

Cathepsin B-Like Activity as a Serum Tumour Marker in Ovarian Carcinoma

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Summary: Serum cathepsin B-like activity was determined in 75 patients with ovarian carcinomas and in control groups. Ovarian cancers were of FIGO stages I–IV. Control groups consisted of 15 healthy women, 20 patients with myomas of the uterus, and 17 with benign ovarian cysts. Preoperative results showed elevated cathepsin B-like activity in 100% of the patients with ovarian cancers in relation to healthy subjects and patients with myomas, and in 78% in relation to benign ovarian cysts. Cathepsin B activity increased progressively with the FIGO stage of the disease, but the differences among particular stages were not statistically significant. In serous tumours cathepsin activity was significantly higher only in comparison to endometrioid ones ($p < 0.001$). Antipapain capacity of cystatins in the sera was also determined. No significant correlation between cathepsin B-like, and antipapain activity of cystatins was found. Serum cathepsin B-like activity may be helpful in the preoperative differential diagnosis between ovarian carcinomas and benign ovarian or uterine tumours.

Introduction

Epithelial ovarian cancer, which is the major cause of death among women with gynaecologic malignancies, produces no readily identifiable or measurable substances that might be useful in the early detection or management of the disease. These are imperative for effective treatment (1).

In an attempt to promote earlier diagnosis and improve monitoring of ovarian cancer, numerous markers have been studied (2–6). To date, none has been found suitable for widespread clinical application.

Pietras et al. (7) have suggested that a cysteine peptidase, cathepsin B¹, is a sensitive marker for ovarian cancer diagnosis and may be of value in monitoring the course of the disease. These conclusions, based on small number of patients, seemed interesting enough to be verified.

Lysosomal cathepsin B, and its extracellularly secreted as well as plasma membrane-associated forms for which the name cathepsin B-like is often used, received great interest regarding their role(s) in biochemical processes underlying tumour growth, invasiveness, and metastasis (8).

The aim of the present study was to examine cathepsin B-like activity in sera of patients with ovarian cancer under aspects of diagnostic value of the enzyme. More-

over, we wanted to find out if there is any correlation (dependence?) between cathepsin B, and its specific inhibitors – cystatins.

Materials and Methods

Patients

Patients attending the Clinic of Obstetrics and Gynaecology, Wrocław University of Medicine, in 1989–1994 were the subject of this study. The group with malignant tumours of the ovaries included

Tab. 1 Classification of ovarian cancers according to histopathological types and FIGO stages.

Histological types	Disease stage				Total	%
	I	II	III	IV		
Serous	4	3	4	12	23	30.7
Mucinous	2	–	–	5	7	9.3
Endometrioid	2	3	3	14	22	29.3
Undifferentiated	2	1	2	7	12	16.0
Clear cell	–	–	3	3	6	8.0
Other	–	2	–	3	5	6.7
Total	10	9	12	44	75	100

75 cases (tab. 1). The control groups consisted of 20 uterine myomas, and 17 benign ovarian cysts (cystis simplex, cystadenoma, cystis dermoidalis, cystis picea, cystis serosa, cystadenoma mucinosum). The age at diagnosis was in the group of cancers 14–82 (median 51 a) in the myomas, and cysts 35–60 (median 48 a), respectively. In all the patients with ovarian or uterine tumours laparotomy was performed. Ovarian tumour mass was removed and the extent of the disease stage according to the FIGO classification (9) was undertaken. Blood was taken before surgery from patients suspected of having ovarian cancer. The blood samples were centrifuged and the serum stored at -20°C until use (up to 2 months).

¹) Enzyme:
Cathepsin B (EC 3.4.22.1); papain (EC 3.4.22.2)

Tab. 2 Preoperative serum cathepsin B-like and antipapain capacity of cystatin levels in patients and controls.

Groups	N	Cathepsin B		Antipapain capacity of cystatins	
		(mU/l)	Range	(mU/l)	Range
Healthy women	15	4.00 ± 3.47	0.00 – 9.5	72.51 ± 7.09	61.17 – 82.58
Myomas of uterus	20	10.82 ± 6.12	0.00 – 21.00	80.17 ± 15.27	61.17 – 107.00
Benign ovarian cysts	17	40.00 ± 31.15 [#]	5.00 – 97.32	87.17 ± 15.37 [#]	56.28 – 110.72
Ovarian carcinoma					
Stage I–IV	75	194.54 ± 161.53*	25.30 – 880.40	99.93 ± 25.32 [#]	30.58 – 174.35
Stage I	10	114.20 ± 40.30*	68.50 – 203.50	106.77 ± 25.14*	67.29 – 145.29
Stage II	9	146.60 ± 58.24*	25.30 – 200.10	107.90 ± 27.57 [#]	74.51 – 162.11
Stage III	12	157.07 ± 72.77*	46.65 – 268.10	96.22 ± 17.37 [#]	72.61 – 137.95
Stage IV	44	232.82 ± 196.74*	34.80 – 880.40	97.49 ± 26.08 [#]	30.58 – 156.00

* Significantly different from: healthy control, myomas of the uterus, and benign ovarian cysts (for cathepsin B-like activity $p < 0.001$, for inhibitory capacity $p < 0.01$).

[#] Significantly different from: healthy control, and myomas of the uterus (for cathepsin B-like activity $p < 0.001$, for inhibitory capacity $p < 0.01$).

The healthy control group, consisting of 15 healthy women at the ages of 28–46 (median 39 a), was obtained from the Silesian Center "Dolmed" in Wrocław.

Methods

Cathepsin B-like activity in diluted (1 : 10) serum was performed with the procedure described by Szego et al. (10) in 40 mmol/l mixed sodium citrate/sodium phosphate buffer, pH 5.7 in the presence of 5 mmol/l dithiothreitol and 0.17 mmol/l substrate (carbobenzoxy-Ala-Arg-Arg-4-methoxy- β -naphthylamide) in a total volume of 2 ml. After a 30 min incubation at 37 °C the reaction was stopped by addition 0.5 ml of 0.1 mol/l HCl. Fluorescence of the reaction product was measured at the emission peak of 410 nm on a Perkin-Elmer Fluorimeter LS-3 B with an activation beam of 292 nm. Fluorescence readings were standardized with 4-methoxy- β -naphthylamine. The results were expressed in mU/l (one mU of activity was defined as the amount of enzyme releasing 1 nmol of 4-methoxy- β -naphthylamine per minute).

Antipapain activity of cystatine was measured after methylamine inactivation of α_2 -macroglobulin, as described by Minakata et al. (11), using casein as a substrate and in the presence of 6 mmol/l cysteine (12). Papain purchased from Calbiochem, USA, was titrated with E-64 (*L*-epoxysuccinyl-leucylamido(4-guanidino)butane) to determine the active concentration of the enzyme (13). One inhibitory unit was defined as the amount which inhibited 1 μ mol of active papain completely. This was determined by extrapolating the titration curves to zero papain activity.

As the upper normal limit (cut-off) of measured analytes we took ovarian cysts mean value plus two standard deviations (SD). Statistical analysis was performed using analysis of variance and the unpaired Student's *t*-test. Linear regressions were computed using the Pearson least-square method.

Results

Table 2 summarizes results of cathepsin B-like and antipapain activity of cystatin determinations in controls and patient groups. Table 3 presents the percentage of the results with value elevated above the cut-off values. Such a limit value was calculated from the mean plus two standard deviations measured in sera of patients with benign ovarian cysts. In healthy women the serum cathepsin activity ranged from 0.0 to 9.5 mU/l, in the myoma patients from 0.0 to 21.0 mU/l, and in the benign

Tab. 3 Incidence of elevated cathepsin B-like activity and antipapain activity of cystatins in sera of patients with benign and malignant tumours.

Cut-off values are calculated from the results for the benign ovarian cyst group ($\bar{x} + 2$ SD).

Groups	Cases with values above cut-off (%)	
	Cathepsin B 102.3 mU/l	Antipapain capacity of cystatins 117.91 mU/l
Healthy women	0.0	0.0
Myomas of uterus	0.0	0.0
Benign ovarian cysts	0.0	0.0
Ovarian carcinoma		
Stage I–IV	78.6	22.6
Stage I	60.0	50.0
Stage II	77.7	22.0
Stage III	75.0	8.3
Stage IV	84.0	22.7

ovarian cysts 5.0 to 97.0 mU/l. The mean value of cathepsin activity in malignant tumours was significantly higher than in all the control groups examined ($p < 0.001$). A wide dispersion of enzyme activity was observed. Range of serum concentrations found for ovarian cancer patients (25.3–880.4 mU/l) showed considerable overlap with those found for benign ovarian cysts patients. Serum levels of cathepsin B-like activity in malignant tumours appear to increase progressively with the stage of the disease. However, because of high standard deviations, differences among groups were not statistically significant. The incidence of elevated cathepsin B-like activity concentrations above the cut-off value (102.3 mU/l) was 60% in patients at stage I, and 84% at stage IV of the disease.

Analysis of the cathepsin B-like activity data in relation to histological cancer types showed significantly higher

activity in patients with serous tumours in comparison to endometrioid types ($p < 0.001$) (data not shown).

Antipain activity of cystatins was significantly raised in the group of benign and malignant ovarian tumors as compared with the healthy control group (tab. 2). The incidence of concentrations elevated above the cut-off value (117.9 mU/l) in the ovarian cancer groups ranged from 8 (stage III) to 50% (stage I). Increase of antipain capacity was similar in all the histopathological cancer types represented in the present study (data not shown). A correlation coefficient equal to 0.2 between serum cathepsin B-like, and antipain activity of cystatins was found.

Discussion

Though serum markers are of limited value in screening the general population for ovarian cancer, they can be used to assist the general gynaecologist in avoiding potentially difficult oncologic surgery. Therefore, it is important that discrimination between benign and malignant disease is made. Cathepsin B overexpression is common to many malignant tumours (14). Elevated serum activity or concentration was observed in patients with breast (15), colorectal (16), gastric (17), laryngeal (18), liver (19), and gynaecological (7, 20, 21) carcinomas.

We examined the usefulness of measuring serum cathepsin B-like activity in the preoperative discrimination of ovarian carcinoma from benign tumours, and in the assessment of the extent of the disease. Our study showed elevated cathepsin B-like activity in 100% of the patients with malignant ovarian tumours in relation to healthy women and those with myomas of the uterus, and in 84% in relation to benign ovarian cysts. Pietras et al. (7) demonstrated marked elevation in cathepsin B-like activity in sera of patients with ovarian cancer of FIGO stage III (+ 2490%) and IV (+ 3890%).

We also examined patients with stage I and II. The level of cathepsin B-like activity in patients with stage I (+ 1130%) and II (+ 1320%) was lower as related to stage IV (+ 2190%). The results were expressed as percent of elevation in relation to levels of enzyme activity in women with myomas. When benign ovarian cysts were used as a control the elevation was four times lower. Benitez-Bribesca et al. (21) reported a significant

increase ($p < 0.001$) of cathepsin B activity in serum and vaginal fluid of all patients with carcinoma of the cervix uteri including that with stage 0 (carcinoma in situ) in comparison to healthy women. In contrast, Makarewicz et al. (20) who used a control group consisting of patients with dysplasia have not found a rise of cathepsin B-like activity in early disease limited to the cervix uteri, while in early cervical carcinoma with metastases to pelvic lymph nodes the activity was markedly increased. Serum levels of the enzyme appear to increase parallel to the activity in tumour tissue. Tissue cytosol cathepsin B-like activity might also appear as a useful independent prognostic marker for survival in human cancers including ovarian ones (14, 22). According to the present study the sensitivity value of cathepsin B-like serum level calculated using the benign ovarian cysts group as a negative control is the same (64%) in ovarian cancer as in cervical cancer, as described by Makarewicz et al. (20). It is not known whether the elevated activity of cathepsin B in serum is due only to its elevated production either to secretion by tumour tissue or to a disorder of its elimination from circulation or to a derangement of its specific inhibitors (7, 19, 21).

Kininogens and cystatin C, together with α_2 -macroglobulin, are believed to be the most potent extracellular inhibitors of lysosomal cysteine peptidases as well as pappain, a model plant protease (23).

Alterations in the balance between endogenous cysteine peptidase inhibitors, and cysteine peptidases have been postulated to contribute to malignant progression (21, 23). This was why we explored a possible correlation between serum cathepsin B-like activity and antipain capacity of cystatins which is mainly due to kininogens (24). We measured the antipain capacity after inactivation of α_2 -macroglobulin with methylamine. The inhibitory capacity was elevated in 36% of patients with ovarian cancer, which is consistent with our earlier observation (25). In spite of the low correlation coefficient between cathepsin B-like and antipain activity the ratio of cathepsin activity to inhibitory activity rose from 0.08 in patients with myomas to 1.46 in patients with ovarian cancer of FIGO stage IV.

It is concluded that the serum cathepsin B-like activity determinations are helpful in the preoperative differential diagnosis between ovarian carcinomas and benign ovarian or uterine tumours.

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