Albumin-binding capacity (ABiC) is reduced in patients with chronic kidney disease along with an accumulation of protein-bound uraemic toxins

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Abstract

Background. Albumin is an important transport protein for non-water-soluble protein-bound drugs and uraemic toxins. Its transport capacity is reduced in patients with advanced chronic kidney disease (CKD) and unbound fractions of uraemic toxins are related to complications of CKD. We investigated whether this reduction could be quantified and how it correlated with the stages of CKD. Albumin-binding capacity (ABiC) is a dye-based method that quantifies the remaining binding capacity of one major binding site (site II) of the albumin molecule.

Methods. Blood samples from 104 CKD patients were incubated with a binding site-specific fluorescent marker and the amount of unbound marker was determined by means of fluorescence detection after filtration. Measurements in a pooled human plasma were used for reference. Glomerular filtration rate and serum indoxyl sulphate (IS) levels were also determined.

Results. Impairment of renal function was associated with a reduction in ABiC (mean ± SD: 118 ± 12; 111 ± 11; 99 ± 8 and 79 ± 9% for Stages 1/2, 3, 4 and 5, respectively; P < 0.001) and an increase in IS (3.9 ± 1.1; 6.2 ± 3.2; 16.3 ± 14.9 and 56.1 ± 28.6 μmol/L for Stages 1/2, 3, 4 and 5, respectively; P < 0.001). In dialysis patients, ABiC was lower in those with urine outputs <500 mL/day than in those with preserved urine output (73.7 ± 6.0 versus 83.8 ± 8.5%; P < 0.001).

Conclusion. Impaired albumin function in CKD patients can be quantified, is related to severity of kidney disease and is associated with an accumulation of uraemic albumin-bound retention solutes.

Keywords: albumin; albumin binding; chronic kidney disease; indoxyl sulphate; uraemic toxins
molecule and their concentrations are only slightly influenced by extracorporeal dialysis treatments [20, 21].

IS, a uraemic toxin, is accumulated in the serum of CKD patients. Part of the dietary protein-derived tryptophan is metabolized into indole by tryptophanase in intestinal bacteria. Indole is absorbed into the blood from the intestine and is metabolized into IS in the liver. Inadequate renal clearance leads to elevated serum levels in CKD [20]. IS has been identified to have a direct toxic effect on renal proximal tubular cells and has been associated with vascular alternations and the progression of renal failure [22–26]. As IS produces reactive oxygen species (ROS) in concentrations found in CKD patients, IS-induced toxicities in CKD patients could be caused by IS-induced oxidative stress [11, 14, 27, 28].

Several reports suggest links between impaired albumin-binding function of distinct ligands and reduced albumin concentration in CKD, elevated concentrations of uraemic toxins and/or chemical modification of the albumin molecule, but no comprehensive assessment of albumin function as CKD progresses from mild impairment to end-stage renal insufficiency has ever been carried out.

Albumin-binding capacity (ABiC) is a simple method for characterizing the site-specific binding functions of the albumin molecule. In in vitro experiments, ABiC was found gradually to decrease as concentrations of albumin-bound substances increased [29, 30], while the therapeutic elimination of albumin-bound agents resulted in an improvement in ABiC [31]. Accordingly, it is plausible to suggest that reduced ABiC in liver failure is linked to an increase in albumin-bound toxins.

To test the hypothesis that the accumulation of albumin-bound uraemic toxins results in a stage-dependent deterioration in albumin-binding function, we determined ABiC in patients with different stages of CKD.

Materials and methods

In this cross sectional study, 120 patients from the nephrology outpatient department or the dialysis department, Internal Medicine II, University Rostock, were planned to be enrolled, stratified into CKD Stage 1 [glomerular filtration rate (GFR) >90 mL/min/1.73 m², 20 patients], Stage 2 (GFR 60–89 mL/min/1.73m²), Stage 3 (GFR 30–59 mL/min/1.73m²), Stage 4 (GFR 15–29 mL/min/1.73m², 20 patients) and Stage 5 (GFR <15 mL/min/1.73m²; 40 patients), 20 of them with urine output ≥500 mL/day) according to the Kidney Disease Outcomes Quality Initiative guidelines [32]. Inclusion criteria were the presence of CKD, though patients with a severe underlying liver insufficiency (CHILD C) and acute renal impairment were excluded.

Patients with CKD were assigned by nephrologists into one of the five stages of CKD [32]. In addition, patients with a GFR <15 mL/min (Stage 5) were further classified according to urine output either into Subgroup 5a (urine output ≥500 mL/day) or Subgroup 5b (oligouria/anuric). A blood sample was taken and plasma was stored at −20°C until ABiC estimation. Laboratory values and other parameters at the time of enrolment were documented in a database. GFR was calculated according to the Modification of Diet in Renal Disease study equation [33, 34].

This study was approved by the local ethics committee (HV-2008-0007) and written informed consent was obtained from all patients and volunteers.

Estimation of ABiC

ABiC was estimated using an indirect method that has been described previously [31, 35]. The method is based on the estimation of the unbound fraction of a specific albumin-bound marker in a plasma sample. By comparing it with the fraction of unbound marker in a reference albumin solution, the site-specific binding capacity of the sample can be expressed semi-quantitatively.

Briefly, plasma samples were diluted to an albumin concentration of 150 μmol/L and incubated with an albumin-binding site II (Diazepam-binding site)-specific fluorescent marker [Dansylsarcosine (DS), 150 μmol/L]. Albumin-free filtrate was obtained in a separation step (Centrisart I, 20,000D; Sartorius GmbH, Goettingen, Germany) and fluorescence was measured (Fluoroscan; Thermo Labsystems, 355/465 nm) after addition of human serum albumin (300 μmol/L) as a fluorescence amplifier.

Parallel to this, the same procedure was performed on a standard albumin for reference. A standardized virus-inactivated human serum preparation from pooled human plasma (Biszeko®, Biotech Pharma GmbH, Dreieich, Germany) was used as a reference for ABiC.

The binding capacity for the marker was quantified according to the following equation:

\[
\text{ABiC} \left( \% \right) = \frac{\text{fluorescence in the filtrate of the reference}}{\text{fluorescence in the filtrate of the sample}} \times 100.
\]

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 15). Data from patients with CKD and a GFR >60 mL/min/1.73m² [Group 1 (n = 6) and Group 2 (n = 121)] were combined in one analysis group. Comparisons between the groups were based on Tamhane’s post hoc analysis for unequal variances and sample size or the non-parametric Mann–Whitney U-test, if appropriate. Correlation between variables was assessed non-parametrically according to Spearman. Logarithmic transformation was applied to GFR and IS to obtain a linear relationship as a prerequisite for Pearson correlation and partial correlation analysis. Data are given in mean ± SD, a P-value ≤0.05 was considered to be significant.

Results

Between November 2008 and October 2009, 105 patients (51 male/54 female) were enrolled. One patient with acute renal failure (IgA nephritis) was excluded from further analysis. Patient characteristics are given in Table 1.

Eighteen of 104 (17.3%) enrolled patients were assigned to CKD Stage 1 or 2, 26 patients to Stage 3 (25%) and 20 patients to Stage 4 (19.2%). Forty patients (36.4%) had a GFR <60 mL/min/1.73m² and 20 (50%) were oliguric or anuric.

Albumin binding of a site II-specific marker, expressed as ABiC, was found to be significantly reduced in the later stages of CKD (P < 0.001, Figure 1 and Table 2). In patients with mild impairment of renal function, normal or slightly reduced ABiC was found without significant differences between Stages 1/2 and 3 (Stage 1/2: 117.8 ± 11.8%; Stage 3: 110.7 ± 11.5%), but as renal function decreased further, ABiC was significantly impaired (Stage 4: 98.8 ± 8.1%, P < 0.001 and Stage 5: 78.8 ± 8.9%, P < 0.001).

Advanced stages of renal impairment were also associated with higher levels of IS than in Stage 1/2 (Figure 2 and Table 2). This increase was significant in post hoc analysis (Stage 3: P < 0.01; Stage 4: P < 0.001; Stage 5: P < 0.001).

The association between renal function (GFR), the serum level of the albumin-bound uraemic toxin IS and albumin-binding function (ABiC) was evaluated further. As expected, a strong correlation between GFR and IS
\( r_s = -0.902; P < 0.001 \) was found. In addition, a strong association was observed between GFR and ABiC \( (r_s = 0.881; P < 0.001, \text{Figure 3a}) \) and between IS and ABiC \( (r_s = -0.862; P < 0.001, \text{Figure 3b}) \).

When considering non-dialysed patients only (69 of 104 patients), there was still a significant relationship between GFR and IS \( (r_s = -0.781; P < 0.001) \) and GFR and ABiC \( (r_s = 0.753; P < 0.001) \).

As a moderate, but significant, correlation was present between ABiC and albumin concentration \( (r = 0.449; P < 0.001) \), partial correlation analysis was performed. The partial correlation coefficient was \( r = -0.378 \) \( (P < 0.001) \), which revealed an inverse relationship between (logarithmic) IS and ABiC independent of (logarithmic) GFR and albumin concentration as control variables.

Creatinine, urea, haemoglobin and albumin values differed significantly in the different stages, as impairment of renal function is accompanied by an increase in creatinine and urea and a decrease in haemoglobin and albumin [5, 37, 38].

No significant differences in liver enzyme activity, body weight, height, body mass index (BMI) nor C-reactive protein were found between groups. Mean age ranged between 56.2 ± 16.6 years in Stages 1 and 2 and 68.9 ± 9.8 years in Stage 4; age differences between groups were significant \( (P < 0.01) \), as was gender distribution in the groups \( (P = 0.03) \).

Considerably reduced ABiC was found in patients assigned to CKD Stage 5 (GFR < 15 mL/min/1.73m²). Twenty of these patients (50%) had a urine output of ≥500 mL/day, while 20 (50%) were oligouric or anuric. Serum creatinine (732 versus 1006 l mol/L, \( P = 0.001 \)) and calculated GFR (6.45 versus 4.17 mL/min/1.73m², \( P = 0.01 \)) were more deteriorated in the oligouric/anuric group. However, albumin concentrations, urea, BMI and liver enzymes were comparable in the two subgroups. ABiC was less reduced in the group with a preserved urine output (83.8 ± 8.5% versus 73.7 ± 6.0%, \( P < 0.001 \)). Inversely, higher serum levels of IS were found in the oligouric/anuric group (68.1 ± 26.0 l mol/L; \( P < 0.01, \text{Figure 4}) \).

Discussion

Impaired albumin binding as well as elevated concentrations of uraemic toxins in CKD are results of numerous investigation within the last 100 years [39, 40]. To our knowledge, this is the first report in which site II-specific albumin-binding function was assessed with respect to all stages of CKD and a relationship between the decrease in albumin-binding function and CKD severity was shown.

In addition, we demonstrate that levels of the albumin-bound uraemic toxin IS increase as CKD progresses. A deterioration in ABiC was found to be associated with GFR and inversely correlated with levels of the uraemic toxin IS.

**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Stage 1/2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFR ≥60 mL/min/1.73m²</td>
<td>GFR 30–59 mL/min/1.73m²</td>
<td>GFR 15–29 mL/min/1.73m²</td>
<td>GFR &lt;15 mL/min/1.73m²</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>18</td>
<td>56.2</td>
<td>16.6</td>
<td>26</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>18</td>
<td>85.7</td>
<td>21.1</td>
<td>26</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>18</td>
<td>172.1</td>
<td>13.1</td>
<td>24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18</td>
<td>28.8</td>
<td>5.9</td>
<td>24</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>18</td>
<td>111.6</td>
<td>46.1</td>
<td>26</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>18</td>
<td>7.0</td>
<td>5.4</td>
<td>23</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>18</td>
<td>43.9</td>
<td>2.3</td>
<td>26</td>
</tr>
<tr>
<td>Haemoglobin (mmol/L)</td>
<td>18</td>
<td>8.3</td>
<td>1.1</td>
<td>26</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>18</td>
<td>0.4</td>
<td>0.0</td>
<td>26</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>6</td>
<td>21.2</td>
<td>4.9</td>
<td>6</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>6</td>
<td>22.6</td>
<td>8.0</td>
<td>7</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>14</td>
<td>7.1</td>
<td>11.5</td>
<td>21</td>
</tr>
<tr>
<td>Gender m/f</td>
<td>7/11</td>
<td>11/15</td>
<td>5/15</td>
<td>27/13</td>
</tr>
</tbody>
</table>

*aALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CRP C-reactive protein.*
Accumulation of albumin-bound substances such as endogenous benzodiazepines and bilirubin is present in hepatic insufficiency as it is in renal failure [41, 42]. ABiC has been reported to decrease in patients with liver failure in line with disease severity. Its cause is likely the increase of albumin-bound toxins associated with hepatic insufficiency [31, 35].

However, the decrease in ABiC in the patients with renal failure in our study was not attributable to accompanying liver insufficiency as liver function test data were comparable between the groups.

The binding of ligands to albumin is affected by competition with other substances that display an affinity to the same molecular-binding site. It has been known since the beginning of the last century that uraemic patients display elevated levels of IS [39], and IS has been associated with impaired albumin binding and the clinical status of uraemia [40, 43].

An association between IS and progression of renal failure in pre-dialysis CKD patients was reported by Wu et al. [15] most recently. Correlation between IS and GFR in this cohort was found to be lesser than in our study. Unfortunately, IS concentrations for different CKD stages were not given.

Effect of haemodialysis therapy on site-specific albumin binding was investigated by Nishio et al. [4]. In accordance with our results, they have found an association between reduction of IS concentrations and an improvement of diazepam binding for albumin-binding site II.

PCS and IS, prototypes of protein-bound solutes which are elevated in renal insufficiency, are covalently bound to the same binding region of the albumin molecule. Meijers et al. [20] were able to show that changes in IS concentration affect free PCS and visa versa through competitive mechanisms.

DS, the marker substance of the ABiC test, has a specific affinity to the same binding region as the uraemic toxins mentioned above [3, 44]. As a result, IS was shown to displace DS in in vitro experiments [13].

In light of these competitive albumin–ligand interactions, the reduction of ABiC in renal insufficiency presented here can be explained in terms of the occupation of binding capacity by endogeneous protein-bound uraemic retention solutes.

Oxidative modification of the albumin molecule is present in CKD patients and might be related to an impaired albumin binding [9, 10, 45, 46]. Impact of elevated levels of oxidized non-mercaptalbumin on ABiC in addition to competitive ligand interactions is possible. Magnitude of such an assumed impact on ABiC has to be clarified in further investigations, as oxidative status of the albumin molecule was not assessed in our study.

ABiC test characterizes the available binding site II-related albumin function without considering the reason for such an impairment (competitive mechanism or alteration of the albumin molecule). IS is a competitive inhibitor of albumin-binding site II-specific ligands and also a pro-oxidant, which induced ROS production through a pathway involving NADPH oxidase or NADPH-like oxidase [27, 28]. Therefore, increasing amounts of oxidized albumin molecules might affect albumin binding too [14]. Measures to reduce IS concentration are associated with a reduction in oxidative stress, as demonstrated by Shimoishi et al. [14, 47]. As IS might influence albumin binding by two different ways, an advantage of the ABiC method approach with a site specific assessment of albumin-binding function becomes apparent (in contrast to estimation of IS concentration or oxidative albumin status).

Reduced albumin concentration has an impact on albumin–ligand binding. Hypoalbuminaemia is frequent in CKD patients and might be related to an impaired albumin binding [9, 10, 45, 46]. Impact of elevated levels of oxidized non-mercaptalbumin on ABiC in addition to competitive ligand interactions is possible. Magnitude of such an assumed impact on ABiC has to be clarified in further investigations, as oxidative status of the albumin molecule was not assessed in our study.

Table 2. IS and ABiC in different stages of CKD

<table>
<thead>
<tr>
<th>CKD Stage 1/2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>IS (μmol/L)</td>
<td>18</td>
<td>3.9</td>
<td>1.1</td>
<td>26</td>
</tr>
<tr>
<td>ABiC (%)</td>
<td>18</td>
<td>117.8</td>
<td>11.8</td>
<td>26</td>
</tr>
</tbody>
</table>

P, Mann-Whitney U-test versus Stage 1/2.

Fig. 2. Elevated serum levels of IS as a representative of uraemic toxins in advanced stages of CKD.
and, therefore, on the concentration ratio of ligand/reagent to binding protein, albumin binding as characterized by the ABiC test is affected by ligand/albumin ratio. In patients with comparable ligand concentration, site-specific binding is reduced in hypoalbuminaemic patients with a higher ratio ligand/albumin. Partial correlation analysis reveals an association between ABiC and IS which, however, is not influenced by renal function or albumin concentration.

What are the consequences of impaired albumin-binding function?

It is generally accepted that only the free fraction of a drug exerts the pharmacological action [48]. The free fraction of highly albumin-bound substances is small but can be influenced significantly by changes in albumin concentration or competitive ligand–ligand interactions [3]. A minimal decrease in protein binding from 98 to 96% results in a 2-fold increase in the pharmacologically active unbound fraction [49]. A lowering of albumin concentrations by 5 g/L in CKD patients was accompanied by an increase in the free fraction of PCS by 39%, although no difference in total PCS concentrations was detected [50].

Free levels of albumin-bound uraemic retention solutes were found to have a negative impact on leucocyte chemiluminescence production and be associated with vascular dysfunction. Clinically more important, however, is the association in patients with CKD between the elevated free fraction of uraemic toxins and outcome, including increased hospitalization for infectious diseases and overall as well as cardiovascular mortality [24, 50–52].

Hypoalbuminaemia is related to mortality and complications of CKD [5, 37, 53]. As already discussed, albumin binding is impaired in hypoalbuminaemia, which causes an increase in the free fractions of uraemic toxins. The negative impact of hypoalbuminaemia on outcome for dialysis patients could be related to the increase in the free fraction and thus the toxicity of protein-bound solutes [3, 54]. Considering that only unbound ligands act pharmacologically, the harmful effects of uraemic toxins are likely to be related to impaired binding and the elevated levels of free fractions it results in. This implies that albumin function corresponds more closely with the clinical effects of a given toxin rather than the concentration of the toxin itself.

Beside competitive mechanism, which can be illuminated by estimation of ligand concentrations, impaired binding as a consequence of oxidative modification of the albumin molecule has to be considered especially in CKD patients. This speaks in favour of the use of ABiC in studies investigating uraemic toxicity.

In accordance with our results, a gradual association between renal function and elevated levels of IS has been recently reported by Baretto et al. [24], albeit to a lesser extent. They were able to demonstrate the importance of the relationship between increased IS levels and vascular dysfunction and cardiovascular mortality.

In dialysis patients with end-stage renal disease, differences were found between patients with urinary output and those with oliguria/anuria. In patients with preserved urine...
production, significantly higher ABiC values and lower IS concentrations were observed than in oligouric/anuric dialysis patients. Our results tie in with the theoretical prediction [3] and clinical observation that the clearance of protein-bound uraemic toxins depends on the residual renal function [55]. Two different mechanisms for disposing of proteins with toxic ligands have been proposed: active renal tubular secretion and/or degradation pathways in proximal tubuli [3, 55–57].

Interestingly, the ABiC values of patients with mild to moderate renal insufficiency were found to be higher than those of the reference sample, a standardized virus-inactivated human serum preparation available as a medicinal product. The preparation is made from pooled human plasma (pools contain >5000 donors) by specific stepwise adsorption of the coagulation factors. Storage-unstable proteins and lipoproteins are removed, while immunoglobulins, transport proteins and inhibitors remain intact. The properties and functions of the proteins closely resemble those of human plasma; the risks of transmitting viral pathogens, however, have been reduced to a minimum [58]. The advantage of a licensed pharmaceutical-grade albumin preparation of this nature is the high number of individual plasma donations it comprises. The preparation provides an unbiased reflection of the albumin function of a large European cohort. Unlike in studies, where reference albumin was found to be comparable to either healthy volunteers or patients with minimal impairment of hepatic function [30, 31], the preparation process of the pharmaceutical protein preparation might have some influence on albumin function. This would explain ABiC values of >100% in nearly healthy individuals in our study. However, our analysis of the association between renal function and ABiC is based on differences between groups and not on absolute ABiC values; values >100% in the Stage 1/2 group do not bias the results of our study.

One limitation of this study is that total IS concentration was measured but free concentration was not. Total and free concentrations of this uraemic toxin, however, are strongly correlated [20]. In light of the association between IS concentration and ABiC presented here, a correlation can be assumed to exist between ABiC and free IS and consequently between ABiC and the clinical effects of unbound IS in patients with CKD.

We acknowledge that only one of the uraemic albumin-bound toxins was investigated in our study. However, IS is considered one of the most representative uraemic toxins not only as an innocent marker molecule but also in terms of its toxic effect. In the light of the large number of known uraemic toxins, the ongoing identification of new ones and the impact of oxidative albumin status on albumin-binding function, the advantage of a site-specific assessment of albumin-binding function independently of concentrations of defined uraemic toxins appears obvious.

In summary, deterioration in site II-specific albumin-binding function as assessed by the ABiC test is related directly and gradually to the progression of CKD. Impairment of ABiC is associated with increasing concentrations of the uraemic toxin IS. Accordingly, since both IS and the marker substance used in the ABiC test compete for the same albumin-binding site, ABiC can be used to assess albumin function in patients with CKD. In dialysis patients, ABiC deteriorates less if residual renal elimination is preserved.

In light of the impact of reduced albumin binding on the free fraction of uraemic toxins, the impairment of ABiC in patients with renal failure presented here and the impact of any increase in the unbound fraction of protein-bound uraemic toxins on outcome in CKD patients, one useful application of the ABiC test in basic research into uraemic toxicity of albumin site II ligands might be to characterize patients and potentially act as a prognostic parameter in renal failure.

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Conflict of interest statement. None declared.

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