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Exhausted Platelets in Patients with Malignant Solid Tumors without Evidence of Active Consumption Coagulopathy*

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Abstract—*Twenty-four patients with various types of tumors and without evidence of consumption coagulopathy (normal routine coagulation tests) were investigated for intraplatelet ATP, ADP, serotonin, beta-thromboglobulin and platelet factor 4; the percentage of light circulating platelets was also determined. Evidence for an acquired storage pool defect was found in seven patients (29%) without any correlation with the clinical status, the presence of metastases, platelet count or fibrinogen level. These results show that exhausted platelets are commonly encountered in cancerous patients even in the absence of consumption coagulopathy. The precise mechanism of this abnormality remains to be established.*

INTRODUCTION

THERE is much evidence suggesting an activation of the platelets and coagulation in cancerous patients. Even in the absence of clinical thrombosis and overt consumption coagulopathy in such patients, several studies have reported an increased fibrinogen and platelet turnover [1–5] and an increased level of fibrinopeptide A [6–8]; in many instances these abnormalities can be improved or normalized by the administration of heparin [2] or platelet-function-inhibiting drugs [9].

In decompensated as well as in compensated cases of consumption coagulopathy, circulating exhausted platelets which have undergone a release reaction phenomenon have been described [10–12]. The present study was aimed at determining whether it is possible to detect such platelet defects in cancerous patients without clinical symptoms of thrombosis or biological evidence of consumption coagulopathy according to the standard coagulation tests. The results show that exhausted platelets were found in about one-third of the investigated patients.

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MATERIALS AND METHODS

Patients

Twenty-four patients hospitalized for various malignant tumors were investigated. Their main clinical features are summarized in Table 1. No patients presented clinical evidence of thrombosis or of disseminated intravascular coagulation; no patients were submitted to radiotherapy or chemotherapy at the time of sampling, nor to any drug known to interfere with coagulation or platelet functions. The results were compared to those of an age-matched population ($n = 16$) composed of healthy blood donors and of elderly people without clinical evidence of cancer or vascular disease.

Table 1. Patient population investigated

Type of tumor	No. of patients	No. of metastases
Rectum	4	2
Bladder	3	3
Breast	5	4
Gynecological tractus	4	2
Miscellaneous (digestive tact, lymphomas, lung)	8	3

Methods

Blood sampling. Blood was taken in citrated vacutainers (B.D. 676608) for routine hemostasis tests and in EDTA-K3 vacutainers (B.D. 606452) for platelet count and estimation of platelet granule content. For platelet density distribution analysis, blood samples were taken in a hyper citrated medium (0.09 M sodium citrate·$2H_2O$, 0.06 M citric acid and 0.11 M glucose, 1 vol. for 4 vol. of blood). Platelet-rich and platelet-poor plasma was obtained by differential centrifugation.

Routine hemostasis investigation. The prothrombin time, activated partial thromboplastin time, thrombin clotting time, platelet count and fibrinogen level were determined according to classical methods [13].

Platelet density distribution. This test allows an easy detection of exhausted platelets [12, 14, 15]. Platelet density distribution was studied according to the method of Corash and Gralnick [16]. The details of the technique have been reported elsewhere [12]; the results were expressed as the percentage of fractions I + II (the lightest platelets) with respect to the sum of the fractions I + II + III + IV, according to Corash's nomenclature.

Platelet dense-granule markers. Intraplatelet ATP and ADP were determined according to the method of Holmsen *et al.* [17] using a kit and reagents purchased for Boehringer Mannheim. The measurement of platelet serotonin was carried out using the fluorimetric technique of Drummond and Gordon [18].

Alpha-granule-specific proteins. Intraplatelet beta-thromboglobulin and platelet factor 4 were measured with the RIA-kits provided by Amersham and Abbott respectively. The total amount of specific proteins contained in the platelets and in the plasma was estimated after seven freeze–thaw cycles in 1 ml of EDTA-platelet-rich plasma with a known platelet concentration. After the final thawing the tube was centrifuged (3000 *g* for 15 min) and the supernatant plasma kept frozen (-20°C) until assayed. Preliminary experiments demonstrated that the assay of each specific protein reached a plateau after the fifth freeze–thaw cycle.

Statistical evaluation. Means and standard deviations of the data were calculated; the comparison between each group of subjects was performed using Student's *t* test. The correlations between ATP/ADP ratio and other platelet parameters were calculated using a linear regression model.

RESULTS

The results of the routine hemostasis tests (prothrombin time, activated partial thromboplastin time, thrombin clotting time) were in the normal range in all the patients. No platelet count was found below 200,000/mm³ except in one case (135,000); plasma fibrinogen level was always above 2.5 g/l and high (>4.5 g/l) in nine cases.

There was a significant increase in the percentage of circulating light platelets [57.6 ± 14

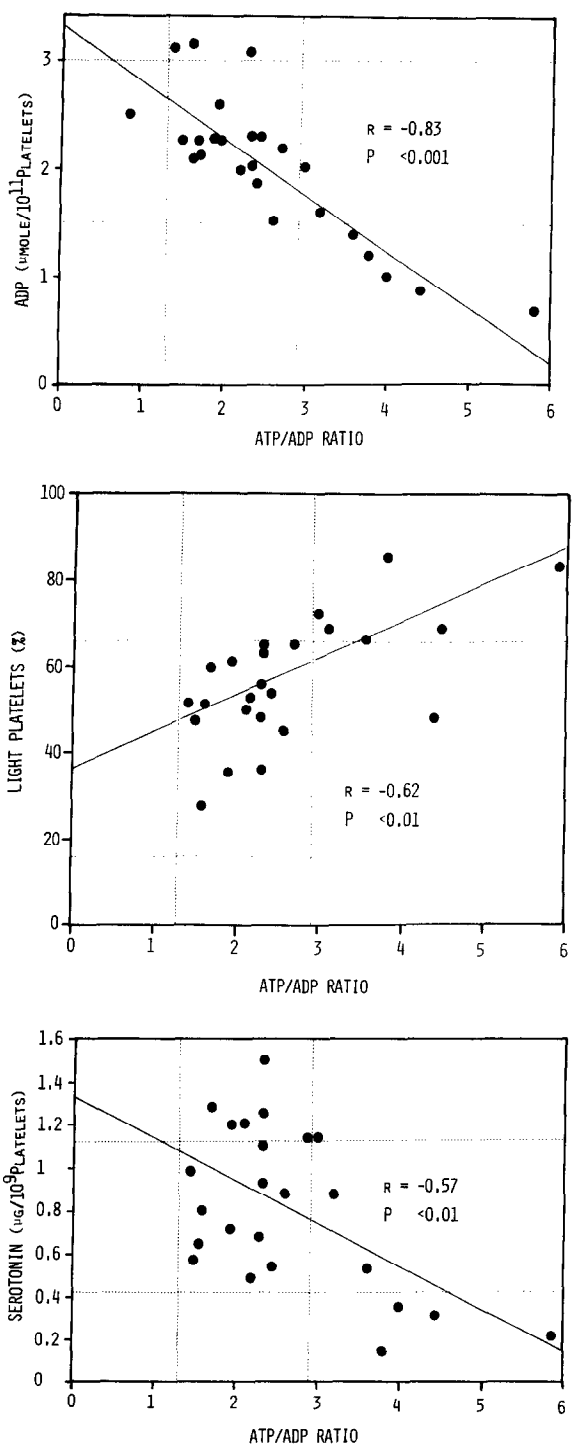

Fig. 1. Correlations between ATP/ADP ratio and intraplatelet ADP (a), percentage of circulating light platelets (b) and intraplatelet serotonin (c). Dotted lines indicate the normal range (mean control values ± 2 S.D.). Solid lines represent the linear regression between the two parameters.

Table 2. Control and patient results classified according to normal or increased ATP/ADP ratio (mean ± S.D.)

	Patients				*P*	
	ATP/ADP >2.95 a	ATP/ADP <2.95 b	Controls c	a–b	a–c	b–c
n	7	17	16			
Platelets ($\times 10^3$/mm^3)	348 ± 90	257 ± 1	/	NS	/	
Fibrinogen (g/l)	4.2 ± 1	3.7 ± 0.7	/	NS	/	/
Light platelet (%)	70.5 ± 12.6	51.2 ± 10.7	41.2 ± 12.3	<0.001	<0.001	<0.05
Serotonin (μg/10^9 platelets)	0.51 ± 0.37	0.94 ± 0.30	0.79 ± 0.19	<0.01	NS	NS
ATP (μmol/10^{11} platelets)	4.7 ± 0.7	4.6 ± 0.9	4.9 ± 0.5	NS	NS	NS
ADP (μmol/10^{11} platelets)	1.3 ± 0.5	2.3 ± 0.5	2.5 ± 0.5	<0.001	<0.001	NS
ATP/ADP	3.9 ± 0.9	2.1 ± 0.4	2.1 ± 0.4	/	/	/
βTG (μmol/10^9 platelets)	30.4 ± 11.2	31.4 ± 7.7	30.6 ± 6.8	NS	NS	NS
PF4 (μmol/10^9 platelets)	9.6 ± 1.5	9.3 ± 2.8	8.6 ± 2.4	NS	NS	NS

Abbreviations: βTG and PF4: intraplatelet beta-thromboglobulin and platelet factor 4; NS: non-significant.

and 41.2 ± 12.3% (mean ± S.D.) for patients and controls respectively, $P < 0.001$]; the intraplatelet level of ADP (μmol/10^{11} platelets) was significantly decreased (1.9 ± 0.6 and 2.5 ± 0.5 for patients and controls respectively, $P < 0.05$). The differences for serotonin, ATP and alpha-granule-specific proteins were not statistically significant. The elevation of the ATP/ADP ratio, a good index to detect platelets which have undergone a release phenomenon [19], was not significant (2.6 ± 1 and 2.1 ± 0.4 for patients and controls respectively, $0.05 < P < 0.10$); however, seven patients presented an increased ATP/ADP ratio above the mean + 2 S.D. of the control values. Significant linear correlations were found between the ATP/ADP ratio and the intraplatelet ADP level (Fig. 1a), the percentage of light platelets (Fig. 1b) and the serotonin level (Fig. 1c).

The patients' results were classified according to the ATP/ADP ratio (Table 2); patients with an increased ratio also presented lower levels of ADP and serotonin, and a dramatic increase in the proportion of light platelets. The alpha-granule-specific proteins, platelet counts and fibrinogen levels were comparable in the two subgroups.

DISCUSSION

This study shows that in the absence of clinical thrombosis or of overt consumption coagulopathy, seven of the 24 investigated patients with malignant tumors presented qualitative platelet abnormalities: a reduced ADP and serotonin content and an increased proportion of light platelets. These abnormalities are highly suggestive of an acquired storage pool defect [12, 19]. The intraplatelet levels of beta-thromboglobulin and of platelet factor 4 were found normal. This does not exclude an alpha granule participation to the release reaction but suggests that this phenomenon is moderate and that the assay of these proteins in total platelets, where their concentration is 3000-fold greater than in the plasma, is not sufficiently sensitive to detect the phenomenon.

The cause of the platelet exhaustion we detected in cancerous patients has not been determined. Only a part of the investigated patients presented qualitative platelet abnormalities without correlation to either the clinical data (tumor histology, presence of metastases) or evidence of an overt consumption coagulopathy phenomenon (an identical and normal or increased platelet count and plasma fibrinogen level in the two subgroups of patients). There are several possibilities to account for these platelet abnormalities: as suggested by various studies [6–8] indicating an increased level of fibrinopeptide A in cancerous patients, thrombin can be generated in the tumor area and induces a platelet release. Several cases of immune-mediated platelet destruction in patients bearing solid tumors have been recently reported [20]. Since an acquired storage pool defect is known to occur in imune thrombocytopenia [21, 22], what was detected in the present study could have been some minor consequences of such a phenomenon upon the

platelets. Finally, using an *ex vivo* model, Marcum *et al.* [23] described the interactions between the subendothelium, the tumor cells and the platelets; such mechanisms can operate in the vascular bed of the tumor, resulting in platelet degranulation. The same group and others [24, 25] also reported an individual sensitivity of platelets from certain donors in their ability to be aggregated by various human tumor cells. This might also explain why platelet abnormalities were found in only a small proportion of the investigated patients.

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REFERENCES

1. Slichter SJ, Harker LA. Hemostasis in malignancy. *Ann NY Acad Sci* 1974, **230**, 252–261.
2. Boneu A, Armand JP, Blasco A *et al.* Demi-vie du fibrinogène marqué à l'iode 131 chez les cancéreux, effet de l'héparine. *Nouv Presse Med* 1976, **5**, 415–418.
3. Lyman GH, Bettigole RE, Robson E, Ambrus JL, Urban H. Fibrinogen kinetics in patients with neoplastic disease. *Cancer* 1978, **41**, 1113–1122.
4. O'Donnell MR, Slichter SJ, Weiden PL, Storb R. Platelet and fibrinogen kinetics in canine tumor. *Cancer Res* 1981, **41**, 1379–1381.
5. Williams CKO, Pineo GF, Gallus AS, McCulloch PB. The relevance of platelet and fibrinogen kinetics and coagulation studies to extent of disease and performance status in patients with adenocarcinoma. *Med Pediat Oncol* 1980, **8**, 367–374.
6. Peuscher FW, Cleton FJ, Armstrong L, Stoepman-Van Dalen EA, Van Mourik JA, Van Aken WG. Significance of plasma fibrinopeptide A (FPA) in patients with malignancy. *J Lab Clin Med* 1980, **96**, 5–14.
7. Yoda Y, Abe T. Fibrinopeptide A (FPA) level and fibrinogen kinetics in patients with malignant disease. *Thromb Haemost* 1981, **46**, 706–709.
8. Mombelli G, Roux A, Haeberli A, Straub PW. Comparison of ^{125}I fibrinogen kinetics and fibrinopeptide A in patients with disseminated neoplasias. *Blood* 1982, **60**, 381–388.
9. Slichter SJ, Weiden PL, O'Donnell MR, Storb R. Interruption of tumor associated platelet consumption with platelet enzyme inhibitors. *Blood* 1982, **59**, 1252–1258.
10. Pareti FI, Capitanio A, Mannucci PM. Acquired storage pool disease in patients during disseminated intravascular coagulation. *Blood* 1976, **48**, 511–515.
11. Pareti FI, Capitanio A, Mannucci L, Ponticelli C, Mannucci PM. Acquired dysfunction due to the circulation of "exhausted" platelets. *Am J Med* 1980, **69**, 235–240.
12. Boneu B, Sié P, Eche N, Caranobe C, Hugo B, Nouvel C. Platelet density analysis: a tool for the detection of acquired storage pool disease in man. *Br J Haematol* 1983, **55**, 523–532.
13. Caen JP, Samama M, Larrieu MJ. *L'Hémostase, Méthodes d'Exploration et Diagnostic Pratique.* Paris, L'Expansion Scientifique, 1975.
14. Cieslar P, Greenberg JP, Rand ML, Packham MA, Kinlough-Rathbone RL, Mustard JF. Separation of thrombin-treated platelets from normal platelets by density-gradient centrifugation. *Blood* 1979, **53**, 867–874.
15. Van Oost BA, Van Hien-Hagg IH, Veldhuyzen BFE, Timmermans APM, Sixma JJ. Determination of the density distribution of human platelets. Methodological aspects and comparison with other tests for platelet activation. *Thromb Haemost* 1982, **47**, 239–243.
16. Corash L, Tan H, Gralnick HR. Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Blood* 1977, **49**, 71–87.
17. Holmsen H, Storm E, Day HJ. Determination of ATP and ADP in blood platelets: a modification of the firefly luciferase assay for plasma. *Anal Biochem* 1972, **46**, 489–501.
18. Drummond AN, Gordon JL. A rapid sensitive microassay for platelet 5 HT. *Thromb Diath Haemorrh* 1974, **31**, 366–367.
19. Holmsen H, Weiss HJ. Secretable storage pools in platelets. *Annu Rev Med* 1979, **30**, 119–134.
20. Schwarts KA, Slitcher SJ, Harker LA. Immune mediated platelet destruction and thrombocytopenia in patients with solid tumors. *Br J Haematol* 1982, **51**, 17–24.
21. Zahavi J, Marder VJ. Acquired storage pool disease of platelets associated with circulating platelet antibodies. *Am J Med* 1974, **56**, 883–890.
22. Weiss HJ, Rosove MH, Lages BA, Kaplan KL. Acquired storage pool deficiency with increased platelet-associated IgG. Report of five cases. *Am J Med* 1980, **69**, 711–717.

23. Marcum JM, McGill M, Bastida E, Ordinas A, Jamieson GA. The interaction of platelets, tumor cells, and vascular subendothelium. *J Lab Clin Med* 1980, **96**, 1046–1053.
24. Bastida E, Ordinas A, Jamieson GA. Idiosyncratic platelet responses to human tumor cells. *Nature* 1981, **291**, 661–662.
25. Grignani G, Almasio P, Pacchiarini L, Ricetti M, Serra L, Gamba G. Interaction between neoplastic cells with different metastasing capacity and platelet function. *Eur J Cancer Clin Oncol* 1983, **19**, 519–525.