieve a final concentration of 20 µM, thus initiating thrombin generation. Hydrolysis of the AMC substrate (at 370/460 nm) was followed over a 3,600 s period. Changes in fluorescence were converted to thrombin concentration using a calibration curve built by sequential dilutions of human thrombin.

Statistical analysis

The study was powered to have a 90% chance of detecting a 10% difference in CLT using a P value of 0.01, based on the previous study (25). In order to demonstrate such a difference, or a greater one, 32 patients or more were required in each group. In turn, to demonstrate such a difference, or a greater one, in K_s using a P value of 0.05 at least 31 patients were required in each group (22).

Variables were presented as mean and standard deviation, median and interquartile range or otherwise stated. The normality of distribution was checked using Shapiro-Wilk test. The Spearman's rho correlation coefficient was computed to measure the relationship between continuous variables. For testing association for categorical variables, the Fischer's Exact test was used. The *t*-test was used for means comparison, whereas the non-parametric U Mann-Whitney or Kruskal-Wallis tests were used for comparison of non-normally distributed variables. For paired data the paired Student's *t*-test or the Wilcoxon signed-rank tests were used as appropriate.

ΔK_s and ΔCLT were calculated as a difference in K_s values after 3-month chemotherapy and baseline, and the difference in the CLT values between baseline and after 3-month chemotherapy, respectively. Higher ΔK_s and ΔCLT denote improved clot permeability and lyzability, respectively. Determinants of ΔK_s and ΔCLT were established in a multiple linear regression model, built by a forward stepwise selection procedure, and verified by F Snedecor's statistics, with F >1. The R^2 was used as a measure of the variance. The models were adjusted for fibrinogen. All statistical analyses were performed with JMP®, version 12.2.0 (SAS Institute INC., Cary, NC, USA).

Results

Patient characteristics

Baseline characteristics of lung cancer patients before and after therapy are summarized in *Table 1*. At enrolment 37 (44.6%) patients were diagnosed with the SCLC and 46 (55.4%) with the NSCLC. Metastatic lung cancer was diagnosed in 53 (63.9%) patients, while in 30 (36.1%) locally advanced inoperable lung cancer was recognized. The study group included 37 patients with limited disease (LD) and 46 patients with extended disease (ED).

Patients with NSCLC before chemotherapy had higher peak thrombin {181 [108–247] *vs.* 135 [97–148] nM, P=0.043} and D-dimer {528 [351–1,085] *vs.* 324 [254–691] ng/mL, P=0.017} levels than those with SCLC, while patients with stage IV/ED were characterized by higher D-dimer {638 [396–1,174] *vs.* 326 [254–495] ng/mL, P<0.0001} and MP-TF activity [1.74 (1.24–2.40) *vs.* 1.30 (0.89–1.90) pg/mL, P=0.032] compared to those with stage III/LD.

Impact of chemotherapy

Among 83 patients who completed at least 4 [median, 5 (range, 4–5)] cycles of chemotherapy, 12 (14.5%) subjects were treated with cisplatin/vinorelbine, one subject (1.2%) with cisplatin/gemcitabine, 5 (6%) subjects with carboplatin/vinorelbine, 13 (15.7%) with paclitaxel/carboplatin, 10 (12%) with cisplatin/pemetrexed, 1 (1.2%) with vinorelbine in monotherapy, 23 (27.7%) with cisplatin/etoposide and 17 (20.5%) with carboplatin/etoposide. In one (1.2%) patient the tyrosine kinase inhibitor was administered. Among lung cancer patients, 54 (65%) individuals responded to treatment, 21 (25%) had disease stabilization and 8 (10%) had cancer progression. Interestingly, CLT assessed before chemotherapy was significantly associated with the clinical response to treatment and was prolonged in patients with the disease progression and shortened in those with the response to chemotherapy (P value for ANOVA =0.038; *Figure 1*). No symptomatic thromboembolic events were observed.

Three months after the onset of chemotherapy we found 17.5% lower red blood cell count, 28.6% lower white blood cell count, 15.5% lower haemoglobin, along with 35.6% higher D-dimer and 22.1% lower MP-TF activity, while fibrinogen was unaltered. Interestingly, we observed 7.5% increased K_s, 8.6% prolonged lag phase, 5.4% shortened CLT and unchanged thrombin generation parameters (*Table 1*). As reflected by the SEM analysis, along with the clot permeability, patients after chemotherapy had more porous fibrin structure (*Figure 2*). Patients with NSCLC compared to those with SCLC had higher glucose levels,

Table 1 Laboratory tests and fibrin clot variables in lung cancer patients before and after chemotherapy

Variable	Lung cancer patients (n=83)	Patients after 3-month chemotherapy (n=83)	P value
Age, years	63.9±7.0	–	–
Male, n (%)	51 (61.4)	–	–
Body mass index, kg/m^2	25.3±4.6	–	–
Current smoking, n (%)	42 (50.6)	–	–
Previous stroke, n (%)	3 (3.6)	–	–
CAD, n (%)	14 (16.9)	–	–
Arterial hypertension, n (%)	41 (49.4)	–	–
Diabetes, n (%)	9 (10.8)	–	–
COPD, n (%)	13 (15.7)	–	–
Statin, n (%)	19 (22.9)	–	–
ACEI, n (%)	19 (22.9)	–	–
Aspirin, n (%)	16 (19.3)	–	–
RBC, 10^6/μL	4.51±0.46	3.73±0.52	<0.001
WBC, 10^3/μL	9.0 [7.2–11.4]	6.5 [5.1–9.0]	<0.001
Platelets, 10^3/μL	313 [256–366]	324 [221–394]	0.92
Haemoglobin, g/dL	12.9±1.5	10.9±1.4	<0.001
Glucose, mM	5.3 [4.9–6.0]	5.5 [5.1–6.1]	0.40
Creatinine, μM	74 [62–84]	77 [67–90]	0.075
Fibrinogen, g/L	3.15 [2.70–3.90]	3.22 [2.65–3.77]	0.83
D-dimer, ng/mL	491 [303–893]	666 [496–940]	0.013
MP-TF activity, pg/mL	1.49 [0.98–2.20]	1.16 [0.83–1.59]	0.034
K_s, 10^{-9} cm^2	6.7 [5.8–7.5]	7.2 [6.7–7.6]	0.00011*
Lag phase, s	39.98±3.96	43.42±4.04	<0.001*
ΔAbs (405 nm)	0.85 [0.80–0.90]	0.84 [0.78–0.88]	0.80*
Clot lysis time, min	98.0±16.8	92.7±15.2	0.031*
Lag time, s	1,177 [1,028–1,483]	1,119 [917–1,301]	0.18
Peak thrombin generated, nM	144 [98–223]	136 [99–205]	0.22
TTPeak, s	2,139 [1,854–2,585]	2,063 [1,740–2,386]	0.97
ETP, nM·s	94,346±37,237	91,744±35,278	0.095

*, adjusted for fibrinogen. Data are given as mean ± SD, n (%), or median [interquartile range]. COPD, chronic obstructive lung disease; CAD, coronary artery disease; HF, heart failure; ACEI, angiotensin-converting enzyme inhibitors; RBC, red blood cells; WBC, white blood cells; MP-TF, tissue factor-bearing microparticles procoagulant activity; K_s, fibrin clot permeability; ΔAbs (405 nm), maximum absorbance of fibrin gel at 405 nm; TTPeak, time to thrombin peak; ETP, endogenous thrombin potential.

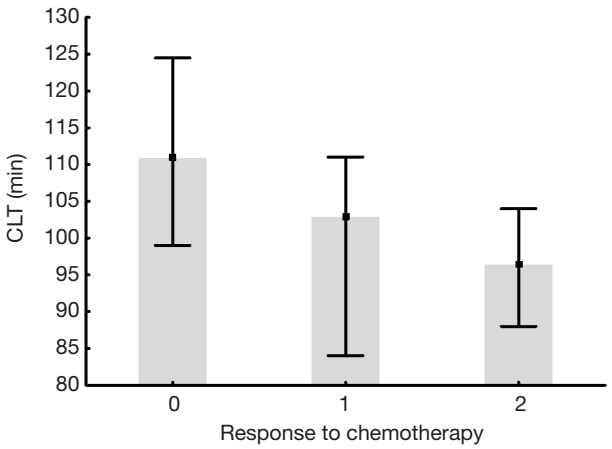

Figure 1 Associations between CLT and response to chemotherapy in lung cancer patients. 0, denotes cancer progression; 1, disease stabilization; 2, any response to treatment. Data are given as median (interquartile range). CLT, clot lysis time.

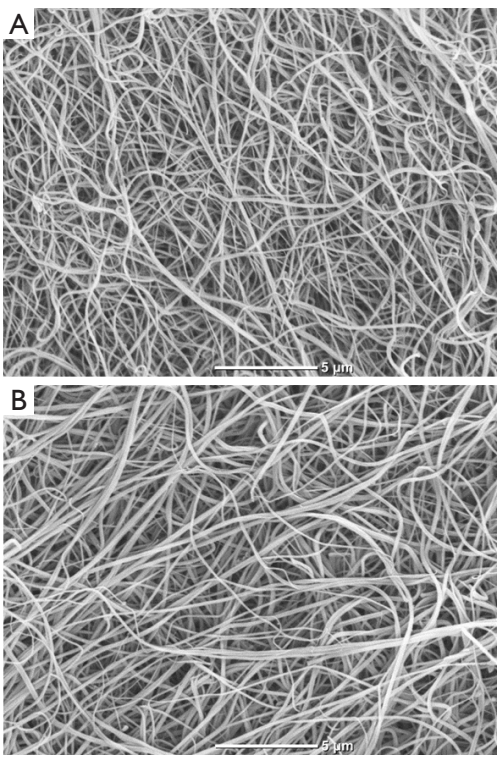

Figure 2 Representative scanning electron microscopy (SEM) images of plasma fibrin networks in a representative lung cancer patient before (A) and after chemotherapy (B). Magnification, 5,000×; fibrinogen concentration, 3 g/L. K_s =6.3×10^{-9} cm^2 before chemotherapy (A) and K_s =7.4×10^{-9} cm^2 after anticancer treatment (B).

peak thrombin generated, and ETP, while subjects with stage ED/IV had higher D-dimer levels and peak thrombin generated than those with LD/III (*Table 2*). None of the analysed variables differed significantly between patients receiving various chemotherapy combinations (data not shown). Fibrin clot properties after chemotherapy were similar in patients with different histological types of lung cancer or cancer stages (*Table 2*). Notably, K_s, but not other fibrin or thrombin generation variables, was higher after chemotherapy in never smoking patients (n=13, 16%) compared with current and former smokers [8.1 (7.5–8.2) *vs.* 7.1 (6.7–7.5) ×10^{-9} cm^2, P=0.046]. Post-treatment K_s was associated with the pack-years (r=–0.29, P=0.016) and with MP-TF activity (r=–0.23, P=0.037). Peak thrombin generated correlated with K_s, WBC count, and D-dimer (r=–0.31, P=0.0091; r=0.46, P<0.001 and r=–0.29, P=0.015, respectively), while ETP was associated with WBC only (r=0.31, P=0.0071).

The multiple linear regression model showed that stage ED/IV (B=–0.31; 95% CI: –0.57 to –0.056; R^2=33, P<0.0001), peak thrombin generated (B per 10 nM =–0.030; 95% CI: –0.059 to –0.001; R^2=39, P<0.0001), and white blood cell count (B per 10^3/µL =–0.082; 95% CI: –0.15 to –0.011; R^2=32, P<0.0001) determined ΔK_s, while active smoking determined ΔCLT (B=–4.95 min; 95% CI: –9.64 to –0.27 min; R^2=0.37, P<0.0001).

Discussion

Recently, we have demonstrated that the prothrombotic plasma clot phenotype, including faster formation of fibrin clots, reduced fibrin network porosity and impaired clot lyzability, characterizes patients with advanced lung cancer, including locally advanced inoperable disease and disseminated disease (Abstract No. PB 912 at the XXVIth Congress of the ISTH). The current study is the first to show that a 3-month chemotherapy in lung cancer patients has a favourable effect on fibrin clot properties, including slower formation of less compact fibrin networks, despite unaltered persistently elevated thrombin generation. Our study provides new insights into the mechanisms of a hypercoagulable state in lung cancer patients during chemotherapy (9), suggesting the presence of additional favourable fibrin-related changes, in part determined by the disease stage, thrombin generation potential, and smoking habit.

A slight but significant improvement in fibrin clot properties assessed after 3 months of standard chemotherapy

Table 2 Characteristics of patients after chemotherapy with regard to histological types of lung cancer and the cancer stage

Variable	Histological type of lung cancer			Cancer stage		
	SCLC (n=37)	NSCLC (n=46)	P value	LD/III (n=37)	ED/IV (n=46)	P value
RBC, 10^6/μL	3.68±0.45	3.80±0.56	0.45	3.62±0.44	3.78±0.56	0.16
WBC, 10^3/μL	6.1 [5.0–8.4]	6.8 [5.3–9.1]	0.30	6.0 [4.7–8.2]	6.8 [5.7–9.1]	0.09
Platelets, 10^3/μL	340 [268–398]	277 [214–383]	0.14	302 [210–378]	340 [241–395]	0.28
Haemoglobin, g/dL	11.3 [10.3–12.0]	11.0 [9.7–12.0]	0.60	11.0 [10.1–11.9]	11.1 [9.9–12.1]	0.39
Glucose, mM	5.3 [5.1–5.6]	5.6 [5.3–6.1]	0.011	5.4 [5.1–5.9]	5.5 [5.1–6.1]	0.28
Creatinine, μM	75 [68–90]	79 [67–90]	0.83	80.5 [68.5–89.5]	74.0 [63.0–90.0]	0.45
Fibrinogen, g/L	3.22 [2.59–3.62]	3.20 [2.87–3.90]	0.33	2.97 [2.60–3.50]	3.35 [2.90–3.90]	0.10
D-dimer, ng/mL	607 [495–912]	667 [499–938]	0.66	498 [409–601]	812 [639–1,033]	<0.0001
MP-TF activity, pg/mL	1.14 [0.80–1.56]	1.30 [0.91–1.70]	0.49	1.14 [0.83–1.34]	1.21 [0.86–1.90]	0.27
K_s, 10^{-9} cm^2	7.29±0.86	7.10±0.72	0.37	7.3±0.9	7.1±0.7	0.28
Turbidimetric lag phase, s	43 [40–46]	43 [40–46]	0.79	43 [41–46]	43 [40–46]	0.52
ΔAbs (405 nm)	0.84 [0.77–0.88]	0.80 [0.80–0.90]	0.50	0.83±0.08	0.84±0.06	0.60
Clot lysis time, min	92.6±14.3	92.7±16.0	0.97	93.1±15.2	92.7±15.1	0.97
Lag time, s	1,161 [943–1,268]	1,099 [865–1,382]	0.10	1,161 [969–1,275]	1,093 [859–1,307]	0.27
Peak thrombin generated, nM	105 [78–190]	152 [111–227]	0.033	120 [78–165]	152 [100–228]	0.017
TTPeak, s	2,072 [1,778–2,352]	2,077 [1,668–2,476]	0.11	2,072 [1,906–2,367]	2,063 [1,654–2,642]	0.35
ETP, nM·s	82,216 [57,872–99,214]	99,554 [68,240–120,914]	0.031	82,216 [62,312–102,120]	99,214 [68,625–121,722]	0.061

Data are given as mean ± SD or median [interquartile range]. RBC, red blood cells; WBC, white blood cells; MP-TF, tissue factor-bearing microparticles procoagulant activity; K_s, fibrin clot permeability; ΔAbs (405 nm), maximum absorbance of fibrin gel at 405 nm; TTPeak, time to thrombin peak; ETP, endogenous thrombin potential.

in advanced lung cancer patients was a surprising finding, since typically anticancer therapy is associated with a hypercoagulable state reflected by increased fibrinogen and D-dimer levels (9,26). In our previous study performed in multiple myeloma patients induction therapy led to higher K_s and shortened CLT associated with a reduction in peak thrombin generated (27). In advanced cancer patients, no such associations were observed. Thrombin and TF have been postulated to represent key mediators of cancer-related thrombosis in general (28). Reitter *et al.* (29) have shown that peak thrombin generated decreased in lung cancer patients at different disease stages over time, while D-dimer levels increased during chemotherapy. The authors examined, however, less than one third of patients with the advanced disease (29). We observed no significant differences regarding thrombin generation in the current study, which might suggest that 3 months of chemotherapy in patients with advanced inoperable lung cancer has a negligible impact on thrombin formation, but MP-TF activity was reduced by 22% after treatment. Increased circulating levels of MPs have been shown in lung cancer patients compared with healthy controls (30). Highly elevated MP-TF activity was also found in patients with metastatic pancreatic cancer, with its moderate inverse association with time to fibrin clot formation (31). Few studies have evaluated MP-TF activity in lung cancer patients (17). We found a weak association of MP-TF with K_s after treatment, which suggests an additional harmful effect of elevated MP-TF activity and its contribution to the formation of denser, more prothrombotic fibrin networks. This observation is novel and might have a large impact in other non-cancer clinical settings. The current study provides additional evidence that a coagulation potential of TF-bearing MPs might contribute to the multifactorial hypercoagulability in lung cancer patients.

We confirmed the strong impact of smoking on plasma clot structure in lung cancer patients, which highlights a major prothrombotic effect of this factor, potent enough to be observed despite a highly prothrombotic state in cancer. Cigarette smoking, in particular in heavy smokers, increases thrombotic risk via multiple mechanisms, including hyperfibrinogenaemia or enhanced oxidative stress leading to impaired fibrin permeability and lysis (32). We demonstrated that the active or former smoking was associated with about 12% lower K_s after chemotherapy, which indicates that negative effects of smoking on clot properties are still detectable during chemotherapy. Of note, these effects of smoking were not related to differences in fibrinogen concentrations or thrombin generation. Our observation regarding smoking underscores the value of recommendations to quit smoking in cancer and non-cancer patients, also in order to positively impact prothrombotic tendencies. We showed that no smoking can determine a more favourable post-treatment clot feature. It has also been reported that never smokers with NSCLC had higher response rates to chemotherapy, lower rates of progressive disease and longer survival than current and former smokers (33). Based on the current evidence, it might be speculated that less prothrombotic fibrin clot phenotype might also be related to a better outcome during anticancer therapy.

Recently, it has been suggested that platelets might play an important role in hypercoagulable state development after chemotherapy (11). Platelets behind blood coagulation are involved in angiogenesis and cancer growth by secretion of multiple growth factors and chemoattractants, including platelet factor 4 (34,35). *In vitro* experiments on platelets treated with cisplatin showed that anticancer drugs can lead to increased production of intrinsic activated factor X, followed by elevated thrombin generation and fibrin formation (11). Thrombin formation and fibrin generation were increased 1 day after chemotherapy and returned to the baseline (day 0) after one week in a model using platelet-free plasma mixed with platelets isolated from blood of lung cancer patients (11). How important is the platelet activation in the development of thromboembolic complications in cancer patients in the light of our data showing favourable changes in fibrin properties after chemotherapy needs further investigation.

This study has several limitations. First, the sample size was limited. The study was, however, adequately powered to detect intergroup differences in clot variables, although the subgroup analyses should be interpreted with caution. Second, the present cohort included various histological types of lung cancer and treatment with a few regimens, however such group is representative for this disease at its advanced stage. Third, an assessment of platelet activation was beyond the scope of this study.

In conclusion, advanced lung cancer unfavourably alters plasma clot properties, including faster formation of more compact clots displaying impaired lyzability, but chemotherapy improves these properties. Clinical relevance of the current findings remains to be explored.

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Footnote

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

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