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Radioimmunoassay (RIA) for Prostatic Acid Phosphatase in Patients with Prostatic Carcinoma

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Summary. In recent years radioimmunological measurements of prostatic acid phosphatase have been proposed for the diagnosis, follow-up and prognosis of prostatic carcinoma. The possibility of screening male populations at risk has even been suggested. The present paper deals with the current position of this method. We studied the specificity and sensitivity of the radioimmunoassay (RIA) for prostatic acid phosphatase in three groups of patients: a normal population, patients with benign prostatic hyperplasia, and patients with untreated prostatic carcinoma. The conclusions of this study are that the RIA is a specific method but the sensitivity is much too low to use the RIA for diagnosis and screening of patients. Comparison with the enzymatic method indicates that under good laboratory conditions the latter is preferable except for patients with metastatic disease and normal enzymatic acid phosphatases.

Key words: Prostatic carcinoma, Acid phosphates, Radioimmuno-assay.

Introduction

Acid phosphatase, a normal component of the seminal fluid, is produced in large quantities by the epithelial cells of prostatic glands. Gutman and Gutman [6] first reported an increase of acid phosphatase levels in the peripheral blood in patients with prostatic carcinoma. Because of the lack of sensitivity, the popularity of the assay was variable. With the development of immunological assays of prostatic acid phosphatase a more sensitive method has become available. Clinical application of the radio-immunology assay (RIA) was first investigated by Cooper and Foti [2]. Later Foti et al. [4] published promising results not only in patients with metastatic disease, but also in the early stages of the disease, offering a simple tool for both early diagnosis and screening. Commercial promotion of the RIA for specific PAP was a direct effect of the first enthusiastic reports. Simple rectal examination seemed to become superfluous in the diagnosis of prostatic carcinoma.

Material and Methods

In 1979 we started a study assessing the value of the RIA for prostatic acid phosphatase in urological practice, in which we studied the sensitivity, the specificity of the RIA for prostatic acid phosphatase and the perspectives for screening patients.

Patients: serum levels of prostate-specific acid phosphatase were measured in 328 patients divided in 3 groups:

1. 15 patients without prostate related disease (men and/or women)
2. 226 patients with BPH, histologically proved in tissue from prostatectomy or transurethral resection
3. 87 patients with histologically proved untreated prostatic cancer.

Laboratory Assay

In 1978 we developed a radioimmunoassay, with which we performed first pilot studies. In 1979 we compared our assay with the RIA from New England Nuclear (RIANEN). The results were similar. Because of technical problems with our own RIA, serum levels of all patients were repeated with RIANEN. The normal range we found was < 3.3 ng/ml prostate acid phosphatase. Blood samples were collected before any manipulation of the prostate or any therapy was started. In 22 patients with proven prostatic cancer, serum acid phosphatase and the prostatic fraction were also measured by the enzymatic method under optimal laboratory circumstances. L (+) tartrate was used as a specific inhibitor and p-nitrophenyl phosphate as substrate.

Staging and Grading of the Patients

Patients with histologically proven prostatic cancer were classified according to the TNM-classification of the UICC [12]. Besides clinical investigation (T-stage) urography and total body scintigraphy (N and M stage) were done. By these criteria, 53 patients (61%) had low stage disease, in which the lesion was confined to the prostatic gland (group I). 10 patients (11.5%) showed local spread of the

Table 1. Stage classification

Group I	T – T – T 0 1 2		
	(N) 0 – x	M 0	53 patients
Group II	T – T 3 4		
	(N) 0 – x	M 0	10 patients
Group III	T – T 1 4		
	(N) 0 – x – +	M +	24 patients

Table 2. Correlation between stage and grade

	patients	G1	G2	G3
Group I	53	30	14	9
Group II	9[a]	–	4	5
Group III	24	2	10	12

[a] Tumor grade not available for one patient

Table 3. Elevation of RIA-PAP in patients without prostatic carcinoma

proven BPH	number of patients	elevated RIA-PAP
benign prostatic hypertrophy	226	9 (4%)
healthy group (control)	15	0 (0%)

Specificity of the test = 96%

Table 4. Elevation of RIA-PAP in patients with histologically proven prostatic carcinoma

	number of patients	elevated RIA-PAP
Group I	53	12 (22.6%)
Group II	10	5 (50 %)
Group III	24	21 (87.5%)
Total	87	38 (43 %)

Sensitivity of the test = 43%

disease outside the prostate (group II); 24 patients (27.5%) had metastases.

The N-classification was done by lymphography, percutaneous lymph node biopsy or pelvic lymphadenectomy. Patients with proved lymphatic spread of the disease (N+) were classified in group III. Patients with no N-classification (Nx) were classified to their T-stage (Table 1).

Histological grading was done according to Mostofi [9]. In this system distinction is made between G I (well differentiated), G II (moderately differentiated) and G III (poorly differentiated adenocarcinoma of the prostate). The tumor of the patients by group is presented in Table 2. 56% of the patients in group I had a well differentiated (G I) tumor while 50% of the M+ patients had a poorly differentiated carcinoma.

Results

1. Radioimmunoassay for Prostatic Specific Acid Phosphatase

Of 226 patients with benign prostatic hypertrophy (BPH) 9 had an increased level of prostatic acid phosphatase in the radioimmunoassay: 4% false-positives, which means a specificity of 96%. In the group of healthy young men and women (15), no increased levels were found (Table 3). The levels in patients with a prostatic carcinoma are shown in Table 4. In group I, 12 out of the 53 patients had increased levels, which means a sensitivity of 22.6% (true positive). In group II the percentage increased to 50% (5 out of 10) and in group III to 87.5% (20 out of 24). Of the total group of 87 patients, 38 showed an increased prostate acid phosphatase by RIA, an overall sensitivity of the test of 43%. Classified according to grade (Table 5), there seems to be a tendency for more true positive results in cases of poorly differentiated tumors. Predominantly in group I with increasing grade an increased number of positives can be found. However, the difference between the percentages of the different groups is not statistically significant.

2. Comparison Between RIA and Enzymatic Method

In 22 patients, the RIA results were compared with the results of the enzymatic method, shown in Table 6. This table shows that only 1 patient had a different outcome comparing the enzymatic method and the RIA. This was a patient staged T2N0M0, with raised levels in RIA, but the enzymatic method gave normal values.

3. Statistical Analysis of the RIA as a Screening Test

The value of a test as a screening method is expressed in the positive predictive value (PVpos%). This value is obtained from three parameters:

1. sensitivity of the test (number of true positives)

Table 5. Correlation between stage and grade of the tumor

total number of elevated RIA-PAP			Grade I		Grade II		Grade III	
			number	RIA	number	RIA	number	RIA
Group I	53	12 (22.6%)	30	4 (13.3%)	14	4 (28.5%)	9	4 (44,4%)
Group II (1 pat. Gx.)	10	5 (50.0%)	—		4	2 (50 0%)	5	2 (40.0%)
Group III	24	20 (87.5%)	2	2 (100%)	10	8 (80.0%)	12	10 (83.3%)

Table 6. Comparison between RIA-PAP and the E (enzymatic) PAP determination

	number of patients	RIA-PAP elevated	RIA-PAP normal	E-PAP elevated	E-PAP normal
Group I	16	4	12	3	13
Group II	3	1	2	1	2
Group III	3	–	3	–	3

2. specificity (% false positive)

3. incidence of the disease in a population.

According to the theory of Bayes, the PVpos% is calculated with the following formula:

$$100 \times \frac{\text{incidence} \times \text{sensitivity}}{\text{incidence} \times \text{sensitivity} + (1\text{-incidence}) \times (1\text{-specificity})}$$

Sensitivity of the RIA for prostatic acid phosphatase in our study is 43% (38 out of 87). Specificity is 96% (9 out of 226). With an incidence of prostatic carcinoma in the Netherlands of 35 out of 100,000 men, the PVpos% with our results would be:

$$100 \times \frac{0.00035 \times 0.437}{0.00035 \times 0.437 + (1-0.0035) \times (1-0.96)} = 0.38\%$$

which would mean that a randomly chosen man, who has a positive RIA for prostatic acid phosphatase in a routine screening with no abnormal findings on rectal examination, had a chance of 1 in 362 of having a prostatic carcinoma.

Discussion

In our study we could not prove the great hopes for the RIA for prostate specific acid phosphatase as a reliable test for early diagnosis, follow-up, therapy and prognosis of the disease. The specificity of the test is sufficient as can be expected with immunochemical measuring techniques. This specificity can also be found in the data from the literature, which make clear that less than 10% false-positive results were obtained in large series [1, 2, 5, 8, 11].

The test obviously lacks sensitivity (57% false-negatives or sensitivity of 43% in our study). Only in high stage disease did we obtain a sensitivity of 87.5%. These results are also comparable with results of other studies, in which self-developed RIA's and commercially available RIA kits were tested (Table 7). In contradiction to Cooper [2] who found 43% positive values in early stage (T0–T2) prostatic carcinoma, no other study could confirm his figures. Without metastases, and with a carcinoma limited to the anatomical boundaries of the prostate, the reliability of the test is obviously lower (± 20% true positive results). In stage T3 this percentage is about 50%.

Almost all investigators found in patients with metastatic disease a positive result in about 80%.

Though in several studies different classifications are used, Table 7 shows that in almost all stages the percentages of true positive results are lower than the initially published data by Cooper. An explanation could be the differences in preparation and purification of the antigen from the tissue. The method used by Cooper [4] is less extensive and accurate than used by other investigators. During development of our own RIA it became clear that the purity of the Cooper antigen was questionable.

Table 7 shows that in spite of the differences in purification method, little difference in sensitivity is seen between the various commercial kits.

Because of the lack of sensitivity, the test is of little value as a screening method illustrated by the calculation of the positive predictive value. Even if the best results to date are used [3], according to Kiesling and Watson [7], the positive predictive value with these figures is no better than 0.43%.

Our results and those of other studies are lower. Even in a selected patient group, for example men over 70 years old, with a higher risk for prostatic carcinoma (the in-

Table 7. Literature data; sensitivity of RIA-PAP (true positive values) related to the stage of disease

Author	Method	T0–T2	T3–T4 (M+)	Total
Cooper (1980)	Own RIA	43%	94%	73%
Griffith (1980)	RIANEN	20%	73%	48%
Wirth et al. (1981)	Serono	0%	68%	50%
	RIANEN	0%	50%	35%
	Biosigma	0%	68%	48%
	Travernol	25%	81%	72%
Tellier et al. (1981)	clin. assay	24%	88%	61%
Quinones et al. (1981)		12%	50%	39%
Bruce et al. (1981)	own RIA	14%	65%	40%
	Mallinckrodt	31%	67%	51%
	RIANEN	20%	60%	41%
Klein and Shapiro (1981)	clin. assay	5%	90%	51%
own investigation	RIANEN	22%	50%	43%

cidence of clinical prostate cancer in men over 75 is estimated on 500 per 100,000), screening would be of little value, because for this group with the specificity and sensitivity we obtained the positive predictive value would be 3.65, so only 1 out of 27 men over 75 years with a positive blood test would be found to have a prostatic carcinoma. This percentage is too low, even in a selected group of patients. (This begs the question of the practical value of detection of asymptomatic T1 prostatic carcinoma in an 80-year-old man).

Improvement of the sensitivity over 95% is needed to make screening possible. Particularly for the RIA for prostate specific acid phosphatase, such improvement in laboratory technique cannot be expected.

Cost-benefit analysis shows another problem for the RIA as a screening test. The RIA for prostate costs Dfl. 70.– per test (US-$ 25). The prevalence of prostate carcinoma in the Netherlands is ± 400 out of 100,000 of all men older than 60 years, which means 1 out of 250 men. Costs of a screening program with the RIA for prostatic acid phosphatase would be Dfl. 70.– x 250 = Dfl. 17,500.– (US-$ 6,250) to detect 1 man with a prostatic carcinoma. Costs of physicians, organisation and administration should be added. This also indicates so far the irreplacable value of the rectal examination as the first and cheapest diagnostic approach to a patient with a possible prostatic carcinoma. The RIA prostatic acid phosphatase is a reliable marker for patients with metastatic disease.

Table 7 shows that nearly 90% of these patients have a raised level of RIA prostatic acid phosphatase. This figure for the enzymatic assay is 60–90% [15]. Fluctuation in pH and temperature and denaturation of the enzyme in time make the enzymatic method less reproducible.

For routine investigation the enzymatic method is less accurate. Tellier et al. [11] found in a given group of patients a sensitivity of 67% if the serum was processed immediately. This figure dropped to 36% if for the same patients the assay was done as a routine laboratory assay.

The RIA, as a stable accurate measurement, would be a better method for therapy control and follow-up of patients with metastatic disease, if it is not possible to perform an accurate enzymatic assay. On the other hand the differences in sensitivity at the expences of the enzymatic method, caused by the inaccuracy of the handling of the serum, can probably be optimised in view of the better cost-benefit analysis.

Our figures show good correlation between the two methods (Table 6). If with improvements in laboratory routing this high degree of correlation can be obtained only a very small patient group will remain in which the RIA is more sensitive than the enzymatic assay. In this group the RIA prostatic acid phosphatase will still be useful.

Conclusions

– The radioimmunoassay for prostatic specific acid phosphatase is a specific method.

– The method lacks sufficient sensitivity to be used as a screening method for prostatic carcinoma. The positive predictive value is too low.

– Under optimal laboratory circumstances, the enzymatic assay of prostate specific acid phosphatase has a sensitivity comparable with the RIA in 80% of the patients with metastatic prostatic carcinoma.

Only a small group of these patients remains with normal results by the enzymatic assay, in whom the RIA for prostate specific acid phosphatase is valuable for diagnosis, therapy, follow-up and in assessing the prognosis.

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