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Original Paper

Longitudinal Evaluation of the Complexed-to-total Prostate Specific Antigen Ratio in Men with Prostate Disease. Effect of Treatment

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The longitudinal changes in the complexed-to-total prostate specific antigen (PSA) ratio were evaluated in 90 men with benign prostatic hyperplasia (BPH) and 50 men with prostate cancer. The influence of treatment on this ratio was studied in 45 BPH patients and 50 patients with prostate cancer. Using a cut-off of 0.80 for the complexed-to-total PSA ratio, the large majority of prostate cancer patients had a ratio above the cut-off before treatment in serial determinations, whereas most BPH patients had a ratio consistently below that value. However, the few prostate cancer patients who had a ratio $\leq$ 0.80 showed this low ratio in serial determinations, as did BPH patients who had a ratio $\geq$ 0.80. During treatment, the ratio significantly decreased in 43 of the 50 patients with prostate cancer in parallel with the decrease in total PSA, and 34 of the 41 patients that had a pretreatment ratio > 0.80 showed a ratio $\leq$ 0.80 during hormonal therapy. Our results show that neither the physiological changes in total and complexed PSA nor the treatment of BPH patients change the diagnostic efficacy of the complexed-to-total PSA ratio, whereas in prostate cancer patients under hormonal therapy, the ratio decreased in parallel with the decrease in total PSA. This suggests that, apart from improving the diagnostic efficacy of total PSA, the complexed-to-total PSA ratio could also be used to monitor BPH patients for newly developed tumours or to monitor therapy in patients with prostate cancer.

Key words: PSA, complexed PSA, prostate cancer, benign prostate hyperplasia, α_1-antichymotrypsin
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INTRODUCTION

SERUM PROSTATE specific antigen (PSA) concentration is routinely measured to diagnose and monitor prostate cancer [1, 2]. However, some patients with benign prostate hyperplasia (BPH) [3, 4] also have increased PSA levels, and a large percentage of patients with clinically localised prostate cancer have serum PSA levels within the normal range [5]. These variations negatively affect the specificity and sensitivity of the PSA assays and make it necessary to find ways to improve this test.

It has been reported that in prostate cancer patients, free non-complexed PSA is a minor fraction in serum [6] and that PSA in blood is bound to α_1-antichymotrypsin (α_1ACT) [6, 7] and α_2-macroglobulin [8, 9]. Several groups have developed assays to evaluate free PSA or PSA complexed to α_1ACT and have investigated their usefulness in discriminating between BPH and prostate cancer patients [7, 10–19]. Their results show that the fraction of PSA bound to α_1ACT in BPH is somewhat less than that bound to PSA in patients with prostate cancer, and that calculation of the free PSA/total PSA or complexed PSA/total PSA ratios provide a better discrimination between BPH and prostate cancer than total PSA alone.

Measuring PSA:α_1ACT complex instead of free PSA has a potential advantage in test samples with low total PSA levels in which only a minimal fraction of PSA is in the free form and most of it is in the form of PSA:α_1ACT complex. We have shown that using a cut-off of 0.80 for the complexed-to-total PSA ratio instead a total PSA of 4 μg/l, the specificity

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increased from 38 to 79% without decreasing the sensitivity (82%) in patients with prostate cancer and a total PSA concentration < 15 μg/l [13]. These results agree with others reported previously [7, 11, 14, 15], and suggest that the use of the complexed-to-total PSA ratio may help urologists to identify patients with organ-confined prostate cancer.

In a recent report, we have shown that the complexed-to-total PSA ratio in BPH patients does not correlate with the age of the patient [20]. This simplifies the use of the complexed-to-total PSA ratio, as the upper limit of normality remains constant and is independent of the patient's age. Oesterling and colleagues [21] also reported that in a healthy male population, the complexed-to-total PSA ratio was independent of the patient's age. However, the longitudinal changes in the complexed-to-total PSA ratio in prostatic patients and the influence of the treatment on this ratio have not been reported previously. To understand better the factors that influence the values of the ratio and potentially improve the use of this valuable clinical marker in men with prostate disease, we evaluated the longitudinal changes in total PSA levels and the complexed-to-total PSA ratio in men with prostate disease before and after appropriate treatment.

PATIENTS AND METHODS

Patients

454 men examined for prostatic disease in the Department of Urology of La Fe University Hospital, in Valencia, Spain, were classified into three groups.

Group 1 comprised 40 men with non-histologically confirmed BPH in whom prostate cancer was excluded because of normal PSA concentrations (< 4 μg/l) and normal digital rectal examination findings, and in whom more than one blood sample was collected before any treatment. In 20 of these patients, samples were drawn before and during therapy.

Group 2 comprised 50 patients with histologically confirmed BPH, 22 of whom had a total PSA ≥ 4 μg/l and prostate cancer was excluded by at least a sextant transrectal ultrasound guided needle biopsy. The other 28 patients had a PSA ≥ 4 μg/l and cancer was excluded because they had a negative histological study in the samples obtained by transurethral prostatectomy or open prostatectomy. In all 50 patients, more than one blood sample was collected before any treatment and, in 25, blood samples were collected before and during treatment.

Group 3 comprised 364 patients with prostate cancer. Diagnosis of prostate cancer was obtained by digital rectal examination, transrectal ultrasonography and at least six ultrasound-guided biopsies, or by examination of tissue specimens following transurethral prostatectomy. In only 50 patients were samples available both before and during hormonal therapy, with 125 patients only providing samples before therapy, and 189 only after therapy had started. Thus, 175 samples were available before therapy and 239 during hormonal therapy. 40 patients had provided more than one sample before treatment.

Hormonal therapy in prostate cancer patients consisted of luteinising hormone-releasing hormone analogues to maintain plasma testosterone within the castrate range (< 50 ng/dl). Treatment of the histologically confirmed BPH patients consisted of transurethral resection of the prostate. Treatment of the non-histologically confirmed BPH patients consisted of laser prostatectomy.

Methods

Citrated blood samples were collected before any prostate manipulation, and the plasma was deep frozen, stored in aliquots at − 80°C and used within 6 months.

Total PSA concentration was determined by a specific sandwich enzyme-linked immunosorbent assay (ELISA), as reported previously [9]. Briefly, the assay uses a polyclonal immunopurified anti-PSA antibody as the primary antibody and the same antibody labelled with peroxidase as the secondary antibody. The assay was calibrated with purified PSA obtained according to the method of Sensabaugh and Blake [22] and had a detection limit of 0.2 μg/l.

The PSA:α_1ACT complex concentration was measured by a specific sandwich ELISA as reported previously [13]. The assay uses as the same primary polyclonal immunopurified anti-PSA antibody as in the total PSA assay, and labelled anti-α_1ACT antibody served as the secondary antibody. The assay was calibrated with purified PSA:α_1ACT complex prepared by incubating the purified components and filtering the incubated mixture on Sephacryl S-200 [13]. A dose–response curve was obtained with concentrations of complexed PSA ranging from 0.1 to 3 μg/l. The detection limit was 0.3 μg/l of complex or 0.1 μg/l of complexed PSA. The results were expressed as nanograms of PSA complexed per millilitre. A cut-off of 0.8 was used [13]. The complexed-to-total PSA ratio was calculated by dividing the concentration of complexed PSA by that of total PSA (measured with our polyclonal assay) in each sample.

Levels of significance were determined by Student's *t* and paired *t* tests and by the Mann–Whitney mean rank non-parametric *U* test. Analysis of variance and the Friedman non-parametric repeated measures (paired) test were used to test variations in parameter levels in the longitudinal study. Proportions were compared using the chi-squared statistic. Unless specified, the values represent the mean ± standard deviation (SD). A *P* value of < 0.05 was considered statistically significant.

RESULTS

Longitudinal study (pretreatment)

Tables 1 and 2 show the mean values of the total PSA and complexed-to-total PSA ratio in all the groups studied before any treatment and on three visits at least 3 months apart (average, 5 months between visits). There were no significant changes in the complexed-to-total PSA ratio in any patient group (Table 2). Total PSA concentration showed no significant variation in BPH patients, but there was a significant increase in total PSA concentration in subjects with prostate cancer. Of the 40 patients with prostate cancer, only 4 had a PSA level < 4 μg/l and 12 patients showed total PSA levels > 20 μg/l. 75 of the 90 BPH patients had complexed-to-total PSA ratios ≤ 0.80. Of 15 BPH patients with a ratio > 0.80 on their first visit, 3 had a ratio ≤ 0.80 on later visits. In contrast, 34 of the 40 patients with prostate cancer had a ratio > 0.80 on their first visit and only 2 showed a ratio ≤ 0.80 on subsequent visits. The other 6 patients with prostate cancer had a ratio ≤ 0.80 on all visits. Figure 1 shows the longitudinal changes of the complexed-to-total PSA ratio in a representative number of patients with BPH or prostate cancer.

Influence of therapy

In 50 patients with prostate cancer, PSA and the complexed-to-total PSA ratio were evaluated before and after

3–12 months of hormonal therapy. Tables 3 and 4 summarise the results obtained. The mean PSA concentration and ratio significantly decreased during hormonal therapy compared with pretreatment values. Following therapy, the total PSA concentration decreased to below 4 μg/l in 85% (43/50) of the prostate cancer patients (Table 3). For the complexed-to-total PSA ratio, 41/50 prostate cancer patients had a ratio > 0.80 before treatment, whilst in 34 of these 41 patients, the ratio decreased to ≤ 0.80 after treatment (Table 4). In the other 7 patients, the ratio remained > 0.80. Furthermore, following treatment, the proportion of patients in whom the ratio had decreased to ≤ 0.80 was significantly higher ($P < 0.0001$) in the group of patients in whom the total PSA concentration had also decreased to below 4 μg/l than in the

Table 1. Plasma concentration of total prostate specific antigen (PSA; μg/l) (mean ± standard deviation (SD), median in parentheses) in men with histologically confirmed (HC) benign prostatic hyperplasia (BHP), non-HC (NHC) BHP or prostate cancer on two or three consecutive visits, before any treatment

Clinical group	No.	First visit	Second visit	Third visit
NHC BPH	40	1.74 ± 1.31 (1.51)	2.01 ± 1.26 (1.75)	1.97 ± 1.01 (1.72)
HC BPH	50	5.11 ± 3.29 (4.82)	5.44 ± 3.09 (5.43)	6.08 ± 3.49 (5.96)
Prostate cancer	40	28.7 ± 35.8 (12.1)	33.2 ± 45.2 (14.6)*	37.4 ± 31.6 (19.17)**

The NHC BPH group included patients with total PSA < 4 μg/l and normal digital rectal examination. The HC BPH group included patients with total PSA ≥ 4 μg/l in whom cancer was excluded by biopsy or histological study following prostatectomy. Statistical significance with respect to the first visit: *$P < 0.01$; **$P < 0.001$.

Table 2. Values of complexed-to-total prostate specific antigen (PSA) ratio (mean ± standard deviation (SD), median in parentheses) in men with histologically confirmed (HC) benign prostatic hyperplasia (BPH), non-HC (NHC) BHP or prostate cancer on two or three consecutive visits, before any treatment

Clinical group	No.	First visit	Second visit	Third visit
NHC BPH	40	0.67 ± 0.12 (0.70)	0.65 ± 0.13 (0.67)	0.66 ± 0.12 (0.68)
HC BPH	50	0.69 ± 0.12 (0.70)	0.66 ± 0.12 (0.66)	0.67 ± 0.10 (0.68)
Prostate cancer	40	0.87 ± 0.12 (0.88)	0.84 ± 0.12 (0.86)	0.84 ± 0.11 (0.87)

The NHC BPH group included patients with total PSA < 4 μg/l and normal digital rectal examination. The HC BPH group included patients with total PSA ≥ 4 μg/l in whom cancer was excluded by biopsy or histological study following prostatectomy.

Table 3. Pre- and post-treatment total prostate specific antigen (PSA) values (μg/l) (mean ± standard deviation (SD), median in parentheses) in men with histologically confirmed (HC) benign prostatic hyperplasia (BPH), non-HC (NHC) BPH or prostate cancer

			Post-treatment	
Clinical group	No.	Pretreatment	First visit	Second visit
NHC BPH	20	2.1 ± 1.2 (2.5)	4.9 ± 3.4 (5.3)*	3.8 ± 2.1 (3.9)
HC BPH	25	4.4 ± 3.4 (3.3)	3.4 ± 2.9 (2.2)	1.9 ± 1.3 (1.8)*
Prostate cancer	50	188.1 ± 696.2 (23.9)	4.2 ± 9.6 (1.2)**	5.3 ± 10.2 (1.1)**

The NHC BPH group included patients with total PSA < 4 μg/l and normal digital rectal examination. The HC BPH group included patients with total PSA ≥ 4 μg/l in whom cancer was excluded by biopsy or histological study following prostatectomy. Statistical significance with respect to the pretreatment value: *$P < 0.05$; **$P < 0.0001$.

Table 4. Pre- and post-treatment values of complexed-to-total prostate specific antigen (PSA) ratio (mean ± standard deviation (SD), median in parentheses) in men with histologically confirmed (HC) benign prostatic hyperplasia (BPH), non-HC (NHC) BPH or prostate cancer

			Post-treatment	
Clinical group	No.	Pretreatment	First visit	Second visit
NHC BPH	20	0.62 ± 0.12 (0.59)	0.72 ± 0.18 (0.74)	0.76 ± 0.15 (0.76)
HC BPH	25	0.70 ± 0.13 (0.69)	0.65 ± 0.20 (0.63)	0.64 ± 0.20 (0.62)
Prostate cancer	50	0.85 ± 0.13 (0.86)	0.63 ± 0.22 (0.64)*	0.62 ± 0.21 (0.65)*

The NHC BPH group included patients with total PSA < 4 μg/l and normal digital rectal examination. The HC BPH group included patients with total PSA ≥ 4 μg/l in whom cancer was excluded by biopsy or histological study following prostatectomy. Statistical significance with respect to the pretreatment value: *$P < 0.001$.

group of patients in whom the total PSA concentration remained above 4 μg/l (94% versus 12%, respectively). 3 patients in whom the ratio decreased to ≤ 0.80 in the first visit after starting hormonal therapy, showed a ratio > 0.80 in subsequent visits in parallel with the increase in their total PSA concentration.

There was a significant decrease in the total PSA concentration in histologically confirmed BPH patients following therapy (Table 3), although the complexed-to-total PSA ratio remained constant. However, a slight increase in total PSA and the ratio was seen in non-histologically confirmed BPH patients following therapy.

Figure 2 shows the individual changes of the complexed-to-total PSA ratio in a representative number of patients with BPH or prostate cancer following therapy.

In a cross-sectional study, we evaluated the influence of hormonal therapy on total PSA and complexed-to-total PSA ratio by measuring total and complexed PSA in 175 samples from prostate cancer patients under no treatment and in 239 samples from patients that were under hormonal therapy for 3–12 months. The mean total PSA concentration in the 239 patients under hormonal therapy was significantly lower (11.0 ± 19.6) than in the 175 prostate cancer patients not treated (49.2.0 ± 155.4; $P < 0.001$). The complexed-to-total PSA ratio was also lower in the treated patients (0.68 ± 0.21) than in patients not treated (0.87 ± 0.09; $P < 0.0001$). 17 patients without treatment showed a total PSA concentration below 4 μg/l and, of these, 15 had a ratio ≥ 0.80. Of the 239 treated patients, 149 (62%) had a ratio ≤ 0.80, whereas of the 175 non-treated patents, only 32 (18%) showed a ratio ≤ 0.80 ($P < 0.0001$). Furthermore, 104 of the 135 (77%) patients under hormonal therapy that had a total PSA concentration < 4 μg/l (responders) had a ratio ≤ 0.80, whereas only 49 of the other 104 treated patients (47%) whose total PSA did not fall below 4 μg/l (non-responders) had a ratio ≤ 0.80 ($P < 0.0001$).

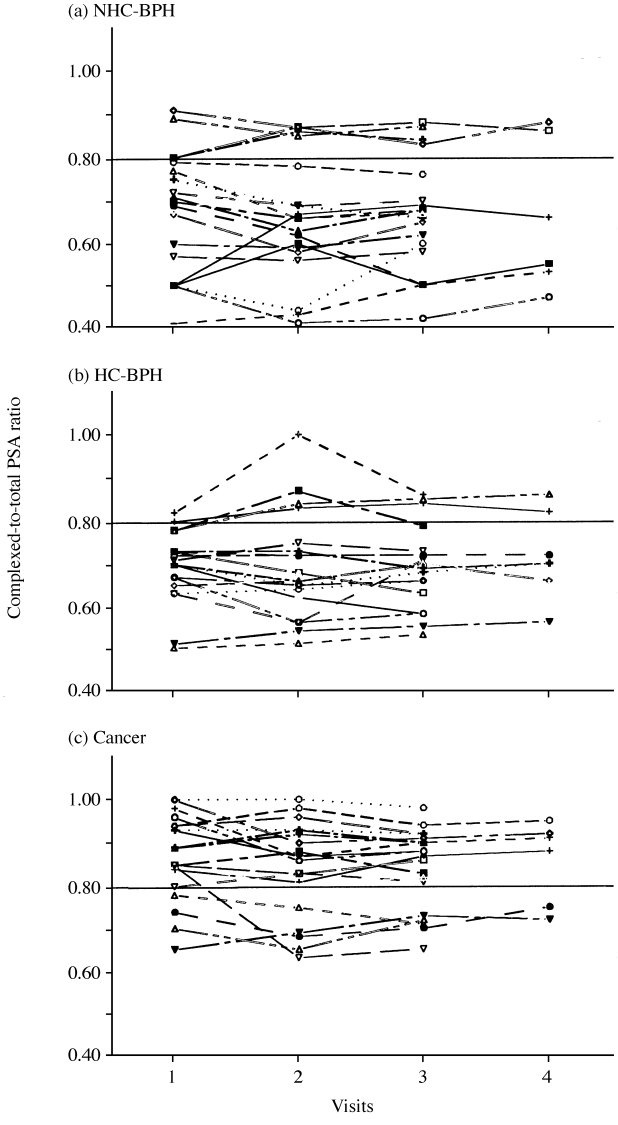

Figure 1. Longitudinal study of the complexed-to-total prostate specific antigen (PSA) ratio in representative groups of patients with histologically confirmed (HC) benign prostatic hyperplasia (BPH), non-HC (NHC) BPH or prostate cancer on two, three or four consecutive visits, before any treatment. A cut-off of 0.80 is indicated [13].

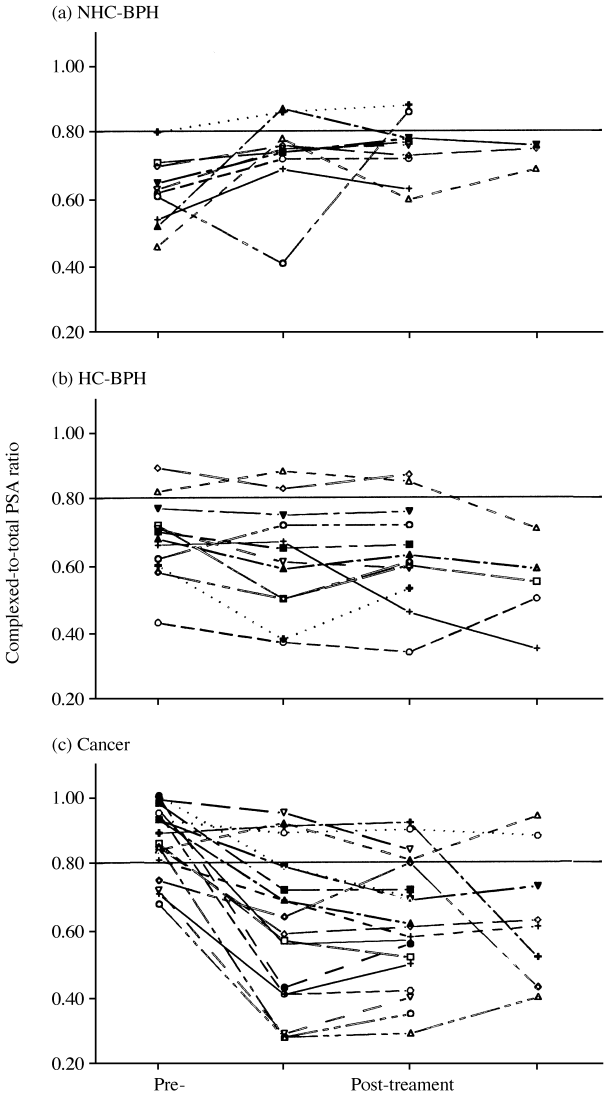

Figure 2. Pretreatment and post-treatment complexed-to-total prostate specific antigen (PSA) ratio in representative groups of patients with benign prostatic hyperplasia (BPH) or prostate cancer. A cut-off of 0.80 is indicated [13].

DISCUSSION

The results of this study show that, without treatment, the complexed-to-total PSA ratio does not significantly change in serial determinations, and confirm previous results showing that the majority of patients with prostate cancer have a ratio > 0.80, whereas most BPH patients have a ratio ≤ 0.80 [6, 7, 11–15, 20]. However, in the few prostate cancer patients that present with a ratio ≤ 0.80, this ratio remains low on all visits, and in the BPH patients that have a ratio > 0.80 it remains constantly high. The fact that some BPH patients show a ratio ≥ 0.80 and maintain this high ratio in serial determinations suggests the presence of an incipient tumour undetectable by biopsy and by digital rectal examination. This view is supported by our finding that 4 BPH patients in whom prostate cancer was excluded by sextant biopsy and who had a complexed-to-total PSA ratio > 0.80 on two or more consecutive visits were found, on subsequent biopsy, to have an organ-confined prostate cancer (F. España, M. Martínez, J.F. Jiménez-Cruz, unpublished observations). These data suggest that BPH patients in whom a ratio > 0.80 is observed in serial determinations should be re-biopsied to confirm the presence of cancer, although further studies with a larger number of patients are required to clarify this point.

In contrast, it is difficult to explain the fact that some patients with prostate cancer show a ratio ≤ 0.80 in serial determinations. This could be due to the presence of a type of tumour cell that produces a PSA isoform less reactive towards α_1ACT. In fact, it has been reported that approximately 30–60% of the total PSA in circulation in patients with prostate disease is in a free, inactive form [23] and that hyperplastic and carcinomatous tissue produces different PSA isoforms [24]. Bjork and colleagues [25] have speculated that the higher proportion of serum PSA complexed to α_1ACT in prostate cancer patients than in BPH may relate to structural changes in the PSA molecule derived from cancer patients compared with that derived from BPH patients. Nevertheless, of the 9 prostate cancer patients that had a pretreatment ratio ≤ 0.80, 7 (78%) showed a decrease in the total PSA concentration to below 4 μg/l, the same percentage found in patients with a pretreatment ratio > 0.80. This means that even if the low pretreatment ratio observed in these patients was due to the occurrence of tumour cells producing a PSA molecular form less reactive toward α_1ACT or a higher proportion of inactive PSA, these tumour cells would still have a normal response to hormonal treatment. Alternatively, it is possible that the location of the cancer in the prostate may influence the proportion of PSA complexed to a α_1ACT that is measured in the blood circulation. It has been suggested that there are morphological and biological differences between prostate cancers originating in the different anatomical zones [26–28]. Bjartell and associates [29] suggested that α_1ACT and PSA released from the prostate epithelium may react with each other in the extracellular compartment to form complexes that then enter the blood circulation. Therefore, different architectural abnormalities in prostatic tumours may account for progressive impairment of complex formation between PSA and α_1ACT before reaching the circulation. Once PSA reaches the circulation, it may react not only with α_1ACT, but also with α_2-macroglobulin [8, 9], thus decreasing the relative concentration of PSA:α_1ACT complex in the circulation.

Another important result of our study is the demonstration of a significant decrease in the complexed-to-total PSA ratio in patients with prostate cancer following hormonal therapy. The great majority (41/50) of patients had a pretreatment ratio > 0.80, and in most of them (34/41, 83%) the ratio decreased to ≤ 0.80 after hormonal treatment. Furthermore, the decrease in the ratio paralleled the decrease in PSA, and all patients in whom the ratio remained above 0.80 during hormonal therapy showed a total PSA concentration ≥ 4 μg/l. Moreover, the 3 patients in whom the ratio decreased to ≤ 0.80 in the initial phase of therapy and then increased to the original ratio value of > 0.80, showed the same pattern of changes in total PSA concentration.

Therefore, this preliminary study suggests that the complexed-to-total PSA ratio may be useful in monitoring patients with prostate cancer under hormonal therapy. However, whether the ratio will improve the information that total PSA already gives the clinicians to monitor therapy and guide the selection of patients for non-hormonal salvage therapy remains to be investigated. The fact that patients with histologically confirmed BPH show no variations in total PSA and complexed-to-total PSA ratio following therapy makes it possible to use the ratio to monitor these patients for newly developed tumours.

The initial increase in total PSA and the ratio seen in patients with non-histologically confirmed BPH following laser prostatectomy could be due to the short time interval between therapy and sampling (approximately 1 month). However, the levels remain increased even after 3 months of therapy. Therefore, in spite of the small number of cases studied, we cannot rule out the possibility that this increase is due to structural changes in the prostate tissue produced by the treatment. Further studies with a larger number of cases and longer follow-up time are needed to confirm this possibility.

In all, 75 of the 90 BPH patients had a complexed-to-total PSA ratio ≤ 0.80 before any treatment, and 143 of the 175 patients with prostate cancer under no treatment had a ratio > 0.80, which gives a sensitivity and specificity of 81.7% and 83.3%, respectively. These data confirm our previous results showing that the use of the complexed-to-total PSA ratio instead of total PSA significantly increases the specificity without decreasing the sensitivity [13].

In summary, our results show that neither the physiological changes in total and complexed PSA nor the treatment of BPH patients change the efficacy of the complexed-to-total PSA ratio, whereas in prostate cancer patients under hormonal therapy the ratio decreased in parallel with the decrease in total PSA. This suggests that the complexed-to-total PSA ratio could also be used to monitor BPH patients for newly developed tumours or to monitor therapy in patients with prostate cancer.

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