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PROSTATE-SPECIFIC ANTIGEN AS A SERUM MARKER FOR ADENOCARCINOMA OF THE PROSTATE

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Abstract To compare the clinical usefulness of the serum markers prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP), we measured them by radioimmunoassay in 2200 serum samples from 699 patients, 378 of whom had prostatic cancer.

PSA was elevated in 122 of 127 patients with newly diagnosed, untreated prostatic cancer, including 7 of 12 patients with unsuspected early disease and all of 115 with more advanced disease. The PSA level increased with advancing clinical stage and was proportional to the estimated volume of the tumor. The PAP concentration was elevated in only 57 of the patients with cancer and correlated less closely with tumor volume. PSA was increased in 86 percent and PAP in 14 percent of the patients with benign prostatic hyperplasia.

After radical prostatectomy for cancer, PSA routinely fell to undetectable levels, with a half-life of 2.2 days. If initially elevated, PAP fell to normal levels within 24 hours but always remained detectable. In six patients followed postoperatively by means of repeated measurements, PSA — but not PAP — appeared to be useful in detecting residual and early recurrence of tumor and in monitoring responses to radiation therapy.

Prostate massage increased the levels of both PSA and PAP approximately 1.5 to 2 times. Needle biopsy and transurethral resection increased both considerably.

We conclude that PSA is more sensitive than PAP in the detection of prostatic cancer and will probably be more useful in monitoring responses and recurrence after therapy. However, since both PSA and PAP may be elevated in benign prostatic hyperplasia, neither marker is specific. (N Engl J Med 1987; 317:909-16.)

IN 1979, after injecting crude extracts of human prostatic tissue into rabbits, Wang et al. isolated and purified a glycoprotein specific for prostatic epithelial cells.[1] Simultaneously and independently, Graves et al., using acrylamide gels, isolated and characterized the same protein in seminal plasma.[2] More recently, Lilja has shown that this prostate-specific antigen (PSA) is a serine protease, belonging to the family of glandular kallikreins, and that its physiologic substrate is the predominant protein of the seminal-vesicle coagulum.[3] The sequence of the 240 amino acid residues forming the single polypeptide chain of PSA has been determined.[4]

In a series of papers from the Roswell Park Memorial Institute (Buffalo, N.Y.), this antigen has been extensively studied as a potential serum marker in patients with prostatic cancer. In their early studies, using an enzyme-linked immunosorbent assay sensitive to 0.1 ng of PSA per milliliter of serum, the Roswell Park group established that in 51 "normal" men, the mean serum value was 0.47±0.66 ng per milliliter (range, 0 to 2.6), and that in patients with prostatic cancer, the mean level was proportional to the clinical stage of the disease.[5] In two recent reports of patient samples obtained from the National Prostate Cancer Project and studied with a double-antibody radioimmunoassay (Pros-Check PSA) developed by one of us (N.Y.), an association with a high level of significance ($P = 0.0002$) was found between serially measured levels of serum PSA and the duration of survival without disease[6]; when the PSA level measured with this assay was compared with levels of other commonly used markers for prostatic tumor, it was the most reliable prognostic indicator of cancer.[7]

Despite these encouraging reports, PSA has not been accepted as a marker for prostatic cancer. Patients were not always separated into treated and untreated groups.[8] Moreover, the observation of elevated serum PSA in the presence of benign prostatic hyperplasia led the Roswell Park investigators to increase the specificity of PSA as a cancer marker (excluding benign prostatic hyperplasia), although this sharply curtailed its sensitivity. For example, they increased their upper limit of normal serum PSA from 2.6 ng per milliliter in 1980[5] to 7.5 ng per milliliter in 1982[8] and later to 10 ng per milliliter.[9]

We have determined the levels of PSA and prostatic acid phosphatase (PAP) in 2200 serum samples from 699 patients, including 1800 samples from more than

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540 patients with prostatic disease, 378 of whom had prostatic cancer. In this paper we report our observations that (1) the PSA concentration determined by radioimmunoassay (the Yang assay) is proportional to the volume of both the intracapsular and extracapsular tumor in untreated prostatic cancer, (2) the concentration is also proportional to the volume of hyperplastic tissue in benign prostatic hyperplasia without prostatic cancer, and (3) the assay is very useful in detecting residual and recurrent disease in patients undergoing radical prostatectomy, radiation therapy, or bilateral orchiectomy.

Methods

PSA and PAP Determination

Blood samples were obtained before rectal examination and processed without delay; serum aliquots were stored at −20°C until assayed.

PSA and PAP concentrations in serum specimens were measured with Pros-Check kit reagents (Yang Laboratories, Bellevue, Wash.).[10,11] Both systems are double-antibody, polyclonal radioimmunoassays based on the principles of competitive binding as described by Yalow and Berson.[12] The performance characteristics of these assays, including their interassay and intraassay precision, have been reported elsewhere.[6,10,11,13]

All statistical variances, with one exception, are reported as standard deviations.

Clinical Stages and Gleason Grading System

The clinical staging system used throughout this study is presented in Table 1.

The Gleason grading system was used for histologic assessment.[14] We report the sum of the primary and secondary Gleason patterns (for example, 4+3). If the entire prostate was available for histologic review, for greater accuracy we determined the percentage of Gleason patterns 3, 4, and 5 present throughout the malignant tumor.

Specimens Obtained at Radical Prostatectomy

Serum PSA and PAP were measured before total prostatectomy in 45 patients. Each prostate was weighed and reconstructed in 3-mm transverse sections perpendicular to the rectal surface; the volume of the cancer was determined in cubic centimeters as previously described.[15] The percentage of total cancer represented by each of the five Gleason patterns was estimated. From this value the percentage of the tumor that was Gleason patterns 4 and 5 was calculated. The outer-surface area of tumor penetrating beyond the prostatic capsule (level III penetration) was then estimated.[15] The presence or absence of seminal-vesicle invasion was determined in each patient. The tissue volume of macroscopic nodular benign prostatic hyperplasia was estimated (in square millimeters) by measuring the transverse section containing the largest cross-sectional area of hyperplastic tissue in each lobe.

Data were transformed logarithmically if skewed distribution or heteroscedasticity was observed. If values for variables included zero, a constant (0.25) was added to each value before logarithmic transformation, as described by Mosteller and Tukey.[16] Three patients for whom one or more values were missing were excluded from regression analysis.

Table 1. Classification for Clinical Staging of Prostatic Cancer.

Stage	Definition
A	Incidental or unsuspected cancer*
A1	≤5% of tissue (Gleason grade ≤7)
A2	>5% of tissue, or any Gleason grade 8–10
B1	Palpable nodule extending into ≤50% of one lobe
B2	Palpable node in Stage >B1, limited to one lobe
B3	Bilaterally palpable cancer
C	Palpable cancer extending into one or both seminal vesicles
D1	Lymph nodes confirmed as positive by pathological examination

Half-Life of PSA

The half-life of PSA was determined in blood drawn from 14 patients within five minutes after the whole prostate had been removed (time zero), at 1, 3, 6, 12, and 24 hours, and every 24 hours thereafter for five to seven days. Serum PSA was then measured at follow-up clinic visits. For each patient the natural log of the PSA concentration (nanograms per milliliter) was plotted against time. In all cases the data fit a two-compartment model of first-order elimination kinetics, in which the plot of the natural log for PSA over time yielded a biphasic straight line. According to this model, the slope of each phase of this line (the alpha and beta phase) equaled the respective elimination constant (K_{PSA}, or the fraction of PSA eliminated per unit of time). These slopes were determined by performing linear regression on all points within each phase of the plot for each patient. Each alpha and beta half-life ($t_{1/2}$) was derived from the equation, $t_{1/2} = 0.693/K_{PSA}$.

Benign Prostatic Hyperplasia

Seven patients underwent simple retropubic prostatectomy for obstructive benign prostatic hyperplasia and 90 had transurethral resection of the prostate. Cancer had not been suspected on rectal examination in any patient. PSA and PAP were measured in all patients on admission to the hospital and in most of them at postoperative evaluation. To exclude incidental cancer in the specimens, all tissue obtained at transurethral resection was studied histologically, regardless of the volume of tissue resected. In the retropubic enucleations, additional tissue from the outer prostate was sampled by either perineal needle biopsies or multiple intracapsular biopsies immediately after enucleation. The enucleated hyperplastic tissue was step-sectioned at intervals of 2 to 3 mm and carefully examined for any gross evidence of cancer; multiple large sections were embedded for histologic study.

Results

PSA and PAP Levels in Normal Subjects

The mean (±SD) PSA concentration in 157 "normal" men 21 to 76 years old was 1.1±0.7 ng per milliliter in serum obtained before rectal examination. These men had been selected from an executive health-screening program after exclusion of potential subjects for the following reasons: abnormal results on rectal examination, including benign prostatic hyperplasia; any history of previous prostatic disease; any urinary symptoms; or abnormal results on urinalysis. The PSA concentrations of five men were more than 3 SD above the mean for the group (i.e., 3.2, 3.3, 3.6, 3.8, and 4.7 ng per milliliter) and thus were excluded from calculation of the mean. PSA was not detected (0.0 ng per milliliter) in 15 of the 157 subjects; 5 of these men belonged to the youngest age group (13 men 21 to 30 years old). The results of PSA and PAP meas-

Table 2. Serum Concentrations of Prostate-Specific Antigen (PSA) and Prostate Acid Phosphatase (PAP) in 157 "Normal" Men, According to Age.

Age Group	No. of Subjects	PSA	PAP
		nanograms per milliliter (means ±SD)	
21–30	13	0.654±0.659	0.969±0.404
31–40	36	1.203±0.654	1.002±0.543
41–50	51	1.192±0.651	1.308±0.530
51–60	44	1.197±0.731	1.107±0.397
61–76	13	1.154±0.683	1.300±0.383
21–76	157	1.148±0.686	1.157±0.490
Observed range		0–3.1	0–2.3
Normal range (mean ±2 SD)		0–2.5	0–2.1

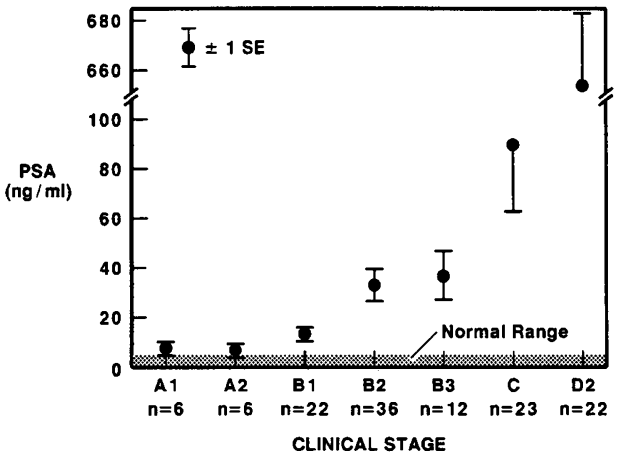
the normal men was 1.2±0.5 ng per milliliter, and the normal range was 0 to 2.1 ng per milliliter.

Reproducibility of PSA and PAP Levels in Patients

Levels of PSA and PAP were measured in two consecutive serum samples obtained within six weeks from 31 ambulatory patients among 127 with newly diagnosed prostatic cancer; during this period, no intervening treatment or prostate manipulation was performed in these 31 patients. In the second sample, the PSA level had increased by 2±22 percent and the PAP level by 2±33 percent. These increases were not significant, as indicated by the P values for any differences from a ratio of 1.00 for the two serum values (P values calculated by the log of the difference between the first and second samples and a one-tailed t-test).

PSA Levels in Untreated Stage A1 to D2 Cancer

We determined serum concentrations of PSA and PAP in the 127 patients with prostatic cancer, all of whom were untreated. Figure 1 shows the mean PSA level (±SEM) at different clinical stages of the disease. Serum PSA appeared to increase with advancing clinical stage. Levels were abnormal (>2.5 ng per milliliter) in 122, or 96 percent, of these patients, including 7 of 12 patients with Stage A disease and all of 115 in Stages B through D. In patients in Stage A, PSA was measured after transurethral resection, which by definition removes part and sometimes all of the cancer. This may have accounted for the relatively low levels of PSA among some of the patients with Stage A disease.



In comparison, serum PAP was elevated (>2.1 ng per milliliter) in only 45 percent (57) of the patients, including none of the 12 in Stage A, 9 percent of those in Stage B1, 39 percent of those in B2, 40 percent of those in B3, 64 percent of those in C, and 96 percent of those in D2. In a comparison on a nanogram-per-milliliter basis, serum PSA was 5 times higher than PAP in Stage A disease, 8 to 16 times higher in Stage B disease, 15 times higher in Stage C disease, and 11 times higher in Stage D2 disease.

PSA Levels in Relation to Tumor Volume within the Prostate

Multivariate regression was used to investigate the relation of the variables in Table 3 to concentrations of PSA and PAP in ambulatory patients before operation. With the log of PSA as the dependent variable, the multiple R-square value was 0.74, whereas with the log of PAP as the dependent variable, the multiple R-square was 0.44. The regression coefficients, t-statistics, and P values for each variable in the regression against the log of PSA are shown in Table 3. The log of cancer volume was the best predictor of the log of PSA, with only a minor contribution from the log of prostate weight and the presence of seminal-vesicle invasion. When the variables were adjusted for cancer volume (all variables, both independent and dependent, were replaced by their residuals after simple linear regression against the log of cancer volume)[16] and regressed with the log of PSA as the response, the multiple R-square fell to 0.35. When all variables were adjusted for the log of cancer volume, no significant collinearity was seen. In the multiple regression against the log of PAP, the significant variables were the log of the Gleason score, the log of cancer volume, and the percentage of Gleason patterns 4 and 5. When the variables were adjusted for cancer volume, none were significant.

Since the relation between cancer volume and the PSA level appeared to be strong, but the relation between cancer volume and the PAP level did not, we investigated the usefulness of combining the preoperative levels of PAP and PSA to predict cancer volume. A multiple regression analysis of the log of PAP and the log of PSA was carried out with the log of cancer volume as the response. All change in cancer volume was related only to change in the log of PSA. There was no significant contribution from the log

Table 3. Multiple Regression Analysis of PSA as a Dependent Variable and Selected Independent Variables.*

Independent Variable	Coefficient	t-Ratio	P Value†
Log cancer volume in cubic centimeters	0.59	4.32	<0.001
Log prostate weight in grams	0.63	1.93	0.1
Seminal-vesicle invasion	0.64	1.88	0.1
Percentage Gleason patterns 4 and 5	0.01	1.66	0.2
Age	−0.02	−1.46	0.2
Log hyperplastic tissue in benign prostatic hyperplasia	0.13	1.38	0.2
Log level-3 capsular penetration	−0.13	−0.97	>0.2
Gleason score, as sum	−0.03	−0.24	>0.2
Intercept	0.62	0.44	>0.2

*Multiple R-square = 0.74, n = 42, degrees of freedom = 33.
†By two-tailed t-test.

volume is shown in Figure 2. Prediction lines (95 percent confidence limits) show the range of cancer volumes that a given preoperative PSA level would reflect. Five of the 45 patients undergoing total prostatectomy had values within the normal range. All five had had transurethral resections (which reduced cancer volumes) before PSA measurement and prostatectomy. The volumes of residual cancer were found to be 0.0, 0.0, 0.1, 1.2, and 1.7 cc at total prostatectomy. By contrast, 36 of the 46 patients had PAP values within the normal range; their cancer volumes ranged from 0 to 19.3 cc.

Effect of Total Prostatectomy on PSA, PAP, and Total Serum Acid Phosphatase

Change at Three Months

Figure 3 compares the concentrations of PSA, PAP, and total serum acid phosphatase (measured with the Bessey–Lowry enzymatic assay[17,18]) immediately before and three months after radical prostatectomy in our first 16 patients in whom PSA was prospectively measured before and after operation. Only PSA fell to undetectable levels, indicating the degree of nonspecificity of the two assays for PAP. Although values for total serum acid phosphatase measured with the Bessey–Lowry assay failed even to reflect the removal of the prostate and the cancer within it, the assay for serum PAP appeared more sensitive to total prostatectomy, since levels in 13 of the 16 patients decreased after operation; however, PAP levels had been elevated before prostatectomy in only 6 patients.

PSA Half-Life and Elimination

Elimination of PSA from the serum followed a biphasic logarith- (alpha phase) had a half-life of 12.6±19.7 hours; this half-life was in effect from time zero (prostatectomy) to 6 hours after operation. The second, beta phase had a half-life of 2.2±0.8 days. Since this half-life was in effect at 12 hours after operation and afterward, it was the main determinant of PSA elimination. Serum PSA was undetectable in most patients by the 14th day after total prostatectomy.

By contrast, PAP fell to normal levels within 24 hours of removal of the prostate, even in patients with residual disease, in whom PSA remained elevated at three weeks.

Effect of Residual and Recurrent Disease on PSA and PAP Levels

Radiation was directed at the pelvis in four patients in whom serum PSA was still detectable at three weeks after radical prostatectomy (7.6, 6.0, 3.4, and 1.7 ng per milliliter at surgical Stage C, D1, D1, and C, respectively). At follow-up 9 to 44 months after radiotherapy, PSA levels were repeatedly found to be undetectable in three patients. In the fourth patient, who had Stage D1 cancer with a volume of 42 cc, the PSA value was 1.7 ng per milliliter after prostatectomy, persisted at this level for 64 days, fell to 0.7 ng per milliliter two months after radiation, but gradually returned to 1.7 ng per milliliter over the following seven months.

Serial determinations of PSA after radical prostatectomy were equally useful in patients in whom serum PSA was undetectable in the early months after surgery but later became detectable. Two patients, in clinical Stages B2 and A2 with cancer volumes of 1.5 and 3.3 cc and capsular penetration into the peripros-

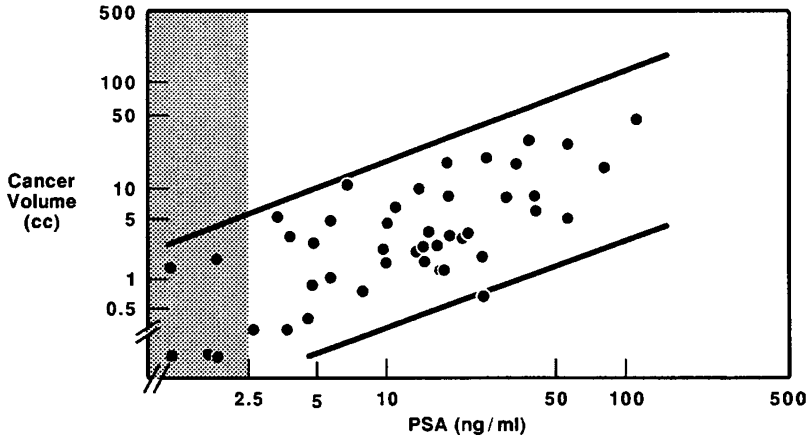


Figure 2. Preoperative Serum Concentration of PSA in Relation to Cancer Volume in

tatic fat, first had detectable PSA (1.3 and 0.4 ng per milliliter) at 12 and 9 months after surgery. They both received 6000 rad of radiation to the pelvis at 16 months after surgery, when their PSA levels were 4.2 and 1.0 ng per milliliter. Levels had become undetectable in both within four months after radiotherapy was completed.

In all six of these patients, all staging methods, including the radioimmunoassay for PAP, computerized tomography, magnetic resonance imaging, bone scanning, and rectal examination, indicated that prostatic cancer was not present.

Effect of Benign Prostatic Hyperplasia on PSA and PAP Levels

Table 4 shows the preoperative and postoperative serum concentrations of PSA and PAP in the seven patients who underwent simple retropubic prostatectomy for benign prostatic hyperplasia. Only one patient (No. 329) was found to have a few microscopic foci of prostatic cancer within nodular hyperplastic tissue in the right prostatic lobe, which had a total volume of about 0.16 cc (Gleason 3+2); these foci were much too small to account for the increase in serum PSA. At surgery, multiple intracapsular biopsy specimens (total, 10 g) were removed from each quadrant of the remaining prostate; all the specimens were negative for cancer. The data in Table 4 present evidence of the elevation of serum PSA (and PAP) in benign prostatic hyperplasia. The levels of PSA in the seven patients ranged from 9.5 to 44 ng per milliliter. PSA, but not PAP, remained elevated at 24 hours and at seven days after prostatectomy. In every patient the PSA level fell below 2.5 ng per milliliter by three weeks after enucleation. PSA and PAP measurement was repeated in all patients 12 to 61 weeks after simple prostatectomy; no patient had elevated levels.

Of 90 consecutive patients undergoing transurethral resection of the prostate for benign prostatic hyperplasia, 17 (19 percent) also had Stage A prostatic cancer; these patients were excluded from the analysis of the effect of the hyperplasia. In the other 73 patients, the weight of the resected tissue varied from 6 to 104 g (mean, 29±19). Preoperative levels of PSA varied from 0.3 to 37 ng per milliliter (mean, 7.9±7.1). Levels were elevated in 63 patients (86 percent) but normal (<2.5 ng per milliliter) in 10, in whom 6 to 36 g had been resected. Postoperatively, levels varied from 0 to 6.7 ng per milliliter (mean, 1.3±1.5). PAP was elevated in 10 of the 73 patients (14 percent), returning to normal levels (<2.1 ng per milliliter) after resection. Levels were elevated only in patients in whom at least 40 g of prostatic tissue had been resected.

The elevation in serum PSA associated with benign prostatic hyperplasia in the patients undergoing transurethral resection was 0.31±0.25 ng per milliliter per gram of hyperplastic tissue; in the seven patients undergoing retropubic prostatectomy (Table 4), it was 0.29±0.09 ng per milliliter per gram of tissue.

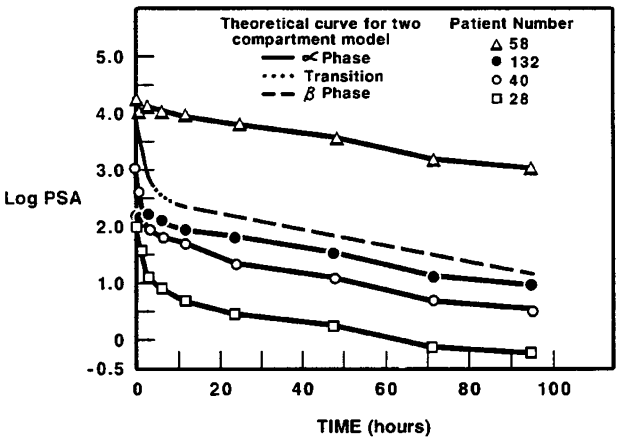


Figure 4. Serum PSA Values (Natural Log) in Four Representative Patients after Prostatectomy.

The alpha-phase half-life is 12.6±19.7 hours, and the important beta-phase half-life is 2.2±0.8 days. PSA should be undetectable three weeks after radical prostatectomy unless residual cancer is present.

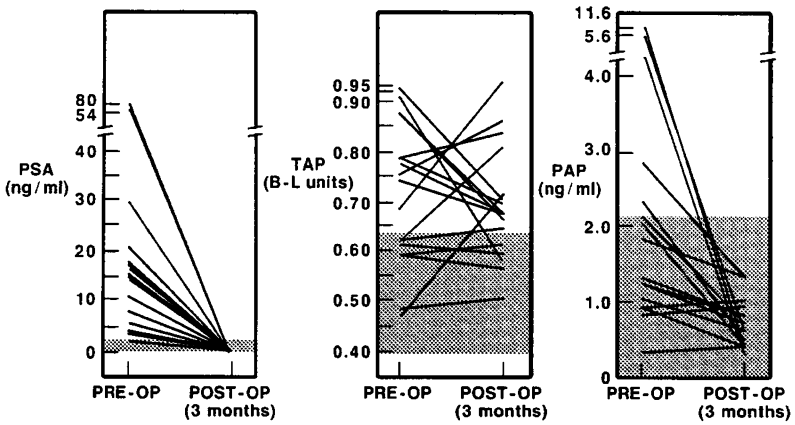


Figure 3. PSA, Total Serum Acid Phosphatase (TAP), and Prostatic Acid Phosphatase (PAP) before and after Radical Prostatectomy.

Effect of Prostate Massage, Perineal Biopsy, and Transurethral Resection

Serum levels of PSA and PAP were determined in 60 patients before and one minute after massage of the prostate, including 7 patients undergoing massage at the time of cystoscopy. In general, levels of both PSA and PAP in post-massage serum samples were increased 1.5 to 2 times; the highest increase oc-

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