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Effects of Chemoendocrine Therapy on the Coagulation-Fibrinolytic Systems in Patients with Advanced Breast Cancer

Japan Advanced Breast Cancer Study Group and Japan Clinical Oncology Group

In order to predict a hypercoagulable state in patients with advanced breast cancer receiving medical treatment, the effects of chemoendocrine therapy on the coagulation-fibrinolytic systems were investigated prospectively. The patients were randomly divided into two groups. The ACT group had 38 patients, who received 20 mg/m^2 adriamycin (ADM) i.v. on days 1 and 8, 100 mg cyclophosphamide (CPA) p.o. on days 1–14, and 20 mg tamoxifen (TAM) p.o. daily. The ACM group had 44 patients, who received 20 mg/m^2 ADM i.v. on days 1 and 8, 100 mg CPA p.o. on days 1–14 and 1200 mg medroxyprogesterone acetate (MPA) p.o. daily. The treatment was repeated every 28 days until there was evidence of progressive disease or until the full ADM dose (550 mg/m^2) had been given. The following 9 hematologic parameters were measured every 4 weeks: alpha 2-plasmin inhibitor plasmin complex (PIC), anti-thrombin-III (AT-III), D-dimer (Dd), fibrinogen (Fg), plasminogen (Pg), protein C (PC), thrombin-antithrombin-III complex (TAT-III), tissue plasminogen activator (t-PA), and factor X (FX). Compared to the ACT group, patients in the ACM group showed significantly higher values of AT-III and PC, which exceeded the normal ranges. The levels of Pg, t-PA and FX were significantly higher in the ACM group than in the ACT group, but were still within the normal ranges. The levels of TAT-III, Dd and PIC decreased in the ACT group and were unchanged in the ACM group after the start of treatment. Fg remained unchanged in both groups after the start of treatment. One patient in the ACM group had thrombophlebitis of the lower extremities with high levels of TAT-III, Dd and PIC and a decrease of Fg, but her condition returned to normal after reduction of the MPA dose. Although these data are not directly indicative of a hypercoagulable state in patients receiving chemoendocrine therapy, changes in AT-III, TAT-III, Dd and PIC should be monitored carefully when this type of treatment is given.

Key words: Tamoxifen — Medroxyprogesterone acetate — Coagulation — Fibrinolysis — Breast cancer

Tamoxifen (TAM) and medroxyprogesterone acetate (MPA) are thought to cause thrombotic complications in patients with advanced breast cancer.[1-4] However, the effects of these drugs on the coagulation-fibrinolytic systems are still unknown. The Japan Advanced Breast Cancer Study Group II (JABCSG II) and the Japan Clinical Oncology Group have carried out a prospective randomized clinical trial in patients with advanced breast cancer in order to compare the effects of a combination of adriamycin (ADM), cyclophosphamide (CPA) and TAM with those of a similar combination containing MPA instead of TAM. We analyzed prospectively the time course of changes in coagulation-fibrinolytic test parameters during treatment with these drugs. The aim of this study was to find indicators useful for predicting a hypercoagulable and/or prethrombotic state in patients with advanced breast cancer receiving chemoendocrine therapy.

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PATIENTS AND METHODS

A total of 232 patients with advanced breast cancer were entered into the JABCSG II trial between December 1988 and December 1991 at 12 institutions. Eighty-two patients who were enrolled into the study between June 1990 and December 1991 were analyzed prospectively for changes in coagulation-fibrinolytic parameters due to chemoendocrine therapy, and the effects of TAM were compared with those of MPA. The patients were all female under 75 years old with measurable and/or evaluable advanced breast cancer lesions. Among patients receiving TAM, MPA, anthracycline or CPA as a postoperative adjuvant therapy, those in whom the cancer recurred more than 6 months after the end of therapy were eligible for this trial. Prior therapy not including TAM, MPA, anthracyclines or CPA for metastatic disease was allowed if the treatment had been stopped at least 4 weeks before entry into the study. The patients were allocated to one of the two groups by permuted block randomization at each institution using the envelope method. The ACT group (38 cases) received 20 mg/m^2 ADM i.v. on days 1 and 8, 100 mg CPA

p.o. on days 1–14, and 20 mg TAM p.o. on days 1–28 in one cycle, and the ACM group (44 cases) received 20 mg/m^2 ADM i.v. on days 1 and 8, 100 mg CPA p.o. on days 1–14, and 1200 mg MPA p.o. on days 1–28 in one cycle. In both groups, patients received the treatment until there was evidence of progressive disease (PD) and/or unacceptable toxicity or until the full dose (550 mg/m^2) of ADM had been given. Patients with a history of diabetes (fasting blood sugar >120 mg/dl), severe heart disease or thromboembolism were excluded from the protocol. If hyperglycemia, hypertension or obesity reached grade 2 by the assessment criteria of the ECOG, or if the values of the coagulation-fibrinolytic test parameters were found to indicate a risk of thrombosis, the MPA dose was reduced to 600 mg/day. If hyperglycemia, hypertension, or obesity reached grade 3 or higher by the ECOG criteria, or if symptoms suggesting thrombosis appeared, MPA administration was discontinued immediately. In both groups, if clinically overt thromboembolism occurred, treatment was discontinued. The following 9 parameters of the coagulation-fibrinolytic systems were analyzed: alpha 2-plasmin inhibitor plasmin complex (PIC, Teijin EIA kit, normal value $<0.8\mu$g/ml), antithrombin III (AT-III, COBAS BIO, normal value 79–121%), D-dimer (Dd, ELISA,[5] normal value <150 ng/ml), fibrinogen (Fg, Auto FI, normal value 200–400 mg/dl), plasminogen (Pg, COBAS BIO, normal value 75–125%), protein C (PC, EIA,[6] normal value 70–150%), thrombin-antithrombin III complex (TAT-III, EIA,[7] normal value <3.0 ng/ml), tissue plasminogen activator (t-PA, ELISA,[8] normal value <7.6 ng/ml), and factor X (FX, chromogenic synthetic substrate method.[9] normal value 56–138%). As a rule, the parameters were measured every 4 weeks on the first day of each treatment course until 40 weeks after the start of therapy. Differences between the ACT and ACM groups were analyzed statistically using two tests: (1) Student's t test (t), used for mean values during each treatment course in both groups, and (2) Wilcoxon's rank sum test (U), used for the actual values during each treatment course in both groups. Differences from the pretreatment levels in each group (time-series difference) were analyzed by t test and U test 4 weeks after the start of therapy. Chi-square test was used for comparison of background factors. The study was approved by the Ethics Committee of the Japan Clinical Oncology Group.

RESULTS

The background factors of the patients are listed in Table I. There was no significant difference between the ACT and the ACM groups with regard to age, menopausal status, performance status, disease-free interval, initial site of recurrence, estrogen receptor status,

Table I. Patients' Characteristics

	A	B
Total number of eligible patients	38	44
Age median	56.5	56.5
range	38–72	35–72
Menopausal status		
pre	8	8
post	30	36
Performance status		
0	19	20
1	11	14
2	5	8
3	3	1
4	0	1
Estrogen receptor		
positive	5	14
negative	11	10
unknown	22	20
Progesterone receptor		
positive	2	6
negative	8	11
unknown	28	27
Systemic adjuvant therapy		
chemoendocrine	7	8
chemo	16	14
endocrine	1	0
none	14	22
Disease-free interval (months)		
mean	80.6	65.6
median	62.9	44.0
range	6.8–231.0	8.6–314.7
Initial site of recurrence		
bone	10	10
soft tissue	13	22
liver	2	2
lung	11	12
pleural effusion	5	0
other	1	1
Number of disease sites		
1	21	27
2	11	11
3	4	4
4	1	1
5	0	1
6	1	0
Total number of evaluable lesions		
bone	16	16
soft tissue	21	33
liver	4	2
lung	13	18
pleural effusion	8	3
others	3	2
Prior systemic therapy after recurrence		
chemoendocrine	1	1
chemo	2	4
endocrine	1	1
none	34	38
		(NS)

progesterone receptor status, total number of evaluable lesions or prior treatment. In the ACM group, the levels of AT-III were significantly higher than the normal range and significantly greater than the pretreatment level between weeks 4 and 16 of administration (t, U; $P<0.01$, Fig. 1). In the ACT group, the level of AT-III decreased from 98.6% to 85.1% during week 4 of administration and the levels of AT-III were significantly different from those in the ACM group between weeks 4 and 48 (t, U; $P<0.01$). However, the mean values of AT-III in the ACT group remained within the normal range during the treatments. The levels of Pg in the ACM group were significantly higher than those in the ACT group between weeks 4 and 40 after the start of treatment (t, U: $P<0.01$), but the values remained within the normal range during the treatments. The levels of t-PA in the ACM group were significantly higher than the pretreatment level during week 4 of administration (t, U; $P<0.01$), but no progressive increase with time was observed. The levels of t-PA in the ACM group were significantly higher than those in the ACT group from weeks 4 to 32 (U; $P<0.05$), but the values also remained within the normal range during the treatments (Fig. 2). The levels of PC in the ACM group increased after the start of treatment and exceeded the normal value, with significantly higher values between weeks 4 and 44 compared to the pretreatment values (t, U; $P<0.05$, Fig. 3). The level of PC in the ACT group, in particular, showed a significant decrease from 111.3% to 99.3% during week 4 of administration, which was the most conspicuous difference from the ACM group (151.4%; t, U; $P<0.01$). The pretreatment levels of TAT-III in both the ACM and ACT groups were higher than the normal value, but despite the absence of any intergroup difference after the start of administration, there was a nonsignificant trend toward a decrease below the pretreatment level in the ACT group (Fig. 4). There were no significant differences between the pretreatment and the post-treatment values in the ACM group. The pretreatment PIC level in the ACT group was higher than the normal value, but returned to the normal range after the start of administration. The levels of PIC in the ACM group were basically unchanged following the treatment (Fig. 5). After the start of treatment, the levels of FX in the ACM group were higher than those in the ACT group, showing a significant difference between weeks 4 and 20 (t, U; $P<0.05$),

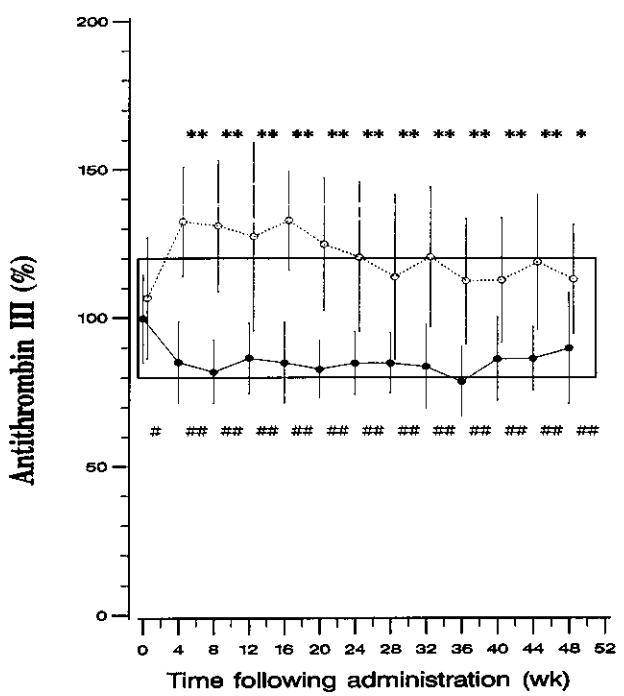

Fig. 1. Time course of changes in antithrombin III in ACT (●)- and ACM(○)-treated patients. The hatched area shows the normal range (79–121%). Symbols and bars are means ± SD. Significantly different at $P<0.05$ (*; t test, #; U test) and at $P<0.01$ (** ; t test, ##; U test).

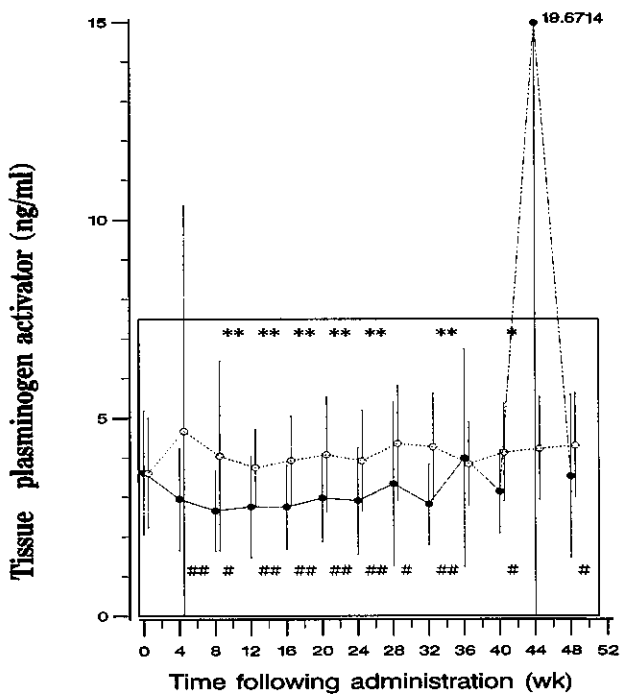

Fig. 2. Time course of changes in tissue plasminogen activator in ACT(●)- and ACM(○)-treated patients. The hatched area shows the normal range (<7.6 ng/ml). Symbols and bars are means ± SD. Significantly different at $P<0.05$ (*; t test, #; U test) and at $P<0.01$ (**; t test, ##; U test).

but the values remained within the normal range during the treatments (Fig. 6). The levels of Fg were within the normal range, and there were no intergroup differences or differences from the pretreatment values. The pretreatment values of Dd were higher than the normal range in both groups. However, the levels tended to return gradually to normal after the start of administration in the ACT group, and did not change significantly in the ACM group. There were no significant differences in Dd levels from the pretreatment values or between the groups except at weeks 8 and 16 (Fig. 7). There were no relationships among the therapeutic effects or recurrence sites and changes in these hematologic parameters. Meanwhile, as shown in Table II, four patients with high TAT-III, Dd and PIC levels were noted in both groups. There was one patient with clinical thromboembolism in the ACM group (61 years old, thrombophlebitis of the lower extremities; case 1). In this patient, high levels of AT-III, TAT-III, Dd and PIC were noted, and the level of Fg decreased 4 weeks after administration of MPA; however, these values returned to normal after reduction of the MPA dose from 1200 to 600 mg and conservative treatment. No other patient developed symptoms. In case 2, a decreased level of Fg (88 mg/dl at 12 weeks) was also noted, but clinical thromboembolism did not develop in this patient.

DISCUSSION

We have reported changes in the laboratory values of parameters associated with the coagulation-fibrinolytic systems, particularly a shortening of APTT (activated partial thromboplastin time) caused by MPA and a decrease in AT-III caused by TAM, when these drugs were given as an adjuvant therapy to postmenopausal breast cancer patients.[10] The nine parameters used in this study were selected for the following reasons. Fg is the key substrate of coagulation,[9] and activation of FX is the most important event in the blood-coagulation cascade.[9] Dd is a specific derivative of cross-linked fibrin, and an increased concentration of Dd is a specific sign of a thromboembolic complication.[5] Pg is the key substrate in fibrinolysis,[8] and t-PA plays an important role in converting Pg to plasmin.[8] We also measured the levels of two kinds of circulating anticoagulant; AT-III, which inhibits the function of thrombin,[11] and PC, which degrades factors Va and VIIIa.[6] The level of TAT-III is associated with the generation of thrombin,[7] while the

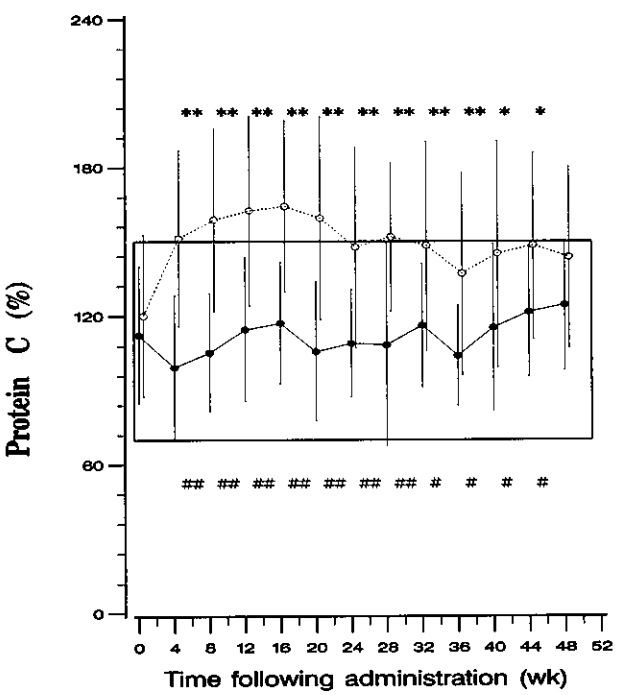

Fig. 3. Time course of changes in protein C in ACT(●)- and ACM(○)-treated patients. The hatched area shows the normal range (70–150%). Symbols and bars are means±SD. Significantly different at $P<0.05$ (*; t test, #; U test) and at $P<0.01$ (**; t test, ##; U test).

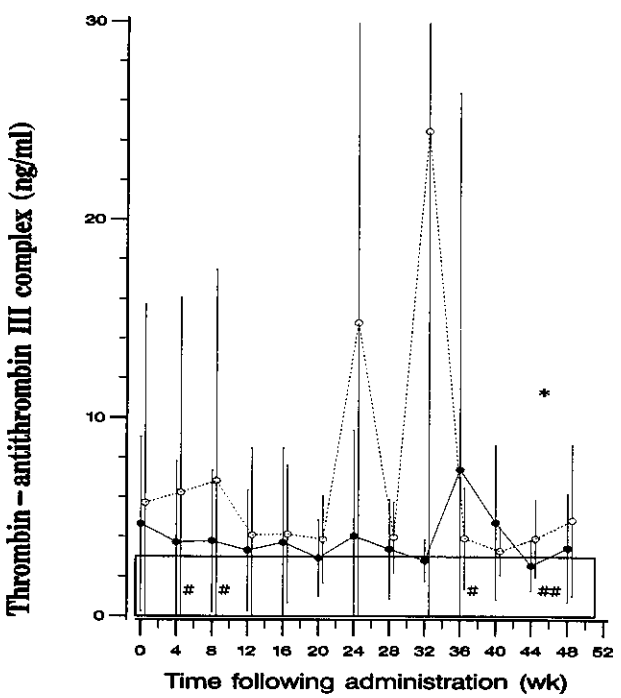

Fig. 4. Time course of changes in thrombin-antithrombin III complex in ACT(●)- and ACM(○)-treated patients. The hatched area shows the normal range (<3.0 ng/ml). Symbols and bars are means±SD. Significantly different at $P<0.05$ (*; t test, #; U test) and at $P<0.01$ (**; t test, ##; U test).

increased concentration of PIC is correlated with increased fibrinolysis.[12] Therefore, significant changes in the levels of TAT-III, Dd and PIC are thought to reflect most sensitively the functional states of the clotting-fibrinolysis systems. In the present study, the levels of PC and AT-III in the ACM group exceeded the normal range after the start of treatment and were significantly higher than those in the ACT group during the treatment. Since these changes associated with MPA treatment are considered to inhibit thrombus formation, they can be attributed to a direct promoting effect on protein synthesis rather than an effect of MPA on the blood clotting-fibrinolytic systems. Love *et al.* also reported a significant decrease of AT-III in TAM-treated patients.[13] The decreased level of AT-III due to TAM might be a useful indicator of a prethrombotic condition. TAT-III, which is thought to be an accurate predictor of the dynamics of the coagulation system, showed a transient decrease after the srart of therapy in the ACT group alone, but there was no significant change after 8 weeks, and no significant intergroup difference. An increase in the level of Dd was reported to be a useful indicator of pulmonary embolism[14]; however, in this series, the levels of Dd, TAT-III and PIC, which were higher than normal before administration in the ACT group, tended to return slowly to normal after the start of therapy. These data suggest that the condition of the patients with advanced breast cancer, who were basically in a hypercoagulable state, may have improved with therapy. However, there was no direct evidence for a relationship between the therapeutic effect and the levels of these hematologic parameters in this series. Furthermore, no correlations were observed between sites of recurrence or prior therapy and the levels of these indicators. As Rosso *et al.*, Yamamoto *et al.*, and we have reported previously, no changes in the levels of Fg and Pg were noted following therapy.[3, 10, 15]

On the basis of the present findings, the changes in the parameters of the coagulation-fibrinolytic systems due to therapy can be separated into 4 groups, as follows. (1) Parameters whose values in the ACM group exceeded the normal ranges after therapy and were significantly higher than those in the ACT group (AT-III and PC). (2) Parameters whose values in the ACM group were higher than those in the ACT group after therapy, but remained within the normal ranges (t-PA, FX, and Pg). (3) Parameters whose values were not changed after therapy (Fg). (4) Parameters whose values decreased in the ACT

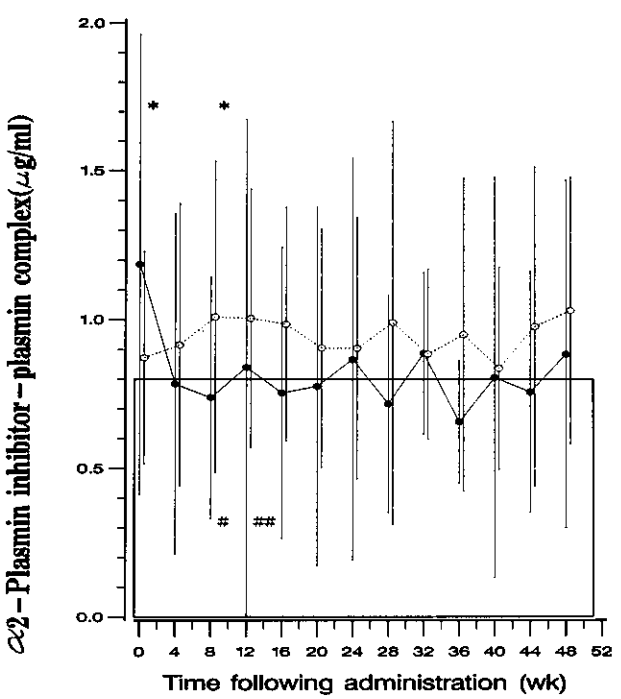

Fig. 5. Time course of changes in alpha 2-plasmin inhibitor-plasmin complex in ACT(●)- and ACM(○)-treated patients. The hatched area shows the normal range (<0.8 μg/ml). Symbols and bars are means±SD. Significantly different at $P<0.05$ (*; *t* test, #; U test) and at $P<0.01$ (##; U test).

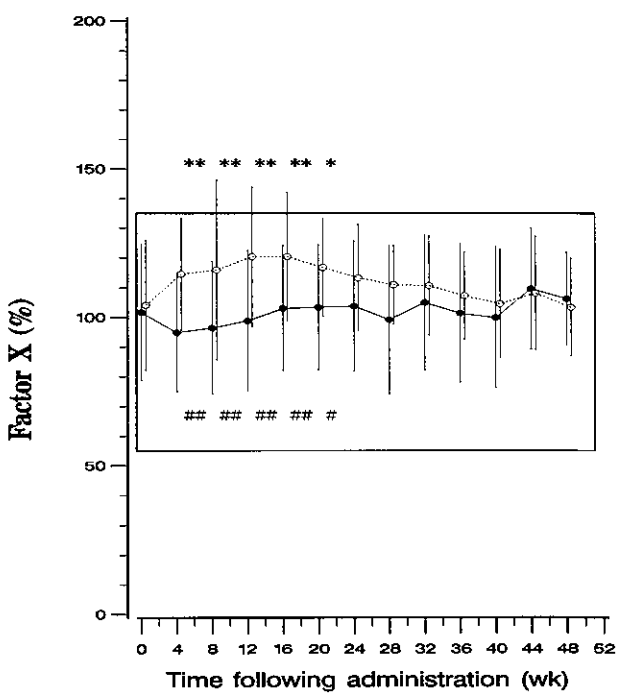

Fig. 6. Time course of changes in factor X in ACT(●)- and ACM(○)-treated patients. The hatched area shows the normal range (56–138%). Symbols and bars are means±SD. Significantly different at $P<0.05$ (*; *t* test, #; U test) and at $P<0.01$ (**; *t* test, ##; U test).

group and were unchanged in the ACM group after therapy (TAT-III, Dd, and PIC).

The ACM group thus showed significant changes in the coagulation-fibrinolytic systems compared with the ACT group, but the increases in AT-III and PC did not indicate a hypercoagulable state. These results indicate that MPA affects liver function and protein synthesis in the liver with or without a therapeutic effect on the lesions. Since, up to now, all reports have agreed that MPA shortens APTT,[3, 10] a shortening of APTT beyond the normal range is considered to be one of the most reliable parameters of a prethrombotic state. In this study, however, there were also some patients with abnormal values of several parameters, and, particularly in the 4 patients listed in Table II, high levels of TAT-III, Dd and PIC were observed simultaneously after the start of treatment. These patients might have had subclinical increases of thrombin generation and microthrombus formation, which were resolved without clinical symptoms.

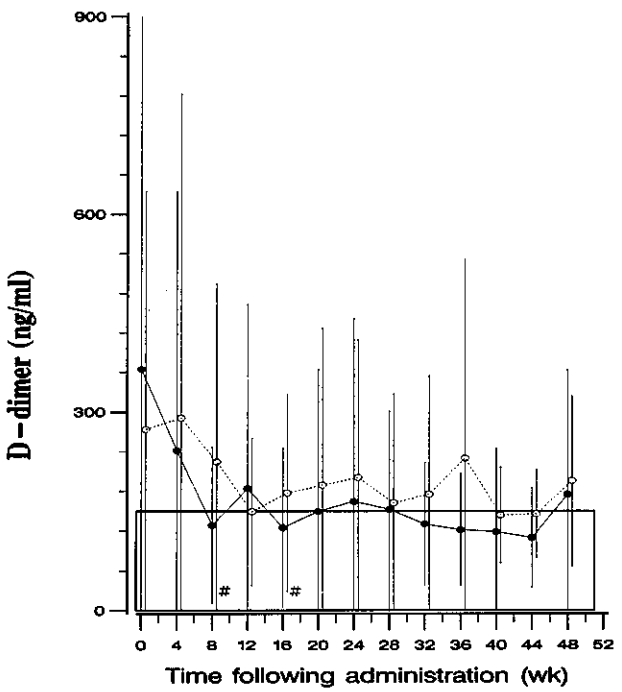

Fig. 7. Time course of changes in D-dimer in ACT(●)- and ACM(○)-treated patients. The hatched area shows the normal range (< 150 ng/ml). Symbols and bars are means± SD. Significantly different at $P<0.05$ (#; U test).

The incidence of thromboembolism is reportedly higher with chemoendocrine therapy than with chemotherapy or endocrine therapy alone.[4] Since in the present study, the same chemotherapy regimen was used in both groups, the time course of changes in the examined parameters was due to differences in the endocrine therapy. However, from the present findings we cannot conclude that thrombosis occurs more often with MPA than with TAM administration.

There is a possibility that the changes in these parameters are the result of (micro)thrombus formation. But even when the changes are statistically significant, they do not seem to be clinically important. Accurate prediction of the prethrombotic state is difficult. Nevertheless, our data suggest that the decreases in AT-III and PC caused by TAM, and the increases in TAT-III, Dd and PIC caused by the drugs should be monitored carefully during treatment.

APPENDIX

Data managers; T. Fukutomi and T. Watanabe. Study chairman; I. Adachi. Data analyzers; N. Aoki (Tokyo Medical and Dental University School of Medicine) and T. Tsukada (Toranomon Hospital). Principal participants (and their affiliations) in the JABCSG II trial were as follows: T. Fukutomi, I. Adachi, T. Watanabe, T. Nanasawa, H. Yamamoto (National Cancer Center Hospital), H. Aoyama (Nagoya National Hospital), M. Sano (Niigata Cancer Center Hospital), Y. Nomura (National Kyushu Cancer Center), M. Ogita (National Sapporo Hospital), T. Tabei (Saitama Cancer Center Hospital), K. Enomoto (Keio University), T. Tominaga (Tokyo Metropolitan Komagome Hospital), H. Murai (Aichi Cancer Center), T. Kitaya (National Matsudo Hospital), S. Takashima (Shikoku Cancer Center Hospital), and T. Wada (Kinki University). We also thank Ms. K. Tajima for information regarding statistical analysis.

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Table II. Patients with Simultaneous High Levels of TAT-III, Dd and PIC after Start of Treatment

Case (Group)	Weeks	TAT-III (ng/ml)		Dd (ng/ml)		PIC (μg/ml)	
		pre	post	pre	post	pre	post
1 (ACM)	4	39.0	31.0	200	1350	1.9	3.7
2 (ACT)	8	5.0	60.0	185	325	0.7	1.9
3 (ACM)	8	1.9	17.9	158	283	0.9	1.7
4 (ACM)	20	2.4	5.7	227	1022	0.5	1.7

REFERENCES

1) Lipton, A., Harvey, H. and Hamilton, R. W. Venous thrombosis as a side effect of tamoxifen treatment. *Cancer Treat. Rep.*, **68,** 887–889 (1984).
2) Hendrik, A. and Subramanian, V. P. Tamoxifen and thromboembolism. *J. Am. Med. Assoc.*, **243,** 514–515 (1980).
3) Yamamoto, H., Noguchi, S., Miyauchi, K., Inaji, H., Imaoka, S., Koyama, H. and Iwanaga, T. Changes in hematologic parameters during treatment with medroxyprogesterone acetate for breast cancer. *Jpn. J. Cancer Res.*, **82,** 420–425 (1991).
4) Saphner, T., Tormey, D. C. and Gray, R. Venous and arterial thrombosis in patients who received adjuvant therapy for breast cancer. *J. Clin. Oncol.*, **9,** 286–294 (1991).
5) Elms, M. J., Bunce, I. H., Bundensen, P. G., Rylatt, D. B., Webber, A. J., Masci, P. P. and Whitaker, A. N. Measurement of crosslinked fibrin degradation products — an immunoassay using monoclonal antibodies. *Thromb. Haemostasis,* **50,** 591–594 (1983).
6) Shimamoto, M., Yoshimura, T., Kimura, N., Kita, M., Hoshino, N., Motoi, S., Suehisa, E., Nishimura, K., Hayashi, C. and Miyai, K. Protein C in human plasma determined by homogeneous enzyme immunoassay with use of a centrifugal analyzer. *Clin. Chem.*, **34,** 1834–1838 (1988).
7) Pelzer, H., Schwarz, A. and Heimburger, N. Determination of human thrombin-antithrombin complex in plasma with an enzyme-linked immunosorbent assay. *Thromb. Haemostasis,* **59,** 101–106 (1988).
8) Bergsdorf, N., Nilsson, T. and Wallen, P. An enzyme-linked immunosorbent assay for determination of tissue plasminogen activator applied to patients with thromboembolic disease. *Thromb. Haemostasis,* **50,** 740–744 (1983).
9) Dati, F., Barthels, M., Conard, J., Flueckiger, J., Girolami, A., Haenseler, E., Huber, J., Keller, F., Kolde, H. J., Mueller-Berghaus, G., Samama, M. and Thiel, W. Multicenter evaluation of a chromogenic substrate method for photometric determination of prothrombin time. *Thromb. Haemostasis,* **58,** 856–865 (1987).
10) Fukutomi, T., Nanasawa, T., Yamamoto, H., Adachi, I. and Watanabe, T. The induction of a hypercoagulable state by medroxyprogesterone acetate in breast cancer patients. *Jpn. J. Surg.*, **20,** 665–670 (1990).
11) Scully, M. F. and Kakkar, V. V. Methods for semimicro or automated determination of thrombin, antithrombin, and heparin cofactor using the substrate, H-D-Phe-Pip-Arg-p-nitroanilide 2Hcl. *Clin. Chim. Acta,* **79,** 595–602 (1977).
12) Harpel, P. C. Alpha 2-plasmin inhibitor and alpha 2-macroglobulin-plasmin complexes in plasma. *J. Clin. Invest.*, **68,** 46–55 (1981).
13) Love, R. R., Surawicz, T. S. and Williams, E. C. Antithrombin III level, fibrinogen level, and platelet count changes with adjuvant tamoxifen therapy. *Arch. Intern. Med.*, **152,** 317–320 (1992).
14) Bounameaux, H., Cirafici, P., De Moerloose, P., Schneider, P. A., Slosman, D., Reber, G. and Unger, P. F. Measurement of D-dimer in plasma as diagnostic aid in suspected pulmonary embolism. *Lancet,* **337,** 196–200 (1991).
15) Rosso, R., Boccardo, F., Canobbio, L., Queirolo, M. A., Zarcone, D. and Brema, F. Effects of high-dose medroxyprogesterone acetate on blood-clotting factors and platelet function. *In* "Proceedings of the International Symposium on Medroxyprogesterone Acetate," ed. F. Cavalli, W. L. McGuire, F. Pannuti, A. Pellegrini and G. Robustelli Della Cuna, pp. 151–157 (1982). Excerpta Medica, Amsterdam-Oxford-Princeton.