Urol Res (1982) 10:75–80

Urological Research

Creatine Kinase Isoenzyme (CK-BB) in Combination to Prostatic Acid Phosphatase Measured by RIA in the Diagnosis of Prostatic Cancer

P. R. Huber, Th. Zaugg, E. Linder, V. Hagmaier and G. Rutishauser

Hormone Laboratory, Department of Gynaecology, Urological Clinic, Department of Surgery and Zentrallaboratorium, Kantonsspital Basel, CH-4031 Basel, Switzerland

Accepted: January 21, 1982

Summary. Creatine kinase isoenzyme (CK-BB) measured by mass was used to determine its value in the early diagnosis of prostatic cancer. Sera of patients with prostatic carcinoma of various stages (treated and untreated) were compared to normal male sera and sera of patients with benign hyperplasia of the prostate (BPH) with respect to CK-BB. The sera were simultaneously tested for PAP content. The sensitivity of the CK-BB-RIA was 1.63 +/– 0.08 μg/l and reproducibility in the higher and lower concentration range 7.6% and 10.5%, respectively. CK-BB alone or in combination with PAP is no marker for early detection of prostatic cancer. In individual cases changes occurred similar to those found with a malignant growth of the prostate.

Key words: Creatine kinase (BB), Prostatic acid phosphatase (PAP), Radioimmunoassay, Prostatic cancer, Benign prostatic hyperplasia.

Introduction

One of the inherent problems with prostatic carcinoma is that it often presents only at a relatively late stage. The clinical diagnosis of prostatic carcinoma by rectal palpation is not always possible. Cytological examination of prostatic tissue can lead to false negative results. For these reasons biochemical tests were introduced in the diagnosis of prostatic tumours. The standard method is to estimate PAP in serum either by an enzymatic or by a radioimmunological technique [1, 2]. However, both of these measurements may give false negative results. Looking for a more reliable or at least supplementary test Silverman [3] found the Creatine kinase isoenzyme BB concentration to be elevated in 15 out of 17 patients with untreated prostatic carcinoma. Creatine kinase BB (brain type) is one of the three Creatine kinase isoenzymes involved in the production of ATP as a source of biochemically available energy.

The other isoenzyme found in sera is called the MM form, originating mainly from muscles. Heart muscle contains the MB form, which is a dimer of "M" and "B" chains. Both the MB and the BB form are practically undetectable in normal human serum, except after myocardial infarction, when the serum MB isoenzyme can be elevated.

The BB form represents 60% of the total Creatine kinase in the prostate [4]. In 1975 Sjoevall [5] described a higher activity of CK-BB in malignant prostate tissue compared with normal. There appeared to exist great differences in the tissue samples from different patients. Several authors [5–10] found CK-BB to be enhanced in sera with several carcinomas. The diagnosis of prostatic carcinoma with CK-BB appeared possible [3, 11–15]. We were interested in the hypothesis that a combination of CK-BB and PAP estimation in the serum of patients suspected of having a prostatic tumour would enhance the accuracy of the diagnosis by biochemical markers.

Material and Methods

The RIA-Quant/TM CK-BB-Test was provided by Mallinckrodt Inc. St. Louis, Mo. It is a radioimmunoassay specific for Creatine kinase isoenzyme BB with a cross reaction at 50% B/B_0 for CK-MB of 0.006% and CK-MB of 41.3%. Saturation technique was used with two individual incubation steps of 2 h at 4 °C. To increase the number of samples assayed in one run we divided the volumes of *all* reagents including the amount of serum by half. At the same time we increased the counting time for the radioactivity in order to increase the accuracy in counting the sample.

The prostatic acid phosphatase (PAP) test of Clinical Assays (Travenol) served as the reference method. This method allowed some differentiation between normal and benign hyperplasia of the prostate (BHP) and/or early stages of prostatic carcinoma, which is not possible with the tests RIA-Quant/TM for PAP (Mallinckrodt) and New England Nuclear RIA for PAP (cf. Section Results and [16]).

Stability Tests for CK-BB

The recommended maximum storage times for the serum samples to be assayed is no longer than 7 days at –20 °C. Since some of our

0300-5623/82/0010/0075/$01.20

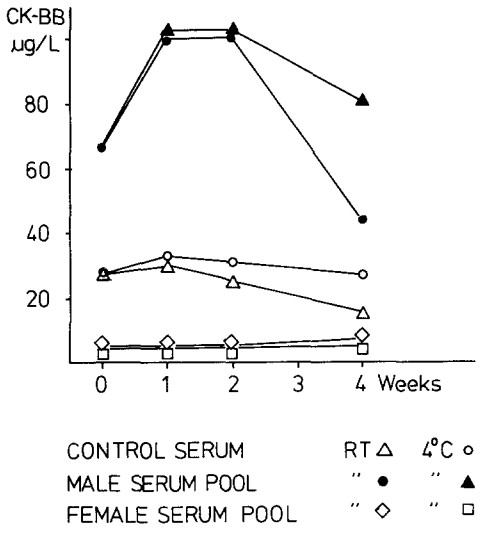

Fig. 1. Influence of freezing and thawing on the immunological stability of CK-BB serum

samples were stored for a much longer time period at −20°C we carried out a stability test.

Samples containing CK-BB were left at 4 °C and at room temerature for up to 4 weeks. At intervals of 1, 2 and 4 weeks the respective samples were brought to −20 °C to be assayed for CK-BB at a later date.

Quality Controls

Control sera provided by the manufacturer were included in each assay.

Mathematical and Statistical Treatment of Data

Raw data obtained from the Gamma-counter (Packard Instruments) were transformed in CK-BB concentrations by use of a 4-parameter curve fitting programme using an Olivetti P6060 desk top computer. Statistical evaluation was done using programmes written by Olivetti using the same computer.

To survey the results obtained for the different conditions we adapted the "Inverse Distribution Function" recently published by Oehr et al. [22] for the computer. The method is as follows: Arrange data in a descending order and associate a rank number, multiply the rank number with the factor 100, divide the obtained numbers by the rank number associated with the smallest concentration. Plot data concentration of antigen vs. percentage (Figs. 2D and 3).

Specimen Collection

Patients were drawn from those presenting to the Urological Clinic at the Kantonsspital Basel, Switzerland, with suspected prostatic disease. Blood was collected in vacutainers (Becton Dickinson) with no additives to obtain serum for both CK-BB and PAP. Sera from patients under surveillance for their prostatic ailment were from the same source.

Male control serum samples were obtained from patients of the Surgery Department, who had no manifest prostatic lesions. Sera

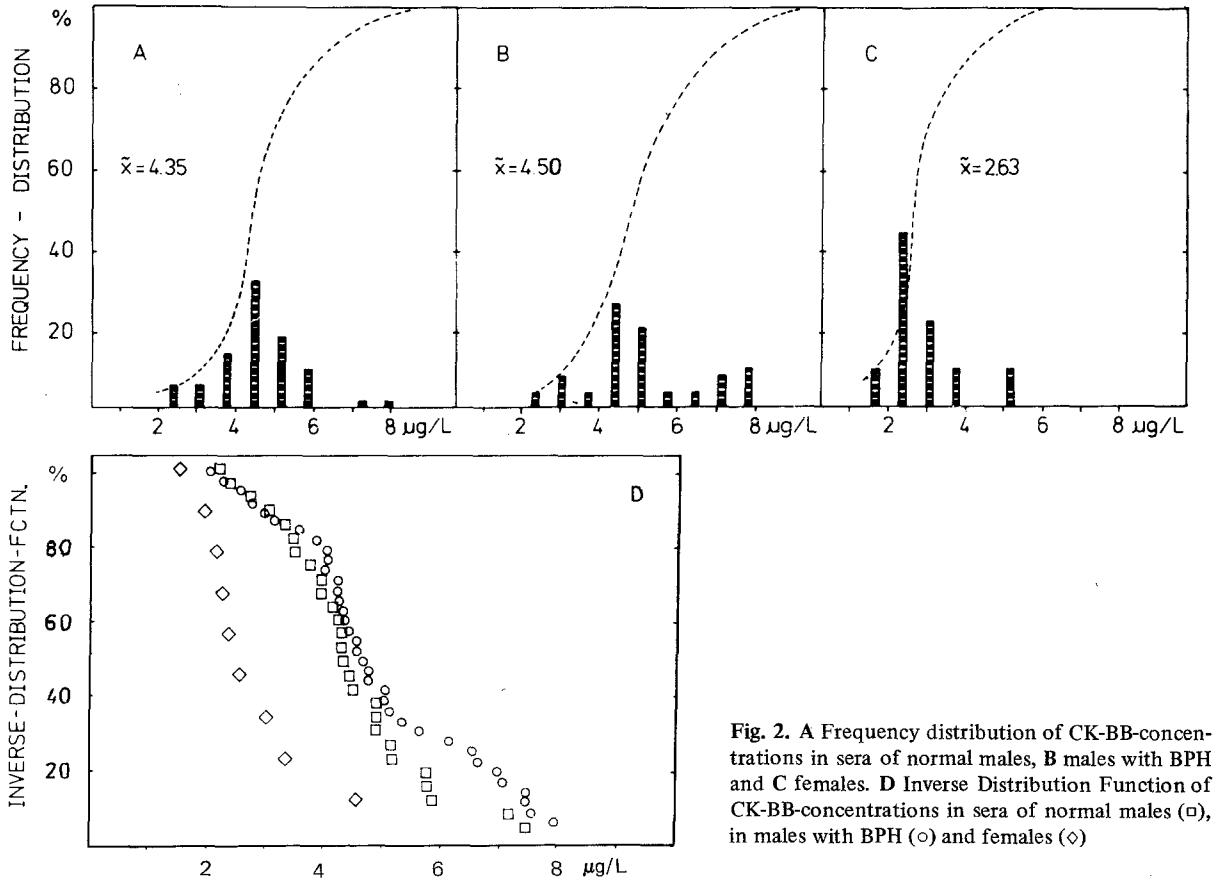

Fig. 2. A Frequency distribution of CK-BB-concentrations in sera of normal males, B males with BPH and C females. D Inverse Distribution Function of CK-BB-concentrations in sera of normal males (□), in males with BPH (○) and females (◇)

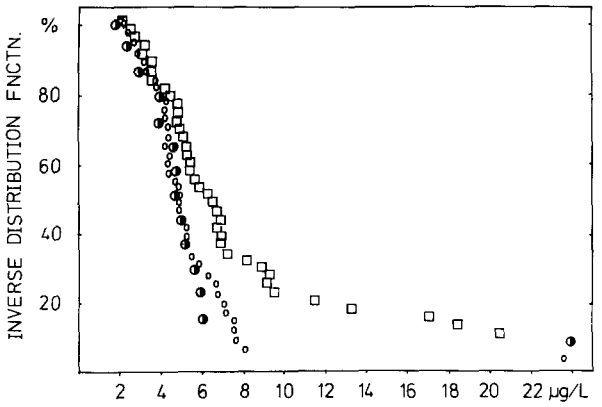

Fig. 3. Inverse Distribution Function of CK-BB-concentrations in sera of patients with BPH (○), patients with untreated (◑) and treated (□) prostatic carcinoma

from females were obtained from patients admitted to the Gynaecology Department of this hospital. Patients suffering from benign hyperplasia (BPH) of the prostate were subjected to transurethral resection of the prostate (TUR-P). The diagnosis of BPH was histologically ascertained in the Pathology Institute of the University of Basel.

Classification of the prostatic carcinomas followed the TNM rules of the "International Union against Cancer (UICC): T0 to T4 category was based on rectal palpation of the prostate. *N*: Lymphnode involvement was determined either by lymphography or lymphadenectomy. *M*: X-ray and Whole-Body-Scintigraphy were used for the detection of bone metastases. *G*: Tissue Biopsy, TUR-P or enucleated specimens were histologically examined by the Patological Institute.

Careful distinction between patients receiving any kind of therapy or no therapy was observed.

Results

Performance of the CK-BB Test

The sensitivity of the test for CK-BB as given by the manufacturer is 1.0 µg/l. In the three experiments carried out to obtain the data presented in this publication we observed a detection limit (mean ± SD) of 1.63 ± 0.08 µg/l.

The quality control sera should yield values in the range of 5.9–9.9 µg/l (Serum A) and 25.2–37.6 µg/l (Serum B). We obtained values of 8.6 ± 0.9 µg/l for Serum A an 29.4 ± 2.25 µg/l for Serum B.

Stability of CK-BB in Serum

Figure 1 shows an interesting and so far unexplained phenomenon where samples of a pool of sera with a high CK-BB content, quality control samples provided by the manufacturer and samples of a pool of female sera were left at 4 °C and at room temperature. An increase in concentration of CK-BB both at 4 °C and at room temperature followed by a plateau with a subsequent decrease were found with the high concentration serum pool, but less so with the quality control serum. The low concentrations in the serum from females remained low and relatively constant throughout.

References Ranges

Creatine Kinase (CK-BB). Figure 2A-C depicts the distribution of CK-BB measured by RIA in a limited number of sera from females, males and males with benign hyperplasia (BPH).

Female sera were lower in their content of CK-BB than were normal male sera. This was obvious when the median values for these two populations (Figs. 2A and 2C) and the concentration of CK-BB corresponding with 50% in the Inverse Distribution Function were compared (Fig. 2D).

The concentration range covered by sera from patients with BPH was the same as that for normal male sera, although a slight shift of the values to the upper end of the range was found (Fig. 2B).

Prostatic acid phosphatase (PAP). In sera from 216 normal male subjects PAP was measured with the RIA test from Clinical Assays. There was an upper limit of 1.8 µg/l (95% confidence) as indicated in Table 1. If the sera of BPH-patients were included, an upper limit of 3.1 µg/l was found [16].

Table 1. Reference ranges for CK-BB and PAP

Subjects	*N*	$\bar{X}$ ± 1 SD	CK-BB µg/l Range	Outliers *N*	Cut-off[a]	PAP µg/l Cutoff[b]
Male	27	4.4 ± 1.3	2.7–7.4	0	7.4	1.8
BPH	36	4.8 ± 1.6	2.0–7.9	1	7.5	3.1
Female	10	2.4 ± 0.6	1.5–4.5	2	3.2	1.2

[a] 95% confidence
[b] Ref. [16]

Table 2. Number of prostate carcinoma patients detected by PAP and CK-BB or their combination

Staging	*N*	PAP ⩾3.1 µg/l		CK-BB ⩾7.5 µg/l		CK-BB and PAP above limit for BPH	
		N	%	*N*	%	*n*	%
Prostate carcinoma *without therapy*	14	5	35	1	7	1	7
T0–T2	6	1	17	0	0	0	0
T3–T4	8	4	50	1	13	1	13
Prostate carcinoma *with therapy*	42	20	54	13	30	9	22
T0–T2	9	4	44	2	22	1	11
T3–T4	33	16	45	11	33	8	24

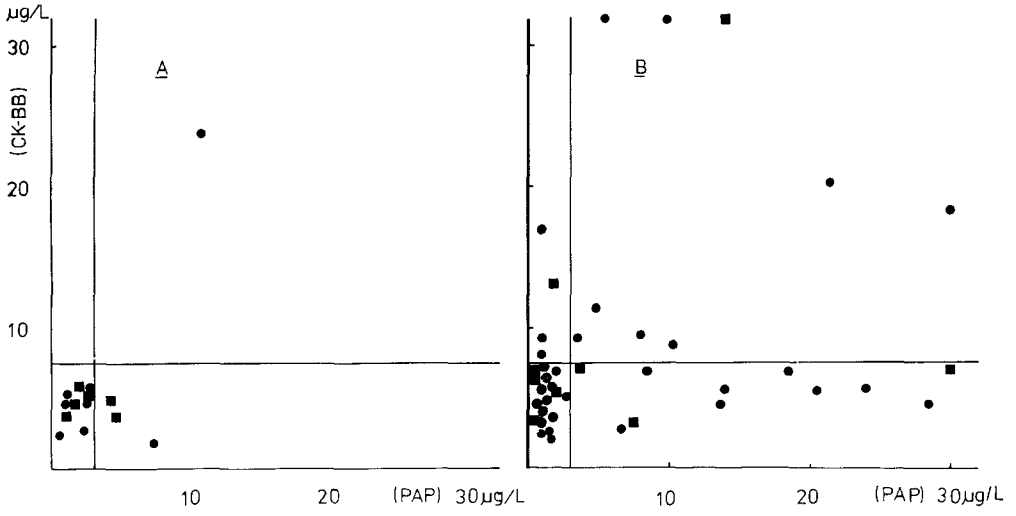

Fig. 4. A Correlation of PAP-with CK-BB-concentrations in sera of untreated prostatic carcinoma. B Correlation of PAP-with CK-BB-concentrations in sera of treated prostatic carcinoma. Stages T0–T2 (▪) and T3–T4 (•)

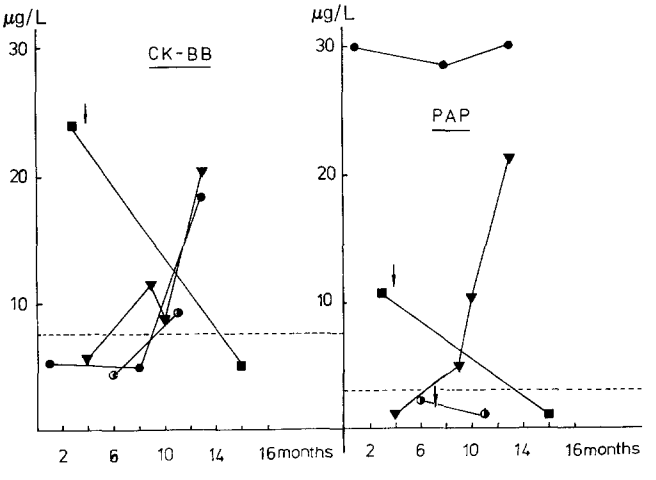

Fig. 5. Response of PAP- and CK-BB-concentrations to therapy in 4 patients with prostatic carcinoma (see text). Patient 1 (▼); Patient 2 (▪); Patient 3 (◑); Patient 4 (•); Normal ranges (– – –)

CK-BB and Prostatic Carcinoma

Out of 14 sera of untreated patients only one had an increased value of CK-BB, which belonged to the group of T3-T4-category (Table 2 and Fig. 3). Thirteen or 30% of the treated patients had increased CK-BB; two belonged to the group T0–T3 and 11 to the more advanced stages T3–T4. The representation of the data in the form of the Inverse Distribution Function (Fig. 3) shows the data in a continuous order separated into the two groups "untreated" and "treated". The two curves are similar. The figure demonstrates that only 10% of the untreated and 25%–30% of treated patients had concentrations of CK-BB above 7.5 µg/l (Table 2). It is not possible to correlate staging of the tumour with CK-BB-concentrations in serum.

Correlation of CK-BB and PAP

Table 2 and Figs. 4A and 4B show a correlation of the CK-BB and PAP-concentrations measured simultaneously in

sera of untreated (Fig. 4A) and treated prostatic cancer patients (Fig. 4B).

Only one serum sample contained increased concentrations of both CK-BB and PAP (Fig. 4A). Three values for PAP alone and none for CK-BB alone were increased. The degree of tumour growth was not reflected either in CK-BB- or PAP-concentrations or in their combination. The same was true for the group of treated prostatic cancer patients. As is evident from the plot, the main contributors for enhanced marker concentrations were the patients with stage T3 and T4.

The concentration changes of either one of the two observed marker substances during the course of tumour therapy can be quite different from each other, as can be seen in four patients receiving similar therapy (Fig. 5):

Patient 1 (T4NxM1G2; status post TUR-P and orchiectomy). CK-BB and PAP were increasing in concentration and increased further even after initiation of a hormone-therapy in the 10th month of treatment.

Patient 2 (T3NxM1G2; TUR-P, orchiectomy and hormone-therapy). Both markers decreased after initiation of therapy (Fig. 5; *arrow*) to normal concentrations.

Patient 3 (T3NxM0; status post TUR-P and hormone-therapy). CK-BB increased from the normal to the pathological range, whereas PAP decreased to within the normal range. The additional orchiectomy (Fig. 5; *arrow*) did not change the course of tumour growth.

Patient 4 (T4N1M1; status post TUR-P, orchiectomy and hormone-therapy). PAP values remained high during the observation period. CK-BB however changed quite markedly after the last examination. The patient died from his illness 4 months later.

Discussion

Most of the data published on CK-BB isoenzymes relate to the enzymatic determination or to the differentiation by electrophoresis. From these studies a "black and white" picture appears since only higher concentrations of CK-BB could be detected.

With the availability of more sensitive radioimmunological methods for assay of CK-BB, very low concentrations could be estimated yielding a better discrimination between normal and pathological levels, in contrast to the older methods. With the new methodology, we encounter difficulty in determining normal ranges and cut-off limits. From these studies we learned that serum CK-BB is lower in female than in normal males (Fig. 2D). Patients with BPH show CK-BB-concentrations largely in the same range as normal males, however, as can be seen from Fig. 2B, there is a slight shift of the values toward the upper end of the normal range.

In certain situations, we found extremely high concentrations of CK-BB in male and female patients. We could not establish whether these patients suffered from malignant growth or whether the increased values were caused by the release of CK-BB from a CK-BB-Immunoglobulin complex as described in the literature [17–19], or whether other sources of CK-BB were reponsible, as is known for PAP.

Another hitherto unexplained phenomenon pointing in the same direction appeared when we measured CK-BB by RIA in the sera kept for different time periods at 4 °C and at room temperature respectively. An increase in CK-BB was detected after 1 week. This was superimposed after a quite long period only by a decline in concentration (Fig. 1). This decline can possibly be explained by a natural decay of CK-BB molecule, whereas the initial rise can be due to an unmasking of the antigenic sites on the enzyme molecule from circulating immunoglobulin.

For this study, an unselected population of patients across the time interval of three months was used, in contrast to other reports [9, 12, 14, 20, 21]. This seems important since CK-BB was intended to be used as an additional marker to PAP in investigating patients for early detection of prostatic cancer. From Table 2 and Fig. 4 we learn that in the limited number of sera in which we studied CK-BB and PAP simultaneously, the first does not give additional information to that already gained by the estimation of PAP alone.

The presentation of the data on CK-BB in sera of prostatic carcinoma patients (Fig. 3) demonstrates that the enzyme is not suitable as tumour marker to detect early stages of prostatic tumours. An ideal tumour marker would yield data from sera of cancer patients, which give an Inverse Distribution Function, that runs from the upper left-hand corner in a convex curve to the lower right-hand corner and would be quite strongly separated from the curve obtained from e.g. BPH patients.

As seen in Figs. 2 and 3 the trace of the Inverse Distribution Function of CK-BB in prostate cancer is similar to the curve established for BPH or normal patients, and the separation is quite insufficient. From Fig. 3 one could speculate that untreated patients yield lower CK-BB values than do treated patients. This phenomenon may be explained by the relatively low number of untreated cases studied.

In single cases however, as can be seen from Fig. 5, above normal concentrations of CK-BB can indicate a tumour where PAP is still normal. This is true for treated and untreated patients,

According to Alleyassine [15], treatment of patients with prostatic carcinoma has no effect on CK-BB-concentrations of these patients. This is in contrast to Silverman [3], who proposes CK-BB as follow-up marker. From our limited data there is no generally applicable pattern arising with respect to follow-up prostatic cancer patients either with CK-BB nor with PAP. But as mentioned above for single patients we found indications for the usefulness of these markers either alone or combined to be used in the diagnosis or follow-up of prostatic carcinoma.

From these results we conclude that CK-BB alone measured by RIA is less valuable as a marker for prostatic carcinoma than is PAP–RIA. The estimation of CK-BB does

not increase the number of cases of prostatic carcinoma detected in a screening protocol. CK-BB has certain merits in single cases where it can help in the follow up therapy and the progress of the disease.

Acknowledgements. We would like to thank the Scientific Foundation of the Kantonsspital Basel, Switzerland, for financial support and Byk-Mallinckrodt Comp. for supplying us with test material. We thank Dr. W. Roos for the establishment of certain computer programmes.

References

1. Quininones GR, Rohner TJ, Drago JR, Demeres LM (1981) Will prostatic acid phosphatase determination by radioimmunoassay increase the diagnosis of early prostatic cancer? J Urol 125: 361
2. Roy AV, Brower ME, Hayden JE (1971) Sodium Thymolphthalein monophosphate. A new acid phosphate substrate with greater specificity for the prostatic enzyme in serum. Clin Chem 17:1093
3. Silverman LM, Dermer GB, Zweig MH, van Steirteghem AC, Tokes ZA (1979) Creatine kinase BB: A new tumor-associated marker. Clin Chem 25:1432
4. Jockers-Wretou E, Pfleiderer G (1975) Quantiation of CK-isoenzymes in human tissues and sera by an immunological method. Clin Chim Acta 58:223
5. Sjoevall K, Rubin S, Muentzing J (1975) CPK in prostatic tissue. Scand J Urol Nephrol 9:181
6. Hoag GN, Franks CR, Earle De Coteau W (1978) CK-isoenzymes in serum of patients with cancer of various organs. Clin Chem 24:1654
7. Lederer WH, Gerstbrein HL (1976) Creatine kinase isoenzyme BB activity in serum of a patient with gastric cancer. Clin Chem 22:1748
8. Coolen RB, Pragay DA, Nosanchuk JS, Belding R (1979) Elevation of brain type creatine kinase in serum from patients with carcinoma. Cancer 44:1414
9. Zweig MH, van Steirteghem AC (1979) Serum CK-Isoenzyme BB as an indicator of active metastatic disease. Clin Chem 25: 1190
10. Coolen RB (1976) The production of brain type Creatine kinase in the serum of patients with oat-cell carcinomas. Clin Chem 22:1174
11. Hoag G, Amies DR, Colquhoun BDP (1978) The production of Creatine kinase isoenzyme BB in sera of a patient with prostatic carcinoma and in tumor homogenates. Clin Biochem 11:38
12. Feld RD, Witte DL (1977) Presence of Creatine kinase BB isoenzyme in some patients with prostatic carcinoma. Clin Chem 23:1930
13. Fortman DT (1979) The significance of Creatine kinase (CK-BB) in metastatic cancer of the prostate. Ann Clin and Lab Sci 9:333
14. Feld RD, van Steirteghem AC, Zweig MH, Wimar GW, Narayana AS, Witte DL (1980) The presence of Creatine kinase BB isoenzyme in patients with prostatic cancer. Clin Chem Acta 100:267
15. Aleyassine H, MacIsaac SG (1980) The diagnostic significance of serum CK-BB isoenzyme in adenocarcinoma of prostate. Clin Biochem 13:109
16. Huber PR, Scholer A, Linder E, Hagmaier V, Vogt H, Wolf T, Christen P, Eppenberger U, Rutishauser G (submitted for publication 1981) Measurement of prostatic acid phosphatase (PAP) by RIA compared to the Thymolphthalein monophosphate based enzymatic test in serum and bone marrow. Clin Chem
17. Yuu H, Tagaki Y, Senju O, Hosoya J, Gomi K, Ishii T (1978) Creatine kinase isoenzyme of high relative molecular mass in serum of cancer patient. Clin Chem 24:2054
18. Urdal P, Landaas S (1979) Macro Creatine kinase BB in serum and some data on its prevalence. Clin Chem 25:461
19. Stein W, Bohner J (1979) Immunoglobulin-bound creatine kinase BB ("Macro CK") in three patients with different diseases. Clin Chem 25:1513
20. Zweig MH, van Steirteghem AC, Schechter AN (1978) Radioimmunoassay Creatine kinase isoenzymes in human serum: Isoenzyme BB. Clin Chem 24:422
21. Zweig MH, van Steirteghem AC (1981) Assessment by Radioimmunoassay serum creatine kinase BB as tumormarker: Studies in patients with various cancer and a comparison of CK-BB concentrations to prostate acid phosphatase concentrations. JNCI 66:859
22. Oehr P, Wustrow A, Derigs G, Borman R (1981) Evaluation and characterization of tumor-associated antigens by the inverse distribution function. Tumor Diagnostik 2:195

Dr. P. R. Huber
Hormone Laboratory
Department of Gynaecology
Kantonsspital Basel
CH-4031 Basel
Switzerland