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Comparison of the percent free prostate-specific antigen levels in the serum of healthy men and in men with recurrent prostate cancer after radical prostatectomy

Andreas Scorilas[a,d], He Yu[b], Antoninus R. Soosaipillai[d], Alkiviades K. Gregorakis[c], Eleftherios P. Diamandis[a,d,*]

[a]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, ON, Canada M5G 1X5*

[b]*Department of Medicine, Louisiana State University Medical Centre, 1501 Kings Hwy, Shreveport, LA 71130-3932, USA*

[c]*Department of Urology, Evangelismos Hospital, 10-12 Glykonos Street, Athens 10675, Greece*

[d]*Department of Laboratory Medicine and Pathobiology, University of Toronto, 100 College Street, Toronto, ON, Canada M5G 1L5*

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Abstract

The percentage of free PSA in serum is currently used to better discriminate between patients with prostate cancer and patients with benign prostatic hyperplasia, in prostate cancer screening programs. We measured using non-competitive immunological techniques, the total PSA and free PSA in post-surgical serum of prostate cancer patients who underwent radical prostatectomy and then relapsed. We compared these data with those of a group of 40 age-matched men with no evidence of prostatic disease. Although in general, patients with prostate cancer had lower percentage of free PSA in serum in comparison to the controls, a subset of these patients (approximately 20%) had percent free PSA significantly higher than the levels considered as exclusive of prostate cancer in screening programs. We also found that percent free PSA does not correlate significantly with most of the standard clinical or pathological indicators of prostate cancer aggressiveness. Only a weak negative association with Gleason Score was observed. The percent free PSA in serum of relapsing prostate cancer patients varies within a relatively wide range and does not correlate significantly with indicators of cancer aggressiveness. The use of

*Correspondence author. Tel.: +416-586-8443; fax: +416-586-8628.

E-mail address: ediamandis@mtsinai.on.ca (E.P. Diamandis)

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percent free PSA for excluding prostate cancer in screening programs must be approached with caution until the mechanism of low percent free PSA in the majority but not all prostate cancer patients is elucidated.

Keywords: Prostate specific antigen; Free prostate specific antigen; Differential diagnosis of prostate cancer from benign prostatic hyperplasia; Tumor markers; Prostate cancer relapse

1. Introduction

Prostate specific antigen (PSA) is widely used for prostate cancer diagnosis and management. PSA is the single most effective test for the diagnosis of prostate cancer but its combination with digital rectal examination further increases the sensitivity of detection [1,2]. Unfortunately, PSA is not a perfect cancer marker since many patients with benign prostatic diseases also show elevations of total serum PSA. The lack of PSA specificity is most pronounced in the intermediate PSA levels, between 4 and 10 μg/l. In this range, only about 25% of men will be diagnosed with prostate cancer while the rest will have benign prostatic hyperplasia (BPH) [3,4]. More recently, the molecular forms of prostate specific antigen in serum have been discovered [5,6]. It is now known that the major immunoreactive forms of PSA in serum are PSA bound to the proteinase inhibitor α_1-antichymotrypsin (about 80% of total PSA) and free PSA (about 20% of total PSA). Many studies have already shown that there is a difference in the percent free PSA between patients with prostate cancer and benign prostatic hyperplasia. Prostate cancer patients have proportionally less free PSA and this difference has been proposed as a tool for the differential diagnosis between prostate cancer and benign prostatic disease, in patients with intermediate levels of PSA [7,8]. The cutoffs of percent free PSA suggested in these studies, to better distinguish between prostate cancer and BPH, range from 15 to 25% and many reasons have been proposed to explain this discordance [9–11]. Among the factors that seem to greatly influence the percent free PSA, apart from the diagnosis of prostate cancer or BPH, are the levels of total serum PSA and the volume of the prostate [7,9,12,13]. Other factors that probably influence the percent free PSA are the age and the histological characteristics of prostate cancer, but the data from the literature are still inconclusive [12–21]. In a recent multicenter trial, Catalona et al. have indicated that the number of prostatic biopsies can be reduced by approximately 20% if the percent free PSA is assessed in addition to total serum PSA [22].

The mechanism by which the percent free PSA is decreased in the serum of prostate cancer patients is currently unknown. Recently, two independent reports

studied the percent free PSA in patients who had undergone radical prostatectomy and then relapsed [23,24]. It was hypothesized in these studies that since the normal prostate tissue has been removed,the percent free PSA should be very low in these patients. Unexpectedly, both studies have found that 18–48% of patients had percent free PSA greater than 15%, which was higher than the cutoffs used to exclude prostate cancer in some series [9]. These data, if confirmed, suggest that the use of percent free PSA to exclude unnecessary biopsies in screening programs should be approached with caution, until the mechanism of production of free PSA by the cancerous cells is elucidated.

In this study, we have measured the percent free PSA in 38 sera from patients who had prostate cancer, undergone radical prostatectomy and then relapsed and compared these values with serum PSA values from 40 age-matched controls without prostate cancer. We have also correlated the percent free PSA of the relapsed patients with various clinicopathological variables.

2. Materials and methods

2.1. Patients

Serum samples from 38 patients who have undergone radical prostatectomy in 1991 or later were collected every six months for the purpose of biochemical monitoring. Patients with confirmed biochemical relapse (with rising PSA over time) were considered eligible for this study. Our criteria for establishing biochemical relapse by use of ultrasensitive PSA analysis have been published previously [25,26]. In short, the relapsed patients had post-operative PSA of < 0.05 μg/l and then had at least two serial elevations which brought their serum PSA to at least 0.2 μg/l. A total post-operative PSA of 0.2 μg/l or higher was necessary since with lower total PSA, the accurate measurement of free PSA is not possible due to limitations in free PSA assay sensitivity. The time between surgery and biochemical relapse in these patients ranged from 5 to 80 months with a median of 52 months. Although multiple serum samples were collected post-operatively, we analyzed only one sample per patient, based on the following criteria: enough residual serum; total PSA > 0.2 μg/l and preferably the one with the highest total PSA; no initiation of treatment at that time.

Clinical and pathological variables were collected from most of these patients. Serum samples were stored at -20°C until analysis. Serum samples from 40 age-matched controls which had no evidence of prostate cancer were also included for comparison. All these men had a negative digital-rectal examination and total serum PSA of less than 2.6 μg/l.

2.2. Immunoassays

The level of total PSA and free PSA in all serum samples was determined by using the Immulite total PSA and free PSA assays, respectively, commercially available by Diagnostic Products Corporation (Los Angeles, CA). The total PSA assay is equimolar, is based on a monoclonal/polyclonal assay configuration and has a detection limit of 0.01 μg/l. The free PSA assay is based on a monoclonal/polyclonal assay configuration and has a detection limit of 0.02 μg/l. Both assays are based on detection of alkaline phosphatase activity by chemiluminescence. The assays were used according to the manufacturer's recommendations.

2.3. Statistical analysis

All statistical analysis, including ANOVA test, Mann–Whitney (*U* Test) and chi-square test as well as Spearman correlation analysis and calculation of odds ratios and their confidence intervals, were performed with SAS statistical software (SAS Institute, Cary, NC, USA).

3. Results

Table 1 summarizes the age, total PSA, free PSA and percent free PSA in the control and case groups. There was no statistical difference in patient age or total PSA between the two groups. However, there was a substantial difference in both free PSA and percent free PSA between the two groups. Patients with relapsing prostate cancer had lower free PSA and lower percent free PSA in comparison to the control group. These differences were evident either by Mann–Whitney (*U* Test) or by chi-square analysis (Table 1). These differences are further graphically depicted in Figs. 1–3. However, it is also evident that among all patients tested ($n = 38$), eight of them have percent free PSA $> 17\%$, and one of them $> 25\%$ (Table 1).

Table 2 summarizes the correlation between total PSA, free PSA and percent free PSA with patient age. As expected, total PSA and free PSA increased with age in the control group. No such correlation was found in the patient group. In both groups (Controls and Cases), there is a strong correlation between total PSA and free PSA concentration.

We have also studied the association between percent free PSA and various clinicopathological variables (Table 3). The only trend for association was noted with the Gleason score; higher Gleason score was associated with lower percent free PSA. In using Spearman correlation analysis, we also noted a weak positive association between pre-operative total PSA and post-operative total PSA, and a

Table 1
Free, total and percentage of free PSA in prostate cancer cases and controls

Variable	Controls	Cases	*P* value
Number	40	38	
Mean Age (S.D.)	67.6 (12.9)	67.2 (5.8)	
Range (year)	50–93	57–77	NS[a]
Total PSA (μg/l):			
Median	0.98	0.67	
Range	0.25–2.52	0.20–15.9	NS[b]
Free PSA (μg/l):			
Median	0.19	0.05	<0.001[b]
Range	0.04–0.99	0.04–2.20	
Free/total PSA (%):			
Median	20.0	8.5	<0.001[b]
Range	7.1–52.6	4.3–25.1	
Total PSA (μg/l):			
<0.60	12 (30.0)	16 (42.1)	
0.60–1.47	15 (37.5)	11 (28.9)	
1.47 +	13 (32.5)	11 (28.9)	
Free PSA (μg/l):			NS[c]
<0.11	13 (32.5)	27 (71.1)	
0.11–0.27	13 (32.5)	6 (15.8)	
0.27 +	14 (35.0)	5 (13.1)	0.003[c]
Free/total PSA (%):			
<17.28	13 (32.5)	30 (79.0)	
17.28–25.14	14 (35.0)	7 (18.4)	
25.14 +	13 (32.5)	1 (2.6)	0.001[c]

[a] ANOVA test: NS *P* value >0.05.
[b] Mann–Whitney (*U* test).
[c] Chi-square test.

weak negative association between pre-operative total PSA and post-operative percent free PSA (Table 4).

4. Discussion

The PSA data presented in this study differ from numerous other literature reports which, in general, study the percent free PSA in patients with prostate cancer, before surgery. In our case, all patients had radical prostatectomy and

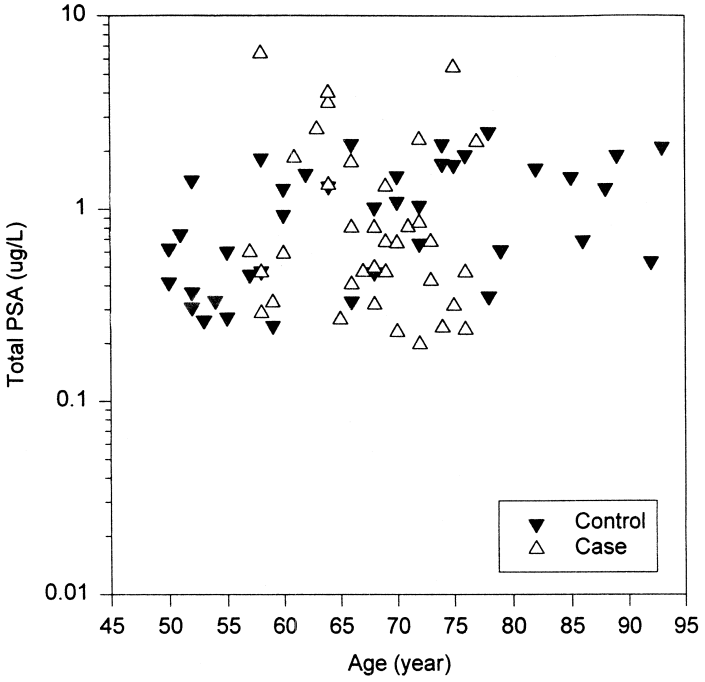

Fig. 1. Distribution of serum total PSA concentration in patients with prostate cancer who relapsed after radical prostatectomy (△) and in age-matched control men without prostatic disease (▼).

circulating PSA represents a product of the cancerous cells after relapse. Since the percent free PSA is decreased pre-surgically in patients with prostate cancer, we expected to see even lower percent free PSA values in patients with relapse.

Until now, only two studies examined in detail the percent free PSA in patients who underwent radical prostatectomy and then relapsed [23,24]. We found that the percent free PSA is significantly reduced in post-surgical serum of relapsing prostate cancer patients, in comparison to the Control group (i.e., men with a negative DRE and a tPSA < 2.6 μg/l). The percent free PSA in our control group had a median of 20%. The patient group had percent free PSA median of 8.5%. Our data closely match the values reported by Wojno et al. [24]. The medians reported by Lin et al. [23] are higher, especially in patients who received radiation and/or hormonal treatment before the blood samples were collected. However, as also reported by Wojno et al. and Lin et al., we found that approximately 20% of our patients had percent free PSA greater than 17%, while one patient in our series had percent PSA greater than 25%. These data are qualitatively similar to those of Wojno et al. and Lin et al., who also demonstrate relatively high percent free PSA values in a subset of prostate cancer patients after recurrence. Wojno et al. further reported that a subset of

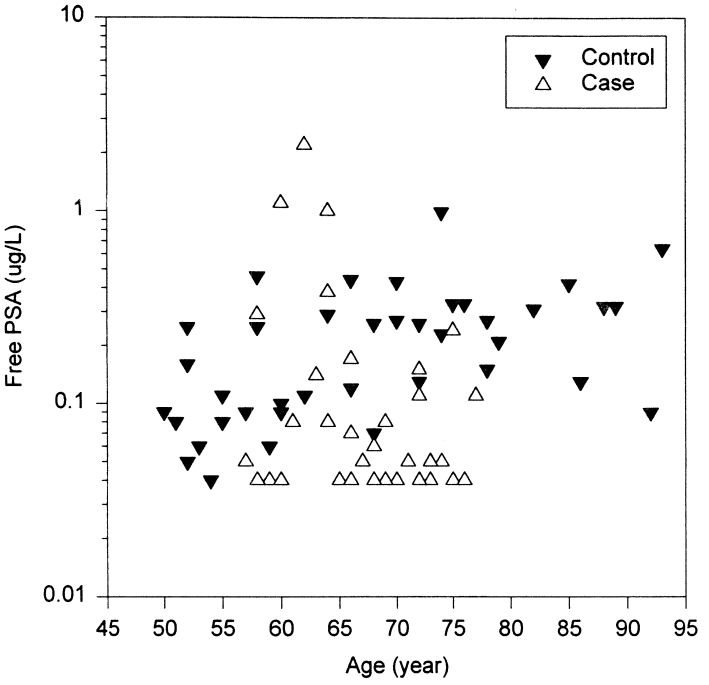

Fig. 2. Distribution of serum free PSA concentration in patients with prostate cancer who relapsed after radical prostatectomy (△) and in age-matched control men without prostatic disease (▼).

patients with free PSA greater than 15% had aggressive prostate cancer, based on pathological staging [24].

We examined if the post-surgical percent free PSA is associated with other markers of aggressiveness of prostate cancer as others have shown for pre-surgical free PSA [7,8,15,16,21]. We found a significant negative association between pre-operative percent free PSA and total post-operative PSA of patients ($P < 0.001$, $r = -0.55$) (data not shown). This may suggest that patients whose total PSA increases more rapidly post-operatively, are likely to have less percent free PSA (Table 2). Among all other pathological variables, including positive margins, apical margin involvement, periprostatic tissue invasion, capsular invasion, seminal vesicle invasion, bladder-neck invasion, tumor volume and clinical stage, none of them was associated significantly with percent free PSA, in accordance with data presented by Lin et al. [23]. However, we found a trend for patients who have lower percent free PSA to have higher histological grade (Gleason Score). This trend is opposite from the trend observed by Lin et al. [23]. Also, our negative association between percent free PSA and total post-operative PSA was not found by Lin et al. We have further found a weak negative association between percent free PSA and pre-operative PSA (Table 4).

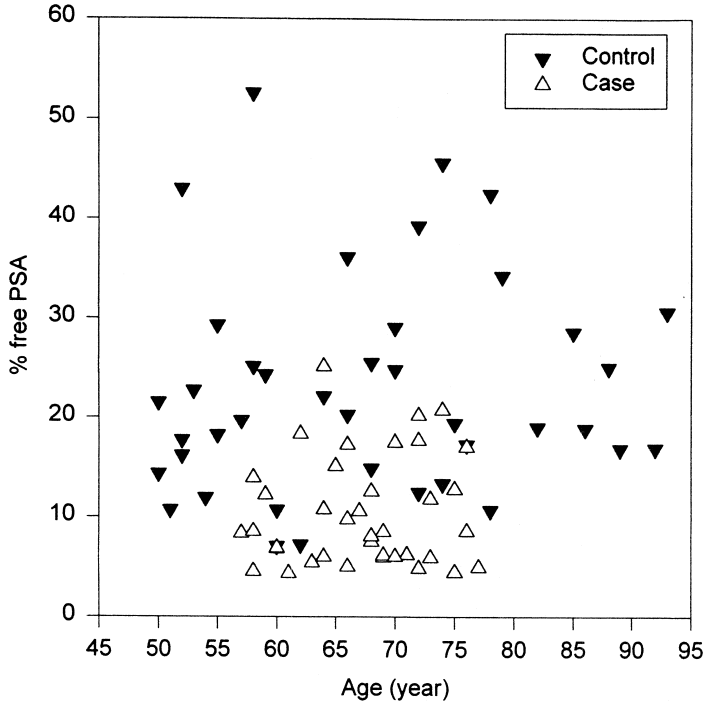

Fig. 3. Distribution of serum% free PSA concentration in patients with prostate cancer who relapsed after radical prostatectomy (△) and in age-matched control men without prostatic disease (▼).

These data combined suggest that low percent free PSA post-radical prostatectomy is associated with high pre-operative PSA, higher histological grade (Gleason Score) and higher total post-operative PSA. Some authors did find an

Table 2
Correlation between free (fPSA) and total PSA (tPSA) between cases and controls

	Spearman Correlation Coefficient (*P* value)					
	Controls			Cases		
	fPSA	tPSA	%fPSA	fPSA[a]	tPSA[a]	%fPSA
Age	0.57	0.50	0.14	− 0.19	− 0.24	0.04
	(< 0.001)	(< 0.001)	(NS)	(NS)	(NS)	(NS)
fPSA		0.82	0.398		0.82	− 0.14
		(< 0.001)	(0.011)		(< 0.001)	(NS)

[a] Post-operative value
[b] NS: *P* value > 0.05

Table 3
Free PSA percentage in clinical and pathological variables

Variable	Number[a]	% free PSA		*P* value[b]
		Median	Range	
Positive Margin				
Negative	11	9.8	4.3–20.7	
Positive	19	7.5	4.5–17.7	NS
Apical Margin Involvement				
Negative	19	8.1	4.3–20.7	
Positive	12	7.7	4.4–18.3	NS
Periprostatic Tissue Invasion				
Negative	5	6.2	4.5–12.8	
Positive	25	8.4	4.3–20.7	NS
Capsular Invasion				
Negative	3	5.9	4.5–7.5	
Positive	27	8.5	4.3–20.7	NS
Seminal Vesicle Invasion				
Negative	20	8.5	4.4–20.7	
Positive	10	6.5	4.3–17.0	NS
Bladder Neck Invasion				
Negative	27	8.4	4.3–20.7	
Positive	4	7.5	4.8–10.6	NS
Tumor Volume				
<30	9	9.8	4.3–20.7	
30–75	12	8.5	4.4–18.3	
75 +	6	7.5	4.8–17.2	NS
Clinical Stage				
T2a	22	8.0	4.3–20.7	
T2b–T1c	9	8.1	5.0–18.3	NS
Histology Grade				
Gleason 6	3	15.1	12.8–18.3	
Gleason 7	17	8.1	4.3–17.5	
Gleason 8	9	7.5	4.4–20.7	
Gleason 9	2	5.1	4.8–5.4	NS

[a] The sum of patients in these categories is less than 38 since for some patients, the clinicopathological variables were not available.
[b] Mann–Whitney (*U* test).

Table 4
Correlation between pre- and post-operative PSA, tumor volume and grade

Variable	Spearman Correlation Coefficient (*P* value)		
	Post-operative PSA[a]		
	tPSA	fPSA	%fPSA
Pre-operative PSA	0.41 (0.024)	0.26 (NS)	− 0.38 (0.042)
Histology grade	0.17 (NS)	0.24 (NS)	− 0.26 (NS)
Tumor volume	0.22 (NS)	0.13 (NS)	− 0.28 (NS)

[a] tPSA, total PSA; fPSA, free PSA; %fPSA, percentage of free PSA.

association between pre-operative percent free PSA and more aggressive cancer [15,16,21] while others did not [17–20,27,28].

5. Conclusions

In accordance with the data of Wojno et al. and Lin et al. [23,24], we found that percent free PSA is indeed lower in the majority of patients with recurrent prostate cancer but approximately 20% of such patients with relapsing disease demonstrate percent free PSA levels $>17\%$ and occasional patients may have percent free PSA $>25\%$. Indeed, a subset of patients with prostate cancer may escape diagnosis in screening programs utilizing total PSA and free PSA, by presenting a high percentage of free PSA. Our data further suggest that percent free PSA does not correlate significantly with most of the standard clinicopathological variables of prostate cancer aggressiveness. The mechanism by which a subset of prostate cancers produce relatively high levels of percent free PSA requires further investigation.

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