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Activity of cancer procoagulant (CP) in serum of patients with cancer of lung, breast, oesophagus and colorectum

Monika Rucińska[1✉], Marian Furman[2], Zdzisław Skrzydlewski[3] and Ewa Zaremba[1]

Department of Oncology[1], Clinic of Thorax Surgery[2], Laboratory of Clinical Analysis[3], Medical Academy in Białystok, Poland

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Activity of cancer procoagulant (CP) was studied in blood serum of 90 patients with cancer of lung, breast, oesophagus and colorectum, and of 15 healthy people. The activity of CP was determined by the coagulation method. Sera of patients with cancer showed higher mean activity of CP than sera of healthy control. Of the 90 cancer patients 78 were identified correctly by this test as having cancer (sensitivity 85%). In the case of lung and colorectal cancers the higher CP activity was observed the more advanced was the clinical stage of cancer, and the test was positive in 100%. After radical removal of malignant tumor of lung, decreased CP activity was found.

A new specific cancer procoagulant (CP) produced by malignant cells was discovered some years ago [1–3], in agreement with the known hypercoagulation of blood derived from patients with malignant cancer. CP was identified as a cysteine proteinase which activates coagulation factor X directly to Xa [3]. The enzyme was found in tissues of malignant cancers and in blood serum of patients with cancers [4, 5]. However, CP is absent in normal tissues except the amnion-chorion tissue of human placenta [6] and its activity should not be observed in the blood of healthy people and patients with benign tumors or with other illnesses. The possibility of using CP as a cancer marker in oncological diagnosis and for monitoring of treatment has been recently considered [4, 5, 7].

The aim of this study was to measure the activity of the cancer procoagulant in blood serum of patients with cancer of lung, breast, oesophagus and colorectum and to evaluate the clinical utility of CP determinations as an aid in the diagnosis of cancer and monitoring of therapy.

MATERIALS AND METHODS

Examinations were carried out on blood sera obtained from 90 patients with cancer prior to treatment. There were 38 patients with lung cancer, 30 patients with breast cancer, 18 patients with colorectal cancer and 4 patients with oesophagus cancer. In the case of lung cancer the measurements were continued on the 10th, 30th and 90th days after the surgery. Blood sera obtained from 15 healthy individuals served for comparison. Blood was taken from the elbow vein in the typical way, left for 30 min at room

✉Corresponding author: Monika Rucińska, Department of Oncology, Medical Academy in Białystok, Ogrodowa 12, 15-027 Białystok, Poland; tel. 048 (085) 320-116.

temperature, centrifuged and then serum was collected. Sera samples were preserved at –25°C for less than 6 weeks.

The activity of CP was determined by the coagulation method of Gordon & Benson [4]. Serum samples were treated with the extraction mixture (aluminium hydroxide gel diluted 1:5 with 20 mM barbital buffer, pH 7.4, containing: 4 mM KCN, 4 mM $FeCl_2$, 4 mM $MgCl_2$, 4 mM $MnCl_2$ and 4 mM $ZnCl_2$) to remove the coagulation factors of prothrombin group (factors II, VII, IX, X), and centrifuged. Procoagulant activity of CP was examined in supernatants using factor VII deficient human plasma. The reaction was initiated by adding 30 mM $CaCl_2$. The coagulation time was measured in seconds. CP activity is inversly proportional to the coagulation time.

RESULTS AND DISCUSSION

In all the examined sera of patients with cancer of lung, breast, oesophagus and colorectum the procoagulant activity of CP was significantly higher than in sera of healthy persons (Table 1). CP is a specific proteinase characteristic only for malignant cells and it should be absent in sera of non-cancer patients. Gordon & Benson [4] showed 92% sensitivity of the coagulation method (34 of 37 cancer patients were identified correctly as having cancer), but specificity of this

Table 1. Activity of cancer procoagulant in serum of patients with cancers as influenced by a histological type

Cancer	Histological type	n	Coagulation (s)	*P*
Lung	Total	38	97 ± 55.34* (35–240)	< 0.001
	Ca planoepitheliale	25	115 ± 62.96 (35–240)	< 0.001
	Ca adenoplanoepitheliale	4	81 ± 20.97 (70–120)	< 0.001
	Ca macrocelullare	4	75 ± 30.33 (40–100)	< 0.001
	Adenocarcinoma	3	71 ± 20.21 (50–90)	–
	Ca microcellulare	2	70 ± 28.28 (50–90)	–
Breast	Total	30	141 ± 93.95** (45–300)	< 0.001
	Ca ductale infiltrans	28	144 ± 96.99 (35–300)	< 0.001
	Ca papillare	2	58 ± 17.68 (45–70)	–
Oesophagus	Ca planoepitheliale	4	88 ± 23.63 (70–120)	< 0.001
Colorectal	Adenocarcinoma	18	152 ± 74.85 (60–300)	< 0.001
Healthy people	Total	15	280 ± 55.60 (210–360)	

*Average value for lung cancer; **average value for breast cancer; ±S.D mean values; a range of experimental values is given in parenthesis.

method in detection of cancer was only 75% (25% false positive results for the people not having cancer) [4]. In our study 78 out of 90 sera samples of cancer patients showed clotting time shorter than 225 s (225 is: the mean value for healthy control minus one SD), so sensitivity of the test was 85%. Three of 15 healthy individuals showed clotting time shorter than 225 s. The 20% false positive results for the normal serum samples is a value too high for this test to be used as a single screening test for cancer, but it could be of use as an aid in diagnosis. Steps have been taken to apply the proteinase as a cancer marker also by other methods: the double antibody immunoassay, sensitivity of which was 81% and specificity 88% [7], and ELISA test, which showed 80% sensitivity and 83% specificity [5]. In all these methods positive results of the test were obtained for some healthy subjects and patients with benign tumors. This may be explained by the inaccuracy of the methods, presence of malignant changes which have not been detected yet or

Table 2. Activity of cancer procoagulant in serum of patients as influenced by the clinical stage of cancer (according to generally accepted classification).

Cancer	Clinical stage	n	Coagulation (s)	
Lung[a]	I	8	159 ± 74.15 (90–240)	
	II	7	89 ± 39.52 (40–140)	($P < 0.02$)*
	III	23	81 ± 33.29 (35–180)	($P < 0.01$)*
Breast[b]	I	7	144 ± 90.67 (50–300)	
	II	13	136 ± 108.66 (35–300)	
	III	10	147 ± 84.40 (40–240)	
Colorectal[c]	B	12	178 ± 76.61 (60–300)	
	C	1	150	
	D	5	88 ± 21.68 (60–120)	($P < 0.02$)**

*According to American Joint Committee for Cancer Staging; [b]according to Steinthal; [c]according to Ducks; *in comparison to clinical stage I; ** in comparison to clinical stage B.

Table 3. Activity of cancer procoagulant in serum of patients with lung cancer before and after removal of the tumor

Type of operation	n	Coagulation (s)			
		before operation	after operation		
			10 days	30 days	90 days
Radical operation	16	126 ± 70.56 (60–240)	219 ± 73.02 (130–350)	247 ± 71.25 (180–355)	243 ± 86.87 (180–360)
Non-radical operation or metastases	22	103 ± 25.62 (35–120)	109 ± 47.27 (45–195)	103 ± 38.73 (50–180)	108 ± 30.62 (60–150)

possibility of elevated CP activity in some kinds of non-cancer conditions.

The highest CP activity was observed in the serum of subjects suffering from papillare breast cancer (58 s), microcellular carcinoma (70 s) and adenocarcinoma of the lung (71 s) (Table 1).

A correlation between the activity of CP and clinical stage was observed in lung cancers and colorectal cancers (Table 2). In the sera of patients with cancer, the more advanced was the clinical stage the higher was the procoagulant activity. Sensitivity of this test for lung cancers at clinical stages II and III and for colorectal cancers at clinical stages D was 100%. However, Kozwich *et al.* [5] using ELISA test observed the highest sensitivity of this test in patients with cancer at clinical stage I. A correlation was found also between the activity of CP and the size of experimental epithelioma in rats [8]. However, the activity fluctuated during tumour growth, at first increasing then decreasing and increasing again at the final stage.

Following of changes in CP activity in blood serum could be of importance in monitoring of cancer therapy as it was already reported for leukaemia [9]. Data in Table 3 show decreased cancer procoagulant activity in serum of patients recovering after radical removal of lung cancer, while this activity was still high when the operation was not radical or metastases were observed. The above results confirm that CP measurement could be useful for monitoring cancer therapy.

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