carcinoma. For this reason, we intend to extend this study, to many more patients of different ages, including younger patients.

Additional data might also provide information useful for theoretical study of the pathogenetic mechanisms for mammary carcinoma, also for evaluating better the genetic hypothesis of the origin of mammary carcinoma, which appears to be consistent with our data obtained in this study.

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Increased Myelosuppression during Cytostatic Treatment and Pleural Effusion in Patients with Small Cell Lung Cancer

Jørn Herrstedt, Paul Clementsen and Ole Paaske Hansen

30 patients with small cell lung cancer (SCLC) and malignant pleural effusion were compared with 30 matched patients with SCLC but without pleural effusion. In the 30 with pleural effusion, white blood cell and platelet counts fell significantly after initial chemotherapy, necessitating dose reduction. Of the patients with pleural effusion, 16 developed severe (WHO grade IV) leukopenia, 7 had severe thrombocytopenia, and 2 patients died of infection. Accordingly, exhaustive aspiration of radiologically verified pleural effusion before starting chemotherapy in patients with SCLC is recommended.
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INTRODUCTION

MYELOTOXICITY IS the dose-limiting factor for the majority of cytostatics. It is well known that during methotrexate treatment pleural effusion may act as reservoirs, resulting in a prolonged presence of methotrexate in the plasma compartment [1]. The plasma concentration and especially the time of exposure of methotrexate correlate well with the myelotoxicity [2]. Such relationships make it important that the physician is aware of the existence of an extra non-physiological compartment. Other cytostatics, e.g. doxorubicin [3], have also been detected in malignant effusions during therapy. Unfortunately, the clinical importance of pleural effusion for the metabolism and toxicity, especially the myelotoxicity, of cytostatics is largely unknown.

The aim of the present study was to compare the toxicity of

combination chemotherapy in patients with small cell lung cancer (SCLC) with and without pleural effusion.

PATIENTS AND METHODS

The study population included 30 consecutive patients who received combination chemotherapy for SCLC at Bispebjerg Hospital and at the time of diagnosis had pleural effusion. The diagnosis of SCLC was histologically confirmed according to WHO criteria. The pleural effusions were in all patients verified by X-ray of the chest. X-rays were evaluated by a radiologist and only patients having obvious pleural effusions were included. In 13 patients diagnostic pleurocentesis was performed with the finding of malignant cells. No patient had previously received chemotherapy or radiotherapy and all had extensive disease (i.e. spread outside one hemithorax, the mediastinum or the bilateral supraclavicular nodes). Patients with pleural effusion were only considered as having extensive disease if malignant cells were proven in the pleural effusion. The patients were treated with combination chemotherapy including two or more drugs toxic to the bone marrow. The regimens were combinations of the following drugs: lomustine, methotrexate, cyclophosphamide, doxorubicin, teniposide and etoposide.

Each patient with pleural effusion was matched to a control patient with SCLC and extensive disease receiving the same combination chemotherapy but without pleural effusion. The patients were matched according to age, sex and bone marrow involvement. Furthermore, there were no significant differences between the two groups as to performance status, haemoglobin, white blood cells (WBC), platelets, lactate dehydrogenase and survival (Mann–Whitney test, Table 1).

The following parameters and adverse reactions were registered and graded according to WHO toxicity grading criteria [4]: haemoglobin concentrations, WBC and platelet counts, fever, infection, haemorrhage, stomatitis, cardiac function and paraesthesia. These parameters were registered (1) before start of the treatment; (2) at the time of the nadir between the first and second cycle; (3) before start of the second cycle; (4) at the time of the nadir between the second and the third cycle; and (5) before start of the third cycle. Also registered were the numbers of blood and platelet transfusions given. Before the start of the treatment all the above-mentioned parameters were normal (WHO grade 0) in the 60 patients.

Table 1. Patients' characteristics

	Study patients	Control patients
No. of patients	30	30
Age (years)		
Median	62.0	61.5
Range	38–73	51–72
Sex		
Male/female	17/13	19/11
Performance status (WHO)		
0	4	6
1	13	15
2	6	7
3	7	2
4	0	0
Haemoglobin (mmol/l)		
Median	8.0	8.4
Range	6.1–10.3	5.6–9.9
WBC (10^9/l)		
Median	8.5	8.5
Range	5.2–18.5	4.4–19.0
Platelets (10^9/l)		
Median	361	363
Range	175–687	149–642
LDH (U/l)		
Median	660	552
Range	278–3381	200–3400
Bone marrow involvement	6	5
Survival (weeks)		
Median	39.0	43.5
Range	2–222	8–341

RESULTS

The WBC counts during the first cycle were significantly lower in the study group than in the control group (median WBC 0.8 and 2.2 × 10^9/l, respectively, $P = 0.035$, Table 2). In the first cycle, 22 of the study patients developed grade III–IV leukopenia compared with 15 controls (Table 3). The leukopenia was life-threatening (WHO grade IV) in 16 patients, accompanied by sepsis in 3, 2 of whom died. None of the 10 control patients with WHO grade IV leukopenia developed sepsis.

The majority of the patients in the study group (18) and of the controls (23) did not develop thrombocytopenia (WHO grade 0), though significantly lower platelet counts were seen in the study group during the first cycle (144 and 325 × 10^9/l respectively, $P = 0.020$, Table 2). In the study patients, 7 developed WHO grade IV thrombocytopenia compared with 2 controls (Table 3). None of the patients died from thrombocytopenia. There was no difference in the number of platelet transfusions.

The median haemoglobin concentrations in the study group and the control group during the first cycle were 7.2 and 7.5 mmol/l, respectively ($P = 0.741$, Table 2). 13 study patients received 36 blood transfusions compared with 9 controls receiving 22 blood transfusions. During the second cycle no significant differences were seen in haemoglobin concentrations, WBC or platelet counts.

The myelotoxicity nadir was observed 12.5 days after start of chemotherapy in the study group compared with 12.9 days in the controls ($P = 0.959$, Table 2). The myelotoxicity lasted 3.0 days and 0.5 days, respectively ($P = 0.139$). The time to onset of myelotoxicity seemed more difficult to predict in the study group, in which myelotoxicity occurred 5–21 days after chemotherapy compared with 7–16 days in the control group. 10 patients from both groups had a prolongation of the interval between the first and the second chemotherapy, but the prolongations were of significantly longer duration in the study group (8 vs. 4 days, $P = 0.018$, Table 2).

Only 3 patients from the control group had WHO grade II infections; none developed grade III or IV infections. In the

Correspondence to J. Herrstedt, Department of Oncology 54 B1, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark.
The authors are at the Department of Internal Medicine C, Bispebjerg Hospital, University of Copenhagen, DK-2400 Copenhagen NV, Denmark.
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Table 2. Haematologic values at first nadir

	Study patients	Control patients	P^*
Hacmoglobin (mmol/l)			
Median	7.2	7.5	0.741
Range	4.4–9.6	5.5–8.8	
WBC (10^9/l)			
Median	0.8	2.2	0.035
Range	0.1–7.5	0.2–8.1	
Platelets (10^9/l)			
Median	144	325	0.020
Range	7–433	7–471	
Time to 1st nadir (days)			
Median	12.5	12.9	0.959
Range	5–21	7–16	
Duration of 1st nadir (days)			
Median	3.0†	0.5	0.139
Range	0–15	0–13	
No. of points with prolongation of chemotherapy intervals	10†	10	1.000
Duration of prolongation (days)			
Median	8†	4	0.018
Range	4–23	2–9	

* Mann–Whitney test.
† 2 patients, who died of infection during the 1st nadir are not included.

study group, 7 patients developed grade II, 2 grade III, and 3 grade IV infections. This difference was not significant (possibly owing to small sample size). Concerning grading of fever and haemorrhage, no differences were seen between the groups in the first or second cycle.

Stomatitis occurred only during the first cycle in 1 patient in the study group and in 2 in the control group. No patients in either group developed cardiac symptoms. No paraesthesias were registered during the first cycle. During the second cycle there were 3 patients with grade I paraesthesias and 8 patients with grade II paraesthesias in the study group. In the control group 5 patients developed grade I paraesthesias and 8 grade II paraesthesias. The difference between the groups was not significant.

Table 3. WBC and platelet nadirs during the first cycle of chemotherapy

WHO grade	WBC $\times 10^9$/l	Study patients	Control patients
0	≥ 4.0	3	6
I	3.0–3.9	2	4
II	2.0–2.9	3	5
III	1.0–1.9	6	5
IV	< 1.0	16	10
	Platelets		
WHO grade	$\times 10^9$/l		
0	≥ 100	18	23
I	75–99	1	2
II	50–74	2	1
III	25–49	2	2
IV	< 25	7	2

DISCUSSION

This study shows that patients with SCLC, who at pretreatment staging had malignant pleural effusion, had significantly lower WBC and platelet counts after initial chemotherapy than patients without pleural effusion. Generally, WHO grade IV adverse reactions are not intended in our treatment protocols for SCLC. It is therefore of major concern that 16 of 30 patients (53%) with pleural effusion developed unexpected severe toxic leukopenia, and 2 patients died of infections. In addition, 23% of the patients developed severe thrombocytopenia. These dose-limiting toxicities, however, were only seen at the time of the first chemotherapy-induced nadir, since the dose of the cytostatics was reduced depending on the haematologic nadir values after the first treatment.

In both groups the risk of grade IV leukopenia was increased in patients with bone marrow involvement. In the study group 5/6 (83%) developed grade IV leukopenia compared with 3/5 (60%) in the control group. This difference (23%) equals the difference seen in patients without bone marrow involvement, since 11/24 (46%) and 6/25 (24%), respectively, developed grade IV leukopenia (difference 22%). Neither of the 2 patients who died of infection had bone marrow involvement.

It has been described that malignant pleural effusion and ascites, after intravenous administration of methotrexate, may act as third-space compartments on methotrexate pharmacokinetics [1, 5]. The retention of methotrexate in massive effusions is associated with a marked decrease in plasma clearance of methotrexate, thereby causing myelosuppression [6]. Although pharmacokinetic parameters were not obtained in this study, our data suggest that pathologically increased third-space compartments as malignant pleural effusions may significantly influence the pharmacokinetics of other chemotherapeutic agents such as lomustine, cyclophosphamide, doxorubicin, etoposide and teniposide. To what extent each agent in our combined chemotherapy regimens adds to the increased myelosuppression has to be clarified in further studies.

In 537 patients with SCLC [7], 11% had pleural effusion at the time of pretreatment staging (K. Østerlind, personal communication).

An obvious therapeutical implication of our results is recommendation of exhaustive aspiration of pleural effusion before starting chemotherapy, (1) to minimise the risk of life-threatening myelosuppression and unexpected toxic deaths in connection with chemotherapy according to a standard schedule, and (2) to reduce the interindividual variability when comparing different drug regimens in clinical trials. Moreover, it seems appropriate to avoid patients with pleural effusion in phase I–II clinical trials of new cytostatics unless simultaneous pharmacokinetic measurements are obtained.

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A Phase I Study of Local Treatment of Liver Metastases with Recombinant Tumour Necrosis Factor

George P. van der Schelling, Jan N.M. IJzermans, Tjebbe C. Kok, Marcel Scheringa, Richard L. Marquet, Ted A.W. Splinter and Johannes Jeekel

15 patients with therapy-resistant liver metastases were treated in a phase I study with recombinant tumour necrosis factor (rTNF). rTNF was injected into a liver metastasis by ultrasound guidance, using a 50 μg escalating dose schedule (3 patients/dosage) ranging from 100 to 350 μg per injection. Influenza-like symptoms such as fever, chills, nausea and vomiting were the main clinical side-effects. 2 patients experienced transient hypotension, probably due to concomitant use of morphine. Other toxicities, as reported after systemic use of rTNF, such as decrease in leucocytes and platelet counts, renal or liver toxicity were not observed. No difference was seen in subpopulations of lymphocytes ($CD3^+$, $CD4^+$ $CD8^+$, $CD16^+$ and $CD19^+$) prior to and after rTNF injection. In 8 patients stable disease occurred in rTNF-treated metastases. The maximal dose used by this route of administration is 350 μg per injection. Based on these observations we conclude that the toxicity of rTNF injected into liver metastases by sonographic control is transient and mild. The results suggest that intratumoral administration of rTNF might play a role in local tumour control.
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INTRODUCTION

TUMOUR NECROSIS FACTOR (TNF) was first identified in BCG-infected mice challenged with endotoxin. This serum factor caused haemorrhagic necrosis of subcutaneously implanted meth A sarcoma in mice [1]. Subsequently it was demonstrated that TNF-alpha is produced by activated cells of the monocyte/macrophage lineage, TNF-beta by lymphocytes [2]. After the isolation of TNF it was possible to identify the gene coding for this polypeptide [3, 4], and with the use of recombinant DNA technology TNF came available in large amounts of highly purified material. It was thought that by the production of TNF on a large scale the beginning was set for a selective form of immunotherapy in patients with advanced cancer.

Correspondence to G.P. van der Schelling, Department of Surgery, General Hospital Stuivenberg, Lange Beeldekensstraat 267, 2060 Antwerp, Belgium.
G.P. van der Schelling, J.N.M. IJzermans and J. Jeekel are at the Department of General Surgery, Erasmus University Hospital, Rotterdam; T.C. Kok and T.A.W. Splinter are at the Department of Oncology, Erasmus University Hospital, Rotterdam; and M. Scheringa and R.L. Marquet are at the Department of Experimental Surgery, Erasmus University Hospital, Rotterdam, The Netherlands.
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The exact antitumour activity of TNF is yet not clearly understood. Its direct cytotoxicity to sensitive tumour cells is mediated by specific cell surface receptors. Interaction of TNF with these receptors leads to membrane perturbations and DNA fragmentation [5]. Malignant cell lines which show no growth inhibition to TNF *in vitro*, can show tumour regression *in vivo*. This implies that other, indirect, mechanisms may be involved in the antitumour activity of TNF *in vivo*, such as activation of host immune defense mechanisms [6]. Recently it was demonstrated that TNF affects the haemostatic properties of vascular endothelium by stimulating procoagulant activity, thereby facilitating the formation of thrombi. TNF can also damage vascular endothelial cells directly. Thus, within a solid tumour TNF might lead to occlusion of its vessels and subsequent diminished perfusion which finally leads to necrosis of the tumour. Not only is the neovasculature of certain tumours sensitive to the activity of TNF, but normal tissue endothelium may also be damaged [7, 8].

Multiple phase I studies with TNF have been carried out. In most studies TNF have been administered intravenously. Overall, it appears that systemic TNF, in the doses and schedules examined, has little single agent activity in the treatment of advanced human malignancy [9–15]. Due to excessive toxicity