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MEASUREMENT OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS IN WOMEN AND IN PROSTATECTOMIZED MEN WITH AN ULTRASENSITIVE IMMUNOASSAY TECHNIQUE

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ABSTRACT

Serum prostate specific antigen (PSA) was measured in 89 prostate cancer patients with no evidence of relapse after radical prostatectomy, in 387 hospitalized women with various diseases and in 674 apparently healthy female blood donors, using an ultrasensitive time resolved immunofluorometric assay with a biological detection limit of 0.01 μg./l. Of the prostatectomized cancer patients 50% had measurable PSA (0.010 μg./l. or greater) and 21% had levels of 0.050 μg./l. or greater. Postoperative PSA was not associated with year of surgery, preoperative PSA or histological grade of the tumors. The distribution of PSA in 1,064 female sera is also reported, of which 83% had no detectable serum PSA, and in 15% PSA was between 0.010 and 0.049 μg./l. Only 27 subjects (2.5%) had PSA levels of 0.050 μg./l. or greater, including 16 (1.5%) with PSA of 0.10 μg./l. or greater. High serum PSA in women was associated with age 50 years or older. Further studies are needed to examine if the difference in PSA levels between women and prostatectomized men is due to residual or recurrent tumor or to other reasons associated with gender.

KEY WORDS: prostatic neoplasms, prostatectomy, immunoassay, antigens

Prostate cancer is becoming the most common cancer in North America and the incidence rate is still increasing.[1] The major reason for the rise in incidence is believed to be the improvement of diagnostic techniques that are capable of identifying more patients with cancer at an early stage.[2] The number of patients treated with radical prostatectomy is also increasing.[3] Since there is no effective way to prevent this cancer, it is important to improve the management of the patients after surgery. Prostate specific antigen (PSA), a 34 kDa. single chain glycoprotein produced by the epithelial cells of the prostate gland and present in prostatic tissue, seminal plasma and serum, is a valuable marker for the management of prostate cancer. The measurement of PSA in serum is used for diagnosis, assessment of therapy and monitoring recurrence or metastasis.[4,5]

Studies have already shown that the postoperative elevation of serum PSA indicates recurrent or metastatic cancer, and that serial evaluation of PSA in the serum of prostate cancer patients after radical prostatectomy is a simple, inexpensive and effective way to identify these lesions.[6–8] However, there are still questions about the efficiency of monitoring.[8–10] The minimum amount of PSA that can be detected by the commercially available methods is about 0.05 μg./l. In most postoperative patients PSA concentrations in serum are below this level if no residual prostatic tissue is left after surgery.[4,5] Therefore, the exact PSA level in serum of these patients is unknown. It has been speculated that residual or recurrent cancer may be identified earlier if a PSA concentration less than 0.1 μg./l. can be accurately measured in the patients postoperatively.[11]

We hypothesized that serum PSA levels in men without any prostate tissue and in women should be similar since women have no prostate. We further speculated that if men undergoing radical prostatectomy have significantly higher levels of serum PSA than women, then these patients may have occult residual prostatic tissue or they have suffered a relapse. To our knowledge these hypotheses have not as yet been examined in the literature because no PSA methods existed that could accurately quantify PSA at levels below 0.05 μg./l. Moreover, no extensive studies on PSA levels in female sera have been conducted for the same reasons. We recently developed a new PSA assay that can accurately quantify PSA in a range of 0.01 to 0.1 μg./l.[12] Using this assay we measured PSA levels in the sera of 89 prostate cancer patients who had undergone radical prostatectomy with no evidence of relapse, and in the sera of 674 healthy and 387 hospitalized women. The serum PSA levels in these populations are described.

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MATERIALS AND METHODS

Serum samples. Sera of prostate cancer patients after radical prostatectomy were provided by the Department of Clinical Biochemistry of the Toronto Hospital. A total of 89 postoperative prostate cancer patients was selected consecutively from a list of patients who had undergone a conventional PSA test for followup from February 1 through May 25, 1993. The selection criteria included histologically confirmed diagnosis of prostate cancer, radical prostatectomy during the last 6 years, no recurrence or metastasis based on clinical information and PSA levels measured by the conventional assay were below the detection limit, that is less than 0.4 μg./l. Information on histological grade (Gleason score) was available for all patients. Of the 89 patients 69 (78%) had undergone a preoperative PSA test.

Sera were provided by the Red Cross Blood Transfusion Service in Toronto from 674 apparently healthy women who donated blood during February through April 1993. Another 387 female sera were obtained from inpatients at The Toronto Hospital during January and February 1993. These patients attended several hospital clinics, including general surgery, neurosurgery, orthopedic surgery, nephrology, urology, dentistry, and obstetrics and gynecology.

PSA assay. The newly developed ultrasensitive PSA assay

described in detail elsewhere[12] was used for the analysis of all serum samples. Briefly, the assay uses 1 monoclonal anti-PSA capture antibody immobilized in white polystyrene microtitration wells, 1 biotinylated polyclonal anti-PSA detection antibody and alkaline phosphatase-conjugated streptavidin. The activity of alkaline phosphatase is measured through the hydrolysis of a substrate, diflunisal phosphate. The dephosphorylated form of diflunisal phosphate reacts with Tb^{3+}-ethylenediaminetetraacetic acid to form a highly fluorescent complex. The fluorescence of the complex is detected by time resolved fluorometry following laser excitation. This assay can accurately quantify PSA in the range of 0.01 to 10 μg./l.

For the assay we used 6 calibrators prepared by diluting a purified seminal PSA preparation in a 60 gm./l. bovine serum albumin solution. These standards were calibrated with the Hybritech Tandem-E PSA kit. The PSA concentrations of these calibrators were 0, 0.025, 0.1, 0.5, 2.0 and 10.0 μg./l. Quality control samples that had PSA concentrations of 0.02, 0.1 and 0.3 μg./l. were also measured in each assay run to monitor performance. The coefficient of variation of the controls for the day-to-day precision was between 10% and 14%. All samples were analyzed in duplicate.

High performance liquid chromatography. To provide more evidence for the presence of PSA in female serum we also examined the molecular weight of PSA in 2 female and 1 male serum samples. We used high performance liquid chromatography to separate the serum components according to their molecular weight and analyzed each fraction with the PSA assay. The detailed method has been described elsewhere.[12]

Statistical analysis. PSA concentrations were categorized into 4 groups: 1) PSA less than 0.010 μg./l., which is the biological detection limit of our PSA assay, 2) PSA between 0.010 and 0.049 μg./l., 3) PSA between 0.050 and 0.099 μg./l., and 4) PSA at 0.100 μg./l. or greater. The distributions of PSA in the 4 groups were compared between the populations with the chi-square test. We analyzed the data using statistical software, and $p \leqq 0.05$ was considered statistically significant.

RESULTS

We studied 89 prostate cancer patients, of whom 49% underwent radical prostatectomy before 1992 and 51% were treated in 1992 or later. Of the patients 91% had moderately differentiated cancer (Gleason score 5, 6 or 7/10), 8% had poorly differentiated cancer (Gleason score 8 or 9/10) and 1% had well differentiated cancer (Gleason score 3/10). Of the 69 patients who had a preoperative serum PSA concentration measured by the conventional assay 43 had a level less than 10 μg./l. and 26 had a level at 10 μg./l. or greater (data not shown).

The postoperative serum PSA concentration of the 89 prostate cancer patients is shown in table 1 along with the data for the 2 female populations. Of all patients 49% had PSA below 0.010 μg./l., 30% between 0.010 and 0.049 μg./l., 10% between 0.050 and 0.099 μg./l. and 11% 0.100 μg./l. or greater. There was no linear correlation between serum PSA and patient age (fig. 1)

TABLE 1. *Serum PSA concentrations in postoperative patients with prostate cancer and in normal and hospitalized women*

Serum PSA (μg./l.)	No. Prostate Ca (%)	No. Normal Women (%)	No. Hospitalized Women (%)
Less than 0.010	43 (49)	561 (83)	321 (82)
0.010–0.049	27 (30)	102 (15)	53 (14)
0.050–0.099	9 (10)	4 (1)	7 (2)
0.10 or greater	10 (11)	7 (1)	9 (2)
Totals	89 (100)	674 (100)	390 (100)

Table 2 shows the association between postoperative PSA and year of surgery, preoperative PSA and histological grade. Patients operated on between 1992 and 1993 or 1988 and 1991 were grouped into 2 categories, according to PSA less than 0.010 μg./l. and PSA 0.010 μg./l. or greater. The difference between the groups was not statistically significant (chi-square 1.53, degrees of freedom 1 and $p = 0.22$). Also, there was no indication that the preoperative PSA level was associated with the postoperative level (table 2).

No statistically significant difference was seen between the postoperative PSA and histological grade after grouping the patients into 2 categories, according to PSA less than 0.010 versus PSA 0.010 μg./l. or greater (chi-square 0.37, degrees of freedom 1 and $p = 0.54$). However, more patients with low histological grade tended to have a postoperative PSA level less than 0.010 μg./l. (53% versus 44%). Furthermore, when the year of surgery was controlled an even larger difference was observed, that is 59% versus 45% in the surgical years 1988 to 1992 and 57% versus 35% in the surgical years 1988 to 1991. However, none of these differences was statistically significant.

PSA was less than 0.010 μg./l. in 83% of the normal women and 82% of the hospitalized women, 0.010 to 0.049 μg./l. in 15% and 14%, 0.050 to 0.099 and 0.10 μg./l. or greater in 1% and 2% (table 1). The highest PSA level in normal women was 0.66 μg./l. and in hospitalized women it was 1.05 μg./l. Figures 2 and 3 show the relationship between PSA and female age. Although there was no linear correlation between PSA and age in either female group high PSA concentrations tended to occur in older women (50 years or older).

Serum PSA concentrations were substantially different between women and prostate cancer patients after radical prostatectomy (table 1). No significant difference was observed between the 2 female groups (chi-square 6.53, degrees of freedom 3, $p = 0.09$). Since prostate cancer affects the elderly and since older women tend to have higher serum PSA, PSA levels in the serum of the prostate cancer patients were also compared with PSA levels of women at age 50 years or older (table 3). PSA was still significantly higher in prostatectomized cancer patients than in either female group (chi-square 26.8, degrees of freedom 3 and $p < 0.001$ for normal women, chi-square 46.0, degrees of freedom 3 and $p < 0.001$ for hospitalized women). Again, no difference was seen between the 2 female groups (chi-square 4.0, degrees of freedom 3 and $p = 0.26$).

Figure 4 shows the results of high performance liquid

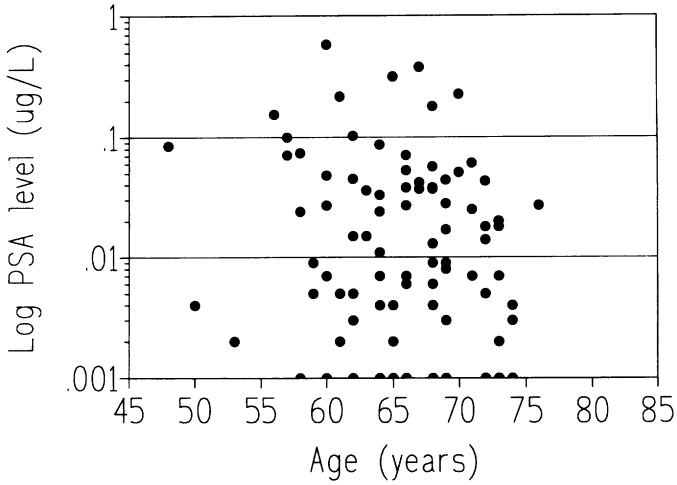

FIG. 1. Serum PSA levels by age in 89 postoperative patients with prostate cancer.

TABLE 2. *Associations between postoperative PSA levels and year of surgery, histological grade and preoperative PSA levels*

	No. (%) Pts. With PSA (μg./l.)				Total No. (%)
	Less Than 0.010	0.010–0.049	0.050–0.099	0.100 or Greater	
Yr. Surgery:					
1988–91	20 (45)	17 (39)	4 (9)	3 (7)	44 (100)
1992–93	23 (51)	10 (22)	5 (11)	7 (16)	45 (100)
Preop. PSA:					
Less than 10	20 (47)	13 (30)	4 (9)	6 (14)	43 (100)
10 or greater	13 (50)	7 (27)	2 (8)	4 (15)	26 (100)
Gleason score:					
Surgery 1988–1993:					
3–6/10	24 (52)	11 (24)	6 (13)	5 (11)	46 (100)
7–9/10	19 (44)	16 (37)	3 (7)	5 (12)	43 (100)
Surgery 1988–1992:					
3–6/10	24 (59)	9 (22)	5 (12)	3 (7)	41 (100)
7–9/10	17 (45)	14 (37)	3 (8)	4 (10)	38 (100)
Surgery 1988–1991:					
3–6/10	12 (57)	6 (29)	2 (10)	1 (4)	21 (100)
7–9/10	8 (35)	11 (49)	2 (9)	2 (9)	23 (100)

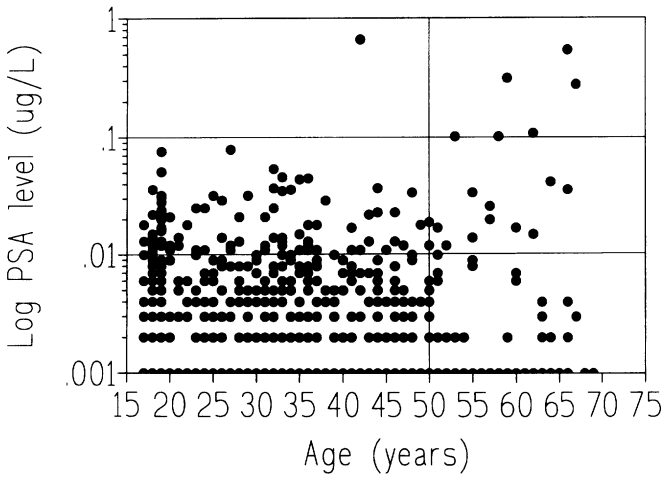

FIG. 2. Serum PSA levels by age in 674 normal women

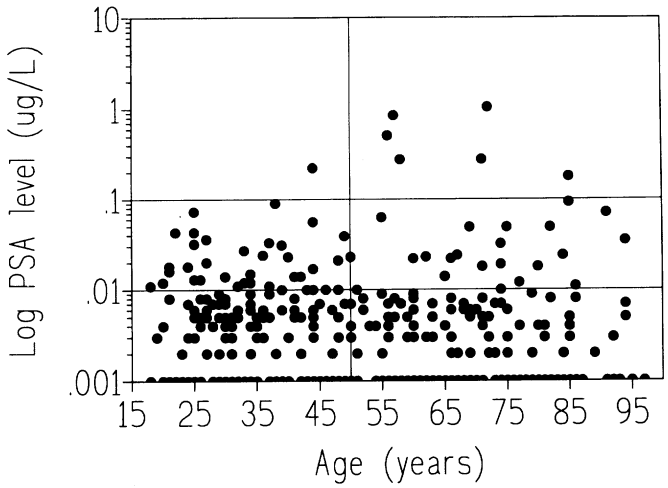

FIG. 3. Serum PSA levels by age in 387 hospitalized women

TABLE 3. *Serum PSA concentrations in postoperative patients with prostate cancer and in normal and hospitalized women age 50 years or older*

Serum PSA (μg./l.)	No. Prostate Ca (%)	No. Normal Women (%)	No. Hospitalized Women (%)
Less than 0.010	43 (49)	83 (81)	178 (86)
0.010–0.049	27 (30)	13 (13)	19 (9)
0.050–0.099	9 (10)	0	4 (2)
0.100 or greater	10 (11)	6 (6)	7 (3)
Totals	89 (100)	102 (100)	208 (100)

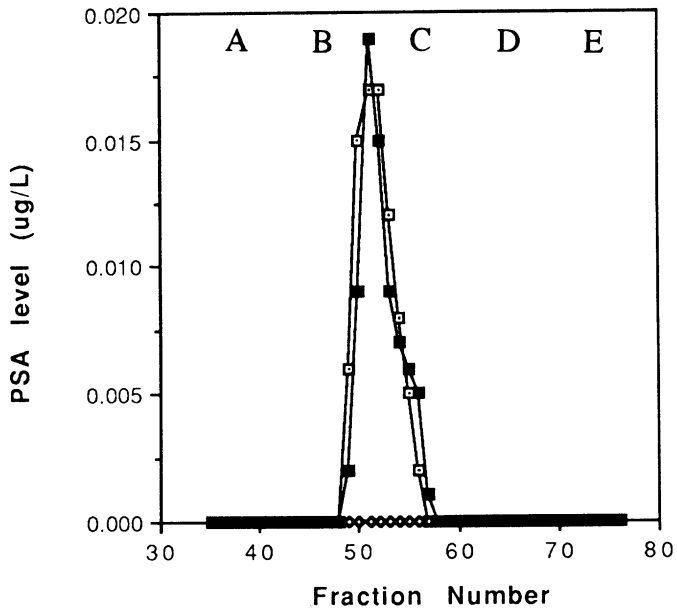

FIG. 4. PSA levels in each high performance liquid chromatography fraction. Lines with closed boxes, open boxes and open diamonds represent 1 male (PSA 0.7 μg./l.) and 2 female (0.5 and less than 0.01 μg./l.) samples, respectively. Fractions that contain gel filtration standard components are thyroglobulin (*A*), gamma globulin (*B*), ovalbumin (*C*), myoglobin (*D*) and cyanocobalamin (*E*) with molecular weights of 670, 158, 44, 17 and 1.4 kDa., respectively.

chromatography separation and analysis of fractions with the PSA assay in 1 male serum sample with a PSA of 0.7 μg./l. and 2 female serum samples with PSA levels of 0.5 and less than 0.010 μg./l. The PSA immunoreactivity in the male sample and in 1 of the female samples (PSA 0.5 μg./l.) was present in the same fraction, which corresponds to a molecular weight of about 100 kDa. No PSA immunoreactivity was detected in any fraction of the other female sample (PSA less than 0.01 μg./l.).

DISCUSSION

In this study PSA in serum of women and post-radical prostatectomy patients was examined at levels not previ-

ously accessible by commercially available techniques. In the PSA range of 0.01 to 0.1 μg./l. substantial differences were observed between women and cancer patients after radical prostatectomy. We found that approximately 50% of post-radical prostatectomy patients, who are free of clinical relapse and have a serum PSA of less than 0.4 μg./l. by a conventional assay have undetectable serum PSA as measured by our ultrasensitive technique (PSA less than 0.01 μg./l.). About 30% of patients have a PSA between 0.01 and 0.049 μg./l. The remaining patients (20%) had a PSA of 0.050 μg./l. or greater. In women only 2 to 4% of the sera had PSA levels of 0.050 μg./l. or greater (table 1).

We do not have as yet enough serial samples from the post-prostatectomy patients to monitor PSA changes with time. We speculate that the presence of PSA in post-prostatectomy patients may be due to the fact that they have residual disease or clinically undetectable recurrent or metastatic lesions, or for some postoperative patients a PSA concentration greater than 0.01 μg./l. represents the physiological level without the presence of a prostate. The traces of PSA in these patients may come from other tissues and may not be associated with a recurrent or metastatic event. Based on recent studies, it is highly unlikely that the prostate gland is the only tissue capable of producing PSA.[13–15] However, some evidence also indicates that PSA from other tissues may not be necessarily secreted into the blood circulation. An open question that can be addressed with studies involving long-term monitoring of patients using an ultrasensitive PSA assay is whether the group of patients with postoperative PSA greater than 0.01 μg./l. will have a higher risk of recurrence or metastasis and shorter overall survival time than those whose PSA is less than 0.01 μg./l.

In this study we found that in 50% of the postoperative patients PSA declined to extremely low levels, that is less than 0.01 μg./l. If patients from this group eventually have relapse our assay could be valuable for efficient monitoring. Provided that the observations and calculations by Schmid et al are applicable to these patients[16] and conservatively assuming that the average doubling time of prostate cancer cells is approximately 24 months, the recurrent or metastatic lesion could be identified, on average, at least 1 year earlier by this method than by other conventional methods. A recent study has already suggested that recurrence could be identified about 1 year earlier using a PSA assay with a detection limit of 0.1 μg./l. instead of 0.4 μg./l.[17] However, it is still not known how many patients who could be identified as having biochemical relapse by an ultrasensitive assay will live long enough for a clinical relapse to develop.

Early identification of relapse is a critical issue in the treatment of postoperative patients. Failure to cure has been thought to be partially attributed to the late discovery of the recurrent or metastatic lesions.[10] Usually, these lesions are small when they are identified earlier, and small lesions may respond better to adjuvant therapy.[9,10] In addition, with small lesions the treatment dosage can be reduced and the improved tolerance will ensure longer continuation of treatment.

Since poorly differentiated cancer cells are more likely to metastasize, the histological grade of prostate cancer should be related to the postoperative PSA levels in patients with metastatic lesions. It has been found that high histological grade was associated with the elevation of postoperative PSA.[18] We did not observe any significant difference in postoperative PSA levels between patients with high and low histological grade. However, more patients (52%) with Gleason scores 3 to 6 had a PSA of less than 0.01 μg./l. compared to those with scores 7 to 9 (44%). When we stratified the patients according to the year of surgery we found that the difference increased (table 2). For patients who were operated on between 1988 and 1991 and who had a postoperative PSA of less than 0.01 μg./l., 57% had Gleason scores 3 to 6 and 35% had Gleason scores 7 to 9. However, this difference did not reach statistical significance (table 3). Serially measuring serum PSA levels in these patients for a long time could determine if those with high Gleason score are associated with high levels of postoperative PSA and higher risks for relapse.

PSA in the serum of women has been measured in several studies mainly to verify if such sera generally give negative values.[5,11] To our knowledge no study has examined PSA levels in large numbers of normal and hospitalized women. In this study we provide a relatively detailed profile of serum PSA levels in women. The 2 distinct female populations, hospitalized and healthy, have a similar distribution of PSA, that is 82 to 83% of women having undetectable PSA in the serum and about 14 to 15% having levels between 0.01 and 0.049 μg./l. Only a few subjects (27 of 1,064 or 2.5%) had a PSA of greater than 0.05 μg./l.

The PSA immunoreactivity in female serum has been suspected to be due to cross-reactivity or nonspecific binding effects. However, new evidence supports the existence of PSA in women.[19] It has been reported that the periuretheral gland in women has prostate-like structure and is positive for PSA staining.[20] No study has yet investigated the molecular weight of the immunoreactive PSA species in female serum because of the low concentrations involved. In our study we examined the molecular weight of the immunoreactive species using high performance liquid chromatography and compared it to PSA in male serum. PSA reactive species in male and female sera presents in the high performance liquid chromatography fractions corresponding to a molecular weight of approximately 100 kDa. This high molecular weight species represents PSA bound to α1-antichymotrypsin and is the major form of circulating serum PSA. No free PSA was detected presumably because of the relatively low levels of total PSA in the female sera used.

Although there is no linear correlation between PSA level and age, graphical representation of PSA levels in female sera by age (figs. 2 and 3) revealed that the majority of women with relatively high PSA (greater than 0.1 μg./l.) were older than 50 years. Of the 1,064 women 310 were 50 years or older, including 13 of 16 women with a PSA of 0.10 μg./l. or greater. The percentage of women with a PSA of 0.10 μg./l. or greater in the 2 age groups (50 years or older, or less than 50 years) were 4.2% and 0.40%, respectively. This phenomenon may indicate that the menopausal status of these women has a role in the production of PSA. PSA production is known to be upregulated by androgen and suppressed by estrogen. After menopause the ratio of estrogen to androgen declines, a situation that may favor PSA production.

We used a new, highly sensitive assay to examine serum levels of PSA in prostate cancer patients after radical prostatectomy and in women. The demonstration of PSA in a significant portion of female sera strengthens the possibility that it may be produced and released by nonprostatic tissue. We speculate that the menopausal status may be associated with the high serum level of PSA in women. Our results suggest that female serum should not be regarded as a PSA-free medium, since some sera have PSA levels comparable to those found in men. It was also demonstrated in the study that about 50% of post-prostatectomy male sera contain measurable amounts of PSA. The significance of this finding should be further examined in long-term followup studies of these patients with serial measurement of serum PSA level. Such studies will allow us to determine if the measurable PSA is released from nonprostatic or remaining normal prostatic tissue, or from residual tumor tissue. In the former case PSA levels should remain stable with time. However, in the latter case PSA levels should increase as tumor cells proliferate. It will also be interesting to investigate if the proliferative potential of residual tumors could be calculated by measuring the changes in PSA levels in the ultrasensitive

range with time and if the proliferative potentials are different between patients.

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