Journal of Clinical Laboratory Analysis 13:75–81 (1999)

Prostate Specific Antigen Molecular Forms in Breast Cyst Fluid and Serum of Women With Fibrocystic Breast Disease

Gudrun H. Borchert,[1] He Yu,[2] George Tomlinson,[3] Maurizia Giai,[4] Riccardo Roagna,[4] Riccardo Ponzone,[4] Luca Sgro,[4] and Eleftherios P. Diamandis[1,5]*

[1]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada*
[2]*Department of Medicine, Section of Hematology/Oncology, LSUMC School of Medicine in Shreveport, Shreveport, Louisiana*
[3]*Department of Preventive Medicine and Biostatistics, University of Toronto, Toronto, Ontario, Canada*
[4]*Department of Gynecologic Oncology, Institute of Obstetrics and Gynecology, University of Turin, Italy*
[5]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada*

We have analyzed matched serum and breast cyst fluid samples for total PSA from 148 patients with fibrocystic breast disease. We have also determined the molecular forms of PSA (free PSA and PSA bound to α_1-antichymotrypsin) in 78 breast cyst fluid samples. We found that total PSA can be detected in all cyst fluids and in about 75% of female sera. The median total PSA concentration in breast cyst fluid (bcf) is about 30 times higher than the median in the corresponding sera. Breast cyst fluid and serum PSA are not correlated with each other. Total serum PSA is inversely associated with patient age but the inverse association between bcf PSA and age is weak. Lower total PSA in bcf was seen in women who breast feed, and higher bcf PSA is associated with multiple cysts. Type I cysts (with a high K^+/Na^+ ratio) tend to have higher total PSA than Type II cysts. All but three of the fractionated cyst fluids (75/78; 96%) had free PSA as the predominant molecular form. The most consistent finding of our study was the positive association between the cyst fluid K^+/Na^+ ratio and the free to bound PSA ratio. This association was confirmed by Spearman correlation as well as by Wilcoxon and chi-square analysis. Secretory/apocrine cysts (Type I) tend to have more total PSA and proportionally more free PSA than transudative/flattened cysts (Type II). J. Clin. Lab. Anal. 13:75–81, 1999.

Key words: prostate specific antigen; breast cyst fluid; fibrocystic breast diseases; molecular forms of PSA; free/bound PSA ratio; electrolyte ratio

INTRODUCTION

It is now widely accepted that prostate specific antigen (PSA) is present in many nonprostatic tissues and especially in the female breast (1–5), breast cancer cell lines after hormone stimulation (6), female serum (7–9), milk of lactating women (10), breast cyst fluid (11–16) and amniotic fluid (17).

We have previously reported that women whose breast tumors are positive for PSA have better prognoses (4). Women with Type-I breast cysts (with high K^+/Na^+ ratio) may have a higher risk of subsequent development of breast cancer (14,18,19). In recent studies, we reported that there are significant differences in the molecular forms of PSA in serum of women with or without breast diseases. We found that the predominant molecular form of PSA in serum of about 50% of benign breast disease and breast cancer patients is free PSA (33kDa) while this PSA molecular form never predominated in sera from blood donors (7,20).

PSA is present in breast cyst fluid (11–16) and its concentration varies widely from undetectable to > 10μg/L. Relatively few studies addressed the issue of PSA molecular forms in this biological fluid (12,15).

The possibility of using the molecular forms of PSA in breast cyst fluid and serum of women with benign breast diseases for clinical applications encouraged us to undertake this study. The type of the cyst (Type I vs. Type II) and the PSA molecular forms were correlated with various clinicopathological features.

*Correspondence to: Eleftherios P. Diamandis, MD, Ph.D, FRCPC., Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, M5G 1X5 Canada. E-mail: ediamandis@mtsinai.on.ca

Received 21 July 1998; Accepted 25 September 1998

MATERIALS AND METHODS

Serum Samples

We examined a total of 148 sera and 148 matched breast cyst fluids from women with fibrocystic breast disease. Serum samples from these patients were collected before initiation of therapy. None of the patients was on hormone replacement therapy. Cyst fluids were collected by needle aspiration.

Seventeen sera and 78 breast cyst fluids containing total PSA > 0.015μg/L were subjected to HPLC fractionation in order to identify the molecular forms of PSA. All samples were stored at –70°C until use.

High Performance Liquid Chromatography (HPLC)

HPLC was performed with a Hewlett Packard 1100 System. The gel filtration column used was a TSK-GEL G3000SW 600× 7.5mm in combination with a guard column (Tosoh-Haas, Montgomeryville, PA). The flow rate was 0.5mL/min and the run was isocratic in all cases. The mobile phase was a 0.1 mol/L sodium sulfate, 0.1 mol/L sodium phosphate, pH 6.8. The molecular weight standard solution was from Bio-Rad and was run daily to ensure column performance. The fraction size collected was 0.5mL. Before injection(50–300μL, usually 100μL), the samples were centrifuged at 15,000*g* for 15 min. Carryover from previous injections was less than 5%. Samples with the lowest PSA concentration were run first and after 3–6 runs the column and the injector were thoroughly cleaned to avoid carryover in subsequent runs.

PSA Immunoassay

PSA was measured with a highly sensitive and specific immunofluorometric procedure described elsewhere (3). The detection limit is 0.001μg/L. All assays were run in duplicate.

All values for free and bound PSA were adjusted to the volume most frequently applied (100μL). A ratio of free to bound PSA was calculated for all separated samples by dividing the areas of the peaks representing free PSA and PSA bound to α_1-antichymotrypsin (PSA-ACT). A ratio of > 1 indicates that the free PSA is the predominant molecular form while a ratio < 1 indicates that ACT-PSA is the predominant molecular form.

RESULTS

In Table 1 we present the number of samples, mean ± standard deviation, median and range of all the continuous variables in our database. In Table 2 we present the frequency distribution of the categorical variables in our database. Consistent with data in the literature, 61% of the cysts are Type I, with a high K^+/Na^+ ratio (14). We have measured total PSA in the 148 serum samples as well as the 148 matched breast cyst fluids of our patients. We have also determined the free PSA and α_1-antichymotrypsin-bound PSA concentration in 78 breast cyst fluids and 17 serum samples from specimens with total PSA ≥ 0.015μg/L. When total PSA is < 0.015μg/L, HPLC fractionation is not feasible since most of the individual fractions will have PSA lower than the detection limit of our method due to dilution. The total PSA distribution in serum and breast cyst fluid samples is presented in Table 3. The median total PSA concentration in breast cyst fluid is approximately 30 times higher than in the serum of these women. In Figure 1 we present examples of sera and breast cyst fluids which had either free PSA or PSA-ACT as the predominant molecular forms.

We have further examined if there is any correlation between total serum PSA, total breast cyst fluid PSA, serum F/B PSA ratio, and cyst fluid F/B PSA ratio with any other variable in our database. Since the data are not normally distributed, Spearman correlation was used throughout. The significant correlations identified ($P \leq 0.05$) are summarized as follows:

1. We found a negative correlation (Spearman r = –0.26) between age and serum total PSA ($P < 0.01$).
2. We found a positive correlation (r = 0.26) between K^+/Na^+ ratio and cyst fluid F/B PSA ratio ($P = 0.02$) and a

TABLE 1. Statistical Description of Continuous Variables in Our Database

Variable	Number of patients	Mean (SD)[a]	Median	Range
Age (years)	148	45 (7)	45	23–76
Day of cycle[b]	127	16 (10)	16	1–81
Serum total PSA (μg/L)	148	0.015 (0.052)	0.003	0–0.48
Serum F/B PSA ratio[c]	17	2.8 (3.3)	1.1	0–9.5
Cyst fluid total PSA (μg/L)	148	0.65 (1.40)	0.095	0–8.3
Cyst fluid F/B PSA ratio	78	34 (39)	14	0.1–100
K^+/Na^+ ratio	148	2.2 (2.2)	2.4	0.02–18
Cyst size (mm)	146	26 (9)	25	10–50

[a]SD, standard deviation.
[b]Refers to the day of cycle the sample was collected. The remaining 21 patients were post-menopausal.
[c]Ratio of free PSA(F) and PSA bound to a_1-antichymotrypsin (B).

TABLE 2. Frequency Distributions of Categorical Variables in Our Database[a]

Variables	Number of patients	Percentage (%)
Number of children		
0	34	23
1	56	38
2	47	32
3	7	5
4	3	2
5	1	1
Breast feed		
No	62	42
Yes	86	58
Abortion		
0	104	70
1	31	21
2	9	6
3	4	3
Menopause		
No	127	86
Yes	21	14
Family history of breast cancer		
No	107	74
Yes	37	26
Number of cysts		
1	52	36
2	38	26
3	24	16
4	15	10
5	4	3
6	6	4
7	4	3
9	3	2
Recurrence		
No	100	68
Yes	46	32
K^+/Na^+ ratio		
≥ 1.5 (Type-I cyst)	90	61
< 1.5 (Type-II cyst)	58	39

[a]Not all 148 patients have all the information listed.

trend (r = 0.15) between K^+/Na^+ ratio and total PSA in cyst fluid. This suggests that there is more total PSA in cyst fluid from Type I cysts and proportionally more free PSA in Type I cysts in comparison to Type II cysts.

3. We found a correlation (r = 0.18) between cyst size and serum total PSA ($P = 0.03$) but not cyst fluid total PSA ($P = 0.18$).
4. We found a weak correlation (r = 0.16) between number of cysts and cyst fluid total PSA ($P = 0.05$).

TABLE 3. Distribution of Total PSA in Serum and Breast Cyst Fluid of the 148 Patients

	PSA, μg/L; Percentiles				
	0	25	50	75	100
Breast cyst fluid	0.011	0.031	0.095	0.45	8.3
Serum	0	0.001	0.003	0.007	0.48

Although there is a trend for a correlation between serum total PSA and breast cyst fluid total PSA (r = 0.14), this did not reach statistical significance ($P = 0.09$). We have further compared the differences between various variables and type of cyst, categorized as Type I and Type II, according to the K^+/Na^+ ratio, as suggested by others (14,16). We found no association between type of cyst and age, number of children, day of cycle for sample collection, cyst size, and number of cysts by ANOVA analysis. By the Wilcoxon test, we also did not find any difference between cyst type and serum total PSA or serum F/B PSA ratio. However, there was a trend for higher total cyst fluid PSA in Type-I cysts and there was a statistically significant association between Type-I cysts and higher cyst fluid F/B PSA ratio ($P = 0.018$). The median total cyst fluid PSA in Type-I cysts was 0.13μg/L and in Type-II cysts was 0.062μg/L (Wilcoxon $P = 0.10$). The median cyst fluid F/B PSA ratio in Type-I cysts was 21 and in Type-II cysts was 6.7 (Wilcoxon $P = 0.018$). This suggests that the proportion of free PSA is higher in Type-I, compared to Type-II cysts.

We also performed chi-square analysis of possible associations between type of cyst and clinicopathological variables. There is no association between type of cyst and number of children, breast feeding, abortion history, menopause, family history of breast cancer, and number of cysts. We only found that Type-I cysts are more prone to recurrence than Type-II cysts ($P = 0.005$).

In Table 4 we present the associations between total serum PSA and various clinicopathological variables using chi-square analysis. We identified only two significant associations: (a) premenopausal women have higher serum total PSA than postmenopausal women ($P = 0.03$), in accordance with the already presented data of the correlation analysis, showing a negative correlation between serum total PSA and age. (b) We here further show that women who breast feed have lower serum total PSA than women who do not breast feed ($P = 0.007$).

We also examined the associations between cyst fluid total PSA and other clinicopathological variables (Table 5) and found the following statistically significant associations:

1. Abortion is associated with lower cyst fluid total PSA ($P = 0.01$).
2. Multiple cysts are associated with higher cyst fluid PSA ($P = 0.02$) in accordance with the already presented data of correlation analysis.
3. Postmenopausal women tend to have lower cyst fluid total PSA than premenopausal women ($P = 0.01$).
4. Breast feeding is associated with lower cyst fluid total PSA, similarly to the serum PSA data ($P = 0.05$).

Although Type-I cysts appear to have more PSA than Type-II cysts, the difference did not reach statistical significance ($P = 0.18$).

In Table 6 we have examined the association between cyst

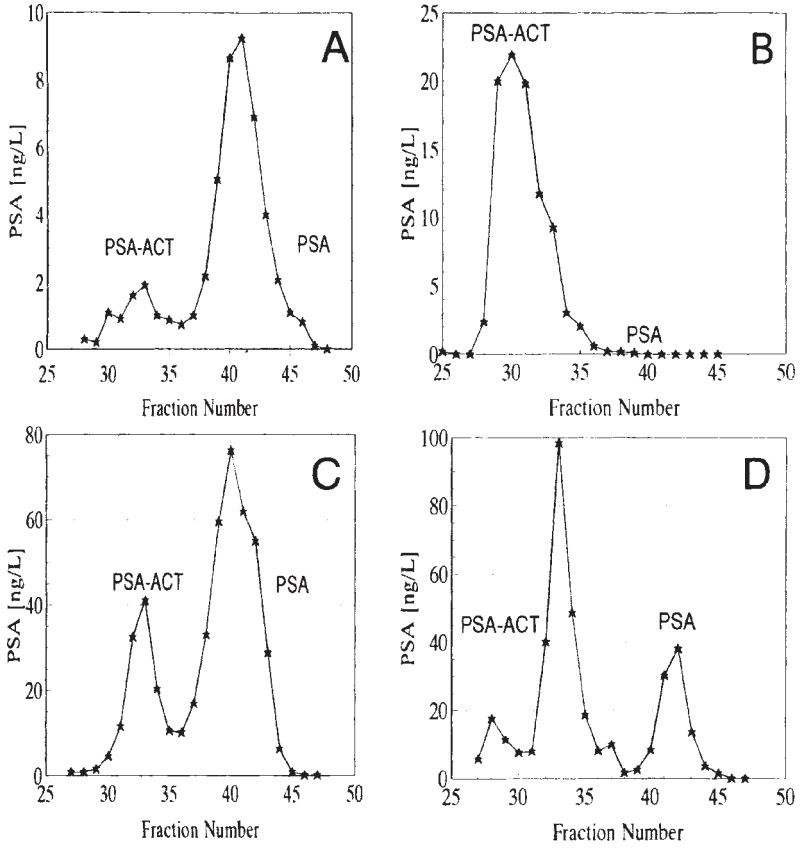

Fig. 1. Separation of free PSA (PSA) and PSA bound to α_1-antichymotrypsin (PSA-ACT) by gel filtration chromatography. Note that the units for PSA is in ng/L. **A** and **B,** female serum samples. **C** and **D,** breast cyst fluid samples. These samples were selected to contain either free or bound PSA as the predominant form and they are not representative of the frequencies of such samples in the patient population studied.

fluid F/B PSA ratio and clinicopathological variables. The only parameter that was associated was the type of cyst. Type-I cysts have proportionally more free PSA ($P = 0.02$), in accordance with the data of Wilcoxon and Spearman analysis already described.

The effect of age on serum total PSA was also confirmed by *t*-test comparison of mean ages of subjects with total PSA < 0.003µg/L (mean age 46.6 years) and > 0.003µg/L (mean age 43.6 years) ($P = 0.005$). The same analysis of cyst fluid total PSA and cyst fluid F/B PSA ratio did not reveal a statistically significant difference.

DISCUSSION

There is now little doubt that prostate specific antigen (PSA) is produced by breast epithelial cells. PSA has been found in serum of women as well as in normal, hyperplastic and cancerous breast tissue, in milk of lactating women, in nipple aspirate fluid, and in breast cyst fluid (9). Our tissue culture and in vivo studies confirm that this molecule is regulated in the breast by steroid hormones (5,6).

A fundamental question that has not yet been answered relates to the physiological function of this molecule in breast tissue. Observations suggest that PSA is a favorable prognostic marker in breast cancer (4) and that its concentration in nipple aspirate fluid is inversely associated with breast cancer risk (21). Recently, it has been suggested that the molecular forms of PSA in female serum, but not total PSA, may have some value for diagnosing breast diseases since it was found that the predominant form of PSA in serum of normal women is always PSA-ACT, while many breast cancer patients and patients with benign breast diseases, have free PSA as the major molecular form (7,20).

A number of independent studies have examined the presence of PSA and its molecular forms in breast cyst fluid (11–16). In our preliminary study, we confirmed presence of PSA in the majority of the breast cyst fluids and demonstrated that in the only sample examined for subfractions, both free PSA and ACT-bound PSA could be detected (11). No clinicopathological data were available for these women. Fillela et al. measured total PSA and free PSA in breast cyst fluids and found that Type-I cysts (with a high K^+/Na^+ ratio) have more PSA than Type-II cysts (with a low K^+/Na^+ ratio) (15). They

TABLE 4. Associations Between Serum Total PSA and Other Variables

Variable	PSA < 0.003 μg/L[a] No. (%)	PSA ≥ 0.003 μg/L No. (%)	*P* value
K^+/Na^+ ratio			
≥ 1.5 (Type I)	43 (48)	47 (52)	0.445
< 1.5 (Type II)	24 (41)	34 (59)	
No. of children			
0	11 (32)	23 (68)	
1 or more	56 (49)	58 (51)	0.085
Abortion			
No	49 (47)	55 (53)	
Yes	18 (41)	26 (59)	0.488
No. of cysts			
1	22 (42)	30 (58)	
2 or more	44 (47)	50 (53)	0.601
Recurrence			
No	46 (46)	54 (54)	
Yes	20 (43)	26 (57)	0.776
Family history of breast cancer			
No	46 (43)	61 (57)	
Yes	28 (49)	26 (51)	0.550
Menopause			
No	53 (42)	74 (58)	
Yes	14 (67)	7 (33)	0.033
Breast feeding			
No	20 (32)	42 (68)	
Yes	47 (55)	39 (45)	0.007

[a]Median value.

TABLE 5. Associations Between Cyst Fluid Total PSA and Other Variables

Variable	PSA < 0.095 μg/L[a] No. (%)	PSA ≥ 0.095 μg/L No. (%)	*P* value
K^+/Na^+ ratio			
≥ 1.5 (Type I)	41 (46)	49 (54)	0.178
< 1.5 (Type II)	33 (57)	25 (43)	
No. of children			
0	16 (47)	18 (53)	
1 or more	58 (51)	56 (49)	0.696
Abortion			
No	45 (43)	59 (57)	
Yes	29 (66)	15 (34)	0.012
No. of cysts			
1	33 (63)	19 (37)	
2 or more	40 (43)	54 (57)	0.016
Recurrence			
No	49 (49)	51 (51)	
Yes	24 (52)	22 (48)	0.722
Family history of breast cancer			
No	48 (45)	59 (55)	
Yes	23 (62)	14 (38)	0.070
Menopause			
No	58 (46)	69 (54)	
Yes	16 (76)	5 (24)	0.010
Breast feeding			
No	25 (40)	37 (60)	
Yes	49 (57)	37 (43)	0.046

[a]Median value.

TABLE 6. Associations Between Cyst Fluid F/B PSA Ratio and Other Variables

Variable	Cyst fluid F/B ratio < 14[a] No. (%)	Cyst fluid F/B ratio ≥ 14 No. (%)	*P* value
K^+/Na^+ ratio			
≥ 1.5 (Type I)	23 (42)	32 (58)	0.025
< 1.5 (Type II)	16 (70)	7 (30)	
No. of children			
0	10 (53)	9 (47)	
1 or more	29 (49)	30 (51)	0.792
Abortion			
No	26 (45)	32 (55)	
Yes	13 (65)	7 (35)	0.120
No. of cysts			
1	11 (48)	12 (52)	
2 or more	28 (52)	26 (48)	0.746
Recurrence			
No	30 (57)	23 (43)	
Yes	9 (38)	15 (62)	0.120
Family history of breast cancer			
No	30 (51)	29 (49)	
Yes	8 (44)	10 (56)	0.634
Menopause			
No	34 (48)	37 (52)	
Yes	5 (71)	2 (29)	0.235
Breast feeding			
No	21 (57)	16 (43)	
Yes	18 (44)	23 (56)	0.257

[a]Median value.

found that breast cysts with high dehydroepiandrosterone sulfate in the fluid were more likely to have higher PSA. These authors found, for a limited number of cyst fluid samples for which the free PSA was quantified, that free PSA was a minor fraction of total PSA, PSA-ACT being predominant. The differences between our data and those of Filella et al. may be due to methodological aspects since we quantified the subfractions after HPLC separation, while the previous authors used a direct immunoassay which was designed for measurement of free PSA in serum. Mannello et al. studied total PSA presence in 64 cyst fluids and found detectable PSA in most samples. These authors also found higher total PSA in Type-I cysts in comparison to Type-II cysts (14). Lai et al. studied 80 breast cyst fluids and found measurable total PSA in most of them. These authors did not observe a significant association between total PSA and cyst type, although there was a weak trend for higher total PSA in Type-I cysts (16).

The present work differs from the previously published reports in several respects. We have analyzed matched serum and breast cyst fluid samples for total PSA from 148 patients and we have determined the free and bound fractions of PSA in 78 breast cyst fluid samples. We had extensive clinicopathological data which allowed us to perform statistical analyses not previously reported. Additionally, we used improved methods for total PSA (our assay being at least 10 times more sensitive than the assays used in previous stud-

ies) and free PSA (our assay based on HPLC fractionation, allowing simultaneous assessment of free PSA as well as PSA bound to α_1-antichymotrypsin).

Our major conclusions are summarized below and are compared with those previously reported by others.

1. With the ultrasensitive PSA assay used, it is possible to detect total PSA in all breast cyst fluids tested (Table 3). In addition, we detected total PSA in about 75% of female sera from benign breast disease patients. The median total PSA in breast cyst fluids (0.095μg/L) is about 30 times higher than the median in the corresponding sera (0.003μg/L). The latter median is in close agreement with the median reported previously in another group of patients with a similar disease (20). Filella et al. could not evaluate accurately serum PSA in women because their method does not detect PSA below 0.004μg/L (12,13,15).
2. In accordance with our previous data (20), we have shown that serum total PSA in women is inversely correlated with age. This finding was verified by correlation analysis as well as by association analysis with the chi-square and *t*-test (Table 4). We speculate that serum total PSA is influenced by the activity of the ovaries through steroid hormones. Postmenopausal women have lower serum total PSA than premenopausal women. We have further seen an effect of breast feeding on total serum PSA, with women who breast feed having lower total serum PSA (Table 4). This effect was not seen in patients who have breast cancer (20). A positive association between total serum PSA and cyst size was also seen in this study.
3. The association between cyst fluid total PSA and patient age is not as clear. Although there is a trend for younger patients to have higher cyst fluid total PSA, the association was not significant with Spearman correlation. This association became significant in chi-square analysis comparing premenopausal and postmenopausal women, the former having generally more total PSA in their cyst fluids (Table 5). Lower total PSA in breast cyst fluid was also seen in women who breast feed (Table 5) and higher total PSA was associated with multiple cysts (Table 5**)**.
4. We could not find a statistically significant association between serum total PSA and breast cyst fluid total PSA in these women.
5. Three previous studies examined whether total PSA concentration is associated with cyst type. Cyst fluids with K^+/Na^+ ratio > 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 (14) although other classifications also exist (12,16). Type-I cysts appear to be associated with higher breast cancer risk although the issue is still controversial. Mannello et al. and Filella et al. found that Type-I cysts have higher cyst fluid PSA while Lai et al. found only a weak trend. In this study, we found with correlation analysis that there is a weak, positive correlation between K^+/Na^+ ratio and cyst fluid total PSA (r = 0.15, P = 0.07) and a trend towards higher total PSA concentration in cyst fluid of Type-I cysts. The median cyst fluid total PSA in Type-I and Type-II cysts were 0.13μg/L and 0.062μg/L, respectively (P = 0.10) (see also Table 5 for chi-square analysis). We can thus conclude, in agreement with the previous studies, that cyst fluid total PSA tends to be higher in Type-I cysts but this association is weak.
6. The most consistent finding identified in this work is the association between type of cyst and the ratio of free/bound PSA in the cyst fluid. We found the free PSA fraction in breast cyst fluid to be the predominant PSA form in the vast majority of cyst fluids (in 75/78 samples). Free PSA was also predominant in the serum of 9/17 HPLC-fractionated samples, in general agreement with our previous data (20). The free/bound PSA ratio in cyst fluid was directly correlated with the K^+/Na^+ ratio of the cyst fluid (r = 0.26, P = 0.02). This correlation, which indicates that Type-I cysts have proportionally more free PSA, was further confirmed by Wilcoxon analysis (P = 0.018) and in Table 6 by chi-square analysis (P = 0.025). We thus propose that the free/bound PSA ratio in breast cyst fluid may be another biochemical marker of breast cyst type, in addition to the K^+/Na^+ ratio.

In Figure 1 we present examples of HPLC separation of various samples, indicating the excellent separation of the two PSA molecular forms with this method. These samples were selected to indicate both molecular forms of PSA in serum or cyst fluid and they are not representative of the frequency of such samples in our database.

The reason for a correlation between the type of the cyst and the proportion of free PSA in the cyst fluid is unknown. We previously found that the proportion of free PSA is increased in serum of patients with benign and malignant breast diseases in comparison to normal controls (7, 20).

In conclusion, we studied PSA presence in breast cyst fluid and found that the free PSA fraction is significantly increased in cysts which belong to the secretory/apocrine type (Type-I cysts). Free PSA fraction may have some value for breast cyst subclassification when quantified in cyst fluid and for breast disease diagnosis when measured in serum. The mechanism by which this fraction is increased in these pathologies is currently unknown.

REFERENCES

1. Yu H, Diamandis EP, Sutherland DJA. Immunoreactive prostate-specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. Clin Biochem 1994;27:75–79.

2. Levesque M, Yu H, D'Costa M, Diamandis EP. Prostate-specific antigen expression by various tumors. J Clin Lab Anal 1995;9:123–128.
3. Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis E. Ultrasensitive detection of prostate-specific antigen by a time-resolved immunofluorometric assay and the Immulite® immunochemiluminescent third-generation assay: potential applications in prostate and breast cancers. Clin Chem 1996;42:675–684.
4. Yu H, Giai M, Diamandis EP, et al. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 1995;55:2104–2110.
5. Yu H, Diamandis EP, Monne M, Croce CM. Oral contraceptive-induced expression of prostate-specific antigen in female breast. J Biol Chem 1995;270**:**6615–6618.
6. Zarghami N, Grass L, Diamandis EP. Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. Br J Cancer 1997;75:579–588.
7. Melegos DN, Diamandis EP. Diagnostic value of molecular forms of prostate-specific antigen for female breast cancer. Clin Biochem 1996;29:193–200.
8. Giai M, Roagna R, Ponzone R, Katsaros D, Levesque MA, Diamandis EP. Prostate-specific antigen in serum of women with breast cancer. Br J Cancer 1995;72:728–731.
9. Diamandis EP, Yu H. New biological functions of prostate-specific antigen? J Clin Endocrinol Metab 1995;80:1515–1517.
10. Yu H, Diamandis EP. Prostate-specific antigen in milk of lactating women. Clin Chem 1995;4:54–58.
11. Diamandis EP, Yu H, Lopez-Otin C. Prostate-specific antigen—a new constituent of breast cyst fluid. Breast Cancer Res Treat 1996;38:259–264.
12. Filella X, Molina R, Alcover J, Carretero P, Ballesta AM. Detection of nonprostatic PSA in serum and non-serum samples from women. Int J Cancer 1996;68:424–427.
13. Filella X, Molina R, Alcover J, Casals E, Carmona F, Ballesta AM. Prostate-specific antigen in non-serum samples from women. Int J Biol Markers 1995;10:238–239.
14. Mannello F, Bocchiotti GD, Bianchi G, Marcheggiani F, Gazzanelli G. Quantification of prostate-specific antigen immunoreactivity in human breast cyst fluids. Breast Cancer Res Treat 1996;38:247–252.
15. Filella X, Molina R, Alcover J, et al. Prostate-specific antigen detection by ultrasensitive assay in samples from women. Prostate 1996;29:311–316.
16. Lai LC, Erbas H, Lennard TWJ, Peaston RT. Prostate-specific antigen in breast cyst fluid: Possible role of prostate-specific antigen in hormone-dependent breast cancer. Int J Cancer 1996;66:743–746.
17. Yu H, Diamandis EP. Prostate-specific antigen immunoreactivity in amniotic fluid. Clin Chem 1995;41:204–210.
18. Bundred NJ, West RR, Dowd JO, Mansel RE, Hughes LE. Is there an increased risk of breast cancer in women who have had a breast cyst aspirated? Br J Cancer 1991;64:953–955.
19. Levitz M, Raju U, Boccardo F, Arcuri F, Castagnetta L. Steroid and cation correlations in human breast cyst fluid: preliminary findings. Cancer Detect Prev 1992;16:57–59.
20. Borchert GH, Melegos DN, Tomlinson G, et al. Molecular forms of prostate-specific antigen in the serum of women with benign and malignant breast diseases. Br J Cancer 1997;76:1087–1094.
21. Sauter ER, Daly M, Linahan K, et al. Prostate-specific antigen levels in nipple aspirate fluid correlate with breast cancer risk. Cancer Epidemiol Biomarkers Prevent 1996;5:967–970.