Eur J Cancer Clin Oncol. Vol. 21, No. 6, pp. 681-685, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Hemostatic Alterations are Unrelated to the Stage of Tumor in Untreated Malignant Melanoma and Breast Carcinoma*

P. M. MANNUCCI,†‡ M. VAGLINI,§ M. MANIEZZO,§ E. MAGNI,† D. MARI† and N. CASCINELLI§

†*A. Bianchi Bonomi Hemophilia and Thrombosis Center and 3rd Institute of Clinical Medicine, Maggiore Hospital and University of Milano, Italy and* §*Oncology Division "E", National Cancer Institute, Via Venezian 1, Milano, Italy*

Abstract—*A study of hemostatic variables was carried out in 80 untreated patients with breast adenocarcinoma or malignant melanoma, chosen as examples of tumors that can be accurately staged for localization or spread. The most marked abnormalities were high levels of clotting factors V and VIII, plasminogen, von Willebrand factor and fibrogen-fibrin degradation products. These abnormalities occurred in both types of tumors, albeit slightly more markedly in melanomas, and were also present in localized tumors. Our data indicate that in tumors, abnormalities of the hemostatic system are an early phenomenon unrelated to the presence of widespread malignancy.*

INTRODUCTION

THERE have been several reports on changes of hemostatic variables occurring in patients with solid tumors [1-3]. The most frequent alterations are elevated levels of clotting factors (particularly fibrinogen, factors V and VIII) and high fibrinopeptide A and fibrinogen/fibrin degradation products, accompanied by changes in platelets ranging from thrombocytopenia to thrombocytosis. A state of low-grade intravascular coagulation with secondary fibrinolysis is thought to cause these alterations, the high levels of clotting factors being usually explained by an increased synthesis overcompensating an accelerated consumption [1-3]. Despite the abundance of reports, there are still a number of unresolved problems in this field. The great majority of studies, for instance, have been carried out in unselected series of patients with a wide range of tumor types that might affect the hemostatic system in various ways. Other causes of heterogeneity in these series were the frequent inclusions of patients with and without metastases and chemotherapeutic or surgical treatment. Therefore, it is not well known how important variables such as metastatization or therapy affect the hemostatic system.

In this study we elected to address these questions by studying a battery of hemostasis tests in a selected series of 80 untreated patients with local or widespread breast adenocarcinoma or malignant melanoma. These tumor types were chosen as an example of the mucin-secreting tumors (breast carcinoma) or the neuroectodermic tumors (melanoma) that can be accurately staged for localization or dissemination. The following hemostatic variables were studied: fibrinogen, factors V and VIII, clotting factors purportedly elevated in unselected tumor patients [1-3]; protein C (PC) and antithrombin III (AT III), the two main naturally-occurring inhibitors of blood coagulation; and fibrinogen—fibrin degradation products (FDP), plasminogen and antiplasmin, the indirect indexes of activation of the fibrinolytic system. Von Willebrand factor and fibronectin were also assayed as examples of adhesive proteins [4, 5] that might be involved in tumor formation and spread, and that are altered in state when accompanied by DIC [6, 7]. Finally, platelets were counted as a rough index of platelet economy.

Accepted 4 January 1985.
*Supported by Associazione Italiana per la Ricerca sul Cancro.
‡To whom requests for reprints should be addressed at: Via Pace 9, 20122 Milano, Italy.

MATERIALS AND METHODS

Patients

Eighty patients were chosen among those referred to the National Cancer Institute of Milano between 1982 and 1983. Selection took place on the basis of the tumor type (breast adenocarcinoma or malignant melanoma). The patients were entered in this study providing they were surgically and medically untreated at the time of blood sampling. Tumor spread was determined by physical examination and appropriate laboratory and radiological tests. Local tumor was defined as a single tumor mass with no detectable regional or distant metastases. Spread tumor was defined by evidence of regional and/or distant metastases. The presence or absence of lymph node metastases was determined microscopically using pathological specimens of regional lymph nodes obtained at surgery. A diagnosis of liver metastases required at least a pathological ultrasound imaging of the liver. The presence or absence of lung metastases was based on chest films, those of brain metastases on CT scans. A diagnosis of bone metastases required evidence by X-ray films or a positive technetium bone scan. Using these criteria, we selected 21 patients with local and 20 with spread breast tumor (11 with regional and nine with regional and/or distant metastases) and 19 patients with local and 20 with spread melanoma (13 with regional and seven with regional and/or distant metastases). None of the patients had clinical or laboratory evidence of liver disease, renal failure or thromboembolic complications. Liver and renal diseases were ruled out on the basis of the normality of standard liver and kidney function tests and on the absence of clinical signs. Thromboembolic complications were ruled out on the basis of a negative clinical history and absence of clinical signs.

Controls

Ninety-three blood donors, matched for sex and age with each subgroup of the tumor patients, were chosen as a control group.

Test

In addition to standard hemocytometry including platelet count (Coulter S Plus) and chemical profile including liver function tests, the following hemostasis tests were performed on plasma samples obtained by centrifugation of venous citrated (3.8%) blood samples. Fibrinogen (fibrin polymerization method) [8] and FDP (staphylococcal clumping test) [9] were measured with commercial kits (Boehringer-Stago, Milano). Antiplasmin [10], antithrombin III [11] and plasminogen [12] were measured spectrophotometrically using the chromogenic substrates S-2238 and S-2251 (Ortho Diagnostic System, Milano). Protein C and fibronectin were assayed by electroimmunoassay [13]: anti-protein C antiserum was raised in rabbits as previously described [14] and anti-fibronectin antiserum was from the Istituto Behring (Scopitto, Italy). Von Willebrand factor was assayed by electroimmunoassay, using the Factor VIII Related Antigen Rocket EID Starter kit (Helena Laboratories, Milano). Factors VIII and V were tested with one-stage assays, using plasmas from congenitally deficient patients as substrates [15].

Statistical analysis

For intergroup comparison of the results of the hemostatic variables, we carried out a multivariate analysis on log-transformed data. Values are reported in the tables as arithmetic means and standard deviations.

RESULTS

Hemostasis tests in tumor patients and controls (Table 1)

When normal controls were compared with all patients irrespectively of tumor type and spread, statistically significant differences were found for platelets (lower in tumors, $P<0.05$), and for factor V, factor VIII, von Willebrand factor, plasminogen and FDP (higher in tumors, P values <0.02, 0.001, 0.01, 0.001 and 0.01 respectively).

Hemostasis tests related to tumor type (Table 1)

When patients with breast carcinoma were compared with those with malignant melanoma irrespectively of tumor spread, statistically significant differences were found only for platelets (lower in melanoma, $P<0.01$) and factor V (higher in melanoma, $P<0.02$).

Hemostasis test related to tumor spread (Table 2)

When all patients with local tumors were compared with normal controls irrespectively of tumor type, statistically significant differences were found for factor V, factor VIII, plasminogen and FDP (higher in local tumors, P values <0.001, 0.001, 0.001 and 0.01 respectively). When all patients with local tumors were compared with those with spread tumors irrespectively of tumor type, only factor VIII, higher in spread tumors, was significantly different ($P<0.02$). When all spread tumors were considered, no test gave significantly different results between 24 patients with regional as opposed to 16 patients with regional and/or distant metastases (data not shown). When patients with localized breast carcinoma were compared with patients with the corresponding spread tumor, no test gave significantly different

Table 1. Hemostasis tests related to tumor type

Hemostasis tests	Normal controls (*n*: 94)	All tumors (*n*: 80)	Breast (*n*: 41)	Melanoma (*n*: 39)
Coagulation factors				
		$P < 0.05$		$P < 0.01$
Platelets ($\times 10^9$/l)	216*	201	211	188
	(39)	(55)	(57)	(53)
Fibrinogen (mg/dl)	272	282	281	284
	(60)	(71)	(80)	(62)
		$P < 0.02$		$P < 0.02$
Factor V (%)	101	114	107	122
	(20)	(29)	(22)	(37)
		$P < 0.001$		
Factor VIII (%)	100	156	157	155
	(23)	(74)	(76)	(72)
Adhesive proteins				
		$P < 0.01$		
von Willebrand factor (%)	123	150	160	137
	(38)	(58)	(71)	(49)
Fibronectin (%)	101	99	99	99
	(25)	(25)	(27)	(21)
Coagulation inhibitors				
Antithrombin III (%)	95	98	99	97
	(10)	(15)	(18)	(14)
Protein C (%)	108	108	112	103
	(19)	(24)	(26)	(22)
Fibrinolysis components				
		$P < 0.001$		
Plasminogen (%)	97	119	120	118
	(26)	(21)	(22)	(18)
Antiplasmin (%)	94	92	93	90
	(14)	(20)	(19)	(20)
		$P < 0.01$		
FDP (μg/ml)	2	8	7	10
	(1)	(8)	(7)	(9)

*Values indicate means, with standard deviations in parentheses.

results; conversely, factor VIII was significantly higher ($P < 0.005$), protein C lower ($P < 0.05$) and FDP higher ($P < 0.05$) in spread melanomas when compared to local melanomas.

DISCUSSION

The differences in the results of hemostasis tests between all tumor patients and controls were small, with a high degree of overlap. However, our findings of significantly high levels of clotting factors V and VIII, the rate-limiting steps of thrombin formation, and of FDP, an index of fibrinogen cleavage by proteolytic enzymes, are consistent with previous results in unselected series of patients with various types of tumors (for reviews see [1-3]). Therefore we demonstrate that a hypercompensated DIC state with secondary proteolysis also occurs in selected and well-characterized patients with breast carcinoma and malignant melanoma. High levels of von Willebrand factor and plasminogen can probably be accounted for by the same mechanism. Acute-phase reactions to tissue injury and neoplastic growth appear to be less plausible causes, because fibrinogen, a sensitive acute-phase reactant, was not significantly increased. In this series we did not find the laboratory signs that usually accompany a more severe, decompensated DIC state in tumors, such as low antithrombin III, protein C, antiplasmin and fibronectin [16-21]. The selected composition of our group, as well as the lack of patients with terminal neoplastic disease, might explain the less severe alterations in these hemostatic tests. The pathogenesis of thrombocytopenia, statistically significant in the entire group of tumor patients but especially in melanoma patients, is not fully consistent with a picture of hypercompensated DIC and remains unexplained. We postulate that thrombocytopenia is due to the direct ability of tumor cells to lower platelet count, well demonstrated in experimental animals [2, 3], and that such an ability is more

Table 2. Hemostasis tests related to tumor spread

Hemostasis tests	Normal controls	All tumors		Breast		Melanoma	
		Local	spread	Local	spread	Local	spread
Coagulation factors							
Platelets (< 10^9/l)	216*	206	193	214	208	196	179
	(39)	(58)	(54)	(60)	(54)	(55)	(52)
Fibrinogen (mg/dl)	272	292	273	285	277	299	270
	(90)	(74)	(68)	(89)	(70)	(53)	(68)
	$P < 0.001$						
Factor V (%)	101	111	118	107	108	116	127
	(20)	(24)	(37)	(15)	(29)	(30)	(42)
	$P < 0.001$	$P < 0.02$				$P < 0.005$	
Factor VIII (%)	100	143	169	157	156	127	182
	(23)	(64)	(81)	(75)	(79)	(45)	(83)
Adhesive proteins							
von Willebrand factor (%)	123	144	154	162	158	123	150
	(38)	(61)	(64)	(69)	(75)	(43)	(51)
Fibronectin (%)	101	98	100	98	100	98	99
	(25)	(20)	(27)	(20)	(33)	(20)	(22)
Coagulation inhibitors							
Antithrombin III (%)	95	98	97	99	98	97	97
	(10)	(17)	(15)	(22)	(13)	(9)	(18)
						$P < 0.05$	
Protein C (%)	108	100	106	108	116	110	96
	(19)	(19)	(32)	(15)	(34)	(13)	(27)
Fibrinolysis components							
	$P < 0.001$						
Plasminogen (%)	97	118	121	118	123	118	118
	(26)	(16)	(24)	(17)	(27)	(46)	(21)
Antiplasmin (%)	94	89	94	91	95	87	93
	(14)	(22)	(17)	(23)	(14)	(21)	(20)
	$P < 0.01$					$P < 0.05$	
FDP (μg/ml)	2	7	10	7	7	6	14
	(1)	(5)	(9)	(5)	(6)	(5)	(10)

*Values indicate means, with standard deviations in parentheses.

pronounced in melanoma than in breast carcinoma.

The main goal of this study was to evaluate whether the results of hemostasis tests are affected by the types or spread of tumors. Two different types of tumor, breast carcinoma and malignant melanoma, gave very similar results, with only two tests (platelet count and factor V) being more altered from normal in melanoma than in carcinoma. Moreover, we found that the extents of alteration of hemostasis tests (with the exception of factor VIII) were similar both in local and in spread tumors, and that only in the subset of patients with melanoma were a few tests (factor VIII, protein C and FDP) more altered in spread tumors than in local tumors. Within all the patients with spread tumors, there was no significant difference between those with local or distant metastases, but these subgroups were small. Hence it cannot be completely excluded that the failure to find more significant abnormalities in patients with spread tumors than in those with local tumors might simply reflect a relative lack of patients with distant metastases as opposed to regional metastases. Moreover, the use of less simple but more sensitive coagulation tests (fibrinopeptide A, fibrinogen kinetics) might be necessary to show any differences between local and spread tumors. However, our findings of significantly altered tests in the carefully defined group of patients with local tumors clearly indicate that activation of the hemostatic system occurring with tumors is an early phenomenon, unrelated to the presence of widespread malignancies. This finding might have general pathophysiological importance, because of the purported role of hypercoagulability in tumor growth and dissemination [1-3].

REFERENCES

1. Bick RL. Alterations of hemostasis associated with malignancy: etiology, pathophysiology, diagnosis and management. *Semin Thromb Hemostas* 1978, **5**, 1-26.
2. Donati MB, Poggi A, Semeraro N. Coagulation and malignancy. In Poller L, *Recent Advances in Blood Coagulation*. Edinburgh, Churchill Livingstone, 1981, Vol. 3, 227-254.
3. Rickles FR, Edwards RL. Activation of blood coagulation in cancer: Trousseau's syndrome revisited. *Blood* 1983, **62**, 14-31.
4. Ronslahti E, Engvall E, Hayman EG. Fibronectin: current concepts of its structure and functions. *Collagen Res* 1981, **1**, 95-128.
5. Zimmerman TS, Ruggeri ZM. von Willebrand's disease. *Progr Hemostas Thromb* 1982, **6**, 203-236.
6. Mosher DF, Williams EM. Fibronectin concentration is decreased in plasma of severely ill patients with disseminated intravascular coagulation. *J Lab Clin Med* 1978, **91**, 729-735.
7. Denson KWE. The ratio of factor VIII-related antigen and factor VIII biological activity as an index of hypercoagulability and intravascular clotting. *Thromb Res* 1977, **10**, 107-119.
8. Vermylen C, De Vreeker RA, Verstraete M. A quick quantitative enzymatic fibrinogen assay method: the fibrin polymerization time (FPT). *Clin Chim Acta* 1963, **8**, 428-434.
9. Hawiger J, Niewiarowski S, Gurewich V, Thomas DP. Measurement of fibrinogen and fibrin degradation products in serum by staphylococcal clumping test. *J Lab Clin Med* 1970, **74**, 93-108.
10. Teger-Nilsson AC, Friberger P, Gizander E. Determination of a new rapid plasmin inhibitor in human blood by means of a plasmin specific tripeptide substrate. *Scand J Clin Lab Invest* 1977, **37**, 403-409.
11. Abildgaard V, Lie M, Odegaard OR. Antithrombin (heparin cofactor) assay with "new" chromogenic substrates (S-2238 and Chromozym Th). *Thromb Res* 1977, **11**, 549-553.
12. Soria J, Soria C, Samama M. A plasminogen assay using a chromogenic synthetic substrate: results from clinical work and from studies of thrombolysis. In: Davidson JF, Samama M, Desnoyers PC, eds. *Progress in Chemical Fibrinolysis and Thrombolysis*. 337-346. New York, Raven Press, 1978, Vol. 3.
13. Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 1966, **15**, 45-52.
14. Mannucci PM, Viganò S. Deficiencies of protein C, an inhibitor of blood coagulation. *Lancet*, 1982, **ii**, 463-466.
15. Ingram GIC, Brozovic M, Slater NGP. *Bleeding Disorders, Investigation and Management, 2nd Edn*. Oxford, Blackwell Scientific Publications, 1982, 264-328.
16. Miller SP, Sanchez-Avalos J, Stefanski T, Zuckerman L. Coagulation disorders in cancer. I. Clinical and laboratory studies. *Cancer* 1967, **20**, 1452-1465.
17. Hagedorn AB, Bowie EJ, Elveback LR, Owen CA. Coagulation abnormalities in patients with inoperable cancer. *Mayo Clin Proc* 1974, **49**, 647-653.
18. Sun NC, MacAffe WM, Hum CJ, Weiner JM. Hemostatic abnormalities in malignancy, a prospective study of one hundred and eight patients. *Am J Clin Pathol* 1979, **71**, 10-16.
19. Kies MA, Posh JJ, Gidma JP, Rubin RN. Hemostatic function in cancer patients. *Cancer* 1980, **46**, 831-837.
20. Honegger H, Anderson N, Hewitt LA, Tullis JL. Antithrombin III profile in malignancy, relationship to primary tumors and metastatic sites. *Thromb Haemostas* 1981, **46**, 500-503.
21. Griffin JM, Mosher DF, Zimmerman TS, Kleiss AJ. Protein C, an antithrombotic protein, is reduced in hospitalized patients with intravascular coagulation. *Blood* 1982, **60**, 261-266.