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Abnormal haemostasis in small cell lung cancer

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ABSTRACT Disorders of haemostasis and altered platelet activity have been documented in patients with malignant disease but their relation to response to treatment and prognosis are not known. Thrombin activity (fibrinopeptide A (FpA), plasmin mediated fibrinolysis (Bβ15-42) antigen), and platelet alpha granule release (β thromboglobulin) were studied in 37 patients with small cell lung cancer to find out whether these indices show a relationship to chemoresponse. There was evidence of considerably increased thrombin activity, with a median fibrinopeptide A concentration of 13·2 (normal <4) pmol/ml, but only modestly increased fibrinolysis, with a median Bβ14–42 antigen concentration of 5·6 (normal <3) pmol/ml. Thus the ratio of fibrinopeptide A to Bβ15–42 concentration (FpA:Bβ) was raised, with a median value of 2·2 (normal $<1·33$). In addition, 57% of patients had increased platelet alpha granule release, the median β thromboglobulin concentration being 50 (normal <50) ng/ml. There was a significant association between increased thrombin generation and lack of response to chemotherapy. Furthermore, non-responders had higher FpA:Bβ ratios. The same haemostatic markers were studied in nine patients who have been in complete remission for at least two years after chemotherapy for small cell lung cancer. There was a significant difference in thrombin activity and also in the ratio of thrombin activity to lysis between the pretreatment group and the group of two year survivors. Lack of response to chemotherapy appears to be related to increased thrombin activity. Such an association has not previously been reported in patients with malignant disease.

Introduction

Disorders of haemostasis[1–8] and altered platelet activity,[9,10] ranging from subtle alterations in coagulability to gross disseminated intravascular coagulation or massive venous thrombosis, have been documented in patients with malignant disease. Increased plasma concentrations of fibrinopeptide A, indicating accelerated fibrin generation and thrombin activity, have been recorded in patients with various malignancies, particularly those with an extensive tumour burden,[6] though the clinical relevance of these observations is not clear. There is little information about endogenous plasmin mediated fibrinolysis, which can now be estimated by measurement of plasma concentrations of the fibrinopeptide Bβ15–42 antigen,[11] and the relative rates of thrombin generation and fibrinolysis in patients with malignancy have not been described. We have therefore used the sensitive markers fibrinopeptide A, Bβ15–42 antigen, and the platelet release product β thromboglobulin to assess the degree of haemostatic disorder in patients with small cell lung cancer. We have attempted to relate changes in these markers to tumour burden at the outset, the subsequent response to chemotherapy, and ultimate survival.

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Methods

PATIENTS

Haemostatic indices were measured in 37 selected patients with histologically proved small cell lung cancer. Initial staging showed disease limited to the thorax in 30 patients, only seven having evidence of widespread metastases. A high proportion of patients with limited disease were studied because they are considered most likely to show a good response to chemotherapy. Twenty six of the patients (24 with limited disease) completed four cycles of intravenous cytotoxic induction chemotherapy with cyclophosphamide, adriamycin, vincristine, and VP–16, and their responses were evaluated. These were assessed by restaging clinical, radiological, and bronchoscopic

examinations. Complete tumour regression was seen in 14 of these 26 patients, partial response (> 50% tumour regression) to treatment in seven patients, and no response in five patients. The response in 11 patients could not be evaluated: five (four of whom had extensive disease) received single agent palliative oral chemotherapy, three died of treatment related myelosuppression, and three died of progressive disease about the time of administration of their first pulse of chemotherapy. The three patients who died of septicaemia due to treatment induced myelosuppression were excluded from survival figures, leaving 34 patients for evaluation.

The same haemostatic markers were also measured in nine patients who entered complete remission after chemotherapy and who have remained disease free for at least two years. This group included four patients whose haemostatic markers had also been estimated at the time of presentation.

BLOOD SAMPLING

Blood for analysis was sampled without stasis via a 21G butterfly needle (Abbott), and immediately transferred to and gently mixed in precooled prepared polystyrene tubes resting in an ice water slurry. Blood for estimation of fibrinopeptide A and Bβ15–42 antigen was drawn into a collecting tube containing Trasylol 1000 IU, heparin 1000 IU, and 0·15M sodium chloride. Blood for β thromboglobulin estimation was drawn into a collecting tube containing prostaglandin E_1, ethylenediamine tetra-acetic acid, and theophylline to prevent in vitro platelet aggregation and release of β thromboglobulin. All specimens were processed and snap frozen within one hour of sampling. Radioimmunoassay kits were used to estimate fibrinopeptide A and Bβ15–42 antigen (IMCO, Stockholm) and β thromboglobulin (Radiochemical Centre, Amersham). Normal ranges for middle aged healthy subjects were obtained from Scottish epidemiological studies that used the same methods as those used in our laboratory (380 subjects for fibrinopeptide A and Bβ15–42 antigen, 50 subjects for β thromboglobulin). Intra-assay and inter-assay precision was always within 10%.

Haemostatic variables in responders and non-responders were compared by the Mann-Whitney U test.

Results

Fibrinopeptide A concentrations were raised in almost all patients (35 out of 37), the median being 13·2 (interquartile range 5·3–65·5) pmol/ml; the upper limit of normal is 4 pmol/ml. There was evidence of increased fibrinolysis in 29 of 37 patients, but the increases were modest, with a median Bβ15–42 antigen concentration of 5·6 (interquartile range 3·6–8·7) pmol/ml (upper limit of normal 3 pmol/ml). Thus a raised ratio of thrombin activity to fibrinolysis (fibrinopeptide A:Bβ15–42 antigen) was found in 23 of 37 patients, with a median fibrinopeptide A:Bβ15–42 antigen ratio of 2·2 (interquartile range 1·0–11·7) compared with a normal ratio of less than 1·33.

The relationship between the haemostatic indices and the response of the patients to chemotherapy is shown in table 1 and figures 1 and 2. Non-responders

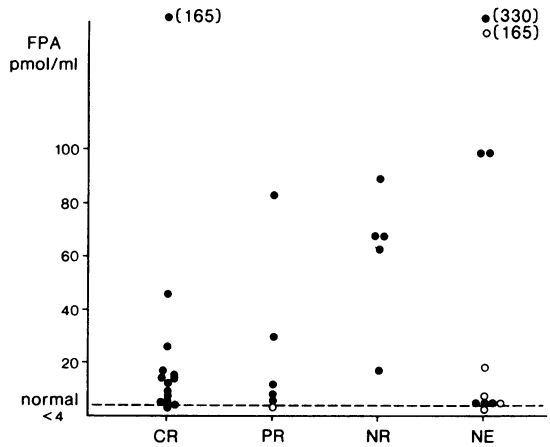

Fig 1 *Fibrinopeptide A (FpA) concentrations in patients with small cell lung cancer grouped according to chemotherapy response. CR—complete response; PR—partial response; NR—no response; NE—non-evaluable. ● limited disease; ○ extensive disease.*

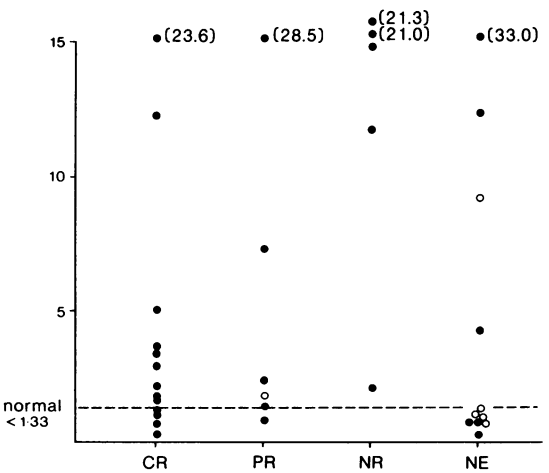

Fig 2 *Ratio of fibrinopeptide A to Bβ15-42 antigen (FpA:Bβ) in patients with small cell lung cancer grouped according to chemotherapy response. CR—complete response; PR—partial response; NR—no response; NE—non-evaluable. ● limited disease; ○ extensive disease.*

Table 1 *Haemostatic indices in patients with small cell lung cancer grouped by response to chemotherapy*

Response		Survival (mo) (median (range))	Haemostatic index (median (interquartile range)) FpA (pmol/ml) [normal <4]	Bβ (pmol/ml) [normal <3]	FpA:Bβ [normal <1·33]	BTG (ng/ml) [normal <50]
Complete	(n = 14)	16 (4 –26)	12·9 (5·3–21·2)	4·5 (3·5– 7·0)	2·2 (1·2– 3·6)	50 (34– 71)
Partial	(n = 7)	8 (4·5–12)	9·6 (5·0–56·1)	4·2 (2·4– 8·7)	2·1 (1·1–17·9)	66 (26– 97)
None	(n = 5)	6 (3 – 9)	68·0 (63·0–68·0)	5·8 (3·2– 6·0)	14·8 (11·7–21·0)	51 (34–120)
Non-evaluable	(n = 11)*	3 (0·5–11)	7·7 (4·3–99·0)	8·0 (5·3–18·5)	1·0 (0·7– 4·1)	50 (27– 81)

*Three deaths from toxicity not included in survival figures.
FpA—fibrinopeptide A; Bβ—Bβ15–42 antigen; BTG—β thromboglobulin.

Table 2 *Haemostatic indices in patients with prolonged remission after treatment of small cell lung cancer*

	Normal range	Median (interquartile range)
FpA (pmol/ml)	< 4	3·3 (2·6–3·3)
Bβ (pmol/ml)	< 3	7·1 (5·6–7·7)
FpA:Bβ ratio	< 1·33	0·41 (0·38–0·56)
BTG (ng/ml)	<50	24 (22–29)

Abbreviations as in table 1.

had significantly higher fibrinopeptide A concentrations than those responding to chemotherapy ($p < 0·01$). Non-responders also had the greatest ratio of thrombin activity to fibrinolysis ($p < 0·05$). In contrast, although there was evidence of increased platelet release in 57% of patients (median β thromboglobulin 50 ng/ml), there was no association between β thromboglobulin concentrations and the response to chemotherapy (table 1). There was also no association between either the degree of haemostatic activation (fibrinopeptide A and Bβ15–42 antigen) or the increase in platelet release (β thromboglobulin) and either disease extent at outset or ultimate survival (data not shown).

The results for the nine patients who were disease free at least two years after treatment are shown in table 2. Although there was a modest increase in fibrinolysis in the long term survivors, there was no evidence of increased thrombin activity (median fibrinopeptide A 3·3 (normal < 4) pmol/ml). The ratio of thrombin activity to fibrinolysis in these patients was also within the normal range, with a median fibrinopeptide A:Bβ15–42 antigen ratio of 0·41 (normal <1·33). The four patients of this group whose haemostatic markers were assessed before chemotherapy had a normal fibrinopeptide A:Bβ15–42 antigen ratio at the time of presentation (median 0·86).

Discussion

In this study we have found evidence of increased thrombin activity in patients with untreated small cell lung cancer. Fibrinopeptide A concentrations in our study group were substantially raised and higher than those found in patients with other malignant disorders (table 3), even in those with apparently limited disease. We have also shown that patients not responding to chemotherapy have higher fibrinopeptide A concentrations than those who responded. In contrast, patients in prolonged complete remission do not have evidence of abnormal thrombin activity.

Several mechanisms whereby malignant processes induce alterations in blood coagulation and platelet release have been proposed. There may be increased procoagulant thromboplastin activity around the tumour itself, or tumour mediated mechanisms may result in increased platelet aggregability and release.[10 12–16] Increased concentrations of thrombin activity and increased platelet activation have been implicated in metastasis formation.[12 17 18] Several studies have shown an antimetastatic effect of anticoagulants and antiplatelet agents in experimental animal models,[19–22] and conditions that enhance coagulation have been shown to increase experimental metastasis formation.[12]

In clinical practice various studies have documented increased thrombin activity and platelet aggregation in malignant disorders and the efficacy of anticoagulants in the treatment of certain malignant tumours has been investigated.[23 24] Studies of haemostasis in lung cancer have been limited[25–27] and the role of anticoagulation remains controversial. Zacharski[28] found a doubling of median survival in patients with

Table 3 *Fibrinopeptide A (FpA) concentrations in various malignancies*

Tumour type		FpA (pmol/ml) Mean	Median
Lung	(n = 3)	4·3*	2·8*
Leukaemia	(n = 3)	4·8*	5·0*
Stomach	(n = 8)	3·0*	2·4*
Mixed	(n = 116)	3·3†	—

*Modified from Yoda and Abe.[5]
†Modified from Peuscher *et al.*[6]

small cell lung cancer treated with warfarin, whereas Stanford[29] found no difference in survival in patients with small cell lung cancer given heparin during chemotherapy and treated with warfarin. Initial studies with heparin[30] suggested benefit, but this finding was not confirmed.[31 32]

Endogenous plasmin mediated fibrinolysis can now be estimated by radioimmunoassay of the Bβ15–42 antigen fragment. We found evidence of increased plasmin mediated fibrinolysis in patients with small cell lung cancer but this was not usually as pronounced as the increase in thrombin activity. This failure of endogenous fibrinolysis to keep pace with increased thrombin activity was most pronounced in non-responding patients, in whom the median FpA:Bβ ratio was 14·8 (normal ratio $< 1{\cdot}33$). Studies of the effects of agents that enhance endogenous fibrinolysis will be needed to clarify whether the present findings are an epiphenomenon or are relevant to clinical management.

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