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Detection of Prostate Cancer Relapse With Prostate Specific Antigen Monitoring at Levels of 0.001 to 0.1 ug/L

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Abstract

Purpose: The development of prostate specific antigen (PSA) assays with detection limits of approximately 0.001 micro g./l. is technically feasible. We examined if serum PSA changes of 0.001 to 0.1 micro g./l. for up to 3 years after radical prostatectomy have any clinical value.

Materials and Methods: We studied 148 patients with a postoperative PSA of less than 0.1 micro g./l. by a conventional PSA assay. At least 3 serial serum samples were collected per patient along with detailed clinicopathological features. Serial serum samples were analyzed for PSA with the ultrasensitive method. Associations between increase in serum PSA and clinicopathological features were analyzed with the unconditional logistic regression model.

Results: After establishing a set of interpretative criteria, we divided the patients into 51 with biochemical relapse, 93 who were free of relapse and 4 with equivocal status. Between the groups with and without relapse there was no difference in year of surgery, age at operation or length of followup. Compared to patients without relapse, those with biochemical relapse were likely to have positive surgical margins ($p < 0.01$), larger tumor volumes ($p < 0.01$), greater preoperative PSA ($p = 0.03$) and disease extending outside the prostate ($p = 0.02$). The relative risks for biochemical relapse estimated by a univariate logistic regression model were 3.1 (95% confidence interval 1.39 to 6.82, $p < 0.01$) for positive surgical margin, 3.4 (95% confidence interval 1.46 to 8.13, $p < 0.01$) for tumor volume, 2.3 (95% confidence interval 1.08 to 5.02, $p = 0.03$) for high preoperative PSA and 2.7 (95% confidence interval 1.12 to 6.26, $p = 0.03$) for extraprostatic tumor extension. At multivariate analysis with the same model the associations between positive surgical margins and biochemical relapse (relative risk 2.95, $p = 0.04$) and tumor volume (relative risk 3.36, $p = 0.03$) remained significant. These associations were still observed when we analyzed a subset of patients classified as having biochemical relapse based on PSA changes of 0.001 to 0.08 micro g./l.

Conclusions: Increases in postoperative serum PSA at levels of 0.001 to 0.1 micro g./l. after radical prostatectomy are associated with clinicopathological features of poor prognosis. Monitoring postoperative cases with a highly sensitive PSA assay (detection limit 0.001 micro g./l.) could offer a simple and effective means of detecting clinically important biochemical relapse early after radical prostatectomy. These patients may be suitable for early intervention when effective treatments for relapse become available.

Key Words: prostatic neoplasms, prostate-specific antigen, neoplasm metastasis

An established clinical application of serum prostate specific antigen (PSA) in the treatment of prostate cancer is the detection of relapse after radical prostatectomy. Clinical studies have shown that relapse can be detected through monitoring of changes in serum PSA concentrations, and that detection by this method can be achieved much earlier compared to other diagnostic tools. [1-4] It also has been demonstrated that sensitive PSA assays can enhance the efficiency of monitoring by widening the window of observation of temporal serum PSA changes. [5-10] For example it has been demonstrated that prostate cancer relapse could be identified approximately 1 year earlier in most patients with recurrence if a PSA assay with a detection limit of 0.1 instead of 0.4 [5] or 0.01 instead of 0.1 [7] micro g./l. is used for monitoring. Early identification of prostate cancer relapse is believed to be crucial for successfully treating patients with recurrent or metastatic disease.

Currently, the most sensitive PSA assays available for postoperative monitoring have biological or functional detection limits of 0.01 to 0.06 micro g./l. [7-10] However, it remains unknown if the monitoring efficiency can be improved further with more sensitive PSA assays. We investigated this possibility with a recently developed assay that can measure serum PSA at a concentration of 0.001 micro g./l. or greater. Serial serum samples from 148 prostate cancer cases after radical prostatectomy were measured for PSA with this new assay. Changes in serum PSA of 0.001 to 0.1 micro g./l. were analyzed in association with patient clinical and histopathological features.

Materials and Methods

Patients and serum collection

Between February 1, 1993 and November 18, 1994, 347 patients with prostate cancer who had undergone radical prostatectomy were enrolled consecutively into the study. Serum specimens were obtained from patients whose PSA values were less than 0.1 micro g./l. as measured at the department of clinical biochemistry with the Abbott IMx* PSA assay. By November 1995, 148 patients had provided 3 or more serial serum specimens (3 samples in 64, 4 in 48, 5 in 19, 6 in 12, 7 in 4 and 8 in 1). For these serial samples there was no fixed interval for serum collection but, on average, the intervals were 3 to 6 months. All sera collected were stored at -70C until analysis. Clinical information, including age, date of surgery, preoperative serum PSA and followup, and histopathological examination of surgical specimens, including information on surgical margin, seminal vesicle involvement, tumor volume, extraprostatic extension, capsular penetration and histological grade (Gleason score), were obtained by reviewing the medical records.

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PSA assay

Previously we developed an ultrasensitive PSA assay with a biological detection limit of 0.01 micro g./l. [11] This assay has been recently modified to improve the biological detection limit to 0.001 micro g./l. [12] Briefly, the assay is a time resolved immunofluorometric method using 2 monoclonal anti-PSA antibodies. The capture antibody is immobilized on the microtiter wells and the detection antibody, incubated with the samples or standards in 1 step, is biotinylated. Streptavidin conjugated with alkaline phosphatase is used as a label in conjunction with diflunisal phosphate as the substrate of alkaline phosphatase. The dephosphorylated diflunisal phosphate forms a complex with a terbium-edetic acid chelate, which emits delayed fluorescence after laser excitation. The fluorescence is measured in a time resolved mode so that the background signal is minimized. The standard curve is constructed with 8 calibrators with PSA concentrations of 0, 0.001, 0.002, 0.005, 0.02, 0.1, 0.5 and 2 micro g./l. The calibrators were prepared by diluting purified seminal PSA in 6% bovine serum albumin in a 50 mmol./l. tris buffer, pH 7.80. This assay recognizes free PSA and PSA bound to alpha 1-antichymotrypsin in an equimolar fashion. [12]

Measurement of serum PSA

All serum samples were measured in triplicate along with the 8 calibrators. Serial serum samples from the same patient were analyzed within the same 96-well microtiter plate to minimize the variation among plates (that is between-run variation). The within-run coefficients of variation were 12.5, 5.4 and 4.9% for the calibrators at concentrations of 0.001, 0.002 and 0.005 micro g./l., respectively. Within-run precision (coefficients of variation) of our assay at serum PSA of 3, 0.3, 0.15, 0.03, 0.003 and 0.002 micro g./l. was 6.1, 6.3, 4.8, 9.8, 10.4 and 8.1%, respectively. The between-day coefficients of variation at the same levels of PSA were 5.2, 5.0, 6.5, 5.1, 8.4 and 15.8%, respectively.

Definition of biochemical relapse

Based on PSA levels in 3 or more serial postoperative serum samples, the biochemical relapse was defined arbitrarily by 1 of several criteria, including 2 or more consecutive increases in serum PSA that resulted in at least the doubling of initial PSA, any increase that resulted in serum PSA greater than 0.1 micro g./l. or a 10-fold increase in serum PSA between 2 serum collections. Patients were considered free of biochemical relapse if changes in serum PSA did not fit any of these 3 conditions (except for 4 patients described). Any value less than 0.001 micro g./l. was considered undetectable.

Calculation of PSA doubling time

PSA doubling time was calculated based on the formula $(PSA)_t = (PSA)_o * e^{Kt}$, where K is the slope of the plot ln(PSA) versus time and doubling time = ln2/K. [7]

Statistical analysis

Patients were classified into 2 groups with or without biochemical relapse based on the criteria described. The associations between the status of biochemical relapse and the clinical or histopathological variables were examined using the chi-square test, and the unconditional logistic regression model was applied at univariate and multivariate levels. [13] In the analysis the clinical and histopathological variables were categorized dichotomously. Classification included patient age younger than 64 years versus 64 years old or older, surgery performed before 1992 versus 1992 or later, preoperative serum PSA less than 10 versus 10 or more micro g./l., positive versus negative surgical margin, with or without seminal vesicle involvement, tumor volume 20 or less versus more than 20% of prostate volume, with or without extraprostatic extension and with or without capsular penetration, and Gleason score less than 7 versus 7 or more. The median levels of PSA doubling time among different histopathological groups were compared with the Wilcoxon rank sum test.

Results

Based on the criteria described 51 patients were classified as having biochemical relapse while 93 were relapse-free. Ten examples of each classification are shown in Table 1. Four patients were excluded from the analysis because the relapse status was difficult to determine. PSA levels in the serial serum samples were 0.028, 0.018 and 0.045 for patient 1; 0.187, 0.088, 0.040 and 0.053 for patient 2; 0.021, 0.024, 0.036 and 0.037 for patient 3, and 0.001, 0.001 and 0.008 for patient 4.

Pt. No.	Serial Postop. Serum PSA Measurements (μg./l.)								Followup (mos.)*
	1	2	3	4	5	6	7	8	
					With relapse				
1	0.000	0.001	0.000	0.001	0.017	1.013	1.802		52
2	0.019	0.039	0.095	0.192					36
3	0.001	0.003	0.009	0.032	0.073	0.118	0.137	0.219	30
4	0.023	0.032	0.063	0.079	0.064				16
5	0.000	0.010	0.077						29
6	0.002	0.009	0.012						43
7	0.003	0.003	0.043						11
8	0.000	0.001	0.003	0.004	0.010	0.026			25
9	0.003	0.019	0.023	0.042					11
10	0.003	0.006	0.011						8
					Without relapse				
11	0.006	0.001	0.001	0.001	0.004	0.003	0.005		28
12	0.010	0.001	0.009	0.000	0.001				29
13	0.045	0.000	0.001	0.000					37
14	0.003	0.000	0.000	0.000	0.000	0.000			50
15	0.005	0.005	0.005	0.024	0.004	0.005			26
16	0.014	0.014	0.012	0.014	0.014				40
17	0.014	0.013	0.023						5
18	0.000	0.000	0.000	0.000	0.000	0.000			22
19	0.003	0.002	0.001	0.003	0.005	0.002			20
20	0.001	0.001	0.001	0.001	0.003				25

* Interval between surgery and last PSA measurement.

Table 1. Examples of classification of biochemical relapse based on changes in serial postoperative serum PSA

(Table 2) summarizes the PSA changes with time in all 148 patients studied. Among the 93 patients without relapse 16 had undetectable PSA (less than 0.001 micro g./l., for example patient 18, Table 1), 57 had PSA less than 0.01 micro g./l. (for example patients 11, 12, 14, 19 and 20) and 20 had PSA less than 0.1 micro g./l. (for example patients 13, 15, 16 and 17) in all serial samples. Of 51 patients with biochemical relapse 15 (for example patients 1, 3, 6, 7, 8, 9 and 10), 11 (patients 4 and 5) and 5 were classified based on increases in serial PSA levels exclusively less than 0.05, 0.08 and 0.1 micro g./l., respectively. The remaining 20 patients had at least 1 PSA measure necessary for classification of more than 0.1 micro g./l.

Serial Serum PSA (μg./l.)	No. Pts.	Followup (mos.)
Without biochemical relapse:		
All less than 0.001	16	8–46
All less than 0.01	57	7–70
All less than 0.1	20	5–73
With biochemical relapse:		
All less than 0.05	15	8–53
All less than 0.08	11	11–59
All less than 0.1	5	18–51
At least 1 more than 0.1	20	10–57
Undetermined relapse status	4	18–29

Table 2. Changes in serial postoperative serum PSA levels among the 148 patients

The distributions of surgical year, patient age and followup between the patients with and without relapse are shown in Table 3. Both groups were similar in the distribution of surgical year (p = 0.73, chi-square test with 4 degrees of freedom). Patient age ranged from 47 to 73 years. Patients without relapse were slightly older than those with relapse (mean age 63 versus 61 years) but the difference was not statistically significant (p = 0.08, 2 sample t test). Followup ranged from 5 to 73 months (median approximately 2 years, that is 26 or 28 months). The patients were followed similarly between the 2 groups (p = 0.69, Wilcoxon rank sum test).

Variables	Biochemical Relapse	
	No	Yes
Yr. surgery (%):		
1990 or Before	10 (10.9)	6 (12.0)
1991	17 (18.5)	9 (18.0)
1992	23 (25.0)	9 (18.0)
1993	32 (34.8)	17 (34.0)
1994	10 (10.9)	9 (18.0)
% Pt. age at surgery (yrs.):		
0	47	47
25	59	57
50	63	61
75	67	66
100	73	72
Mean age	63	61
% Followup (mos.):*		
0	5	8
25	18	17
50	28	26
75	39	42
100	73	70
Mean mos.	30	30

* Interval between surgery and last PSA measurement.

Table 3. Clinical and histopathological information on the 148 patients

(Table 4) shows the associations between pathological features and patient biochemical relapse status. Biochemical relapse was significantly associated with positive surgical margin (p <0.01), large tumor volume (p <0.01), lesions not confined to the organ (p = 0.02) and higher preoperative serum PSA (p = 0.03). No significant association was noted between biochemical relapse and seminal vesicle involvement, capsular penetration or Gleason score, although there was a trend for the patients with relapse to have a Gleason score of 7 or more, capsular penetration and seminal vesicle involvement.

Variables	Total No. Pts.	No. Pts. (%)		p Value
		No Relapse	Relapse	
Surgical margin:	124			
Neg.		44 (78.6)	12 (21.4)	
Pos.		37 (54.4)	31 (45.6)	<0.01
Seminal vesicle involvement:	124			
No		70 (66.7)	35 (33.3)	
Yes		11 (57.9)	8 (42.1)	0.46
% Tumor vol.:	109			
20 or Less		38 (79.2)	10 (20.8)	
More than 20		32 (52.5)	29 (47.5)	<0.01
Organ confined:	123			
Yes		33 (78.6)	9 (21.4)	
No		47 (58.0)	34 (42.0)	0.02
Capsular penetration:	123			
No		15 (75.0)	5 (25.0)	
Yes		65 (63.1)	38 (36.9)	0.31
Gleason score:	137			
Less than 7		40 (72.7)	15 (27.3)	
7 or More		50 (61.0)	32 (39.0)	0.16
Preop. PSA (μg./l.):	122			
Less than 10		56 (71.8)	22 (28.2)	
10 or More		23 (52.3)	21 (47.7)	0.03

Table 4. Associations between biochemical relapse and clinical or histopathological features

(Table 5) demonstrates the strength of the associations between the pathological features and biochemical relapse using the relative risks, estimated with the use of unconditional logistic regression models. Estimation was done at univariate and multivariate levels. Patients with positive surgical margins or large tumor volume had more than a 3-fold increased risk for biochemical relapse compared to those with negative surgical

margins or smaller tumors. More than a 2-fold increase in risk of biochemical relapse was also noted for patients with lesions extended outside the prostate or with preoperative serum PSA greater than 10 micro g./l. After adjusting for patient age, followup time and other histopathological features, we were still able to observe a significant increase in risk of biochemical relapse in patients with positive surgical margins and large tumor volume but not in those with extraprostatic extension or high preoperative serum PSA Table 5.

Variables	Univariate Analysis				Multivariate Analysis (98 pts.)		
	No. Pts.	Relative Risk	95% Confidence Interval	p Value	Relative Risk	95% Confidence Interval	p Value
Surgical margin:	124						
Neg.		1.00			1.00		
Pos.		3.07	1.39–6.82	<0.01	2.95	1.05–8.32	0.04
Seminal vesicle involvement:	124						
No		1.00			1.00		
Yes		1.46	0.54–3.94	0.46	1.05	0.25–4.24	0.94
% Tumor vol.:	109						
20 or Less		1.00			1.00		
More than 20		3.44	1.46–8.13	<0.01	3.36	1.15–9.78	0.03
Organ confined:	123						
Yes		1.00			1.00		
No		2.65	1.12–6.26	0.03	2.00	0.51–7.85	0.32
Capsular penetration:	123						
No		1.00			1.00		
Yes		1.75	0.59–5.21	0.31	0.39	0.07–2.03	0.26
Gleason score:	137						
Less than 7		1.00			1.00		
7 or More		1.71	0.81–3.58	0.16	0.47	0.15–1.44	0.19
Preop. PSA (μg./l.):	122						
Less than 10		1.00			1.00		
10 or More		2.32	1.08–5.02	0.03	1.26	0.46–3.47	0.65

Univariate analysis used an unconditional logistic regression model that contains only 1 variable. Multivariate analysis used an unconditional logistic regression model that contains all variables listed plus followup and age.

Table 5. Univariate and multivariate analyses of relative risk of biochemical relapse associated with clinical and histopathological features

Since the purpose of our study was to examine if changes in serum PSA at extremely low levels would have any clinical implication, we reanalyzed the data with exclusion of the patients whose relapse status was determined based on a PSA of more than 0.08 micro g./l. The associations between biochemical relapse and positive surgical margin or large tumor volume remained Table 6. The interval from surgery to the detection of biochemical relapse is shown in Table 7. Median detection time was 18 months and 75% of the cases could be detected within 28 months postoperatively.

Variables	Relative Risk	95% Confidence Interval	p Value
Surgical margin:			
Neg.	1.00		
Pos.	4.48	1.27–15.87	0.02
% Tumor vol.:			
20 or Less	1.00		
More than 20	4.75	1.38–16.35	0.01

Using an unconditional logistic regression model that also contains variables of age, followup, preoperative PSA and Gleason score.

Table 6. Multivariate analysis of relative risk of biochemical relapse associated with surgical margin and tumor volume among 84 patients whose serial serum PSA changed to less than 0.08 micro g./l.

% Pts.	Biochemical Relapse (mos.)	PSA Doubling Time (days)
0	4	33
25	11	77
50	18	151
75	28	239
100	61	619

Table 7. Distribution of time for biochemical relapse and PSA doubling time

PSA doubling time was calculated for 50 patients with relapse who had information on all of the dates of serum collection. PSA doubling time among these patients ranged from 33 to 619 days (median 151, Table 7). The medians and means of PSA doubling time in relation to surgical margin, tumor volume, Gleason score and preoperative PSA are shown in Table 8. Patients with positive surgical margins tended to have a shorter PSA doubling time compared to those with negative surgical margins (p = 0.06). Tumors with a higher Gleason score had a significantly shorter PSA doubling time (p = 0.03). The doubling time was not statistically associated with tumor volume and preoperative PSA (p >0.33).

Variables	PSA Doubling Time (days)		p Value	
	Median	Mean	Wilcoxon Rank Sum Test	2-Sample t Test With Equal Variance
Surgical margin:				
Neg.	193	223		
Pos.	122	152	0.06	0.07
% Tumor vol.:				
20 or Less	129	143		
More than 20	157	186	0.43	0.33
Gleason score:				
Less than 7	218	241		
7 or More	123	150	0.03	0.02
Preop. PSA (μg./l.):				
Less than 10	167	203		
10 or More	151	164	0.66	0.34

Table 8. PSA doubling time in association with histopathological features

Discussion

Prostate cancer relapse is now evaluated with serial PSA measurements after radical prostatectomy. In a patient with a PSA of less than 0.1 micro g./l. postoperatively disease is considered to be in remission. Once PSA is elevated to more than 0.1 micro g./l. biochemical relapse is suspected. Recently, a few studies have shown that earlier detection of relapse can be achieved by monitoring PSA changes in the range 0.01 to 0.1 micro g./l. [6,7] An unanswered question is whether there is any clinical use in monitoring PSA changes after radical prostatectomy at levels lower than 0.01 micro g./l. This question can now be addressed with the availability of a method that can measure reliably PSA levels of at least 0.001 micro g./l. [12] A prospective study examining PSA changes of 0.001 to 0.1 micro g./l. in association with patient disease-free and overall survival would answer the question but this will require at least 5 to 10 years of followup. To provide preliminary data on this issue we examined if PSA changes in this range are associated with established histopathological features known to be associated with patient survival, including status of surgical margins, Gleason score, tumor volume, tumor confinement to prostate, seminal vesicle involvement, capsular penetration and preoperative PSA. [14-24]

We found that postoperative PSA varied widely from less than 0.001 to 0.1 micro g./l. In many cases the postoperative PSA was less than the measuring ability of any available PSA method. Clearly, no postoperative PSA level will guarantee that the patient will not have relapse. Many patients who eventually have relapse had a postoperative PSA of less than 0.001 micro g./l. Table 1. On the other hand, in patients with a PSA of more than

0.001 micro g./l. disease may remain in remission for long periods. We assume that the measurable but not changing PSA in these patients may originate from remaining normal prostatic tissue or from nonprostatic sources as described previously. [25] However, we do not exclude the possibility that many of these patients will have relapse with longer followup.

Our data suggest that the most effective way to detect biochemical relapse is by evaluating serial PSA changes with time. [26,27] We designed a set of simple criteria to judge if a change in PSA concentration with time is significant. Using these criteria we defined a sizeable group of postoperative prostate cancer patients (51 of 144, or 35%) who had biochemical relapse within a relatively short monitoring interval (median 18 months). For half of these patients (26 of 51, or 51%) biochemical relapse was detected with PSA measurements of less than 0.08 micro g./l. Table 2.

When we compared year of surgery, patient age and followup between patients with and without biochemical relapse there was no difference Table 3. However, the biochemical relapse group was strongly associated with positive surgical margins ($p < 0.01$), large tumor volume ($p < 0.01$), nonorgan confined disease ($p = 0.02$) and a preoperative PSA of 10 micro g./l. or more ($p = 0.03$, Table 4 and Table 5). The same group also tended to have seminal vesicles involvement, capsular penetration and higher Gleason score but the differences did not reach statistical significance. The associations between biochemical relapse and positive surgical margin or large tumor volume were sustained when we reanalyzed the data at multivariate levels Table 5 or when we included patients whose serum PSA remained less than 0.08 micro g./l. during the monitoring period Table 6.

The question of how soon the biochemical relapse can be detected with ultrasensitive PSA assays compared to conventional assays has been addressed previously. [6,7] Besides PSA assay sensitivity, 3 other factors also are important in the earlier diagnosis of relapse: 1) frequency of serum collections (in our study we did not control this parameter), 2) tumor doubling time (for prostate cancer this varied widely from 60 to 600 days [7]) and 3) criteria applied to determine relapse. In our study the criteria were set arbitrarily and they were generally conservative. We plotted serial PSA values of 3 representative patients (see Figure 1). Clearly, the lead time for relapse detection can be shortened by 6 to 18 months in agreement with previous data. [6,7]

With use of this highly sensitive PSA assay, the majority of patients with biochemical relapse could be identified within 28 months postoperatively Table 7. This early detection may provide an opportunity for early intervention. A randomized clinical trial is needed to examine this possibility.

The association between shorter PSA doubling time and higher Gleason score provides further evidence for the validity of using PSA doubling time as an indicator of tumor proliferating potential Table 7 and Table 8. Calculating the PSA doubling time for patients with relapse may be useful for patient subclassification and selection of therapy. These possibilities have not as yet been examined with prospective studies.

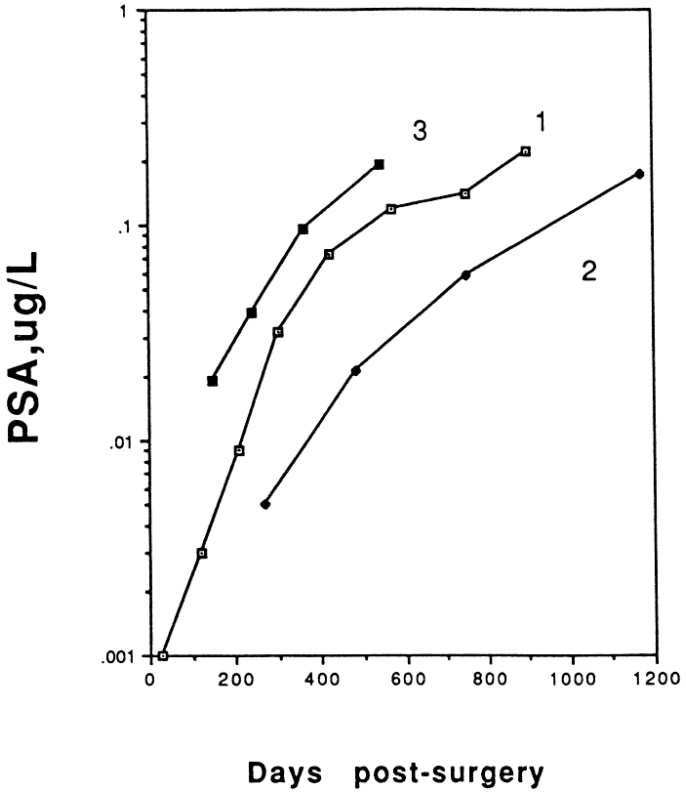

Figure 1. Changes in PSA with time in 3 patients who underwent radical prostatectomy. Difference in biochemical relapse time between ultrasensitive assay and conventional PSA assay (0.1 micro g./l. detection limit) was 360, 419 and 180 days for patients 1, 2 and 3, respectively. PSA doubling times calculated by using first 3 points of graph as described by Yu et al [7] were 54, 138 and 91 days, respectively.

Conclusions

Our data suggest that approximately 35% of prostate cancer patients who underwent radical prostatectomy and disease is believed to be in remission based on PSA values of less than 0.1 micro g./l. have biochemical relapse as determined by an ultrasensitive PSA assay with a detection limit of 0.001 micro g./l. Many of these patients could be identified by serial PSA measurements, even when the increase in serum PSA remained exclusively less than 0.08 micro g./l. Patients with biochemical relapse were likely to have positive surgical margin large tumor volume, nonorgan confined disease and high preoperative PSA. We speculate that a proportion of patients will eventually have clinical relapse and will require further treatment. Based on the premise that minimal residual disease can be treated more effectively than an overt relapse, we propose that patients with biochemical relapse should be enrolled in clinical trials to examine if an earlier intervention will result in prolonged survival and better quality of life.

Purified seminal PSA was donated by Dr. T. A. Stamey, Stanford University, Stanford, California.

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