Percent-free prostate specific antigen is elevated in men on haemodialysis or peritoneal dialysis treatment

Laila Bruun1, Thomas Björk2, Hans Lilja3, Charlotte Becker3, Ove Gustafsson4 and Anders Christensson1

1Department of Nephrology and Transplantation, 2Department of Urology and 3Department of Laboratory Medicine, University Hospital, Malmö and 4Department of Urology, University Hospital, Huddinge, Sweden

Abstract

Background. Men with chronic renal failure evaluated for transplantation are often tested for prostate specific antigen (PSA) to detect prostate cancer. PSA occurs in several different molecular forms in serum: free PSA (fPSA) and complexed PSA (cPSA), the sum of which corresponds to total PSA (tPSA). In addition to tPSA, percent fPSA to tPSA (%fPSA) is widely used to enhance discrimination of benign disorders from prostate cancer. The low molecular mass of fPSA suggests elimination by renal glomerular filtration and that renal failure may significantly influence %fPSA. We evaluated whether established reference levels for %fPSA are applicable also to patients treated with haemodialysis or continuous ambulatory peritoneal dialysis (CAPD).

Methods. The study included 20 men on intermittent haemodialysis with low-flux membranes and 25 men on CAPD, without known history of prostate cancer. The control group included 3129 men without known prostate cancer. We analysed fPSA and tPSA in serum by dual-label immunofluorometric assays, from which we calculated %fPSA and cPSA. Serum levels of different PSA forms were adjusted for age and presented as geometric means.

Results. Percent fPSA was significantly higher in patients on either haemodialysis (39.5%) or CAPD (39.6%) compared with controls (28.1%). Haemodialysis patients, but not CAPD patients, had significantly higher mean levels of fPSA. Levels of tPSA and cPSA for haemodialysis or CAPD patients did not differ significantly compared with controls.

Conclusions. Recommended reference ranges for %fPSA, based on men with normal renal function, do not apply to uraemic men on dialysis. In these men, a high %fPSA should not be considered as a sign of benign disease. This is clinically important in the evaluation of dialysis patients for transplantation, as %fPSA is often used as a tool for detection of prostate cancer.

Keywords: haemodialysis; peritoneal dialysis; prostate cancer; PSA; renal failure; renal transplantation

Introduction

In the evaluation of patients for transplantation, it is important to exclude malignant diseases. One reason is that immunosuppressive drugs may influence the natural history of malignancies unfavourably. Prostate cancer is one of the most common forms of malignancy in men in western countries [1]. Furthermore, patients on haemodialysis have been reported to be at increased risk for prostate cancer [2] and are often subject to transplantation with subsequent immunosuppression. It has been shown that immunosuppression in renal transplant recipients means a risk for recurrence of prostate cancer [3]. For detection and screening of prostate cancer, measurement of serum prostate specific antigen (PSA) has become an important tool [4]. PSA occurs in several molecular forms in blood: mainly as a free non-complexed PSA form (fPSA) with a molecular mass of 28 kDa; and as PSA complexed to α1-antichymotrypsin, complexed PSA (cPSA), with a mass of 90 kDa [5]. Total PSA (tPSA) comprises the sum of fPSA and cPSA. The most commonly exercised clinical cut-off point for serum tPSA in detecting prostate cancer is ≥4 μg/l [6]. However, only 25–35% of men with tPSA levels of 4–10 μg/l are diagnosed as having prostate cancer, as there is significant overlap in tPSA levels in men with prostate cancer and those found to have benign prostatic hyperplasia (BPH). The percentage of free-to-total PSA (%fPSA) was introduced due to its capacity to enhance discrimination of prostate cancer from BPH, since the %fPSA is

Correspondence and offprint requests to: Laila Bruun, MD, Department of Nephrology and Transplantation, University Hospital Malmö, SE-205 02 Malmö, Sweden. Email: laila.bruun@klkemi.mas.lu.se

© 2003 European Renal Association–European Dialysis and Transplant Association
lower in prostate cancer than in BPH [7]. By using a combination of both tPSA and %fPSA, the specificity for early detection of prostate cancer has been improved, especially in the diagnostic grey zone [8].

The transport of proteins across the glomerular barrier depends on the charge, shape and size of the molecule, as well as the charge and pore-size of the glomerular capillary wall [9]. Low molecular weight proteins (with molecular mass <40–50 kDa) are readily eliminated from plasma by glomerular filtration followed by reabsorption and catabolism in proximal tubule cells. Patients with renal failure have a decreased clearance of low molecular weight proteins and their plasma level becomes elevated as renal function decreases. There are several factors that determine levels of plasma proteins in haemodialysis patients. Residual renal function and permeability of dialysis membranes are the main factors involved in the elimination of low molecular weight proteins [10,11]. In patients on continuous ambulatory peritoneal dialysis (CAPD), the efficiency of the dialysis per se, but also residual renal function, is of great importance for clearance of low molecular weight proteins [12]. Clearance of low molecular weight proteins over the peritoneum takes place through large pores. The shape, size and electrical charge of the proteins determine clearance rates.

PSA is a glycoprotein consisting of a single polypeptide chain with an isoelectric point ~7. The low molecular mass of fPSA, its predicted globular conformation, and the neutral charge at physiological pH suggest elimination by glomerular filtration. Data from several different studies of fPSA elimination have reported half-lives of 12–18 h, which also supports the hypothesis of renal elimination [13]. The route of elimination for cPSA is still uncertain, but reported data suggest mechanisms other than renal clearance due to the high molecular mass and remarkably slow elimination rates [13].

Some previous reports have suggested that patients with severe renal failure treated with haemodialysis may have elevated serum fPSA and higher %fPSA [14, 15], but contradictory findings have also been reported [16,17]. Furthermore, it has been shown that haemodialysis may decrease the concentration of fPSA in serum following dialysis sessions performed with high-flux membranes, but not with low-flux membranes [18]. However, there are no previously reported data on CAPD patients and their levels of fPSA and cPSA forms.

Percent fPSA is often used in combination with tPSA to detect prostate cancer in patients with chronic renal failure before they are considered for transplantation and subsequent immunosuppression. We hypothesized that these patients may have a falsely elevated %fPSA, which could diminish the diagnostic usefulness of this parameter in the detection of prostate cancer. The purpose of the present study was to evaluate whether the reference ranges for %fPSA are applicable in patients on haemodialysis with low-flux membranes or CAPD.

Subjects and methods

Patients

All subjects receiving treatment by chronic haemodialysis with low-flux membranes or CAPD at our dialysis unit between January 1999 and September 2001 were considered for inclusion in this study. We excluded one patient on haemodialysis and four patients on CAPD with a diagnosis of prostate cancer, and five men on haemodialysis and four men on CAPD who were under 40 years of age. All patients included presented tPSA levels <10 µg/l.

The haemodialysis group consisted of 20 men (median age 66 years; range 42–84) on treatment for at least 1 month with low-flux membranes (Sureflux®, Nipro, Japan; Dicea®, Baxter, USA; Pro® and GFS®, Gambro, Sweden). Dialysis was performed three times weekly. All patients had a Kt/V >1.2. The duration of each dialysis session was from 4 to 5 h and dialysate flow was 500 ml/min, which gives a total dialysate amount of 120–150 l during each treatment. The median accumulated treated blood volumes were 81 l (range 56–120). Residual renal function was measured in 13 of 20 patients by iohexol clearance, showing a median residual renal function of 0 ml/min/1.73 m² (range 0–1.4). Seven men with low urinary production were not evaluated by iohexol clearance. The CAPD group included 25 men (median age 65 years; range 41–79) on dialysis for at least 1 month. Median residual renal function was 2.8 ml/min/1.73 m² (range 0–8.9). The daily treatment regimen included four to five exchanges of 2–2.5 l of dialysis fluids, resulting in 8–12.5 l of dialysate per 24 h.

Informed consent was obtained from all patients.

Controls

In order to obtain a sufficiently large number of controls, we used a total of 3129 healthy men (median age 57 years; range 33–80). We used male controls from three different studies to cover the age range of our dialysis patients in this study.

One control group consisted of 1680 healthy men (median age 63 years; range 55–70) who had been randomly selected and volunteered to participate in 1988–1989 in a prostate cancer screening in Stockholm, Sweden [19]. Biopsy criteria were abnormal findings on either digital rectal examination or transrectal ultrasound, or tPSA >10 µg/l. In this group, 307 men underwent prostate biopsy without carcinoma being detected.

The second control group included 63 randomly selected elderly men (median age 73 years; range 70–80) from a population study of men in Olmsted County, Minnesota, at the University of Michigan, USA. These men with no history of voiding problems underwent digital rectal examination, serum PSA testing and transrectal ultrasound without prostate cancer being detected [6].

Finally, a third group of controls came from a population study in Malmo, Sweden (the Preventive Medicine Project) enrolling 1386 younger men (median age 47 years; range 33–61) who were without any diagnosis of prostate cancer, registered in the Swedish Cancer Registry, up to 23 years after base-line blood sampling [20].

Blood collection and pre-analytical work-up of samples

One blood sample was collected from each CAPD patient. Blood samples were taken both immediately before and after the haemodialysis treatment. The blood samples were allowed
to clot for 30 min at room temperature and subsequently centrifuged at 2000 g for 15 min. The serum was then immediately frozen and stored at −20°C, pending analysis. Dialysate was collected 5 min after the start and 5 min before the end of a haemodialysis session. Dialysate was obtained from each bag during a 24 h CAPD session. The collected volumes of dialysate were concentrated 10–30-fold before analysis by ultrafiltration on Diaflo membranes with cut-off ranges of 10 kDa (Amicon Corp., Danvers, MA, USA). All dialysates were stored at −20°C.

**Analytical methods**

A time-resolved fluoroimmunoassay (ProStatus™ PSA Free/Total assay) from Perkin-Elmer Life Sciences (Turku, Finland) was used to measure tPSA and fPSA from which we calculated %fPSA and cPSA (tPSA–fPSA = cPSA). This commercial assay is based on a dual-label detection technique, which uses three distinctly uniquely binding and carefully characterized monoclonal anti-PSA antibodies. The lower limit of detection is 0.04 μg/l for fPSA and 0.05 μg/l for tPSA. The patients in all control groups were analysed with similar assay methods using the same antibody combinations as those used in this study. Correlation coefficients for tPSA vs Tandem-R PSA in the control groups and in our study ranged from 0.97 [6] to 0.99 [19,21].

There were no available measurements of cPSA in all samples from the control groups. However, in our study patients, cPSA was analysed with the commercially available Automated Chemiluminescence System (ACS:180® cPSA assay) from Bayer Diagnostics (NY, USA). We performed simple linear regression analysis in the study group to evaluate whether calculated cPSA (tPSA minus fPSA) correlated with measured cPSA, in order to use calculated cPSA throughout the study.

**Residual renal function**

Glomerular filtration rates in haemodialysis patients were determined by measuring the plasma clearance of iohexol according to a one-compartment model [22]. Iohexol was analysed by a HPLC technique [23]. The coefficient of variation was 4.0–3.5% from the lowest standard (31 mg/l) to the highest standard (133 mg/l), respectively.

Patients on CAPD were evaluated regularly by individual peritoneal dialysis capacity (PDC™). Residual renal function was obtained thereby from creatinine clearance.

**Statistical methods**

Logarithmic transformation of all variables was performed to fulfill the assumption for normality. For the same reason, our results are presented as geometric means.

As PSA levels are age dependent [6], the values of the different PSA forms were adjusted for age and thereafter expressed as percentage of controls. Statistical analysis comparing the differences between dialysis patients and controls was performed using an analysis of covariance (ANCOVA). A paired t-test was used in the comparison of PSA forms before and after a haemodialysis session.

**Results**

Age-adjusted geometric means and 95% confidence intervals (CIs) for the different molecular forms of PSA and %fPSA in patients on haemodialysis, CAPD and controls are shown in Table 1. Using our control material, consisting of 3129 subjects, <14%fPSA was considered to be abnormal, according to the 5th percentile.

Percent fPSA was 40% higher in haemodialysis patients and 41% higher in CAPD patients than in the controls, which was statistically significant (P < 0.0001) (Figure 1). The tPSA levels did not differ significantly in haemodialysis patients or in CAPD patients compared with the controls, nor did they differ between the two dialysis groups. The geometric mean level of fPSA in haemodialysis patients was 55% higher (P = 0.005) than for the controls, whereas fPSA levels in CAPD patients were not significantly increased (23% higher) compared with the controls. There was no significant difference in fPSA concentrations in haemodialysis compared with CAPD patients.

We also estimated the 95th percentile for fPSA levels in our 3129 controls and found the upper normal level to be 0.62 μg/l. From this we evaluated the proportion of the CAPD and haemodialysis patients with an elevated level of fPSA. Five of 25 CAPD patients and seven of 20 haemodialysis patients presented with fPSA > 0.62 μg/l.

Simple linear regression analysis between measured cPSA and calculated cPSA showed an excellent correlation (r = 0.98) with the equation y = 0.063 + 0.981x, supporting the use of calculated cPSA throughout this study to substitute for the lack of measured cPSA levels. The levels of cPSA in either haemodialysis patients (P = 0.69) or CAPD patients (P = 0.086) were not significantly different as compared with the controls, but there was a tendency toward lower levels of cPSA in CAPD patients.

Haemodialysis patients were found to have significantly higher serum concentrations of both tPSA and cPSA when serum was collected immediately after termination of the dialysis session, but %fPSA did not differ in samples collected before treatment as compared with post-treatment (data not shown). Finally, we found no detectable levels of the different molecular forms of PSA in patients on haemodialysis with low-flux membranes, CAPD patients and controls.

**Table 1.** Age-adjusted geometric mean of the different molecular forms of PSA in patients on haemodialysis with low-flux membranes, CAPD patients and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Geometric mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haemodialysis patients (n = 20)</td>
</tr>
<tr>
<td>tPSA</td>
<td>1.05 (0.74–1.51)</td>
</tr>
<tr>
<td>fPSA</td>
<td>0.42 (0.31–0.56)</td>
</tr>
<tr>
<td>cPSA</td>
<td>0.60 (0.40–0.90)</td>
</tr>
<tr>
<td>%fPSA</td>
<td>39.5 (33.4–46.7)</td>
</tr>
</tbody>
</table>

* tPSA, fPSA and cPSA (tPSA minus fPSA) are given in μg/l. The values for haemodialysis patients represent results before the haemodialysis session.

* Statistical difference from controls at P < 0.01.

* Statistical difference from controls at P < 0.0001.
fractions of PSA in dialysates, either from patients on haemodialysis or CAPD.

Discussion

Measurements of %fPSA in blood have found widespread use during the past 10 years to increase the diagnostic specificity for early detection of prostate cancer. Since the publication of the original report [7], it has been confirmed extensively that high %fPSA indicates a lesser risk of cancer as compared with low %fPSA. Analysis of tPSA and %fPSA in blood is used in the evaluation of patients with chronic renal failure who are considered for renal transplantation. Our results show that the recommended reference ranges for %fPSA are not applicable to patients on dialysis for the detection of prostate cancer, whether they are patients on haemodialysis or CAPD.

Haemodialysis patients have been studied by other groups who also noted higher %fPSA [14,15]. However, other researchers have reported conflicting data [16,17] showing unchanged levels of %fPSA in the same category of patients. Yet another study recently presented findings that tPSA, fPSA and %fPSA were unchanged during haemodialysis and that the reference ranges for these parameters were applicable to patients on chronic haemodialysis [24]. It is noteworthy that these authors did not present any comparisons with controls.

As %fPSA involves both tPSA and cPSA, the ratio is dependent on the mechanisms and rates of elimination of these two molecular forms. Increased levels of tPSA as well as decreased cPSA levels result in increased %fPSA. Previously reported elimination kinetics data and the low molecular mass (28 kDa) of fPSA suggests clearance from plasma by glomerular filtration and that decreased renal function may lead to an increased serum level of fPSA, resulting in higher %fPSA. In this study, we show that patients on haemodialysis with low-flux membranes have significantly higher levels of fPSA in serum, indicating decreased elimination of fPSA in patients with severe renal insufficiency and no elimination over low-flux membranes. This is in accordance with the permeability of low-flux membranes considering their low cut-off point (5 kDa). We also found higher levels of tPSA and of cPSA in samples collected immediately post-vs pre-dialysis, which is most likely due to haemoconcentration during haemodialysis. Elimination by the kidneys was excluded due to the findings of very low residual renal function in our haemodialysis patients.

Sasagawa et al. [14] suggested that decreased levels of binding proteins might be the cause of the increased levels of fPSA and %fPSA reported by their group. Douville and Tiberi [15] found both increased levels of fPSA and decreased levels of cPSA as an explanation for the higher %fPSA. We could not demonstrate any significant decrease in cPSA compared with controls in this study as an explanation for the higher %fPSA. In addition, occurrences of up to 10-fold molar excess of α1-antichymotrypsin relative to PSA also makes it less likely that minor differences in blood levels of these binding proteins could significantly increase the fPSA levels in blood.

Our findings are the first to describe levels of fPSA and cPSA in patients on CAPD. The increased %fPSA in CAPD patients may be caused by several factors. The peritoneal membrane has a great variability in pore size. Molecular transport over the peritoneal membrane takes place through different pores; ultra small, small and large pores [12]. Low molecular weight proteins are eliminated through large pores. Our results show that fPSA in serum is not significantly increased in patients on CAPD. This may be explained by either elimination of fPSA over the peritoneal membrane or by renal elimination, or both. It is well known that CAPD patients usually preserve a minor residual renal function for a long time on dialysis compared with haemodialysis patients. This was also noted in our patients, although the residual renal function was very low. The significantly increased %fPSA in CAPD is most likely attributable to a combination of some decrease in levels of cPSA and increased levels of fPSA, even although the differences in either fPSA or cPSA levels were not found to be significant.

Our control material (n = 3129) was obtained from three different cohorts. The reason for this is that we wanted to cover the entire age spectrum of our dialysis patients and be able to make age-adjusted comparisons of the serum levels of PSA forms between the dialysis patients and the controls. All analyses of the different PSA isoforms in our study patients and controls were made with the same assay technique and men with diagnosis of prostate cancer were excluded from the control groups. Based on the 5th percentile, the %fPSA considered abnormal is <15% according to Oesterling et al. [6]. This is very close to the %fPSA we found to be considered abnormal (<14%) using our control
material of 3129 subjects. Based on our estimates of the 95th percentile for tPSA levels in our control material, five of 25 CAPD patients and seven of 20 haemodialysis patients presented with abnormal fPSA levels.

Our findings are important for patients with chronic renal failure, for several reasons. Patients on haemodialysis have been reported to be at increased risk for prostate cancer [2] and are often considered for renal transplantation. Immunosuppression of transplanted patients is associated with an increased risk of malignancies. Before transplantation, an extensive evaluation of the potential recipient is performed to detect the presence of cancer and in most cases tPSA and %fPSA are used in screening for prostate cancer. However, our findings show that in patients with chronic renal failure on dialysis, a high %fPSA should not be considered as a sign of benign disease. The recommended reference range for %fPSA is based on men with normal renal function and thus not applicable to dialysis patients. In contrast, tPSA is not affected by either haemodialysis or peritoneal dialysis. In these patients, the detection of prostate cancer must be based on tPSA, the image at transrectal ultrasound and digital rectal examination.

There are no reports on the effect of different degrees of renal failure on PSA levels. Further studies are required to determine the level of renal function that is significant for reduction of fPSA elimination, and to obtain reference ranges for tPSA and %fPSA for these patient categories.

Acknowledgements. We thank Jan-Åke Nilsson for statistical assistance, Gun-Britt Eriksson and Kerstin Häkansson for laboratory work and the staff at the dialysis unit, University Hospital Malmö. We also express our thanks to Steven J. Jacobsen for assistance with control subjects. Bayer Diagnostics and PerkinElmer Life Sciences provided reagents for the analyses. This investigation was supported by the Swedish Research Council (Medicine, project no. 7903); the Faculty of Medicine, Lund University; the Research Fund and the Cancer Research Fund of the University Hospital, Malmö; the Foundation Federico S.A. and the Regional Research Foundation.

References

Received for publication: 16.5.02
Accepted in revised form: 23.9.02