

# Quantification of Pepsinogen C and Prostaglandin D Synthase in Breast Cyst Fluid and Their Potential Utility for Cyst Type Classification

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**Objective:** To quantify pepsinogen C (PEPC) and prostaglandin D synthase (PGDS) in breast cyst fluid and examine if these two parameters can be used for breast cyst type classification.

**Design and Methods:** We quantified PEPC and PGDS in 92 and 50 breast cyst fluids, respectively, using previously established immunofluorometric procedures. We then examined if the levels of PEPC or PGDS correlate with the type of cyst or with other clinicopathological variables.

**Results:** Quantitative analysis of the breast cyst fluids indicated that PEPC is present in all cyst fluids at various concentrations ranging from 3 to 31,000 ng/mL. PGDS positivity was confined to 30% of the cyst fluids. PEPC and PGDS levels were correlated with the breast cyst fluid cation ratio and were associated with the type of the cyst. Increased PEPC levels in breast cyst fluids were significantly correlated with a  $\geq 1.5$  K<sup>+</sup>/Na<sup>+</sup> ratio and were associated with the secretory/apocrine type of cyst (Type I) ( $p = 0.011$ ). Immunoreactive PGDS levels were highly correlated with a low cation ratio and were associated with the transudative/flattened type of breast cyst (Type II) ( $p = 0.0003$ ). A weak association was observed between PEPC levels in breast cyst fluid and menopausal status ( $p = 0.093$ ). No significant associations were observed for either PEPC or PGDS concentration in breast cyst fluid and number of cysts, recurrence of the disease, family history of breast cancer, number of children, abortion, and breast feeding.

**Conclusions:** Quantification of PEPC and PGDS in breast cyst fluid may be useful in the subclassification of cyst type in patients with gross cystic disease. Copyright © 1999 The Canadian Society of Clinical Chemists

**KEY WORDS:** pepsinogen C; prostaglandin D synthase; breast cyst fluid; electrolyte ratio; benign breast disease.

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## Introduction

The etiology of gross cystic disease of the human breast remains elusive (1). The biochemical composition of breast cyst fluid has been the subject of many studies in an attempt to elucidate the mechanisms involved in cyst formation and to define their possible role in carcinogenesis. The protein, hormone and electrolyte content of breast cyst fluid has been examined previously and discussed in the context of understanding the pathophysiology of breast disease (2–4).

Proteolytic enzymes expressed by human mammary epithelium are thought to be involved in the development of breast diseases, and could be of potential interest as biochemical markers of the hormonal imbalance underlying such pathologies (5). Prostate specific antigen (PSA) is a serine protease currently used for prostate cancer diagnosis and management (6). Although the involvement of PSA in the physiologic and pathogenic mechanisms of tissue development and tumorigenesis has not been elucidated yet (7), we have provided evidence that qualifies PSA as a prognostic indicator in breast cancer and supports its use for patient prognosis and selection of therapy (8–11). Recently, we have quantified the PSA immunoreactivity in breast cyst fluid and suggested that this marker has some value for cyst subclassification (12,13).

Pepsinogen C (PEPC), such as PSA, is a proteolytic enzyme that is upregulated by androgens, glucocorticoids, and progestins (14). Breast carcinomas, in addition to PSA, have the ability to synthesize and secrete PEPC (15). Clinical studies have indicated that PEPC is associated with favorable prognosis of breast cancer (16). It has been previously documented that women with gross cystic breast

TABLE 1  
Distribution of PEPC and PGDS Concentration in Breast Cyst Fluid

Analyte (ng/mL)	N <sup>a</sup>	Mean	SD <sup>b</sup>	25th percentile	Median	75th percentile	Range
PEPC	92	4,448	5,727	932	2,284	5,479	3–31,005
PGDS	50	62	296	0	0	3.4	0–2,078

<sup>a</sup>Number of samples/patients.

<sup>b</sup>Standard deviation.

disease accumulate PEPC in their cyst fluid (17). We have recently developed an immunofluorometric assay for PEPC in order to determine its distribution in various body fluids (18).

Prostaglandin D synthase (PGDS) is one of the major proteins of cerebrospinal fluid, and functions both as an enzyme and as a lipophilic transporter (19,20). A recent preliminary study was conducted to determine the distribution of PGDS in breast cyst fluids and breast tumor extracts with a newly developed immunofluorometric assay (21). Although we identified presence of PGDS in breast cyst fluid, no study has as yet been conducted to associate PGDS immunoreactivity with clinicopathologic features of cystic disease of the breast.

The objectives of this study were to quantitate PEPC and PGDS concentration in breast cyst fluid with our newly, highly specific and sensitive immunofluorometric assays, and associate these findings with various patient clinicopathologic variables. In this report, we provide evidence that supports the

use of PEPC and PGDS for breast cyst type subclassification.

## Materials and Methods

### CLINICAL SPECIMENS

Breast cyst fluids were collected by needle aspiration from 92 women with fibrocystic breast disease prior to initiation of therapy. Cyst fluids were centrifuged and the supernatants were collected and stored at  $-20^{\circ}\text{C}$  until analysis. Our clinical specimens were leftovers from a previous study examining if free (F-PSA) or total PSA levels in cyst fluids and matched sera associate with the type of breast cyst (13).

### PEPC AND PGDS DETERMINATIONS

Breast cyst fluids were analyzed with two highly sensitive and specific immunofluorometric assays

TABLE 2  
Associations Between PEPC in Breast Cyst Fluid and Other Variables

Variable	PEPC < 2,284 ng/mL <sup>a</sup> No. (%)	PEPC $\leq$ 2,284 ng/mL No. (%)	<i>p</i> Value
<i>K<sup>+</sup>/Na<sup>+</sup> Ratio</i>			
$\leq 1.5$ (Type I)	25 (40)	38 (60)	0.011
< 1.5 (Type II)	21 (68)	10 (32)	
<i>No. of children</i>			0.276
0	13 (59)	9 (41)	
1 or more	33 (46)	39 (54)	
<i>Abortion</i>			0.294
No	31 (46)	37 (54)	
Yes	15 (58)	11 (42)	
<i>Family history of breast cancer</i>			0.390
No	33 (46)	38 (54)	
Yes	12 (57)	9 (43)	
<i>Menopause</i>			0.093
No	38 (46)	45 (54)	
Yes	8 (73)	3 (27)	
<i>Breast feeding</i>			0.865
No	19 (50)	19 (50)	
Yes	27 (48)	29 (52)	
<i>No. of cysts</i>			0.452
1	15 (56)	12 (44)	
2 or more	31 (47)	35 (53)	
<i>Recurrence</i>			0.731
No	29 (51)	28 (49)	
Yes	17 (47)	19 (53)	

<sup>a</sup>Median value.

for PEPC and PGDS, described in detail elsewhere (18,21). Both assays utilize monoclonal capture and detection antibodies, and are used in a one-step procedure. All assays were performed in duplicate.

#### STATISTICAL ANALYSIS

For group comparisons, chi-square analysis was used throughout for continuous and noncontinuous variables. When the number of observations in one or more cells was 5 or less, the Fisher's exact test was used. Spearman correlation coefficients were determined for continuous variables that had a skewed distribution. For median comparisons, we used the Mann-Whitney rank sum test. A *p* value of less than 0.05 was considered statistically significant.

#### Results

The distribution of PEPC concentration in breast cyst fluid was determined for 92 women who were diagnosed with fibrocystic breast disease. We additionally examined the immunoreactivity of PGDS in 50 breast cyst fluids corresponding to 50 females from the same patient group. The remaining 42 samples were exhausted. PEPC was detectable in all the breast cyst fluids, unlike the situation with PGDS (Table 1). PGDS was detectable in 15 breast cyst fluids or approximately 30% of the patients.

PEPC values in breast cyst fluid were correlated with various patient clinicopathological variables (Table 2). Contingency tables constructed using the median PEPC concentration of the breast cyst fluids, indicated that the  $K^+/Na^+$  ratio is associated with the respective PEPC concentration (Chi-square = 6.5, *df* = 1, *p* = 0.011). Patients with Type I breast cysts or with an increased  $K^+/Na^+$  ratio tend to have higher PEPC concentration in their breast cyst fluids. The median PEPC concentrations in Type I and Type II cysts were 3,128 and 955 ng/mL, respectively (*p* = 0.002 by Mann-Whitney rank sum test). The data are graphically shown in Figure 1. Breast cyst PEPC concentration was not associated with the number of children, abortion history, family history of breast cancer, breast feeding, number of cysts or recurrence, but there was a trend for premenopausal women to have more PEPC in the cyst fluid.

We have also examined the association between PGDS positivity in the breast cyst fluid and various clinicopathological variables using chi-square analysis (Table 3). The association of PGDS positivity with  $K^+/Na^+$  ratio in the breast cyst fluids from patients with fibrocystic breast disease was statistically highly significant (chi-square = 13.2, *df* = 1, *p* = 0.0003). The majority of patients with Type II cysts have immunodetectable PGDS concentration in their breast cyst fluid while undetectable levels of PGDS were observed in the vast majority of patients with low breast cyst  $K^+/Na^+$  ratio or with Type I cysts. The median PGDS concentrations in Type I

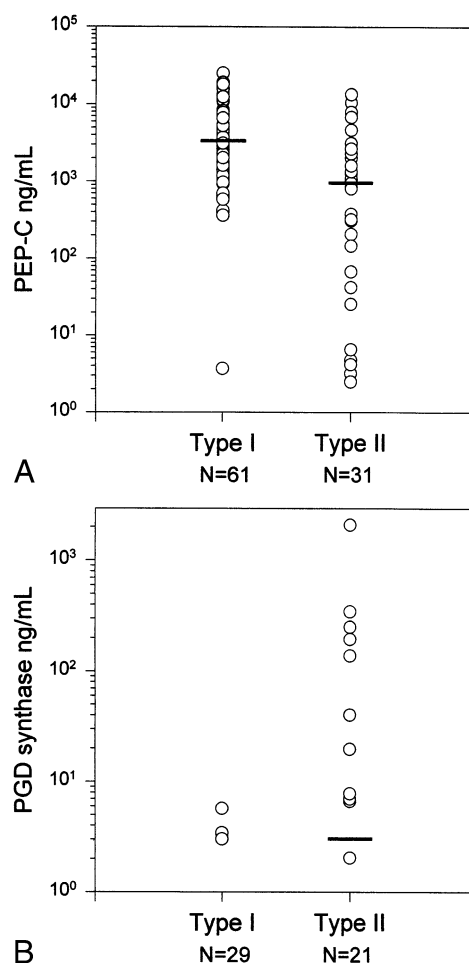


Figure 1 — Distribution of PEPC (A) and PGDS (B) concentration in the breast cyst fluid of patients with fibrocystic breast disease. Concentration values are plotted on a logarithmic scale for both Type I and Type II breast cysts. The medians are represented by horizontal bars. Concentration and median values of zero are not plotted. For Type I cysts, 26 samples and zero PGDS concentration. For Type II cysts, 9 samples and zero PGDS concentration.

and Type II cysts were 0 and 2.0 ng/mL, respectively (*p* = 0.006 by Mann-Whitney rank sum test). The data are graphically shown in Figure 1. Breast cyst PGDS concentration was not associated with the number of cysts, recurrence of fibrocystic breast disease, family history of breast cancer, number of children, abortion, history, breast feeding, and menopausal status.

Spearman correlation analysis was performed to examine if the PEPC concentration is associated with the PGDS concentration in breast cyst fluid. For 41 paired specimens, our results indicate that there is no statistical association between PEPC and PGDS levels in breast cyst fluid (*r* = -0.238, *p* = 0.134). The same analysis was also used to determine the presence of an association between patient age, day of menstrual cycle for sample collection, and cyst size and PEPC and PGDS. A significant negative correlation was observed between PEPC

TABLE 3  
Associations Between PGDS in Breast Cyst Fluid and Other Variables

Variable	PGDS = 0 ng/mL No. (%)	PGDS > 1 ng/mL No. (%)	<i>p</i> Value
<i>K<sup>+</sup>/Na<sup>+</sup> Ratio</i>			
≤1.5 (Type I)	26 (90)	3 (10)	0.0003
<1.5 (Type II)	9 (43)	12 (57)	
<i>No. of children</i>			
0	10 (71)	4 (29)	1.0
1 or more	26 (70)	11 (30)	
<i>Abortion</i>			
No	29 (76)	9 (24)	0.164
Yes	7 (54)	6 (46)	
<i>Family history of breast cancer</i>			
No	22 (65)	12 (35)	0.174
Yes	13 (87)	2 (13)	
<i>Menopause</i>			
No	32 (74)	11 (26)	0.213
Yes	4 (50)	4 (50)	
<i>Breast feeding</i>			
No	17 (74)	6 (26)	0.637
Yes	19 (68)	9 (32)	
<i>No. of cysts</i>			
1	12 (80)	3 (20)	0.507
2 or more	24 (69)	11 (31)	
<i>Recurrence</i>			
No	23 (74)	8 (26)	0.659
Yes	13 (68)	6 (32)	

and day of menstrual cycle for sample collection ( $n = 83$ ,  $r = -0.247$ ,  $p = 0.024$ ). No other significant association was observed with the other parameters ( $p > 0.213$ ).

In order to examine if an association is present between PSA levels and PEPC or PGDS levels, we have performed a number of Spearman correlations and chi-square tests as follows:

1. Breast cyst fluid PEPC vs breast cyst fluid total PSA and serum total PSA. A significant association was found between PEPC and total PSA concentration in breast cyst fluid ( $n = 94$ , Spearman correlation coefficient  $r = 0.265$ ,  $p = 0.010$ ; chi-square = 8.4,  $df = 1$ ,  $p = 0.004$ ). No significance was observed when correlation analysis was performed with the PSA subfraction ratio in the cyst fluids (F-PSA/PSA-ACT), ( $n = 57$ , Spearman correlation  $r = 0.17$ ,  $p = 0.211$ ; chi-square = 2.5,  $p = 0.117$ ). Correlations between breast cyst fluid PEPC vs serum total PSA and free to bound PSA ratio did not reach statistical significance ( $p > 0.147$  for all tests).
2. Breast cyst fluid PGDS versus breast cyst fluid PSA and serum PSA. No association was found when PGDS and PSA levels were correlated in the breast cyst fluids ( $n = 41$ , Spearman correlation  $r = 0.05$ ,  $p = 0.753$ ; chi-square = 0.021,  $df = 1$ ,  $p = 0.884$ ). Although the association between PGDS and free to bound PSA ratio was statistically significant in the breast cyst fluids ( $n = 17$ , Spearman correlation  $r = -0.482$ ,  $p = 0.05$ ), no significance was found when a chi-

square test was used ( $n = 25$ , chi-square = 1.7,  $p = 0.189$ ). Correlations between breast cyst fluid PGDS concentration versus serum PSA, and free to bound PSA ratio were not significant when both Spearman and chi-square tests were used ( $p > 0.104$ ).

## Discussion

Recently, it became apparent that a number of molecules that are present at high concentrations in seminal plasma can also be found in breast secretions and breast tissue extracts. Among these molecules are PSA, PEPC, and PGDS. No report has as yet elucidated the pathologic functions, if any, for PEPC and PGDS. In this report, we describe for the first time the quantitative distribution of PEPC and PGDS in breast cyst fluids of patients with gross cystic disease. In previous studies conducted with various body fluids, we observed that PEPC distribution is highest in seminal plasma followed by the immunoreactive levels in breast cyst fluid (18). PEPC concentration in serum was found to be lower to levels found in breast cyst fluid. Our quantitative results indicate that PEPC is present in all of the breast cyst fluids, even though its variable distribution seems to cover a wide range of immunoreactive values from 3 to 31,000 ng/mL. However, this is not the case with the PGDS distribution in the breast cyst fluids examined, where we observed a 30% positivity. Recent studies indicate that PGDS is



present in all normal human sera with a range of approximately 300–600 ng/mL (21).

Cyst fluids with a  $K^+/Na^+$  ratio  $\geq 1.5$  are classified as Type I or secretory/apocrine cysts while those with a  $K^+/Na^+$  ratio  $< 1.5$  are classified as Type II or transudative/flattened cysts (22,23). Quantitative analysis of breast cyst fluids for PEPC and PGDS indicated that these molecules may be of value in discriminating the type of breast cyst. Our results indicated a positive relationship between Type I cysts and PEPC, and between Type II cysts and PGDS. Taken into account that PEPC levels are generally greater in the cyst fluid than those found in the serum, it seems that PEPC is produced by the apocrine cells and secreted into the Type I cysts. This view is also in agreement with immunohistochemical results for PEPC by others (15,17). The presenting symptom of a rapidly developing cyst is often localized pain, which is thought to result either from the distention of the surrounding tissue or possibly from the escape of cyst fluid into the surrounding breast tissue causing a sterile inflammatory reaction (1,2). We believe that the PGDS positivity observed with Type II cysts could be attributable to a possible local inflammatory reaction that resulted from a rapid cyst enlargement or cyst fluid escape, and that the PGDS presence could have originated ectopically (e.g., from the pool of circulating PGDS).

PEPC and PSA are known to be upregulated by androgens and progestins (6,10,14). To examine the hormonal influence on PEPC concentration in breast cyst fluids, statistical analysis was performed with various clinical variables. Our results indicate that cyst fluid PEPC concentration is weakly associated with the menopausal status of the patient. Also, high PEPC levels in breast cyst fluid were found in women who were aspirated on the first half of their menstrual cycle. Recently, it was demonstrated that the production and intracystic accumulation of PSA, and in particular the free form of PSA, was associated with the apocrine/Type I cyst (13,24). In this report, we also associated increased levels of PEPC in cyst fluid with the apocrine/Type I cyst. Moreover, we found a positive correlation between PSA and PEPC levels in breast cyst fluids. We suggest that PEPC may be upregulated similarly to PSA in benign breast tissue.

To summarize, we provide quantitative data for PEPC and PGDS presence in cyst fluids from women with gross cystic disease. Increased PEPC concentration was found in cyst fluid belonging to the secretory/apocrine type of breast cyst (Type I cyst). PGDS presence in breast cyst fluid was significantly associated to the transudative/flattened breast cyst (Type II cyst). PEPC and PGDS maybe be useful for breast cyst subclassification when quantified in breast cyst fluid. The biochemical and pathologic significance of cyst fluid PEPC and PGDS in breast disease is currently unknown.

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