Haemostasis Activation Markers in Plasma of Patients with Benign and Malignant Gynaecological Tumours: A Pilot Study

By *Jan W. J. van Wersch*[1], *Claire Peters*[2] and *Hans M. H. Ubachs*[2]

[1] *Department of Haematology*
[2] *Department of Gynaecology*
De Wever Hospital, Heerlen, The Netherlands

Summary: Coagulation and fibrinolysis activation have been investigated in fifty-eight women with recently detected gynaecological tumours. Twenty-six were benign and 32 malignant: of the last group nine patients had metastases. A control group consisted of 31 age-matched healthy women. Prothrombin fragment 1 + 2, thrombin-antithrombin III and D-dimer were measured. The median values of all analytes were significantly higher in the malignant tumour group, but not in the benign tumour group, as compared to the control group. The group of patients with a gynaecological tumour and metastases differed from the non-metastasized tumour group in prothrombin fragment 1 + 2, thrombin-antithrombin III and D-dimer. In the non-metastasized malignant tumour group solely prothrombin fragment 1 + 2 was significantly higher than in the benign tumour group. Coagulation and fibrinolysis appeared to be activated in the patients with a metastasized gynaecological tumour, fibrinolysis predominating as can be derived from the elevated D-dimer/thrombin-antithrombin III and D-dimer/prothrombin fragment 1 + 2 ratios. The studied constituents do not enable a differentiation between the benign and malignant processes. However, as the differences of these values in both malignant tumour groups were significant, this might be used to trace the existence of metastases in gynaecological tumours. Investigation of these analytes in several specific types of gynaecological tumours might be clinically relevant.

Introduction

Activation of coagulation and fibrinolyis has been demonstrated in several cancer types (1—5), sometimes accompanied by overt bleeding or thrombosis even in patients without metastasis, necrosis or inflammation (6). Much effort has been made to identify procoagulants on tumour cells themselves (7), as a wide variety of these cells induce local fibrin deposition by increasing microvascular permeability and by clotting extravasated fibrinogen (2). An increase in fibrinolytic activity has been observed in patients with metastases and evidence of disseminated intravascular coagulation (8).

Thromboembolism can be demonstrated at autopsy in 50 percent of all cancer patients although at most 10 percent of these patients actually have significant clinical signs of thrombosis (9). Episodes of haemorrhage may occur in patients with tumour invasion (10). Routine blood coagulation tests display abnormalities in up to 92% of patients with cancer, although this percentage varies with the distinct types of malignancy (11, 12). Little is known about the haemostatic behaviour of patients with gynaecological cancer. Examination of fibrin degradation products has only been presented for patients with ovarian tumours in the early seventies for diagnostic and follow-up reasons (13, 14). Therefore, in the present study the balance between coagulation and fibrinolysis markers was investigated in patients with benign and malignant gynaecological tumours. Coagulation activation was measured by the sensitive clotting turnover products thrombin-antithrombin III complex and prothrombin fragments 1 + 2. The fibrinolytic system was evaluated by measuring D-dimer.

Materials and Methods

Patients

Plasma samples were collected from 58 patients with suspicion of a benign or malignant gynaecological tumour and subsequent diagnostic confirmation by histology. On admission to the hospital no patient showed clinical signs of bleeding or thrombosis. Twenty-six patients had a benign disease (tab. 1) and 32 patients a malignancy (tab. 2). In the latter group nine patients had metastases histologically proven mostly in lymph nodes or peritoneal tissue. The use of medication was excluded. A reference group consisted of 31 age-matched ostensibly healthy women.

Samples

Blood samples were drawn between 8.30 and 9.00 a.m. after fasting overnight for 10 h and a resting period of 20 min.

The various constituents were determined in citrated plasma. This was prepared by centrifugation of a mixture of nine volumes freshly drawn blood with one volume trisodium citrate (0.11 mol/l) during 30 min (1600 g) at 25 °C. The plasma was stored at −70 °C in plastic tubes and thawed at 37 °C for 5 min before batch analysis.

Methods

The prothrombin fragment 1 + 2 test was determined with an ELISA test kit (Behring Corporation, Marburg, Germany). For the thrombin-antithrombin III determination an ELISA test kit was used (Behring, Marburg, Germany). The fibrin degradation products were measured by means of the D-dimer test of Boehringer-Mannheim (Mannheim, Germany). The D-dimer/thrombin-antithrombin III and the D-dimer/prothrombin fragment 1 + 2 ratio were calculated on molar basis as arbitrary measures of the fibrinolysis/coagulation balance.

The Mann-Whitney U test and the χ^2-test, where appropriate, were used for statistical analysis.

Results

In table 1 the diagnoses of the patients with a benign disease are given. Ovarian cysts occur most in this group.

Table 2 shows the various tumour types in the patients with a malignancy. Most of the patients appeared to have a cervix or an ovarian carcinoma. Metastases were especially present in the ovarian carcinoma group.

In table 3 a comparison of the benign tumour group, the total malignant and an age-matched control group is given. Except for the age, all of the test results showed

Tab. 2 Diagnoses and metastasis status of the malignant tumour group.

Malignant tumour type	All patients	Patients without metastases	Patients with metastases
Carcinoma cervicis	12	11	1
Ovarian carcinoma	10	3	7
Endometrium carcinoma	6	6	0
Carcinoma of vulva	2	2	0
Leiomyosarcoma uteri	1	1	0
Sarcoma uteri	1	0	1

a non-*Gauss*ian distribution, and the results are presented as median values and interquartile ranges. The group with a benign gynaecological tumour showed statistically significant higher median values of D-dimer/thrombin-antithrombin III ratio in comparison with the age-matched control group. Comparing the malignant tumour group and the age-matched control group, statistically significantly increased median values were found for prothrombin fragment 1 + 2, D-dimer and D-dimer/thrombin-antithrombin III ratio.

In table 4 patients of the malignant group with and without metastases are compared with the benign tumour group. The values of prothrombin fragment 1 + 2, thrombin-antithrombin III, D-dimer, D-dimer/thrombin-antithrombin III ratio and D-dimer/prothrombin fragment 1 + 2 ratio were significantly increased in the group with metastases.

Table 5 gives the percentages of decreased and increased values of the various coagulation and fibrinolysis constituents in the three patient groups based on the laboratory reference ranges.

Between the benign and the non-metastatic tumour group significantly different proportions of increased values were found for prothrombin fragment 1 + 2 and for D-dimer. The percentages of increased values between the non-metastatic and metastatic tumour groups were significantly different for thrombin-antithrombin III and D-dimer.

Discussion

The patients with a benign gynaecological tumour did not differ significantly in comparison with the control group with the exception of the D-dimer/thrombin-antithrombin ratio. The malignant tumour group however showed, compared to the control group, significant differences for all measured quantities. Subdivision of the malignant tumour group in a group with

Tab. 1 Diagnoses of the benign tumour group.

Benign tumour type	Patients
Ovarian/adnex cyst	15
Ovarian fibroma	3
Ovarian cyst and myoma uteri	2
Endometrioma	2
Myoma uteri	2
Cyst of unknown origin	2

and a group without metastases revealed that the deviations in the total malignant tumour group were mainly due to the patients with metastases.

The results reflect a substantial activation of blood coagulation (prothrombin fragment 1 + 2, thrombin-antithrombin III) with reactive fibrinolysis (D-dimer).

Tab. 3 Comparison of the benign and malignant tumour groups with an age-matched control group.

	Patients with benign gynaecological tumour (G1)	Patients with malignant gynaecological tumour (G2)	Age matched control group (G3)	Significance p-value		
				G1 vs G2	G1 vs G3	G2 vs G3
	Median (25−75 percentiles)	Median (25−75 percentiles)	Median (25−75 percentiles)			
Number	26	32	31	−	−	−
Age	53 (46−65)	57 (48−68)	51 (49−54)	n. s.	n. s.	n. s.
Prothrombin fragment 1 + 2 (nmol/l)	1.00 (0.86−1.50)	1.50 (1.23−1.95)	1.10 (0.84−1.35)	0.001	n. s.	0.001
Thrombin-antithrombin III (μg/l)	2.5 (1.8−3.9)	3.3 (2.4−8.3)	2.3 (2.0−3.2)	0.007	n. s.	0.03
D-dimer (μg/l)	340 (220−580)	570 (300−2138)	240 (200−360)	0.003	n. s.	0.0001
D-dimer/thrombin-antithrombin III ratio	77.3 (66.6−156.2)	108.8 (76.0−199.8)	66.1 (37.5−78.8)	n. s.	0.047	0.0003
D-dimer/prothrombin fragment 1 + 2 ratio	1.6 (1.3−4.2)	2.3 (1.6−6.7)	1.4 (1.2−2.1)	n. s. (0.058)	n. s.	0.003

Tab. 4 Comparison of the malignant tumour groups with and without metastases, and the benign tumour group.

	Patients with benign gynaecological tumour (G1)	Patients with malignant gynaecolgcial tumour without metastases (G2)	Patients with malignant gynaecological tumour and metastases (G3)	Significance p-value		
				G1 vs G2	G1 vs G3	G2 vs G3
	Median (25−75 percentiles)	Median (25−75 percentiles)	Median (25−75 percentiles)			
Number	26	23	9	−	−	−
Age	53 (45−65)	60 (45−70)	54 (49−65)	n. s.	n. s.	n. s.
Prothrombin fragment 1 + 2 (nmol/l)	1.00 (0.86−1.50)	1.35 (1.20−1.70)	2.00 (1.85−2.70)	<0.02	0.002	0.03
Thrombin-antithrombin III (μg/l)	2.5 (1.8−3.9)	2.6 (2.0−3.6)	8.5 (6.4−10.0)	n. s.	<0.0001	0.002
D-dimer (μg/l)	340 (220−580)	420 (270−680)	5000 (1375−6000)	n. s.	<0.0001	0.0002
D-dimer/thrombin-antithrombin III ratio	77.3 (66.6−156.2)	84.5 (71.2−125.8)	236.7 (160.2−348.4)	n. s.	0.003	0.003
D-dimer/prothrombin fragment 1 + 2 ratio	1.6 (1.3−4.2)	1.8 (1.4−2.5)	7.8 (6.1−13.1)	n. s.	0.00005	0.0002

Tab. 5 Percentage of decreased and increased values of various coagulation and fibrinolysis constituents based on laboratory reference ranges. The significant values are indicated (χ^2-test):

[1] p = 0.001;   [2] p = 0.001;   [3] p = 0.026;   [4] p = 0.045; [5] p = 0.002; [6] p = 0.0005; [7] p = 0.0034

	Patients with benign gynaecological tumour (G1)		Patients with malignant gynaecological tumour without metastases (G1)		Patients with malignant gynaecological tumour and metastases (G2)		Laboratory reference range
	Decreased values (%)	Increased values (%)	Decreased values (%)	Increased values (%)	Decreased values (%)	Increased values (%)	
Prothrombin fragment 1 + 2	0	44.4[1],[2]	0	87.0[1]	0	88.9[2]	0.44– 1.11 nmol/l
Thrombin-antithrombin III	0	18.5[3]	0	21.7[6]	0	88.9[3],[6]	1.0 – 4.1 µg/l
D-dimer	0	33.3[4],[5]	0	43.5[4],[7]	0	100[5],[7]	0 –450 µg/l

This phenomenon has also been reported in a number of other cancer types (14–16). The elevated thrombin-antithrombin III levels are consistent with an earlier study on this topic, in which thrombin-antithrombin III was considered a marker of malignant gynaecological disease (17). In general, an explanation for the activation of blood coagulation in tumours is thought to be a direct interaction of the coagulation factors with tumour cells (18, 19) or an indirect production of cytokines (20). The evidence for both of these etiologies are however based on immunohistological investigations. An alternative, well-documented explanation is the evidence for the production of "cancer procoagulants", which have been shown to be produced by a large number of cell lines tested (21).

We calculated the D-dimer/thrombin-antithrombin III ratio and the D-dimer/prothrombin fragment 1 + 2 ratio as arbitrary measures for the balance of the fibrinolysis/coagulation system. Both ratios were increased in favour of fibrinolysis, being the highest in patients with metastases. The D-dimer/prothrombin fragment 1 + 2 ratio showed a more pronounced increase than that of D-di-

mer/thrombin-antithrombin III, probably because prothrombin fragment 1 + 2 and thrombin-antithrombin III differ in production and elimination rates. Fibrinolysis seems to dominate over the coagulation system, therewith possibly preventing thrombotic events. Nevertheless, when the quantities of fibrinolysis and coagulation are enhanced the risk for such an event may be increased (1–3, 22–24).

In conclusion, it is not possible to differentiate between a benign and a malignant process on the basis of the constituents studied. The haemostasis quantities seem, however, to give an indication of the existence of metastases in patients suspected of malignant gynaecological tumours, although we are aware that the studied patient groups are rather heterogeneous. Therefore a follow-up study is ongoing focussing on the more frequent gynaecological cancer types e. g. ovarian cancer and cervix cancer. Moreover, more research will be necessary to evaluate the clinical impact of the elevated level of the fibrinolysis/coagulation balance in patients with the mentioned gynaecological cancer types.

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Dr. J. W. J. van Wersch
De Wever Hospital
P. O. Box 4446
NL-6401 CX Heerlen
The Netherlands