Adjuvant CMF-chemotherapy and haemostasis. Effect of "classical" and "modified" adjuvant CMF-chemotherapy on blood coagulation fibrinolysis in patients with breast cancer

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Summary

Effects of "classical" and "modified" adjuvant CMF-chemotherapy on haemostasis were studied in 22 patients with breast cancer receiving cyclophosphamide (100 mg/m² p.o.; days 1-14 or 600 mg/m² i.v.; days 1,8), methotrexate (40 mg/m² i.v.; days 1,8) and 5-fluorouracil (600 mg/m² i.v.; days 1,8). Blood collection was done prior to chemotherapy on day 1 and 8.

A significant decrease of protein C antigen and activity associated with cumulative effects was observed from day 1 to 8. This effect was similar with "classical" and "modified" CMF-chemotherapy but the reduction of protein C was more pronounced with the oral application of cyclophosphamide. In absence of any significant cumulative decrease of other vitamin K-dependent blood coagulation proteins (factor VII, protein S), the simultaneous decrease of protein C activity and antigen indicates a specific influence of CMF-chemotherapy on vitamin K-dependent protein C-synthesis in the liver.

Key words: Adjuvant chemotherapy; Breast cancer; Cyclophosphamide; Haemostasis; Protein C; Thrombosis.

Introduction

Combination chemotherapy containing cyclophosphamide, methotrexate and 5-fluorouracil (CMF) is a standard adjuvant treatment in patients with breast cancer [1, 2]. Several clinical studies report an increased incidence of thromboembolic complications associated with CMF-chemotherapy. During adjuvant treatment the incidence of thrombosis ranges from 2% to 10% [3-6]. The highest rate of thrombosis (13.6%) was observed with the combination of adjuvant CMF-chemotherapy and tamoxifen [7]. In metastatic disease the rate of venous thrombosis was 4.4% to 17.6% [8, 9].

Some authors have suggested alterations of the haemostatic system as a possible mechanism leading to the elevated risk of thrombosis [10-13]. A significant decrease of vitamin K-dependent haemostatic proteins (factor VII, protein C and protein S) was observed, contributing to an enhanced thrombotic state in women receiving CMF-therapy for breast cancer [11-13]. In most studies presenting a decrease in coagulation inhibitions, cyclophosphamide was given orally (80-100 mg/m²) for a treatment period of two weeks or more [11, 12]. Only Rella *et al.* studied the effect of standard intravenous CMF [13].

Cyclophosphamide is an alkylating agent, that is inactive until it undergoes hepatic transformation to form 4-hydroxycyclophosphamide, which then breaks down to form the ultimate alkylating agent, phosphoramide mustard [14].

Since cyclophosphamide is activated by the liver, there is evidence that it might undergo a first-pass hepatic metabolism resulting in higher levels of alkylating metabolites when given orally. Pharmacokinetics describe an increased alkylating activity with oral over intravenous administration [15]. Supporting the assumption of an increased alkylating activity using the oral administation of cyclophosphamide, Engelsmann *et al.* found a higher response rate with the "classical" CMF schedule than with intravenous CMF (48% vs. 29%) in patients with advanced breast cancer. Also the different appearance of adverse side-effects indicates a higher dose intensity achieved per unit time with "classical" CMF [16]. The aim of this study was to consolidate data on the effect of adjuvant CMF-chemotherapy on the haemostatic system in patients with breast cancer. In addition to other authors who investigated only single courses of chemotherapy [11, 12], we observed a complete 6-month treatment period, to measure possible long-term effects. Because of the clinical and pharmacological experience with various routes of administration, a second aim was to assess potential different influences of the oral and the intravenous application of cyclophosphamide.

Patients and Methods

Twenty-two patients with breast cancer were eligible for the study. All patients enrolled gave informed consent. Patients with evidence of metastatic disease, impaired liver function, concomitant endocrine therapy or ongoing anticoagulant or antiplatelet therapy were excluded. All patients had received postoperative anticoagulation with 5,000 IU of standard heparin

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Table 1. — *Patient characteristics (n=22) at time of diagnosis, separated by treatment groups.*

Characteristic	No of patients (Treatment - Group A) [n=11]	No of patients (Treatment - Group B) [n=11]
Age (years)*	50 (31-69)	48 (33-66)
Bodyt weight (kg)*	67 (50-95)	68 (42-80)
Menopausal status:		
Pre-	8	9
Post-	3	2
Surgery:		
Tumorectomy	2	3
Mastectomy	9	8
Extent of primary tumor#:		
T_1	1	3
T_2	7	6
T_3	1	1
T_4	2	- * -
T_x	- * -	1
No. of positive lymph nodes:		
0	- * -	4
1-3	5	4
4-6	3	1
7-9	1	1
>9	2	1
Histopathological grading#:		
Grade 2	6	5
Grade 3	5	6
Histology:		
Ductal	10	8
Lobular	1	1
Medullary	- * -	2
Hormone receptors:		
ER+/PR+	5	3
ER+/PR-	2	3
ER-/PR+	2	1
ER-/PR-	2	4

* median value and range
# according to: UICC TNM Classification of Malignant Tumors, 5th Edition, 1997
ER: estrogen receptor; PR: progesterone receptor; +: positive; -: negative

(3x daily s.c.) for the time of hospitalization. Patient characteristics at time of diagnosis are summarized in Table 1. After primary surgery 11 patients received the "classical" adjuvant CMF-chemotherapy (Treatment group A: cyclophosphamide 100 mg/m², orally days 1 to 14, methothrexate 40 mg/m² and 5-fluorouracil 600 mg/m², intravenously days 1 and 8, repeat day 29), 11 patients were treated with a "modified" intravenous schedule (Treatment group B: cyclophosphamide 600 mg/m², methotraxate 40 mg/m², 5-fluorouracil 600 mg/m², intravenously days 1 and 8, repeat day 29). The first course of chemotherapy was given at a mean of 21 days after surgery. Patients with breast conserving treatment received concomitant radiotherapy.

Although screening for thrombosis was not included in the study, patients with a previous history of thrombosis underwent a pretreatment angiologic examination including doppler-ultrasonography. In patients with clinical evidence of a thromboembolic event during chemotherapy, the diagnosis was verified by phlebography, angiography or a pulmonary ventilation-perfusion scan. These patients were excluded from further examination.

Blood sampling procedures

Blood collection was performed in each single treatment course on days 1 and 8 prior to intravenous application of the chemotherapy during a 6-month treatment period.

Procedures of blood collection and handling were performed by standard protocols [17-19]. Since in vivo coagulation may be initiated by poor venipuncture, and particularly by prolonged stasis and multiple injury of the vessel wall, blood samples were taken only by specially trained staff. Because of circadian variation of fibrinolytic activity, blood collection was restricted to the early morning hours.

Laboratory Tests

Fibrinogen (Fbg), partial thromboplastin time (PTT) and thromboplastin time (TPZ) were measured using coagulometric techniques (Boehringer Mannheim, Germany). Trombin-antithrombin III (TAT) complex, D-dimer fibrin degradation products (Ddimer), free protein S (PS) and protein C antigen (PC Ag.), as well as tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor (PAI-1) antigen, were assessed by enzyme-immunoassays (Behring Werke Marburg, Germany; Boehringer Mannheim, Germany; Organon Teknika Oss, NL;

Table 2. — *Global clotting times and parameter of procoagulation during 6 months of adjuvant CMF-chemotherapy (course I-VI, days 1,8). Analysis of the total treatment-group (n=20). Data are presented as mean value ($\bar{x}$), standard deviation (STD) and median value (M).*

Parameter [Reference range]		Course I Day 1	Course I Day 8	Course II Day 1	Course II Day 8	Course III Day 1	Course III Day 8	Course IV Day 1	Course IV Day 8	Course V Day 1	Course V Day 8	Course VI Day 1	Course VI Day 8
Global clotting times													
PTT	$\bar{x}$	33	32	30	31	30	31	34	32	31	30	39	36
[26-42 sec]	STD	4.3	3.7	4.5	6.6	5.4	3.8	9.4	4.8	4.3	3.8	23	16
	M	33	32	29	30	29	30	33	32	30	30	32	31
TPZ	$\bar{x}$	92	91	95	88	97	89	95	89	96	89	95	87
[70-120%]	STD	11	8.5	13	11	12	9.6	13	8.7	11	9.3	9.9	19
	M	42	91	97	89	98	89	92	89	95	91	96	91
Procoagulation													
Fbg	$\bar{x}$	312	280	293	275	314	276	301	285	309	293	312	293
[150-450ng/ml]	STD	97	76	63	58	82	65	57	63	67	53	41	57
	M	278	278	292	278	292	265	287	272	295	295	314	289
F VII	$\bar{x}$	133	108	123	112	127	99*	110	90*	139	120	126	113
[70-120%]	STD	44	33	36	29	36	24	43	26	37	35	35	38
	M	134	104	130	106	135	103	103	83	139	122	119	109
TAT	$\bar{x}$	5.0	5.5	4.3	4.5	5.9	3.5	4.0	3.2	3.3	3.4	2.8*	3.2
[1.0-4.1 ng/ml]	STD	3.9	8.3	2.4	3.5	5.7	1.5	3.4	1.8	3.0	1.9	1.7	1.8
	M	3.4	3.1	3.2	3.0	2.7	3.2	3.0	2.8	2.5	2.7	2.6	2.8

* = significant ($p<0.05$) change compared to pretreatment (course I, day 1)

Table 3. — *Parameter of anticoagulation during 6 months of adjuvant CMF-chemotherapy (course I-VI, days 1, 8). Analysis of the total treatment-group (n=20). Data are presented as mean value ($\bar{x}$), standard deviation (STD) and median value (M).*

Parameter [Reference range]		Course I Day 1	Course I Day 8	Course II Day 1	Course II Day 8	Course III Day 1	Course III Day 8	Course IV Day 1	Course IV Day 8	Course V Day 1	Course V Day 8	Course VI Day 1	Course VI Day 8
AT III	$\bar{x}$	105	103	107	104	106	108	113	112	115	113	112	110
[80-120%]	STD	14	20	18	14	19	17	20	16	14	15	12	14
	M	104	103	105	102	109	112	117	114	120	119	114	111
PC Ag	$\bar{x}$	93	76**	82	67**	76*	61**	72**	62**	72**	64**	72**	66**
[70-140%]	STD	18	12	21	14	20	13	20	16	10	13	13	13
	M	91	77	83	71	74	61	75	63	74	62	71	64
PC Act	$\bar{x}$	111	88**	96*	81**	89**	72**	83**	71**	88**	84**	87**	78**
[70-140%]	STD	18	12	21	14	23	13	19	16	23	20	20	19
	M	113	87	89	84	81	72	83	70	86	82	85	78
PS Ag	$\bar{x}$	89	81	81	80	81	77	93	85	88	88	93	89
[70-140%]	STD	22	16	23	16	17	16	28	17	21	20	20	18
	M	93	78	73	77	78	79	86	82	82	85	88	84

* = significant ($p<0.05$) change compared to pretreatment (course I, day 1)
** = significant ($p<0.005$) change compared to pretreatment (course I, day 1)

Table 4. — *Parameter of fibrinolysis (Plg, t-PA), antifibrinolysis (PAI-1) and fibrin turnover (D-dimer) during 6 months of adjuvant CMF-chemotherapy (course I-VI, days 1,8). Analysis of the total treatment-group (n=20). Data are presented as mean value ($\bar{x}$), standard deviation (STD) and median value (M).*

Parameter [Reference range]		Course I Day 1	Course I Day 8	Course II Day 1	Course II Day 8	Course III Day 1	Course III Day 8	Course IV Day 1	Course IV Day 8	Course V Day 1	Course V Day 8	Course VI Day 1	Course VI Day 8
Plg	$\bar{x}$	101	97	106	102	107	98	108	102	110	103	107	101
[80-120%]	STD	24	24	21	22	17	21	23	20	23	21	21	22
	M	97	93	107	107	109	102	110	108	111	104	105	110
t-PA	$\bar{x}$	7.4	5.5	7.0	5.4	5.3	4.2*	6.2	5.1	6.0	5.8	6.0	5.6
[1-12 ng/ml]	STD	6.7	4.9	8.4	4.75	3.3	2.5	7.4	4.9	6.0	5.1	5.6	4.9
	M	4.8	4.2	3.8	4.5	5.3	2.8	3.2	2.9	4.2	4.4	4.9	4.1
PAI-1	$\bar{x}$	43	37	51	42	45	29	46	42	47	42	46	49
[4-43 ng/ml]	STD	36	35	48	49	34	20	52	54	45	47	46	49
	M	37	27	25	21	32	24	34	25	30	27	36	35
D-dimer	$\bar{x}$	720	756	761	839	937	961	496	482	606*	591	528	557*
[<400 ng/ml]	STD	466	582	1004	881	1470	1016	312	278	752	623	417	537
	M	580	646	505	590	593	576	422	419	346	370	421	343

* = significant ($p<0.05$) change compared to pretreatment (course I, day 1)

Chromogenix Mölndal, Sweden). Factor VII (FVII), antithrombin III (ATIII), plasminogen (Plg), protein C activity (PC Act.) were measured by chromogenic substrates (Boehringer Mannheim, Germany; Chromogenix Mölndal, Sweden).

Statistical analysis

All statistical analysis was done using the SAS® statistical software package [20]. Median value, 25th percentile, 75th percentile, mean value, standard deviation and minimum/maximum values were calculated. A first analysis was done on the total treatment group of 20 patients (2 patients were excluded because of thromboembolic complications). The results are summarized in Tables 2 to 4. A second analysis was performed by separating treatment group A ("classical" CMF, n= 10) and treatment group B ("modified" CMF, n=10). Statistically significant results are presented as box-plot diagrams displaying median value, 25th percentile, 75th percentile, mean value and data outside the 10th and 90th percentile (Figures 1 and 2).

Wilcoxon's test for unpaired and paired samples was used to assess significant changes to baseline values and to compare differences between the two treatment groups. Values were considered significant at the $p<0.05$ level.

Results

The analysis of the pretreatment data (measurements from day 1 of the first course of chemotherapy) demonstrated values within the reference range for global clotting times (PTT, TPZ), fibrinogen, factor VII, anticoagulation (AT III, PC Ag., PC Act., PS) and fibrinolysis (Plg, t-PA, PAI-1). TAT and D-dimer fibrin split products were elevated. On therapy no significant change for PTT, Fbg, TAT, AT III, PS, Plg, t-PA and PAI-1 was observed. The mean values of D-dimer remained outside the reference range.

Our main finding was a reduction of F VII and protein C from day 1 to day 8 within each course of chemotherapy. This effect was associated with an insignificant decrease of the thromboplastin time. Within the treatment-free interval between the single courses of chemotherapy the plasma levels of F VII and TPZ returned to pretreatment values. In contrast, the significant decrease in PC Ag. and Act. was associated with a distinct cumulative effect over the 6-month treatment period and demonstrated plasma concentrations, and acti-

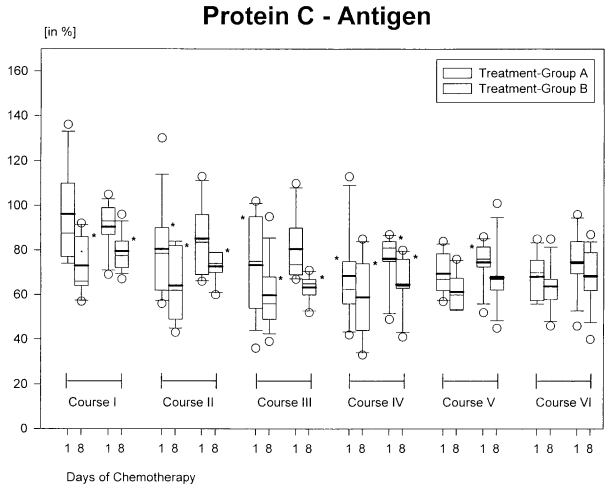

Figure 1. — Effect of adjuvant CMF-chemotherapy (course I-VI, days 1,8) on protein C antigen separated by treatment groups (A="classical", n=10; B="modified", n=10). Data are presented as a box-plot-diagram displaying median value, 25th percentile, 75th percentile, mean value and data outside the 10th and 90th percentile.
*=significant ($p<0.05$) change compared to pretreatment.

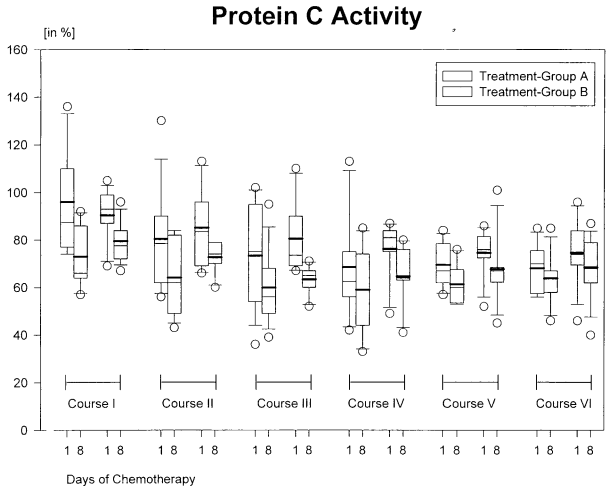

Figure 2. — Effect of adjuvant CMF-chemotherapy (course I-VI, days 1,8) on protein C activity separated by treatment groups (A="classical", n=10; B="modified", n=10). Data are presented as a box-plot-diagram displaying median value, 25 th percentile, 75th percentile, mean value and data outside the 10th and 90th percentile.
* = significant ($p<0.05$) change compared to pretreatment.

vity below <60% (minimum value: 40%). The maximum effect was seen during the third course of chemotherapy. The comparison of the wo treatment groups demonstrated no significant differences for the parameters of blood coagulation, anticoagulation and fibrinolysis. The continous reduction off protein C activity and antigen was seen irrespectively to the route of application. Although the reduction was more pronounced with the oral application of cyclosphamide, the difference did not reach significance.

During the first 3 courses of chemotherapy, one patient within each treatment group developed a pulmonary embolism associated with a deep vein thrombosis. The analysis of haemostatic parameters in these patients demonstrated a distinct tendency towards an acquired protein C-deficiency, but at the time of diagnosis the plasma levels of protein C antigen and activity were found within the reference range. The medical histories report additional risk factors for thrombosis (obesity, smoking, varicosis of the lower extremities).

Discussion

Our results demonstrate no significant effects of adjuvant CMF-chemotherapy on global clotting times, procoagulation and fibrinolytic activity.

Prior to chemotherapy elevated plasma concentrations of thrombin-antithrombin III complex and D-dimer fibrin degradation products were measured. Elevated plasma levels of D-dimer, the end product of the fibrinogen-fibrin metabolism, indicate an increased fibrin turnover. The increase of D-dimer is a well-known phenomenon in patients with malignant tumors, but occurs also after major surgery [21-23].

TAT was determined to evaluate the thrombin generation rate. The elevated mean values of TAT measured at baseline and on therapy, were caused by extremely high plasma levels in single patients. The short half-life time of TAT (<10 min) and ex vivo thrombin activation (during venepuncture and blood collection) may cause methodical problems. Therefore the elevated plasma levels of TAT represent no reliable results.

Our main finding was a significant reduction of protein C activity and antigen with a distinct cumulative effect within six months of chemotherapy. Pathological plasma levels below 60% were observed, which usually occur in inherited deficiencies of coagulation inhibitors.

The results of our study confirm those of several published studies in patients with breast cancer treated with adjuvant CMF-chemotherapy. Feffer *et al.* reported a significant reduction of protein C activity during 2 months of treatment with CMF in nine patients with breast cancer. The values normalized after termination of chemotherapy [11]. Rogers and co-workers found a significant decrease of protein S, protein C and factor VII during 15 courses of chemotherapy. For ten patients the examinations were done during the first course of chemotherapy. Therefore the results demonstrate only a marginal reduction of protein C [12]. Due to the study-design and the short time of observation both studies could not demonstrate a cumulative reduction of protein C. Similar results are reported in the study of Rella *et al.* During six courses of intravenous CMF they could demonstrate that a reduction in protein C was more prominent if CMF was given on days 1 and 8 and repeated every four weeks in comparison with a single application repeated every three weeks [13].

Protein C is a vitamin-K-dependent plasma protein synthezised in the liver. Activated protein C cleaves and

inactivates the membrane-bound activated forms of coagulation factors V and VIII [24, 25]. Inherited and acquired deficiencies of protein C are associated with a high risk of thromboembolic diseases. Resistance to activated protein C caused by a mutation of factor V (factor V_{Leiden}) is the most common hereditary thrombophilia; 50% of family members with hereditary protein C deficiency having had thrombosis before the age of 30-40 yars [26, 27].

Liver toxicity is a frequent complication of oncotherapeutic agents. Accumulation or retainment of the agents in the liver, bioconversion to toxic metabolites and the reduction of hepatic defence mechanisms against damage are possible mediators of liver toxicity [28, 29].

Damage of hepatocytes but also effects on protein synthesis in the liver are possible explanations for reduction of vitamin K-dependent blood coagulation proteins during CMF-chemotherapy. If damage of hepatocytes induced by cytotoxic drugs is the reason for the observed decrease, a reduction of all vitamin K-dependent blood coagulation proteins should have been present. Moreover, we would expect to find some increase in liver enzymes. Our data demonstrate clinically significant effects of CMF-chemotherapy only for protein C. No patient exhibited elevated serum levels of liver enzymes.

The simultaneous reduction of protein C plasma concentration (demonstrated by reduced antigen levels) and decreased functional activity supports the concept of a specific influence of CMF-chemotherapy on protein C-metabolism in the liver.

The comparison of oral and intravenous application of cyclophosphamide demonstrates no different effect on the haemostatic system. The reduction in protein C was more pronounced in patients treated with the "classical" CMF-chemotherapy schedule, but the difference did not reach statistical significance.

Our results suggest that adjuvant CMF-chemotherapy provokes a prethrombotic state that might be a possible explanation for the observed increased incidence of thromboembolic complications during the clinical CMF-chemotherapy trials.

Using the medical history of the patients who developed thromboembolic complications during the six month period of CMF-chemotherapy, it can be shown that – aside from the acquired protein C deficiency – individual risk factors play a decisive role in the clinical manifestation of thromboembolic complications.

The recognition of primary and secondary risk factors by means of precise patient and family medical histories and supplementary determination of protein C before beginning the treatment of high-risk patients could possibly increase the safety of the adjuvant CMF-chemotherapy.

Clinical trials are performed to determine whether prophylactic anticoagulation (i.e. with low molecular weight heparin) can ameliorate the safety of adjuvant CMF-chemotherapy in the treatment of breast cancer.

The clinical research of Levine *et al.*, who achieved a significant reduction of thromboembolic complications during chemotherapy in patients with stage IV breast cancer with very-low-dose warfarin treatment, represents the first step in this direction [9].

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