# The activity of cancer procoagulant in cases of uterine leiomyomas

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## Summary

*Purpose:* It is currently believed that cancer procoagulant (CP), an enzymatic protein, is a product of malignant neoplastic cells. The present study was designed to test whether it is also synthesized by benign neoplastic cells, namely uterine leiomyomas.

*Materials and methods:* We determined the activity of CP in the blood serum of women with uterine leiomyomas (N = 24), normal women (N = 15), and genital cancer patients (N = 6) by the coagulative method according to Gordon and Benson. Also, the CP activity in 10% tissue homogenates of uterine leiomyomas, normal uterine muscle and tissues of cervical and endometrial carcinoma was determined by the chromogenic method according to Colucci *et al.*

*Results:* The mean CP activity in the sera of women with uterine leiomyomas was 181.1 seconds (s) ± 19.9 s, in healthy women - 293.2 s ± 33.8 s, and in genital cancer patients - 78.8 ± 18.5 s (all differences: $p < 0.001$). Similarly, in homogenates of uterine leiomyomas the CP activity was 19.6 ± 3.8 nmoles pNa/ml, in normal uterine muscle it was 13.2 ± 2.2 nmoles pNa/ml, and in cancerous tissue - 28.0 ± 6.6 nmol pNa/ml (all values being significantly different from each other). There was a strong correlation (r = -0.8122; $p < 0.001$) between the CP activity in uterine leiomyomas and serum activity, suggesting that the source of the serum CP activity was from the leiomyoma. The coagulation time of 120 to 240 s by the Gordon and Benson method supported the diagnosis of uterine leiomyoma, and a value below 120 s - the suspicion of genital cancer.

*Conclusions:* Uterine leiomyomas, representing benign genital neoplasia, synthesize CP and are the likely origin of CP activity in blood, as has been described for malignant tumors, but to a lesser degree. There may be a role for CP as a tumor marker of genital neoplasia.

*Key words:* Cancer procoagulant; Clotting system; Uterine leiomyomas.

## Introduction

Cancer markers allow early detection of neoplastic disease, thus enhancing the chance of a complete cure. One such relatively new marker is a sulfhydryl proteinase known as cancer procoagulant, or CP [1]. The enzyme is thought to be primarily synthesized and expressed by malignant neoplastic cells and by the cells of fetal membranes, whereas its activity is low in the blood serum of healthy subjects [2, 3]. Consequently, increased CP activity in serum of non-pregnant women has been interpreted as being associated mainly with the presence of a malignant proliferative process in the host body [4]. On the grounds of this distinction, there have been attempts to examine the possible usefulness of CP in cancer detection and monitoring of oncotherapy [5-7]. In fact, in comparison with other cancer markers, the activity of CP has been demonstrated to be a sensitive and reasonably specific marker for distinguishing between advanced cancer patients and normal patients [5, 6]. To date, high CP activities have been found in serum and cancerous tissues in patients suffering from gastric, esophageal, colorectal, lung and breast cancer [7-9]. However, there has been no systematic study of CP activity in cases of genital neoplasia, nor in benign proliferative disorders. Recently, we found in a limited number of observations, the presence of CP activity in the sera of women with uterine leiomyomas [10]. This result now requires confirmation since the unique role of malignant cells in CP production and the potential usefulness of CP as a malignant tumor marker need to be defined.

The present investigation was designed as a pilot study to verify the synthesis of CP by benign neoplastic cells and the possibility of measuring CP activity to assist the clinical diagnosis. For this purpose, we measured the CP activity in uterine leiomyoma tissues and in the serum of women with uterine leiomyomas, and compared these data with those obtained from both cancer patients and healthy subjects.

## Patients and Methods

### *Patients*

The study was approved in advance by the Institutional Review Board, Medical University of Bialystok, and all participants gave an informed consent.

The material for the study consisted of uterine myomas and adjacent normal uterine muscle specimens obtained immediately postoperatively from 24 women aged 40 to 58 years (mean 49.1 ± 5.1 years). The indication for surgery was the presence of a uterine myoma at pelvic examination, confirmed by ultrasound examination. All specimens were subjected to histopathological confirmation of both leiomyoma and normal uterine muscle. In all cases, the distance from the leiomyma to the site where the normal uterine muscle was sampled was above 1 cm, so that both tissues were not directly in contact.

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Cancer patients were three patients with cervical carcinoma and three patients with endometrial carcinoma. Cervical carcinoma patients were aged 38 to 59 years (mean 47.0 ± 10.8 years), had Stage IB disease according to the FIGO classification [11], and histopathologically confirmed invasive squamous cell carcinoma. Endometrial carcinoma patients were aged 54 to 68 years (mean 61.7 ± 7.1 years), had Stage IB disease according to the FIGO classification [11], and histopathologically confirmed invasive adenocarcinoma.

Peripheral blood was sampled preoperatively in a sterile manner from an antecubital vein at the moment of insertion of an IV line. Thus, with such an approach, the sampling was performed before the action of anesthetic agents had occurred, and was not associated with any additional pain or discomfort to the patient. Blood from the reference group (N = 15) was derived in an identical manner in the morning after an overnight fast. These women were volunteers aged 21 to 24 years (mean 21.9 ± 1.0 years), healthy and with regular menses. With such an approach, the time of blood sampling, circadian phase, and the fasting state were similar in the examined and reference groups.

The clinical exclusion criteria from the study were: history of viral hepatitis, presence of clinical symptoms of hiperbilirubinemia, and history or presence of symptoms of coagulopathy. The histopathological exclusion criteria were: endometrial polyp(s), endometrial hyperplasia, and internal or external endometriosis.

*Analytical methods*

The CP activity in 10% tissue homogenates (in saline) of uterine leiomyomas and normal uterine muscle was determined in duplicate by the chromogenic method according to Colucci et al. [12]. This method is specific for the enzyme, the activity of which is expressed in nmoles of p-nitroanilin (pNa) released by the color reaction per ml. Concerning the interpretation of results, the higher the result, the more pronounced the CP activity.

The CP activity in blood serum was determined in duplicate using the coagulative method according to Gordon and Benson [5], and was expressed as coagulation time in seconds (s). This procedure was specifically developed for the evaluation of CP in serum. The test was performed as originally described [5], except that we used a commercially available preparation of factor VII-deficient plasma. As to the interpretation of results, the shorter the coagulation time, the more pronounced the CP activity.

Our care to minimize the effect of hiperbilirubinemia on the results stems from the fact that bilirubinoids demonstrate their maximum absorbance at a wavelength of 450 nm, whereas by the Colucci et al. method the spectrophotometric reading is performed at a wavelength of 405 nm.

*Reagents*

In the Colucci et al. method [12], the substrate for the color reaction producing pNa was Bz-Ile-Glu-Arg-pNa (S-2222) obtained from Chromogenix (Milan, Italy; catalogue no. 82 03 16-39). For the Gordon and Benson method [5], we used Clotting Factor-VII Deficient Plasma from Sigma (St. Louis, MO, USA; catalogue no. F7D-I). Clotting Factor X (catalogue no. F 4634), TRIS-HCl, dimethyl sulphoxide, and ethylenediaminetetraacetic acid, or EDTA, were also from Sigma. Sodium veronal was obtained from Loba Chemie – Fischamend (Vienna, Austria; catalogue no.19756). All other reagents came from P.O.Ch. Gliwice, Poland.

*Statistical analysis*

The data are presented as means ± standard deviation (SD). Statistical analysis was performed using the SPSS 8.0 PL statistical package (SPSS Inc., Chicago, IL, USA). The data were verified for normal distribution using the Kolmogorov-Smirnov goodness of fit test, and their distributions were found to be normal, except for age. Consequently, the Student's-t test for unpaired observations was used to determine the significance of differences, with the exception of age difference which was determined using the Mann-Whitney U test. Correlations were examined using Pearson's linear coefficient. A $p$ value of less than *0.05* was considered significant.

## Results

The mean CP activity in the serum of women with uterine leiomyoma was 181.1 ± 19.9 s, and in healthy women – 293.2 ± 33.8 s *($p < 0.001$).* Similarly, the mean CP activity in homogenate of uterine leiomyoma (19.6 ± 3.8 nmol pNa/ml) was higher than in the homogenate of normal uterine muscle (13.2 ± 2.2 nmol pNa/ml; *$p < 0.001$*). Moreover, in every patient examined, the ratio of CP activity in leiomyoma to that in normal uterine muscle exceeded 1.0.

There was a significant difference in age between the uterine leiomyoma and reference groups *($p < 0.001$).* There was no significant difference in age or CP activity in serum or cancerous tissue between cervical and endometrial carcinoma patients. These data were combined (N = 6) to derive mean values of 54.3 ± 11.5 years for age, 78.8 ± 18.5 s for CP activity in serum, and 28.0 ± 6.6 nmol pNa/ml for CP activity in cancerous tissue. Again, there was a significant difference in age between the cancer and reference groups *($p < 0.001$)*, but not between the cancer and uterine leiomyoma groups.

Figure 1 demonstrates significant differences in mean CP activity in the blood serum of women with uterine leiomyoma, women with cancer and normal women. Figure 2 delineates significant differences in mean CP activity between tissues of uterine leiomyoma, normal uterine muscle and genital cancer. Clearly, both in serum and tissue, CP activity was lowest in normal serum and uterine muscle, moderate in cases of uterine leiomyoma, and highest in cases of genital cancer.

No correlation of CP activity with age in any tissue was found. In contrast, there was a strong negative correlation ($r = -0.8122$; *$p < 0.001$*) between the CP activity in homogenates of uterine leiomyoma and the CP activity in serum of women with uterine leiomyoma (Figure 3). The correlation between CP activity in serum of women with uterine leiomyoma and CP activity in the homogenate of normal uterine muscle was also negative but less marked ($r = -0.4861$; *$p = 0.016$;* Figure 4). However, the correlation between CP activity in the homogenate of uterine leiomyoma and in the homogenate of normal uterine muscle was direct and surprisingly strong ($r = 0.7608$; *$p < 0.001$*; Figure 5).

Of interest, the serum values of CP activities in cases of cancer ranged from 53 s to 101 s. In cases of uterine leiomyomas these values ranged from 151 s to 233 s, and in normal subjects they ranged from 244 s to 367 s. Thus, the value of approximately 240 s could be considered as being a borderline value which distinguishes sera of

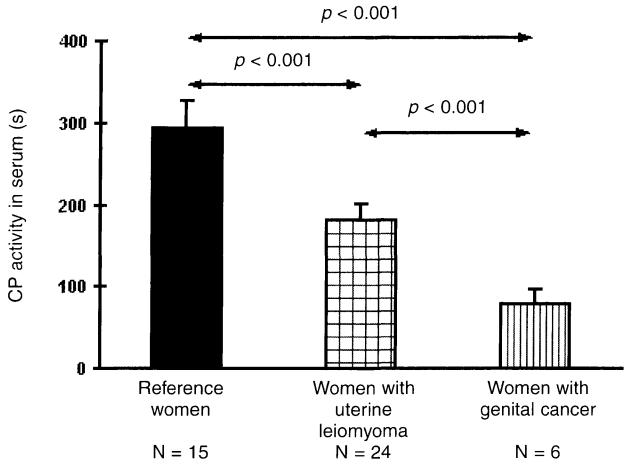


Figure 1. — Comparison of mean CP activity in the blood serum of women with uterine leiomyoma, women with genital cancer and normal women; *p*-values were determined by the Student's t-test for unpaired samples.

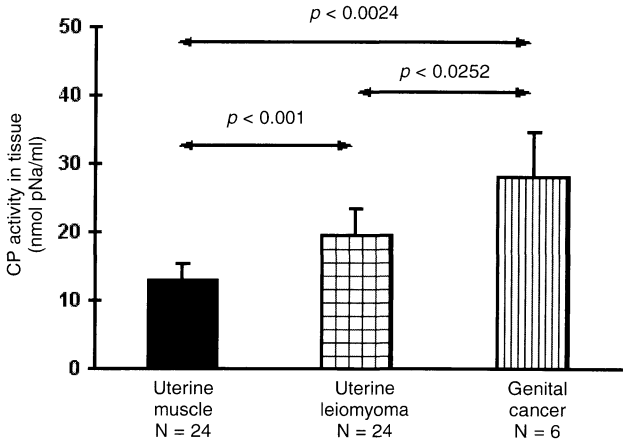


Figure 2. — Comparison of mean CP activity in tissues of uterine leiomyoma, normal uterine muscle and genital cancer; *p*-values were determined by the Student's t-test for paired samples comparing uterine leiomyoma to normal uterine muscle, and by the Student's t-test for unpaired samples for other comparisons.

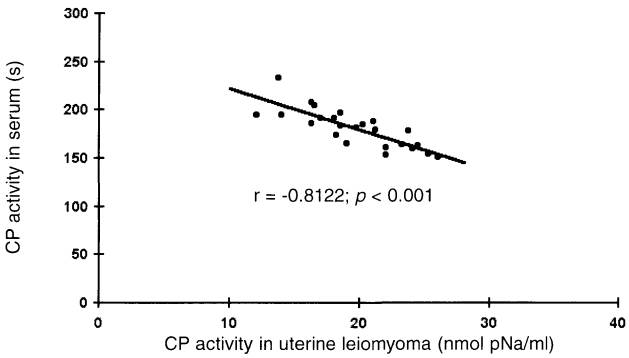


Figure 3. — The correlation between CP activity in 10% homogenate of uterine leiomyoma and CP activity in serum of women with uterine leiomyoma. Number of observations – 24. Correlation was assessed with Pearson's linear coefficient.

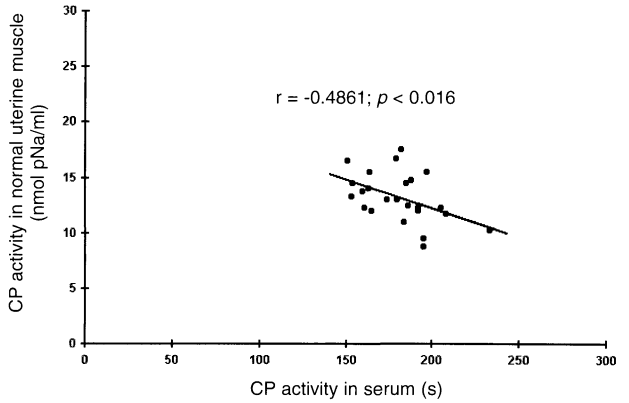


Figure 4. — The correlation between CP activity in the serum of women with uterine leiomyoma and CP activity in 10% homogenate of normal uterine muscle. Number of observations – 24. Correlation was assessed with Pearson's linear coefficient.

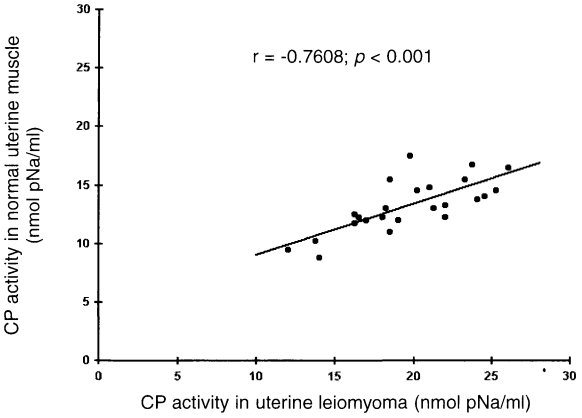


Figure 5. — The correlation between CP activity in 10% homogenate of uterine leiomyoma and normal uterine muscle. Number of observations – 24. Correlation was assessed with Pearson's linear coefficient.

normal women and women with uterine leiomyomas, and the value of approximately 120 s could be considered as a borderline value which distinguishes sera of women with uterine leiomyomas and women with cancer.

## Discussion

In the present study, our intention was to focus on uterine leiomyomas. In 24 carefully selected subjects blood samples were taken together with samples of uterine leiomyoma and normal uterine muscle to enable strictly paired observations. With similar care, subjects were selected to form the reference and genital cancer groups. Our results for healthy women are typical of those reported for normal subjects using the Gordon and Benson method [5]. There was a significant difference in CP activity between uterine leiomyoma and adjacent

normal muscle. Based on our results, normal uterine muscle near a myoma is perfused by blood displaying CP activity. Thus, the results obtained for normal uterine muscle are likely to be a little higher than for normal uterine muscle with no neighboring myoma.

To the best of our knowledge, this is the first study to demonstrate the presence of CP activity within benign neoplastic lesions, uterine myomas. Thus, not only malignancies are capable of synthesizing and expressing the CP protein, but also benign proliferative processes other than myomas may be associated with CP production. The correlations examined suggest that myomas are sites of CP synthesis and the source of CP activity in blood. Recently, an efficient transport of biologically active substances in the female genital tract by means other than arterio-venous circulation has been described [13]. The strong relationship of CP activity within the myoma and in uterine muscle indicates the possibility that CP may be transferred transcellularly or transmurally by proximity (i.e. from the tumor), or via the lymphatic system.

In oncotherapy, an aspect of paramount importance is tumor detection at the earliest possible stage, which validates an important role of cancer markers in the process of oncological diagnosis. Currently used markers lack sensitivity and specificity [14]. Our study strengthens the information on the possible role of CP as a cancer marker, as there was a good distinction between the presence of malignant and non-malignant tumors, as well as normal tissue, based on the determination of serum CP activity. In the present study, the coagulation time of 120 to 240 s by the Gordon and Benson method supported the diagnosis of uterine leiomyoma, and a value lower than 120 s - the suspicion of genital cancer. Now the sensitivity and specificity of CP as a marker of genital neoplasia should be assessed.

Finally, the observation of a decreased coagulation time in patients with uterine leiomyoma compared with young healthy women (Figure 1) indicates a need for the evaluation of the possible coexistence of uterine leiomyomas with hypercoagulability. This finding points to a pathophysiological role of CP in leiomyoma patients: the enzyme directly activates coagulation factor X, exerting its action without the participation of factor VII or factor VIII [1, 4, 15-17]. This is one of the means of activation of the clotting system, frequently observed in the course of cancer [18]. Our review of the literature revealed a paucity of data on the incidence of thromboembolic events in women with uterine leiomyomas. To date, it has been known that leiomyomas of varied, also extrapelvic, localization are capable of producing erythropoietin or an erythropoietin-like substance, thus inducing poliglobulia [19-21]. Myomas are commonplace disorders in middle-aged peri- and postmenopausal women, such as our patient population. The implementation of hormone replacement therapy is not indifferent to the clotting system [22, 23]. Women with uterine myomas may deserve special care regarding an evaluation of their clotting system prior to hormone replacement therapy.

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