Analysis of haemostasis biomarkers in patients with advanced stage lung cancer during hypofractionated radiotherapy treatment

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Abstract

Objective: To investigate the relationship between changes in inflammatory and coagulatory biomarkers before and after short palliative radiotherapy in patients with advanced stage lung cancer.

Methods: This prospective observational single-centre study enrolled patients with histologically- or cytologically-confirmed lung cancer who were eligible for palliative radiotherapy. Inflammatory and coagulatory biomarkers including complete blood count, D-dimer and fibrinogen levels were evaluated before and after short hypofractionated radiotherapy.

Results: Seventy-two patients with advanced stage lung carcinoma were enrolled in this study. Metastases were associated with an increase in white blood cells, neutrophils and mean platelet volume. Increased volume of the primary tumour had a borderline level of correlation with white blood cell and neutrophil counts. After radiotherapy, white blood cells, neutrophils, haemoglobin and lymphocyte counts were decreased. After radiotherapy, the change in fibrinogen and mean platelet volume were borderline significant.

Conclusion: The levels of inflammatory and coagulatory biomarkers can be used to monitor treatment.

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Introduction

Armand Trousseau first identified the relationship between cancer and coagulopathy in 1865.[1] Since then, research has suggested that activation of the coagulation cascade pathway may play an essential role in the regulation of tumour growth and metastatic spread.[2] The activation of the haemostatic system occurs in two different ways; either leading to coagulation via thrombin formation or activation of the fibrinolytic system via plasminogen activators.[3–5] The process of coagulation and fibrinolysis in cancer patients may be triggered by the tumour cells or by tumour-associated inflammatory cells.[6] The smallest degradation product of fibrin is fragment D-dimer (DD) and it is a sensitive indicator of the proteolytic actions of plasmin on fibrin.[5,7] Many researchers have suggested that DD might be a valuable marker for prognosis and for measuring treatment response in patients with lung cancer.[8–10] The present study investigated the relationship between changes in inflammatory and coagulatory biomarkers before and after short palliative radiotherapy in patients with advanced stage lung cancer.

Patients and methods

Patient population

This prospective observational single-centre study enrolled patients with histologically- or cytologically-confirmed lung cancer who were eligible for palliative radiotherapy and who were treated at the Regional Clinical Hospital, Zielona Gora, Poland between 1 September 2015 and 31 August 2016. The exclusion criteria were as follows: (i) a history of secondary tumour(s); (ii) active infection; (iii) familial coagulopathy; (iv) peripheral vascular disease (thrombophlebitis and thromboembolism); (v) treatment with anticoagulants or anti-aggregants; (vi) treatment with chemotherapy during the 3 months prior to the study; (vii) World Health Organization performance status of 4 (i.e. completely disabled, unable to undertake any selfcare, totally confined to a bed or chair). The disease stage was defined based on clinical and physical examinations as follows: thoracic computed tomography (CT), brain CT or magnetic resonance imaging, abdominal ultrasonography, bone scintigraphy and/or positron emission tomography-CT. The volume of the primary tumour was measured using the information from CT scans and using the model for an ellipsoid V=4/3 n (abc). The histopathological data were determined according to the Union for International Cancer Control TNM classification.[11] This study was approved by the Ethics Committee at the Medical Council in The Regional Medical Chamber, Zielona Gora, Poland (no. 2/57/2015). Patients participating in the study provided written informed consent.

Radiotherapy treatment

The patients were divided into two groups: group 1 consisted of patients with distant metastases; and group 2 consisted of patients with locoregional disease. All patients had palliative short

hypofractionated radiotherapy (hRT) treatment split into five doses over five days to a total dose of 20 Gy. Three–dimensional computer planning was used in all cases. All patients underwent CT scans (SOMATOM CT scanner; Siemens Healthcare, Erlangen, Germany) and the Eclipse™ Treatment Planning System (Varian Medical Systems, Palo Alto, CA, USA) was used for hRT treatment planning. The clinical target volume (CTV) included the primary tumour as detected by CT scans.

Biochemistry assays

Before and after radiotherapy, venous blood samples were drawn from peripheral blood on the morning after an overnight fast and evaluated immediately. Blood drawn into vacutainer tubes containing 3.2% sodium citrate was used for assays on haemostaseology and blood treated with 1.6 mg/ml ethylenediaminetetra-acetic acid was used to determine the complete blood count with a CELL-DYN RUBY haematology analyser (Abbott Diagnostics, Abbott Park, IL, USA). The plasma DD level (reference range: 0–278 μg/l), fibrinogen (reference range: 200–472 mg/dl), haemoglobin (reference range: 12–18 g/dl), platelets (reference range: $140-420 \times 10^9$/l), white blood cells (reference range: $4.0-10.2 \times 10^9$/l), red blood cells (reference range: $4.2-6.5 \times 10^6$/μl), neutrophils (reference range: $2.0-6.9 \times 10^9$/l), lymphocytes (reference range: $0.6-3.4 \times 10^9$/l), mean corpuscular volume (reference range: 80–97 fl) and mean platelet volume (reference range: 7.0–12.0 fl) were measured. The study investigated the relationship between the biochemical results, the disease stage and tumour volume.

Statistical analyses

Exploration of a prospective design for new data collection (a longitudinal observational study) lead to repeated measurements'

statistical analysis. Since a change of concentrations of the analysed biomarkers over time was assessed, multilevel (hierarchical) modelling was required (in these current models, additional random effects were structured as longitudinal randomized experiments). In particular, the multilevel models recognized the existence of such data hierarchies by allowing for residual components at each level in the hierarchy and they were generalizations of linear models relying on nested random analysis of variance (mixed models).

In the assumed concept, concentrations of biomarkers with time divided by groups of patients with distant metastases or with locoregional disease were analysed using a stratified linear regression following a simple linear relation (concentration ~ time + group), and the regression with an interaction term (concentration ~ time * group).[12] The statistically significant results of the regression coefficients ($P < 0.05$) and those on the borderline of the statistical significance ($P < 0.1$) were considered in the study. The statistical computation was performed using the R platform.[13]

Results

There were 97 patients eligible for palliative radiotherapy, but 25 were excluded. The remaining 72 patients with lung cancer were enrolled in the study and they were divided into two groups: group 1 consisted of 51 patients with distant metastases; and group 2 consisted of 21 patients with locoregional disease. The clinical and demographic characteristics of the study cohort are shown in Table 1.

The complete blood count, fibrinogen level and DD level before and after radiotherapy are shown in Table 2. After short hRT, the following parameters decreased: white blood cells ($P = 0.0012$), neutrophils ($P = 0.01$), haemoglobin ($P = 0.03$), lymphocyte counts ($P = 0.04$) and mean platelet

Table 1. The clinical and demographic characteristics of patients with lung cancer who were eligible for palliative radiotherapy (*n* = 72) who were enrolled in this study to investigate the relationship between haemostasis biomarkers, disease stage and tumour volume.

Characteristic	Cohort $n = 72$
Age, years	
Median (range)	68 (41–86)
Sex	
Male	40 (56)
Female	32 (44)
Tumour histology	
Non-small cell carcinoma	15 (21)
Adenocarcinoma	32 (44)
Squamous	15 (21)
Small cell carcinoma	10 (14)
World Health Organization performance status	
0–1	10 (14)
2–3	62 (86)
Tumour/Node/Metastasis classification	
T1a/T1b/T2/T3/T4	2/3/12/17/38
N0/N1/N2/N3	22/18/18/14
M0/M1a/M1b	21/4/47
Tumour volume, mm^3	
Mean (range)	37.8 (0.6–152.4)

Data presented as *n* of patients (%), median (range) or mean (range).

volume (P = 0.0573) was on the border of statistical significance. After radiotherapy, the change in fibrinogen was borderline (P = 0.08). Metastases were associated with an increase in white blood cells (P = 0.01), neutrophils (P = 0.02) and mean platelet volume (P = 0.04). Whereas, the increase of the volume of the primary tumour had a borderline level of correlation with white blood cells (P = 0.07) and neutrophils (P = 0.07). Interpretations of the remaining results are presented in Table 3.

In the entire study cohort (n = 72), median DD levels did not change significantly after radiotherapy (1070 μg/l before hRT versus 1131 μg/l after hRT).

Discussion

The aim of any anticancer therapy, such as radiotherapy, is to reduce the tumour volume and limit further growth of the tumour. Targeted radiotherapy results in endothelial damage and is followed by continuous induction of an inflammatory response that contributes to the mechanisms of coagulation activation.[14] This present study investigated whether various inflammatory and haemostatic parameters

Table 2. The complete blood count, fibrinogen level and D-dimer level for patients (*n* = 72) with lung cancer who underwent short hypofractionated radiotherapy (hRT).

Variable	Before hRT low/high/median	After hRT low/high/median
White blood cells, ×10^9/l	3.2/23.0/10.5	3.3/22.0/8.5
Neutrophils, ×10^9/l	1.6/20.5/7.7	1.9/19.0/6.3
Neutrophils, %	43.0/89.0/70.0	46.2/90.9/71.3
Lymphocytes, ×10^9/l	0.5/4.0/1.7	0.2/27.0/1.2
Lymphocytes, %	5.3/42.5/19.9	1.4/41.5/17.0
Haemoglobin, g/dl	7.2/15.2/12.0	8.6/15.5/11.9
Red blood cells, ×10^6/μl	2.8/5.3/4.0	2.7/5.6/4.0
Mean cell volume, fl	73.0/100.0/89.3	76.0/101.0/89.7
Platelets, ×10^9/l	77.6/760.0/311.9	85.4/731.0/290.0
Mean platelet volume, fl	5.0/17.1/6.7	4.7/16.2/6.5
Fibrinogen, mg/dl	218.0/819.0/428.2	186.0/866.0/429.2
D-dimer, μg/l	116/6524/1070	111/6675/1131

Table 3. Multilevel modelling of biomarker concentrations.

Response variable	Regression coefficient analysis (risk factor)[a]	Mean	SE	P-Value
Mean platelet volume	Intercept	5.03	1.22	$P = 0.0001$
	Age	0.03	0.02	NS
	Time	−0.27	0.14	NS
White blood cells	Intercept	8.47	2.06	$P = 0.0001$
	Tumour	0.90	0.45	$P = 0.0496$
	Time	−1.67	0.49	$P = 0.0012$
Neutrophils	Intercept	5.70	1.90	$P = 0.0038$
	Tumour	0.75	0.41	NS
	Time	−1.09	0.45	$P = 0.0184$
White blood cells	Intercept	11.1	1.0	$P < 0.0001$
	Tumour volume	0.03	0.01	NS
	Time	−1.46	0.47	$P = 0.0030$
Neutrophils	Intercept	7.97	0.94	$P < 0.0001$
	Tumour volume	0.02	0.01	NS
	Time	−0.95	0.43	$P = 0.0345$
Haemoglobin	Intercept	14.2	0.7	$P < 0.0001$
	Female	−1.33	0.47	$P = 0.0062$
	Time	−0.66	0.30	$P = 0.0315$
	Female*Time	0.34	0.20	NS
Lymphocytes	Intercept	−1.55	2.43	NS
	Tumour	0.81	0.55	NS
	Time	3.17	1.56	$P = 0.0478$
	Tumour*Time	−0.76	0.36	$P = 0.0360$
White blood cells	Intercept	7.11	2.29	$P = 0.0028$
	Metastases	2.17	0.91	$P = 0.0195$
	Time	1.24	1.23	NS
	Metastases*Time	−1.25	0.49	$P = 0.0133$
Neutrophils	Intercept	4.39	2.10	$P = 0.0406$
	Metastases	1.89	0.83	$P = 0.0265$
	Time	1.48	1.13	NS
	Metastases*Time	−1.11	0.45	$P = 0.0172$
Mean platelet volume	Intercept	5.62	0.73	$P < 0.0001$
	Metastases	0.60	0.29	$P = 0.0422$
	Time	0.30	0.36	NS
	Metastases*Time	−0.25	0.15	NS
Fibrinogen	Intercept	561	70	$P < 0.0001$
	Time	−63.1	36.3	NS

[a]Correlations between factors: time (before and after short hypofractionated radiotherapy) and tumour volume and metastases.
NS, no significant association ($P \geq 0.05$).

changed during the process of hypofractionated radiotherapy in patients with lung cancer. Following hypofractionated radiotherapy, basic inflammatory markers decreased, whereas there were no significant changes in the DD levels and fibrinogen levels. DD levels were very high in both groups of patients but did not significantly

change after radiotherapy. Previous studies have reported no significant difference in DD levels between stage IIIB and IV advanced disease.[8,15] It was observed that despite the high DD levels in the present study, none of the patients developed any clinical events. A previous study reported significantly higher DD levels in patients with advanced lung cancer compared with those patients with local disease.[16] Another study reported higher DD levels in patients with metastasis compared with those without metastatic disease.[17] In several studies,[15,17–20] the levels of DD and fibrinogen were significantly decreased after anticancer treatment in patients that underwent complete remission. In the present study, following palliative radiotherapy treatment, fibrinogen changes were borderline significant. It is not clear why the DD levels remained high.

Fibrinogen is an acute-phase protein and its concentration changes in response to the inflammatory status. A process of cell ionization takes place during radiotherapy. Proteins and DNA are very radiosensitive, hence free organic radicals react with them and destroy them. It is possible that fibrinogen is more sensitive than its breakdown product DD, which is generated by the thrombin–fibrinogen reaction. It is possible that the structural changes to DD take longer to complete after radiotherapy. In the current study, the median concentration of DD was increased following hRT. There are many studies that have described changes in inflammatory and haemostatic parameters, but most of the changes are observed after systemic treatment or after surgery.[21–23] In a study of paediatric patients with acute lymphoblastic leukaemia, changes in haemostatic parameters during treatment were observed; both DD and fibrinogen levels decreased during treatment.[24] Another study on patients with ovarian cancer observed a continuous decrease in fibrinogen after surgery and

chemotherapy.[25] Tumour cells can interact with the damaged endothelial cells, leukocytes and platelets, which is the cause of the activation of the thrombotic process.[3,26–30] Fibrinogen is a soluble plasma glycoprotein that is synthesized by hepatocytes. Excess fibrinogen is associated with an increased risk of clotting. During the blood coagulation process fibrinogen is converted into fibrin by the enzyme thrombin. Some published data suggest that fibrinogen promotes cancer progression. For example, one study showed a correlation between higher levels of fibrinogen and metastasis.[31] The authors reported that fibrinogen is a factor that facilitates the enhanced adherence of metastatic foci in the vasculature of organs.[31] In contrast, another study showed the opposite results; with fibrinogen fragments suppressing tumour angiogenesis by binding to endothelium and down regulating the expression of integrins on vascular endothelial cells.[32] Research demonstrated that a high plasma fibrinogen level before treatment (chemoradiotherapy) was a negative predictor of tumour response; and was significantly associated with a poor prognosis for patients and with reduced survival time.[33–35] Borderline significant changes in the level of fibrinogen and decreased inflammatory biomarkers after radiotherapy were shown in this current study. However, it is difficult to define the mechanism of action.

This study had two main limitations: a small sample size and a heterogenous study population.

In conclusion, the evaluation of inflammatory and coagulatory biomarkers in this present study supports the importance of palliative radiotherapy as it led to a reduction in parameters that might be contributing to the progression of cancer.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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