Original Article

Serum levels of beta-trace protein and its association to diuresis in haemodialysis patients

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Abstract

Background. Beta-trace protein (BTP) has been proposed as an alternative endogenous marker of the glomerular filtration rate. However, possible determinants of BTP in ESRD patients undergoing regular renal replacement therapy have not been evaluated.

Methods. Serum levels of BTP, beta-2-microglobulin, creatinine and urea were analysed before and after dialysis treatment in 73 patients [haemodialysis (HD) n = 52; haemodiafiltration (HDF) n = 21]. Patients were categorized into four groups with residual diuresis (RD) < 0.5 l/day (group 1; n = 24), 0.5–1 l/day (group 2; n = 18), 1.1–1.5 l/day (group 3; n = 12) and >1.5 l/day (group 4; n = 19). Subsequently RD was compared to pre-treatment levels of BTP.

Results. HD treatment did not affect BTP serum levels [pre-treatment 8.1 ± 4.1 mg/l (mean±SD) vs post-treatment 7.7 ± 4.1 mg/l; -0.6 ± 16.1%; ns]. However, in 6 out of 21 patients undergoing HDF BTP levels were reduced by more than 20%. Overall, the resulting decrease in serum concentration was minuscule (9.6 ± 6.2 vs 8.3 ± 4.9 mg/l; -14 ± 21.9%; P = 0.03). BTP serum levels were tightly associated to RD of the four groups. Comparison of BTP levels showed significant differences between patients of groups 1 vs 3 and 4 as well as 2 vs 4.

Conclusions. BTP serum levels may serve as a surrogate marker for residual renal function since HD and HDF do not exert clinical relevant alterations on them. Furthermore, BTP serum concentrations appear strongly associated to RD.

Keywords: beta-trace-protein; beta-2-microglobulin; haemodiafiltration; haemodialysis; residual renal function

Introduction

Beta-trace-protein (BTP) (MW 25.2 kDa) was primarily isolated as prostaglandin D2 synthase from cerebrospinal fluid and found to be increased in the serum of patients with renal failure [1]. The half-life of BTP is approximately 1.2 h and it is virtually exclusive excreted via the kidneys [2]. Therefore, it has been proposed as a new endogenous marker of glomerular filtration rate (GFR) [3–5].

Markedly increased serum levels of BTP have been reported in patients undergoing haemo- and peritoneal dialysis [6]. Although the mode of renal replacement therapy (RRT) importantly affects removal of middle molecules and small-molecular weight proteins [7], the impact of major determinants of RRT on BTP has not been investigated to date. The aim of this study was to evaluate the serum levels of BTP in patients on chronic RRT, with special emphasis on patient and treatment characteristics as well as residual diuresis (RD).

Patients and methods

Seventy-three patients (24 female, 49 male) on continuous intermittent RRT aged 59 ± 15.2 years (mean ± SD) were included in the study.

Treatment consisted of haemodialysis (HD) in 52 and of haemodiafiltration (HDF) using the post-dilution method in 21 patients. Further treatment characteristics are given in Table 1.

Five different high flux dialyzers were used throughout the study: polysulfone membranes: Braun HI-PS 18 (effective surface 1.8 m², UFC 55 ml/h/mmHg; Braun, Germany) (HDF n = 6; HD n = 2), FX60 (effective surface 1.4 m², UFC 46 ml/h/mmHg; Fresenius, Germany) (HDF n = 6; HD n = 15), F60S (effective surface 1.35 m², UFC 40 ml/h/ml/mmHg; Fresenius, Germany) (HDF n = 1; HD n = 20) and polyamide membranes: Arylane H 6 (effective surface 1.57 m²; UFC 69 ml/h/mmHg; Hospal, Switzerland) (HDF n = 2; HD n = 5), Polyflux 14S (effective surface 1.4 m², UFC 62 ml/h/mmHg; Gambro, Germany)
(HDF $n=6$; HD $n=10$). Single pool $Kt/V$ was calculated using the Efficacy$^\circledR$ software (Riegel, Kuhlmann et al., Homburg, Germany).

The study was approved by the local ethics committee and informed consent was obtained from all patients enrolled in the study.

Parameters and assays

Before and after the dialysis treatment, 5ml serum was drawn for determination of the analytes as described in detail elsewhere [8].

Serum creatinine (crea) was measured with a modified Jaffe-method using the Dimension™ Clinical Chemistry System (Dade Behring, Marburg, Germany). Serum beta-2-microglobulin (B2MG) and BTP were analysed by fully automated, latex-enhanced immunonephelometry (N latex B2 mg, N latex BTP, respectively, on a Nephelometer II; Dade-Behring). Total 95% reference intervals established from a representative cohort of 100 female and 100 male healthy blood donors (median age, 31 years; 2.5–97.5 percentiles, 19.0–60.5 years) were 0.402–0.738 mg/l for BTP, 1.085–2.015 mg/l for B2MG and 0.6–1.2 mg/dl for crea as described elsewhere [4]. The reduction of the analytes by treatment was corrected for body weight loss as described by Bergström and Wehle [9] to counteract ultrafiltration effects.

Data on RD were documented at study inclusion. In out-patients attending the study, volumes of RD were determined by a 24 h urine collection during a ‘long interval’ as routinely performed at home quarterly, whereas in in-patients the urine was collected by the nurse at the ward.

Statistical analysis

Results were expressed as the arithmetic mean and standard deviation (mean ± SD). Statistical analysis was performed using the StatView 5.0™ Software (Version for Windows; SAS Institute Inc., Cary, NC, USA).

Differences between two groups (HD vs HDF) were analysed by the Mann–Whitney–U-test or $t$-test for unpaired data and pre- vs post-session data by the Wilcoxon Sum Rank test or paired $t$-test, where applicable. Differences between BTP serum levels in the four groups categorized by RD and the groups with different dialysers were analysed by ANOVA (+ Bonferroni/Dunn post-hoc test). $P$-values <0.05 were considered significant.

Results

Age, body weight, duration of the dialysis sessions, the solution-flow rate, the fluid removal rate and $Kt/V$ did not differ between HD and HDF groups (Table 1). On average, the blood flow rate was some 24 ml/min higher in the HDF group. The mean volume of replacement fluid in the HDF group was 17.5 ± 51 per session.

Pre-treatment levels of BTP

Pre-treatment levels of BTP were markedly elevated in all patients studied. The mean serum level was 8.5 ± 4.8 mg/l being 11.5-fold the upper normal range derived from a control group of 200 healthy blood donors (0.402–0.738 mg/l). Pre-treatment levels of all analytes are given in Table 1.

There was no significant correlation between predialysis serum levels of BTP and age, body weight or time since start of RRT. BTP serum concentrations measured in male patients were not statistically different from those measured in female patients (data not shown).

Influence of haemodialysis and haemodiafiltration on serum concentration of analytes

As shown in Figure 1 and Table 2 HD and HDF treatment reduced crea, urea and B2MG significantly. In contrast, BTP levels were not altered significantly by HD treatment (pre-treatment 8.1 ± 4.1 mg/l vs post-treatment 7.7 ± 4.1 mg/l, $P=ns$), whereas a small reduction vs baseline was found for BTP levels after HDF treatment (9.6 ± 6.3 vs 8.3 ± 4.9 mg/l, $P=0.03$). Figure 2 shows in detail the pattern of BTP serum-level reduction delineating a distinct range that was more pronounced in HDF patients ($−0.6 ± 16$ vs $−14 ± 22$%; $P=0.027$).

Table 1. Treatment characteristics and pre-treatment levels of crea, BTP and B2MG

<table>
<thead>
<tr>
<th></th>
<th>All ($n=73$)</th>
<th>HD ($n=52$)</th>
<th>HDF ($n=21$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.0 ± 15.3</td>
<td>57.3 ± 16.4</td>
<td>63.9 ± 11.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70.1 ± 19.1</td>
<td>69.1 ± 20.2</td>
<td>72.5 ± 16.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Dialysis time (h)</td>
<td>4.4 ± 0.57</td>
<td>4.3 ± 0.51</td>
<td>4.6 ± 0.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Blood-flow rate (ml/min)</td>
<td>262.4 ± 38.8</td>
<td>255.4 ± 39.5</td>
<td>279.8 ± 31.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Solution-flow rate (ml/min)</td>
<td>506.8 ± 53.6</td>
<td>503.8 ± 48.4</td>
<td>514.3 ± 65.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Fluid removal rate (l)</td>
<td>1.6 ± 1.3</td>
<td>1.6 ± 1.2</td>
<td>1.6 ± 1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>$Kt/V$ single pool</td>
<td>1.33 ± 0.46</td>
<td>1.32 ± 0.48</td>
<td>1.35 ± 0.42</td>
<td>0.4</td>
</tr>
<tr>
<td>Pre-treatment crea (mg/dl)</td>
<td>7.8 ± 3.2</td>
<td>7.6 ± 3.4</td>
<td>8.3 ± 2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Pre-treatment B2MG (mg/dl)</td>
<td>23.8 ± 12</td>
<td>23.2 ± 10.8</td>
<td>25.2 ± 14.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Pre-treatment BTP (mg/dl)</td>
<td>8.5 ± 4.8</td>
<td>8.1 ± 4.1</td>
<td>9.6 ± 6.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Pre-treatment urea (mg/dl)</td>
<td>119.2 ± 42</td>
<td>118.7 ± 44.3</td>
<td>120.4 ± 36.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Statistical significant differences between the groups are indicated where appropriate. Data are given as mean (±SD).
Influence of treatment determinants on reduction of analytes

Reduction of BTP was analysed in accordance to the characteristics of dialysers used (Figure 2).

UF-corrected reduction of BTP correlated weakly but significantly to the dialysers surface ($r = 0.283; P = 0.023$) and the membranes ultrafiltration-coefficient ($r = 0.254; P = 0.042$). Applying ANOVA analysis the UF corrected percental BTP reduction in patients treated with Braun HI-PS dialysers (22.9 ± 26.5%) was significantly larger compared to treatments performed with F 60 S (1.5 ± 13.1%; $P = 0.0016$) dialysers. No differences were observed between all other filters. Although blood flow-($r = 0.103; P = 0.9$) and solution-flow rate ($r = 0.108; P = 0.4$) were not correlated to BTP reduction, there was a weak but significant correlation to the duration of the dialysis session ($r = 0.264; P = 0.027$). Single-pool $K_t/V$ as a measure of dialysis dose was significantly correlated to the reductions of crea, urea and B2MG, but not to that of BTP (Table 3).

Pre-treatment levels of analytes and RD

Patients were categorized into four groups with RD $<0.51$/day (group 1; $n = 24$), 0.5–1/day (group 2; $n = 18$), 1.1–1.5/day (group 3; $n = 12$) and $>1.51$/day (group 4; $n = 19$).

As shown in Figure 3A, BTP levels were significantly different between patients of groups 1 vs 3 and 4 as well as 2 vs 4. In contrast, no differences were found for pre-dialysis B2MG levels between the groups (Figure 3B). Pre-treatment crea and urea levels were significantly different between patients of groups 1 and 4 only (Figure 3C and 3D).

By applying ROC analysis, a serum BTP concentration of 8.2 mg/l was identified as criterion [sensitivity of 75.5% (95% CI: 61.1–86.6), specificity of 70.8% (48.9–87.3)] to distinguish between no relevant RD and more than 0.51 diuresis per day. The positive predictive value was calculated as 84.1%, the negative predictive value tested as 59.3%.

Discussion

In this study, we show that serum levels of BTP are not altered significantly by HD. Although HDF treatment on average results in a small diminution of BTP this appears to be without clinical relevance in comparison to the other analytes studied. Furthermore, we found a striking association of BTP serum concentration with the amount of RD, suggesting BTP as a surrogate marker of residual renal function (RRF).

On average, we found an 11.5-fold increase of pre-treatment BTP serum levels in our cohort compared to the upper normal range of healthy blood

![Fig. 1. Percent reduction of the studied analytes in comparison to pre-treatment levels shown as the dotted baseline. Data are given as box plots indicating 25th and 75th percentile and the median; the whiskers designate 10th and 90th percentile.](https://academic.oup.com/ndt/article-abstract/23/1/309/1922995)

![Fig. 2. Post-treatment BTP reduction (%) depicted according to the dialyse membrane and mode of treatment applied.](https://academic.oup.com/ndt/article-abstract/23/1/309/1922995)

**Table 2.** Pre- and post-treatment levels of crea, urea, BTP and B2MG divided in HD and HDF

<table>
<thead>
<tr>
<th></th>
<th>HD Pre-Post $P$</th>
<th>HDF Pre-Post $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment crea (mg/dl)</td>
<td>7.8 ± 3.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-treatment crea (mg/dl)</td>
<td>3.1 ± 1.4</td>
<td>3.4 ± 1.6</td>
</tr>
<tr>
<td>Pre-treatment B2MG (mg/dl)</td>
<td>23.8 ± 11.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-treatment B2MG (mg/dl)</td>
<td>11.6 ± 5.2</td>
<td>10.5 ± 6.5</td>
</tr>
<tr>
<td>Pre-treatment BTP (mg/dl)</td>
<td>8.1 ± 4.1</td>
<td>0.83</td>
</tr>
<tr>
<td>Post-treatment BTP (mg/dl)</td>
<td>7.7 ± 4.1</td>
<td>8.3 ± 4.9</td>
</tr>
<tr>
<td>Pre-treatment urea (mg/dl)</td>
<td>118.7 ± 44.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-treatment urea (mg/dl)</td>
<td>40.3 ± 19.9</td>
<td>40.3 ± 25.9</td>
</tr>
</tbody>
</table>

Data are given as mean (±SD). Statistical significant differences between the pre- and post-treatment values are indicated where appropriate.
donors. In accordance with previous findings in non-ESRD patients [10], clinical characteristics like gender, age and body weight were not significantly connected to pre-dialysis BTP levels.

Melegos and colleagues reported increased serum levels of BTP in patients undergoing HD and PD [6], however, little is known about the exact impact of renal replacement on this protein. In contrast, the impact of HD on serum levels of B2MG, a representative of the LMWP-class, has previously been well studied by Kabanda and coworkers [11]. In accordance with this study, both high-flux HD and HDF, reduced B2MG levels significantly in our cohort. However, we could demonstrate that in contrast to B2MG, BTP is not significantly eliminated by high-flux HD. During HDF treatment we exclusively used the post-dilution mode, which has been shown to be superior to the predilution mode with respect to the removal of LMWP in the 12–33 kD range [12–14]. Post-dilution HDF treatment leads to the expected plunge in serum levels of B2MG, which is in line with previous findings [11].

![Fig. 3A–D. Pre-treatment levels of the analytes in four groups of patients categorized by RD (l/24 h).](https://academic.oup.com/ndt/article-abstract/23/1/309/1922995)

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>P</th>
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<tr>
<td>Creatinine reduction, spKt/V</td>
<td>HD 0.730</td>
<td>0.562</td>
<td>0.840</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>HDF 0.905</td>
<td>0.771</td>
<td>0.962</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urea reduction, spKt/V</td>
<td>HD 0.909</td>
<td>0.838</td>
<td>0.950</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>HDF 0.858</td>
<td>0.670</td>
<td>0.943</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B2MG reduction, spKt/V</td>
<td>HD 0.505</td>
<td>0.258</td>
<td>0.690</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>HDF 0.613</td>
<td>0.234</td>
<td>0.830</td>
<td>0.0032</td>
</tr>
<tr>
<td>BTP reduction, spKt/V</td>
<td>HD 0.266</td>
<td>-0.511</td>
<td>0.020</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>HDF 0.383</td>
<td>-0.072</td>
<td>0.706</td>
<td>0.096</td>
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concentrations of B2MG, crea and urea but only to a far smaller reduction of BTP serum concentration which seems to be not clinically relevant in everyday practice.

ANOVA analysis showed considerable differences in BTP reduction ability by different filters. These results suggest that the observed drop in serum BTP levels in patients undergoing HDF treatment are solely attributable to the BRAUN HI-PS membrane, preferably used in this cohort. Since we found only a poor correlation to UFC and dialyser surface, this effect must be attributed to other factors currently not known. It may be conceivable that our observed results for HDF treatment are confounded by the small number of patients undergoing HDF and the heterogeneity of membranes used. Increase of BTP serum levels after treatment in some patients may be attributed to concentration effects not completely counterbalanced by the Bergström formula applied in our study.

Since BTP is not substantially cleared by RRT and to a large extent independent from important determinants of RRT and dialysis dose—calculated as single-pool Kt/V—it was tempting to analyse its relation to RRF. Unfortunately, when we designed our study the measurement of RRF by urea-clearance was not implemented, which adds a drawback to our investigation. We therefore analysed BTP pre-treatment levels in sight of RD, the latter serving as a surrogate marker of RRF. After categorization of patients into groups according to their RD, BTP levels showed an inverse association with the RD. Furthermore, BTP allowed for a far better differentiation of patients with different RRF than pre-treatment creatinine and urea. Despite the recommendation to measure RRF in regular intervals in patients on chronic haemodialysis [8], the best clearance measure is still under debate. Both, creatinine and urea clearance have little accuracy estimating GFR at very low levels, especially of less than 1 ml/min [15]. Therefore, an established relation between BTP serum levels and RRF could ease patient management.

Our data may provide an explanation as to why lipoprotein-type prostaglandin D synthase, which has been shown to be the same protein as BTP [16], has previously been linked to cardiovascular injury [17]. This might only reflect the relation of impaired renal function and cardiovascular risk which is well-established [18]. Moreover, BTP is also under suspicion to contribute to the progression of renal failure [19], while underlying mechanisms have not been reported. Thus, one might speculate that the observed increase of BTP serum levels in patients with increasing impairment of renal function reflects only accumulation.

This is the first study to show that BTP serum levels are associated with RD of HD patients. Furthermore, we found that BTP, an endogenous marker of GFR, is virtually not cleared by RRT to a clinically important extent. This property of BTP may favour its use in the surveillance of patients on chronic RRT, but also in patients resolving acute renal failure while being on RRT. Due to limitations of our study, namely small sample size and heterogeneity of filters and treatment modes, additional systematic investigations are warranted to further elucidate the value of BTP as a diagnostic tool in patients on RRT.

References

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