

Perspectives on Serum Acid Phosphatase in Prostatic Disease

An evaluation of two methods

S. Dedorson,¹ Å. Fritjofsson,¹ B. J. Norlen¹ and G. Ronquist²

*From the Departments of Urology¹ and Clinical Chemistry,²
University Hospital, Uppsala, Sweden*

ABSTRACT

Acid phosphatase in serum was measured in 116 patients with prostatic disease, benign in 59 and malignant in 57 cases. Comparisons were made between radioimmunoassay (RIA) and an enzymatic method. The correlation coefficient between the respective values was 0.96 in patients with untreated prostatic cancer, indicating that no significant difference between results with the two methods was to be expected. The correlation coefficient between RIA values and cancer stage was 0.48, and between catalytic activity and cancer stage it was 0.50. The validity of the two methods consequently was equal. RIA, however, was the more sensitive method, giving elevated values in 10 of 11 patients with untreated stage III or stage IV prostatic cancer, as compared with only 4 of the same 11 in the enzymatic assay. This seeming paradox most probably was attributable to differing intrinsic properties of the methods when the upper limits of normal range were established. Neither RIA nor enzymatic analysis discriminated early prostatic cancer (stages I and II) from benign lesions.

INTRODUCTION

Measurement of acid phosphatase in serum has been employed for more than 40 years to detect and monitor malignant prostatic disease (8). The prostate gland is the main, but not the sole source of this enzyme in man. Research on enzymatic assay of acid phosphatase has therefore been focused on making the procedure more prostate-specific. Refinements in substrates and inhibitors for the reaction, however, have not so far led to absolute organ specificity.

The enzymatic activity of the acid phosphatase molecule is sensitive to pH, with optimum at pH 5.5. The molecule dissociates into its two subunits at pH 2.0 or less and also at pH 7.4 or higher. The dissociation results in inactivation of the enzyme, and the two subunits immediately begin to aggregate (4). Inherent difficulties thus limit the usefulness of enzymatic assay of acid phosphatase.

A competitive-binding, 'labelled antigen' technique for isotopic assay of PAP (prostatic acid phosphatase) was therefore evolved as an alternative procedure (6). Such assay offered improvements with regard to prostate-specificity, stability, sensitivity and precision (7). Despite controversy on particular aspects of radioimmunoassay for PAP, reports have continued to emphasize the advantages of this principle over the enzymatic technique (5,9,13).

The aims of the investigation here presented were to measure PAP concentrations in sera from patients with benign or malignant prostatic disease and to compare results from radioimmunoassay (RIA) with those from a conventional enzymatic procedure for determining PAP activity.

PATIENTS AND METHODS

Radioimmunoassay

All radioimmunoassays of PAP were run in duplicate, using 0.1 ml serum samples and a commercially available RIA kit (GammaDab^R, Clinical Assays, Travenol Laboratories, Cambridge, Mass., USA) in accordance with manufacturer's instructions. After incubation and centrifugation, however, it was found necessary to remove traces of supernatant fluid in the tubes, using filter paper, before making counts of the sedimented radioactive material in the tubes.

Enzymatic assay

The catalytic activity of serum acid phosphatase was measured as recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology using paranitrophenyl phosphate as substrate with L(+)-tartrate as inhibitor. The upper limit of the reference range for this method is $58 \text{ nkat} \cdot \text{l}^{-1}$.

Patients

The studies were made on 116 patients consecutively referred to this department of urology because of prostatic disorder. Prostatic cancer was found in 57 of the men and benign prostatic disease in 59. Table I surveys the numbers of patients and their mean age distribution according to diagnosis.

Table 1 Distribution of patients according to age and diagnosis

Diagnosis	No of patients	Mean age (years)
Prostatic cancer	57	72.6
Stage I	3	79.0
Stage II	15	74.4
Stage III	23	73.3
Stage IV	16	68.7
Benign prostatic disease	59	63.3
Hyperplasia	39	68.1
Prostatitis	13	50.8
Other benign process	7	59.1

In all cases of prostatic cancer the diagnosis was confirmed by transrectal aspiration biopsy for cytological study. Intravenous pyelography and X-ray examination of the lungs were also performed. Bone scan was done in all but two patients of this group (1 with stage II and 1 with stage IV tumour), with skeletal roentgenography when indicated. Since PAP- concentration was the issue under study, it was not included in the clinical staging of malignancy, which otherwise was in accordance with recommendations by the Veterans Administration Cooperative Urological Research Group (12): Stage I thus implied clinically unsuspected cancer in a transurethral resection specimen, stage II a palpable nodule confined to the prostate gland, stage III periprostatic growth of the tumour and stage IV known distant metastases, irrespective of PAP level in the serum and findings at rectal examination.

Of the 57 patients with prostatic cancer, 20 were untreated at the time of the study. The treatment in the other 37 patients varied - orchiectomy, oestrogen/chemotherapy (estramustin, Estracyt^R) or irradiation, alone, in combinations or in succession. The duration of therapy and the response were highly variable at the time of the investigation.

In the group of 59 patients with benign prostatic disorders, histological or cytological examination was done only if surgery was performed or if the clinical findings aroused suspicion of malignancy.

Statistical methods

A Hewlett-Packard 9820 A calculator, Model 20 with standard programmes was used for registering data and for the statistical analyses.

The reliability of RIA was measured as the correlation coefficient between the duplicate sets of values (n/set = 116). The validity of the respective methods was assessed in the patients with untreated prostatic cancer (n=20) as the correlation between cancer stage and PAP concentration according to RIA or to the enzymatic assay (Fig. 3). The validity was additionally calculated as correlation between

results with the two methods in the 59 patients with benign prostatic disease (Fig.1), in the total 57 with prostatic cancer (Fig.2), and in the 20 patients with untreated prostatic cancer (Fig.3). For calculation of correlation coefficients and testing of significance, we used the methods described by Snedecor & Cochran (10).

The sensitivity of the method was judged from the proportion of elevated readings among the patients with prostatic cancer. The specificity was calculated as the proportion of non-elevated readings in patients with benign prostatic disease. The significance of differences in sensitivity and specificity was calculated with the z test (one-tailed, with correction for continuity) as described by Snedecor & Cochran (10).

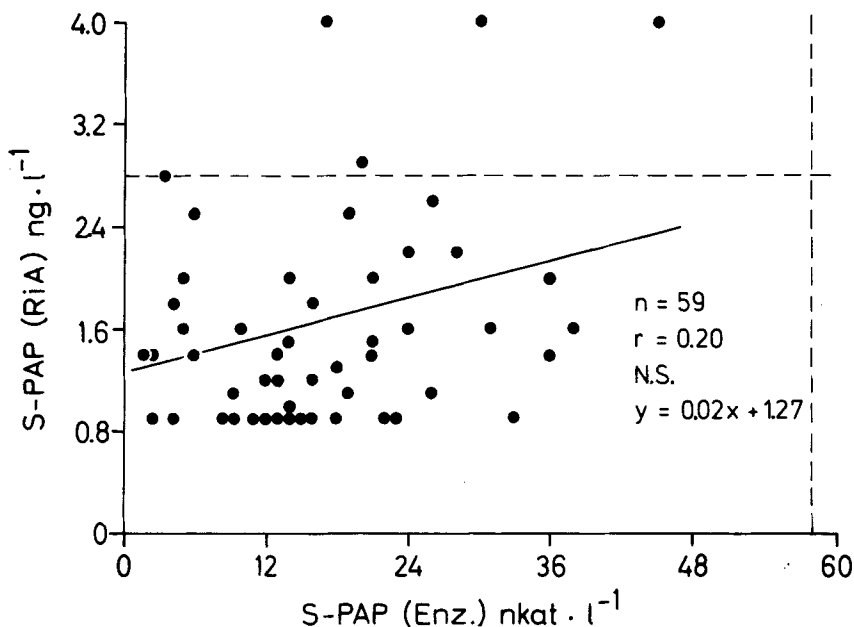


Fig.1 Correlation between values from enzymatic analysis (Enz.) and from radioimmunoassay (RIA) of serum phosphatase (S-PAP) in patients with benign prostatic disease.

_____ Regression line
 - - - - - Upper limit of normal range

RESULTS

To determine the upper normal limit for RIA results, PAP was measured in serum sampled from 58 male blood donors. The mean value was $1.20 \mu\text{g} \cdot \text{l}^{-1}$, S.D. 0.80. The upper limit of the reference range therefore was set at $2.80 \mu\text{g} \cdot \text{l}^{-1}$. The values from enzymatic analysis showed a weak positive correlation ($r=0.20$) with the RIA values in the patients with benign prostatic disorders, despite the relatively narrow range of readings in that group (Fig 1). The correlation between the two variables was appreciably better among the 57 patients with malignant disease (Fig 2), and it was particularly high (0.96) in the 20 patients with untreated prostatic cancer (Fig 3).

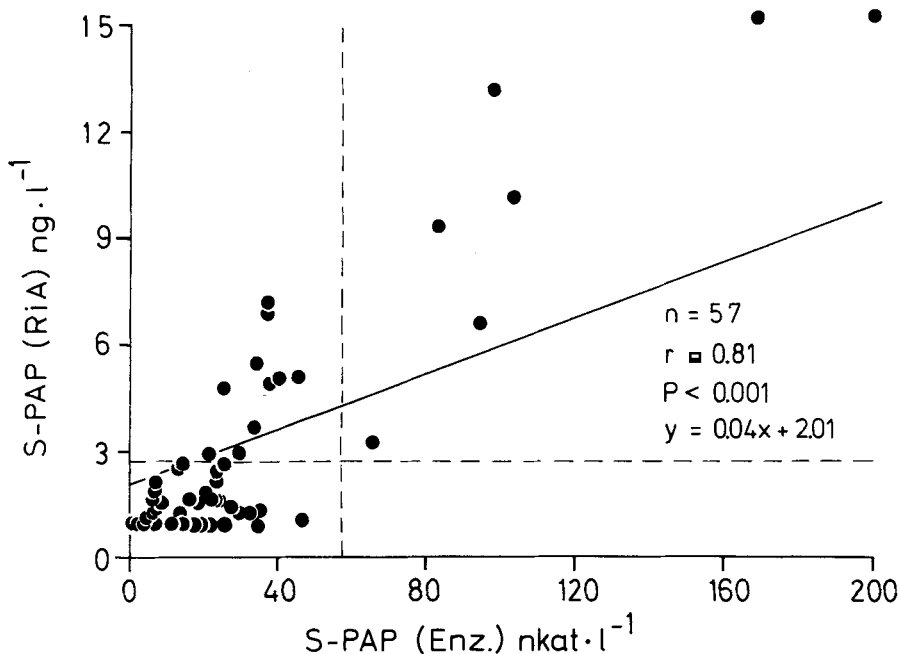


Fig 2. Correlation between Enz. and RIA values in all patients with prostatic cancer.

————— Regression line
 - - - - - Upper limit of normal range

The correlation between the duplicate runs of RIA ($r=0.997$) expressed high reliability of the method. The stage of cancer, moreover, showed significantly ($p<0.05$) positive correlation with results from enzymatic analysis ($r=0.50$) and RIA values ($r=0.48$). Between the two methods, therefore, no difference in validity was detectable.

RIA gave elevated values in 4 of 59 patients with benign prostatic disease (Fig 1, Table 2). The enzyme tests showed no elevated values in the same group. The specificity, i.e. the ability to recognize benign nature of prostatic lesions, thus was lower with RIA (0.93) than with enzymatic assay (1.00), though the difference was not statistically significant.

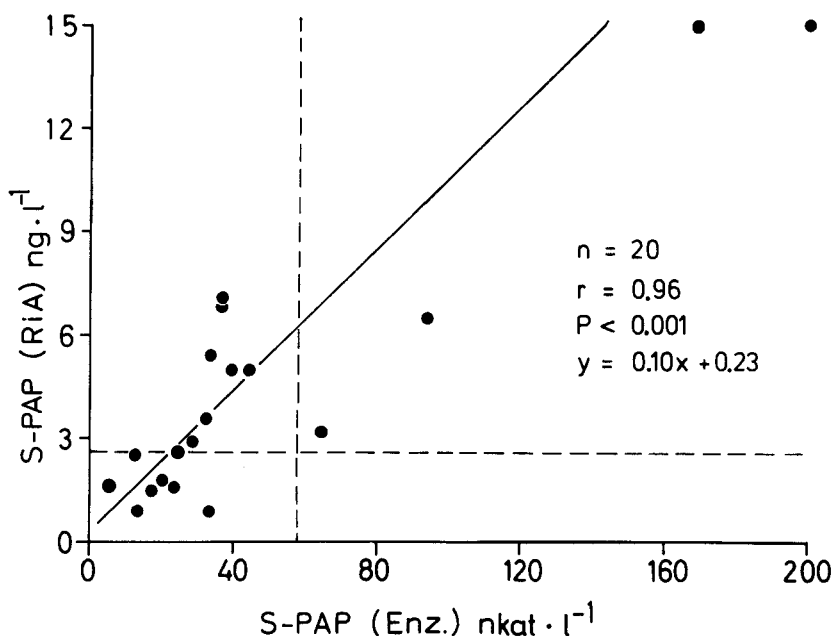


Fig 3. Correlation between Enz. and RIA values in patients with untreated prostatic cancer.

_____ Regression line
 - - - - - Upper limit of normal range

Table 2 Radioimmunoassay (RIA) and enzyme assay of S-PAP in prostatic disease (means and S.D.)

Diagnosis	No of patients	Serum PAP		Enzyme assay ($\text{nkat}\cdot\text{l}^{-1}$)	Values above upper range limit	
		RIA ($\mu\text{g}\cdot\text{l}^{-1}$)	RIA ($\mu\text{g}\cdot\text{l}^{-1}$)		RIA	enzyme assay
Prostatic cancer	57	4.0 \pm 6.2	4.0 \pm 6.2	49.8 \pm 127.5	19	7
Stages I and II	18	1.7 \pm 1.5	1.7 \pm 1.5	17.9 \pm 11.4	1	0
Stage III	23	3.5 \pm 4.6	3.5 \pm 4.6	33.8 \pm 37.0	9	3
Stage IV	16	7.3 \pm 9.5	7.3 \pm 9.5	108.6 \pm 230.7	9	4
Benign prostatic disease	59	1.7 \pm 1.4	1.7 \pm 1.4	17.5 \pm 11.3	4	0
Hyperplasia	39	1.9 \pm 1.7	1.9 \pm 1.7	16.4 \pm 12.5	4	0
Other disorders	20	1.3 \pm 0.5	1.3 \pm 0.5	19.9 \pm 8.4	0	0

The means from RIA and from enzymatic assay were almost the same in the patients with stage I or II prostatic cancer as in those with benign prostatic disease. The proportion of patients with values above the upper limits of normal was also similar (Table 2). Both parameters, however, distinguished cancer stages III and IV from the benign conditions, especially when RIA was used. Thus, elevated values were given by RIA in 9 of the 23 patients with stage III cancer, but by the enzyme method in only 3 patients. The corresponding figures in stage IV cancer were 9 and 4 of 16 patients.

The sensitivity, i.e. the ability to recognize prostatic disease as malignant, was 0.33 with RIA and 0.14 with the enzymatic assay when all 57 cancer patients were included in the analysis. This difference was significant ($p < 0.01$) and was explained solely by the superior sensitivity of RIA in tumours of stage III or IV.

The enhanced sensitivity of RIA was still more evident in untreated prostatic cancer (Table 3). Of the 11 patients with stage III or stage IV tumour, 10 showed elevated values in RIA, but only 4 in enzymatic assay.

Table 3. Radioimmunoassay (RIA) and enzyme assay of S-PAP in untreated prostatic cancer

Diagnosis	No of patients	Serum PAP		Values above upper range limit	
		RIA ($\mu\text{g}\cdot\text{l}^{-1}$)	Enzyme assay ($\text{nk}\cdot\text{l}^{-1}$)	RIA	enzyme assay
Prostatic cancer	20	5.6 ⁺ 7.4	51.8 ⁺ 68.5	11	4
Stages I and II	9	2.1 ⁺ 2.0	19.9 ⁺ 12.1	1	0
Stage III	7	6.9 ⁺ 6.8	57.4 ⁺ 51.6	6	2
Stage IV	4	11.2 ⁺ 12.6	113.5 ⁺ 125.9	4	2

DISCUSSION

RIA measures antigen determinants of the analyzed enzyme, whereas the various methods of enzymatic analysis reflect catalytic activity. When RIA was introduced for determination of prostatic acid phosphatase in serum it was hoped that this assay, based on an antibody against a purified isoenzyme, would increase diagnostic acuity. Such specificity should contribute to early detection of prostatic cancer and also to the more appropriate tumour staging that is essential for optimum choice of treatment and for accurate monitoring of tumour response. These hopes remain widely held, although it is generally recognized that no known isoenzyme of acid phosphatase is tumour-specific or even tumour-associated.

The present study showed good correlation between results with RIA and those with the enzymatic method of determining serum acid phosphatase in patients with prostatic cancer, particularly before treatment had been instituted. A priori, therefore, no clear difference was to be expected between the two methods in the continued investigations. Their validity, accordingly, proved to be similar. In sensitivity, on the other hand, the methods clearly differed. The most probable explanation of this apparent paradox was intrinsic difference in the properties of the methods when the upper normal limits were determined. If RIA is a completely organ-specific test, the upper limit of its normal range should be 'absolute'. The catalytic activity in the enzymatic analysis, however, is assumed not to derive exclusively from the prostate. Acid phosphatases from other organs may contribute to the results of the analysis, and such extra-prostatic 'contaminating activities' may diminish with advancing age. The mean age was higher in the cancer patients than in those with benign prostatic disease. Intergroup comparisons of RIA and enzymatic analysis data (Table 2) suggest that the extraprostatic contribution to the latter values could have been inversely related to age. Use of an age-matched reference group might well have eliminated this discrepancy.

The false positive RIA results occurred only in patients with benign prostatic hyperplasia. The 10 per cent of results

above upper normal limit in this group is close to the figure reported by Foti et al. (6). In dealing with the problem of false positive results, these writers introduced +4 S.D. as upper normal limit, which reduced the proportion to 5 per cent but decreased the sensitivity of the assay in stage I cancer.

In our series the two assay techniques did not differ in regard to detection of early cancer. Among the total 18 patients with stage I or stage II cancer, only one showed an elevated RIA value (1 of the 9 with untreated early cancer). We therefore conclude that neither assay is useful as a screening procedure for early detection of prostatic carcinoma. Contradictory findings were reported by other authors (2,3,6,11,13). On the other hand, Bruce et al (1) found elevated RIA values in only 8 per cent of patients with stage I or II prostatic cancer.

Between intracapsular and extracapsular cancer, however, RIA discriminated reasonably well in our study. Thus in stage III or IV, RIA gave elevated values in 10 of 11 untreated patients, while the enzymatic analysis showed only 4 with such values. RIA therefore appears to be useful for the staging of prostatic cancer.

Standardized methods are essential when PAP determination is used in the classification of prostatic disease. Extended studies of large series and repeat analyses in individual patients, both RIA and measurement of catalytic activity, are required to clarify if the two methods continue to reflect the same circumstances with regard to tumour progression, therapeutic response and prognosis.

REFERENCES

1. Bruce, A.W., Mahan, D.E., Morales, A., Clark, A.F. & Belville, W.D.: An objective look at acid phosphatase determinations: A comparison of a biochemical and immunological methods. *Br J Urol* 51(3), 213-217, 1979.
2. Carroll, B.J.: Radioimmunoassay of prostatic acid phosphatase in carcinoma of the prostate. *N Engl J Med* 298(16), 912, 1978.
3. Cooper, J.F. & Foti, A.G.: A radioimmunoassay for prostatic acid phosphatase. *Natol Cancer Inst Monogr* 49, 235-237, 1978.
4. Derechin, M., Rustum, Y.M. & Barnard, E.A.: Acid phosphomonoesterase of human prostate. Molecular weight, dissociation and chemical composition. *Biochim Biophys Acta* 250, 143-154, 1971.
5. Dewers, L.M.: Prostatic acid phosphatase: Assay Comparisons. *Laboratory Management* 19, 8-16, 1981.
6. Foti, A.G., Cooper, J.F., Herschman, H. & Malvaez, R.R.: Detection of prostatic cancer by solidphase radioimmunoassay of serum prostatic acid phosphatase. *N Engl J Med* 297(25), 1357-1361, 1977.
7. Griffiths, J.C.: Prostate-specific acid phosphatase:reevaluation of radioimmunoassay in diagnosing prostatic disease. *Clin Chem* 26, 433-437, 1980.
8. Gutman, E.B., Sproul, E.E. & Gutman, A.B.: Significance of increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. *Am J Cancer* 28, 485-495, 1936.
9. Lundholm, G.R., Stirton, S., Liedtke, R.J. & Batjer, J.D.: Prostatic acid phosphatase by radioimmunoassay. Sensitivity compared with enzymatic assay. *JAMA* 244(18), 2071-2073, 1980.
10. Snedecor, G.W. & Cochran, W.G.: *Statistical Methods*. Sixth Edition. The Iowa State University Press. Ames, Iowa, U.S.A., 1967.
11. Wajzman, Z., Chu, T.M., Saroff, J., Slack, N. & Murphy, G.P.: Two new, direct and specific methods of acid phosphatase determination. *Urology* 13(1), 8-11, 1979.
12. The Veterans Administration Cooperative Urological Research Group. Cancer of the prostate: treatment comparisons. *J Urol* 98, 516-522, 1967.

13. Vihko, P., Sajanti, E., Jänne, O., Peltonen, L. & Vihko, R.:
Serum prostate-specific acid phosphatase: development and
validation of a specific radioimmunoassay. Clin Chem 24(11),
1915-1919, 1978.

Address for reprints:
Gunnar Ronquist, M.D.
Department of Clinical Chemistry, University Hospital
S-751 85 Uppsala