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The use of 'ultrasensitive' prostate-specific antigen assays in the detection of biochemical recurrence after radical prostatectomy

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Objective To determine the gain in lead time obtained when using ultrasensitive prostate-specific antigen (PSA) assays in the diagnosis of biochemical progression after radical prostatectomy.

Patients and methods The post-operative PSA serum concentrations of 137 patients who had undergone radical prostatectomy were evaluated retrospectively. From these patients, 12 were selected who showed biochemical recurrence, as measured by the Hybritech Tandem-E Singlepoint PSA assay. Samples of the serum frozen at the time of the initial analysis were thawed and PSA values were remeasured by the Abbott IMx PSA assay and the Tandem-E Multipoint PSA assay. Analytical thresholds (zero-dose + 3 SD) for the Tandem-E Singlepoint, IMx and Tandem-E Multipoint assay were 1.0, 0.04 and 0.04 ng/mL, respectively. The lead time to the detection of a recurrence obtained when using the IMx and the Tandem-E Multipoint PSA assay was compared with that attained using the Tandem-E Singlepoint PSA assay. As a control, PSA values were determined in

58 serum specimens of nine patients having no evidence of recurrence after radical prostatectomy.

Results All 58 control specimens had PSA levels below the analytical thresholds of the three assays, except one which had a PSA serum concentration of 0.08 ng/mL, estimated by the IMx assay. When compared with the lead time obtained with the Tandem-E Singlepoint assay, the 12 patients with a biochemical recurrence had a median gain in lead time of 327 days (range 60–627) with the IMx assay and of 369 days (range 60–639) with the Tandem-E Multipoint assay.

Conclusion A PSA value >0.04 ng/mL after radical prostatectomy heralds further biochemical progression. The use of the ultrasensitive IMx and the Tandem-E Multipoint assays provided more lead time, but there is no clear evidence that this gain is necessarily of benefit to the patient.

Keywords Radical prostatectomy, prostatic carcinoma, prostate-specific antigen, ultrasensitive assay

Introduction

The monitoring of patients after radical prostatectomy performed for localized prostate carcinoma has been significantly enhanced by the widespread application of assays for serum prostate-specific antigen (PSA). If the entire gland is extirpated by radical prostatectomy then the concentration of PSA should decline to less than the level of detection. Assuming that all benign tissue is removed, the presence of PSA is highly specific for cancer during the follow-up of patients after radical surgery. A biochemical recurrence (i.e. an increase of serum PSA above the analytical threshold) is a reliable harbinger of eventual clinical failure [1,2]. Schild *et al.* [3], in a study of 27 patients, found that an isolated increase in serum

PSA of >0.3 ng/mL after prostatectomy indicated the presence of residual or recurrent disease. Conversely, tumour recurrences in patients with undetectable PSA concentrations after prostatectomy are infrequently reported [4,5]. None of the 1058 patients studied by Partin *et al.* [6] demonstrated local recurrence or distant metastases after radical prostatectomy, if the serum PSA level was <0.2 ng/mL.

A PSA assay of increased sensitivity could permit the earlier detection of rising serum PSA concentration and therefore of tumour recurrence. Recent reports have demonstrated the earlier detection of residual cancer after radical prostatectomy using PSA assays of increased sensitivity, which have been termed 'ultrasensitive assays' [7–9]; the time between prostatectomy and detection of a significant increase in PSA is designated 'lead time' [10].

Accepted for publication 14 November 1995

The present study was conducted to assess any gains in lead time obtained using ultrasensitive PSA assays when compared to those obtained using the Tandem-E singlepoint assay.

Patients and methods

The records of 150 patients who underwent a radical prostatectomy in our clinic for prostatic cancer between April 1986 and October 1994, and had a follow-up of at least one year, were reviewed. This period covers the time during which the Tandem-E PSA assay with a singlepoint calibration (cat #4813, Hybritech Inc, San Diego, CA, USA) was used routinely in our laboratory. To obtain sufficient precision, the analytical threshold used for this assay was 1.0 ng/mL, although the manufacturer claims a minimum detectable concentration of 0.5 ng/mL.

Of the 150 patients, 137 (median age 65 years, range 47–73) had post-operative PSA measurements performed in our hospital. Pre-operatively, there was no clinical evidence of disseminated disease in any patient, as determined by a history, a physical examination, chest X-ray, a radionuclide bone-scan, computed tomography and, eventually, magnetic resonance imaging of the abdomen and pelvis. All patients underwent a retropubic radical prostatectomy. Post-operatively, 35 patients had a biochemical recurrence and of these, nine patients had detectable PSA levels throughout the follow-up, whereas the other 26 patients had undetectable PSA levels in the early post-operative period, as measured by the singlepoint assay, followed by a rise in PSA level to >1.0 ng/mL. In 12 of these 26 patients, a median of four PSA determinations were performed each year and these 12 patients (median age 61 years, range 51–68) constituted the study group.

The serum determinations by the singlepoint assay were performed on freshly collected venous blood samples processed routinely to serum. Samples of serum were stored frozen at -35°C and were later thawed as required for the determination of PSA by two assays of increased sensitivity; the IMx PSA assay (Abbott Laboratories, Abbott Park, Ill, USA) and the Tandem-E PSA assay with multipoint calibration (cat #4824, Hybritech Inc, San Diego, CA, USA). In both, the minimum detectable concentration was 0.04 ng/mL, as determined by replicated measurements of the zero-dose of the calibration standard and taking the PSA concentration at the mean response rate $+3\text{ SD}$ of these measurements. All three assays used are double-determinant, 'sandwich-type' enzyme immunoassays; sandwich assays use a capture antibody and an immunologically different tracer antibody. The IMx assay uses a polyclonal tracer antibody, whereas both the Tandem-E

assays apply a monoclonal tracer antibody. The multipoint assay uses five calibration standards to obtain a calibration curve of greater precision. The 'zero' diluent of the multipoint assay is a bovine-protein matrix, which possibly creates less 'noise' from interfering proteins than does the human-protein matrix of the singlepoint assay. Furthermore, samples in the multipoint assay are diluted less during the procedure and the incubation time with the substrate reagent is 10 min longer than in the other assays. These differences give an increased analytical sensitivity. As there was (and still is) no internationally recognized PSA calibration standard when the IMx assay was designed, it was calibrated against the Hybritech assays to achieve equivalent values in comparable clinical situations [10].

Because the half-life of serum PSA *in vivo* is 2.2–3.15 days [1,11], measurements of PSA within the month after prostatectomy are unsuitable for detecting or excluding residual disease [12]. Thus, only serum that had been taken more than one month after prostatectomy was used. All measurements of PSA were duplicated. The time at which the PSA level became detectable was defined as that halfway between consecutive PSA measurements where the first was below and the second above the analytically detectable threshold. The Wilcoxon test was used to compare this period from operation to biochemical recurrence.

The residual disease detection limit (RDDL) was defined as the lowest serum PSA concentration that indicates disease after radical prostatectomy [13]. The RDDL should be higher than the concentration of PSA resulting from contamination from extra prostatic sources of PSA, e.g. the peri-urethral glands or immunologically related interfering proteins. To ensure that a measurement above the analytical threshold signified residual disease and lay above the RDDL, the PSA levels of a control group of 58 serum specimens were measured using the three assays. These specimens were taken from nine patients who underwent a radical prostatectomy for prostatic cancer, had negative surgical margins, had no capsular penetration on pathological examination and were free of clinical and biochemical recurrence, as measured by the Tandem-E singlepoint assay, for at least 2 years post-operatively.

Results

Pathological examination of the initial 137 patients revealed positive lymph nodes in eight patients and capsular penetration in 50% of the specimens. The median follow-up was 36 months and the 5-year biochemical and clinical recurrence-free rate was 68% (Kaplan-Meier statistic, SEM 6%). In the 58 control samples, the PSA value was undetectable with all three

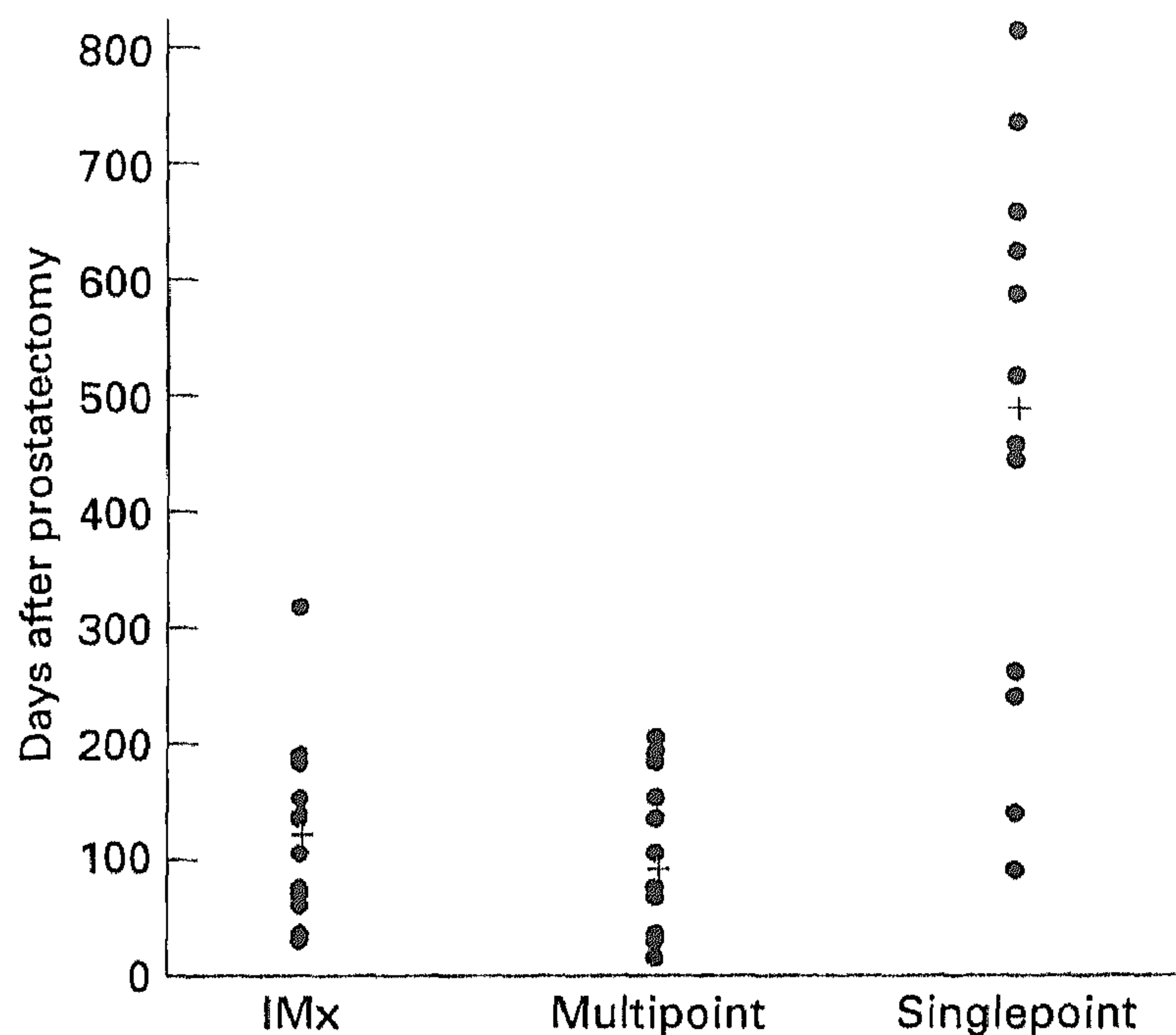


Fig. 1. Number of days after prostatectomy on which serum PSA was detectable for the IMx, the multipoint and the singlepoint assay.

assays (<1.0 ng/mL for the Tandem-E singlepoint assay and <0.04 ng/mL for both the ultrasensitive assays) except for one sample which measured 0.08 ng/mL by the IMx assay.

In the 12 patients selected for further study, a histological examination showed involvement in the seminal vesicle in seven, positive margins in eight, tumour growth penetrating the capsule in nine and a Gleason sum grade of ≥ 7 in four of them. The median follow-up of these patients was 3.4 years (range 2.5–4.1). Post-operative PSA values remained above the analytical threshold of the IMx and multipoint assays in three and five patients, respectively. Figure 1 shows the number of days after surgery on which the serum PSA level rose above the analytical threshold of each of the three assays. PSA was detectable at a median of 484 days (range 89–810) with the singlepoint assay, which was significantly longer ($P < 0.0025$) than the median of 120 days (range 29–318) with the IMx assay and 92 days (range 16–202) with the multipoint assay; the lead times with the ultrasensitive assays were not significantly different. When compared to the singlepoint assay, the median gain in lead time was 327 days (range 60–627) for the IMx assay and 369 days (range 60–639) for the multipoint assay. Figure 2 gives an example from a patient with increasing PSA levels as measured by the three assays after radical prostatectomy. Compared to the singlepoint assay, the serum PSA was detectable earlier using the IMx assay and earlier still using the multipoint assay.

Discussion

PSA is a very sensitive tumour marker for monitoring patients after definitive therapy. The immunometric

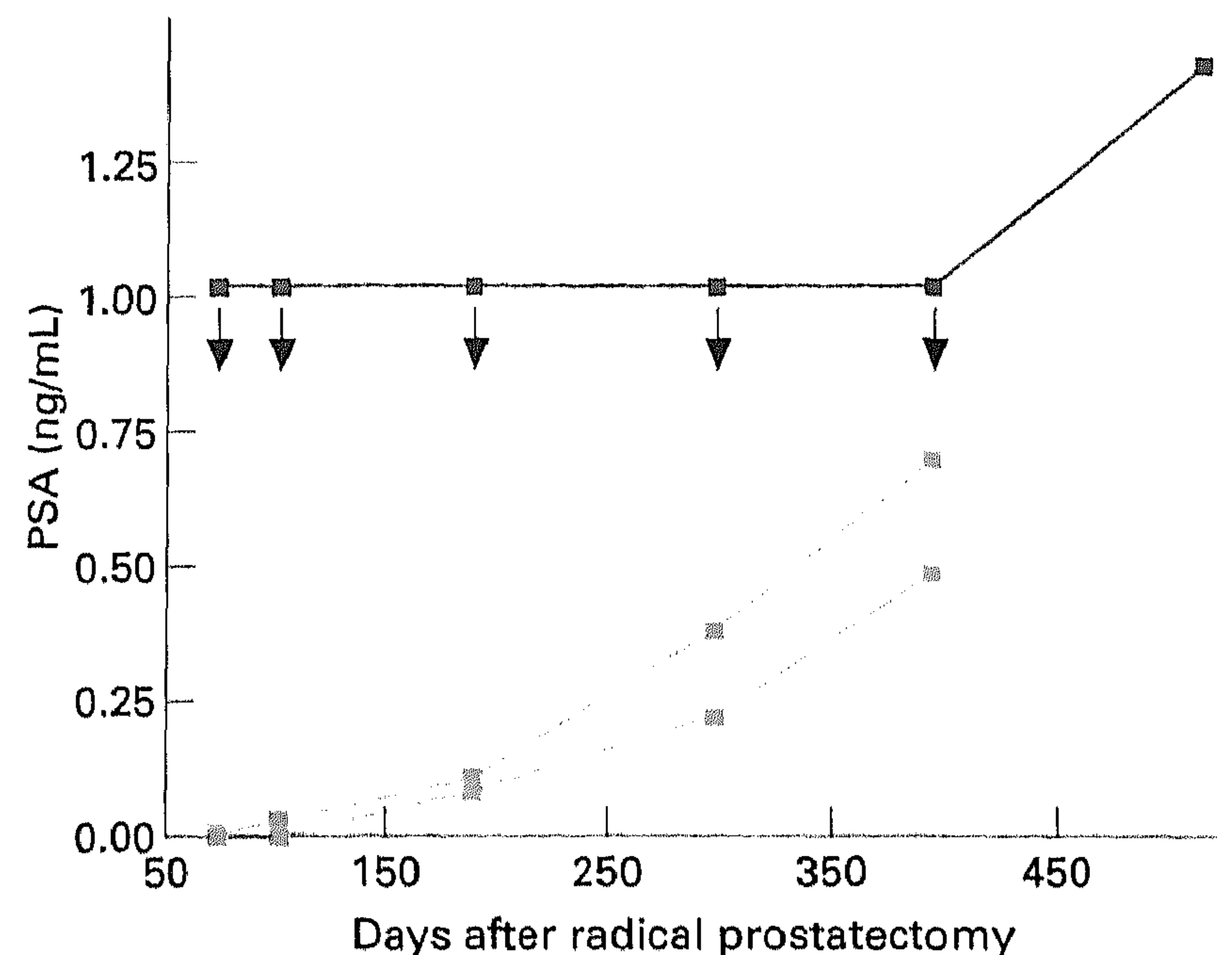


Fig. 2. An example of a patient with a gain in lead time in the detection of progression obtained using the ultrasensitive assays. Dark green, Single-point; ↓: <1.0 ng/mL. Light green, Multipoint. Light red, IMx.

technique used for the 'ultrasensitive' PSA assays is already used in most PSA assays in the clinical setting. Optimizing this existing technique has improved the sensitivity. Several problems should be resolved before recommending the use of ultrasensitive assays during the follow-up of these patients:

- (i) Before a radical prostatectomy, PSA circulating in the serum originates mainly from the prostatic gland. After a radical prostatectomy, the contamination by PSA produced in extraprostatic sources [14] could cause false-positive results in the biochemical detection of residual prostate cancer. However, Elgamal *et al.* [15] and Oesterling *et al.* [16] found no significant contribution to serum PSA from the peri-urethral glands at detection thresholds of 0.1 ng/mL and 0.07 ng/mL, respectively.
- (ii) The amino acid sequence of PSA is 77% homologous with human glandular kallikrein-1 (hGK-1) and also has significant homology with other proteases of the kallikrein family, possibly because the gene for hGK has been duplicated during evolution to form the PSA gene [17]. The homology of hGK with PSA may cause cross-reactivity of kallikreins with the anti-PSA antibodies used in the assays and may diminish the clinical utility of the ultrasensitive assays. However, from the results obtained with the present serum samples from control patients, it is clear that the PSA 'noise' produced by extraprostatic sources and the presence of cross-reacting substances did not influence the analytical specificity of the Tandem-E PSA immunoassay at values >0.04 ng/mL. With the development of even more sensitive PSA assays [18], this matter should be reconsidered.
- (iii) It has not been established whether the early

Table 1 Actuarial clinical recurrence and prostate-specific antigen progression-free rates 5, 10 and 15 years after radical prostatectomy

	Reference						Present study
	[21]	[22]	[23]	[24]	[25]	[26]	
Number of patients	232	2371	955	601	500	925	137
Stage \leq pT2 (%)	22	47	37	48	45	65	50
PSA threshold (ng/mL)	0.2	0.2	0.2	0.4	0.4	0.6	1.0
Clinical and biochemical recurrence-free rate at:							
5 years (%)	51	70	83	69	76	78	68
10 years (%)	38	52	70	47	73		
15 years (%)	40						

detection of biochemical recurrence of prostate cancer has any clinical benefit. A complete remission after adjuvant radiation therapy can only be achieved in patients with a recurrence localized to the pelvis. However, according to Sanders *et al.* [19] PSA, as well as DRE and TRUS, are poor predictors of the results of biopsies taken from the anastomosis after radical prostatectomy. Metastases might respond better to adjuvant therapy when they are still small. Any value of the early detection of biochemical recurrence to obtain a more optimal adjuvant hormonal treatment will remain unproven until the current debate on early versus delayed treatment is resolved.

(iv) Knowing that there is a biochemical recurrence and thus an inevitable clinical recurrence can have a considerable impact on the quality of life of the patient. Currently, this knowledge is only of theoretical use to the clinician, while it is a burden for the patient. A hormonal treatment started after the detection of a biochemical recurrence can diminish the quality of life of the patient concerned. In the study of Kurth *et al.* [20] on the quality of life of patients who either did or did not receive hormonal treatment, the group without hormonal therapy had better psychological and sexual function.

The 5-year recurrence-free rate of 68% in the patients in the present study compares with that in other studies (51–83%, Table 1). As all but one of the serum PSA measurements in the control group were <0.04 ng/mL, an increase above this level can be considered as indicative of tumour recurrence. A considerable gain in lead time was obtained if the post-operative serum PSA concentration was measured with an ultrasensitive assay. The precision of the estimate of lead time depends on the frequency at which serum PSA measurements are performed. Stamey *et al.* [8] reported a median gain in lead time of 202 days when comparing an ultrasensitive PSA assay with a threshold of 0.1 ng/mL and a standard assay with a threshold of 0.3 ng/mL. Although the use of ultrasensitive assays to detect recurrence

sooner remains unproven in daily clinical practice, the ultrasensitive assays can be beneficial when the value of adjuvant therapy is assessed. Because a rise in PSA is indicative of recurrence or metastasis of prostate cancer [3] a biochemical recurrence can be used as an intermediate endpoint in clinical studies after radical prostatectomy; using ultrasensitive assays, these endpoints can be reached sooner.

Acknowledgement

The authors thank Mr. H. Ariaëns for excellent technical assistance.

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