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Carbamylation of albumin is a cause for discrepancies between albumin assays

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ABSTRACT

Background: Several investigators have reported discrepancies between the bromocresol-purple (BCP), bromocresol-green (BCG) and immunonephelometric (INP) assays in dialysis patients. This study compared the abovementioned assays and investigated whether hemodialysis itself or carbamylation of albumin is the cause for this discrepancy.
Methods: Samples obtained from hemodialysis patients were analyzed by BCP, BCG and INP. Furthermore, albumin was carbamylated in vitro using isocyanate. Isocyanate converts lysine to homocitrulline.
Results: No differences were observed between samples of the pre- and post-hemodialysis groups for BCP. In the control group, BCG averaged 6 g/L higher than INP. BCP did not statistically deviate from INP. In the dialysis group BCG averaged 5 g/L higher when compared to INP, whereas BCP averaged 2 g/L lower. BCP was affected by carbamylation of albumin. BCG and INP measurements were affected to a much lesser extent. Homocitrulline content of hydrolysates was increased in both the carbamylated albumin as well as in the dialysis population.
Conclusion: In a hemodialysis population albumin concentrations are not consistently estimated by both BCG and BCP methods. Relative to INP measurements BCG overestimates the albumin concentration (4–10 g/L), whereas BCP leads to an underestimation (0–4 g/L). Carbamylation of albumin is the main attributor to the discrepancy found with BCP.

1. Introduction

Analysis of plasma albumin is generally performed using either a bromocresol-green (BCG) or a bromocresol-purple (BCP) based colorimetric method. BCG and BCP are the most frequently utilized methods, since they are cost effective, quick and readily available on a variety of chemistry platforms. Other techniques, such as immunonephelometry (INP) or immunoturbidimetry are used less frequently, since these are more time-consuming and expensive. Main advantage of the use of immunonephelometry is the absence of cross-reactivity with non-albumin proteins [1].

In patients with end-stage kidney disease, albumin is an important predictor of mortality and morbidity. Furthermore, it is required for the interpretation of total calcium levels in patients requiring dialysis in case of hypoalbuminemia [2,3]. Adjustment of total calcium for low albumin levels is advised by the Nephrology Guidelines (KDIGO; [4]) and several correction formulas have been suggested [5,6].

Several publications have reported discrepancies in albumin concentration between the BCP, BCG and INP assays [1,5,7–13]. In healthy subjects, BCP has been reported to closely agree with INP, whereas BCG reveals a positive bias when compared to BCP or INP, especially at low albumin levels [8]. Carfray et al. demonstrate that BCG has a positive bias up to 10 g/L when compared to either BCP or INP and also show that this discrepancy increases with hypoalbuminemia [14]. Ueno et al. describe that acute phase proteins such as haptoglobin may interfere in the BCG assay [13]. Xu et al. described that serum globulins are the main contributor to the discrepancy between BCG and BCP [7]. Pinell et al. report that immunoglobulins do not affect the BCP assay [15].

Interestingly, several investigators have also reported that differences found between BCP, BCG and INP are more pronounced in a population of patients with chronic renal failure requiring hemodialysis [9,10,16]. A discrepancy of up to 9 g/L has been observed between BCP and BCG [9,10]. Previous studies have hypothesized that the reported increase of albumin in plasma of uremic patients could be attributed to either uremic toxins [17] or possibly to an unidentified chemical modification of albumin, that inhibits the binding of BCP to albumin, but not that of BCG or INP [8,10]. Jaisson et al. and Kraus et al. have

Abbreviations: BCP, bromocresol purple; BCG, bromocresol green; INP, immunonephelometry; CKD, chronic kidney disease; KDIGO, the Kidney Disease Improving Global Outcomes guidelines.

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previously reported on the occurrence of modification of proteins coined carbamoylation or carbamylation. Carbamylation results from the covalent binding of isocyanic acid (or isocyanate) to the ε-amino group of lysine to form homocitrulline proteins. Isocyanate may be formed under normal physiological conditions but is amplified in a hyperuremic state, such is the case for patients suffering from (end stage) renal failure. Furthermore, isocyanate may also be formed by oxidation of thiocyanate by myeloperoxidase [18,19]. Recently, carbamylated proteins have shown to be associated with increased mortality and morbidity rates in patients suffering from end stage renal disease [20–22]. Furthermore Gillery et al. described that carbamylation products are considered potential toxins [23].

Ito et al. showed that approximately two binding sites for BCP are present on albumin. Interestingly, they also showed that both binding sites contain a lysine moiety, which may be available for modification by isocyanic acid [24]. We hypothesize that modification of this specific amino acid may prevent binding of BCP to albumin and leads to falsely lower results when measuring albumin.

In this study, we evaluated whether hemodialysis itself influenced the BCG, BCP and INP by comparing samples obtained from 80 dialysis patients pre- and post-hemodialysis. Furthermore, we investigated whether in vivo carbamylation of ε-amino-end groups is a cause for the differences between BCP and BCG or INP. To this aim, purified human albumin was carbamylated by adding isocyanate. The carbamylated albumin as well as samples of patients were analyzed for homocitrulline content.

2. Materials and methods

2.1. Patient samples

To investigate the discrepancy in measurement of albumin in hemodialysis patients between BCP and BCG, both methods were compared with INP. To this aim, heparin plasma of 80 hemodialysis patients was collected pre- and post-dialysis. Furthermore, control-group heparin plasma samples were collected from 35 patients not diagnosed with kidney disease. In the control group, subjects creatinine and urea levels were within the reference interval.

2.2. Measurement of albumin/total protein

Heparin plasma was used for analysis. All methods were validated according to the standard protocols provided by CLSI. Quality of the measurements was assessed by measuring both internal and external (Dutch Foundation for Quality Assessment in Medical Laboratories, SKML, www.skml.nl) quality control standards. Proficiency testing was performed according to the scheme for the 'Combi Synchron' and the 'Combi General Clinical Chemistry'. Measurements of total protein and albumin by means of BCP and INP were performed by de Laboratory for Clinical Chemistry, Hematology and Immunology of the Medical Center Alkmaar, Alkmaar, The Netherlands. Both the BCP (Beckman, ALBm albumin, REF467858) and the rate biuret based total protein assay (Beckman, TPm, total protein, REF465986) were performed on a Beckman Synchron Dx860i using a modular chemistry cartridge based assay. Albumin INP measurements were performed on a Siemens Prospec 2 using N antiserum to Albumin (Siemens, NAS ALB). Albumin measured by means of BCG (Roche, Albumin gen. 2, ALB2) was performed by the Laboratory for Clinical Chemistry of the Westfriesgasthuis, Hoorn, The Netherlands on a Roche COBAS 6000. All measurements were performed on the same day.

2.3. KCNO experiment

To verify whether carbamylation affected the BCP and BCG assay 2 mL of human albumin (200 g/L, Albuman, Sanquin Blood Supply, The Netherlands) was modified by adding 4 mL isocyanate (KCNO; Merck, Darmstadt, Germany) in demineralized water at a concentration of 225 mM, 225 μM and 225 nM, to obtain a final albumin concentration of approximately 70 g/L and a concentration of respectively 150 mM, 150 μM and 150 nM of KCNO. As a control, the KCNO solution was substituted by an equimolar solution containing KCl (Merck, Darmstadt, Germany) in demineralized water. After addition of the KCNO or KCl solution, both total protein and albumin were measured instantaneously. Subsequently, the samples were incubated at 37 °C. After 24 h of incubation, total protein and albumin were measured. Samples were stored at −30 °C and thawed before further analysis. Carbamylation of albumin was verified by electrophoresis as was previously described by Jaisson et al. [25]

2.4. Measurement of homocitrulline

In total 15 patient samples (6 hemodialysis patients and 9 control patients) and 2 albumin samples (KCNO experiment and control) were analyzed. For every sample 500 μL of plasma was hydrolyzed for 24 h at 102 °C using 37% HCl (Merck, Darmstadt, Germany) to obtain a final concentration of 6 M. The amino acid mixture was filtered using a 0.2 μm low protein binding filter (AcroDisc, Pall Lifesciences, NY, USA). The filtrate was analyzed by UPLC–MS/MS (Acquity — Quattro Premier XE, Waters) using stable isotope dilution (SID) technique at the Laboratory Genetic Metabolic Diseases of the Academic Medical Center of the University of Amsterdam, The Netherlands according to Waterval et al. [26]. For homocitrulline, the limit of detection (LOD) was 0.6 μmol/L. The limit

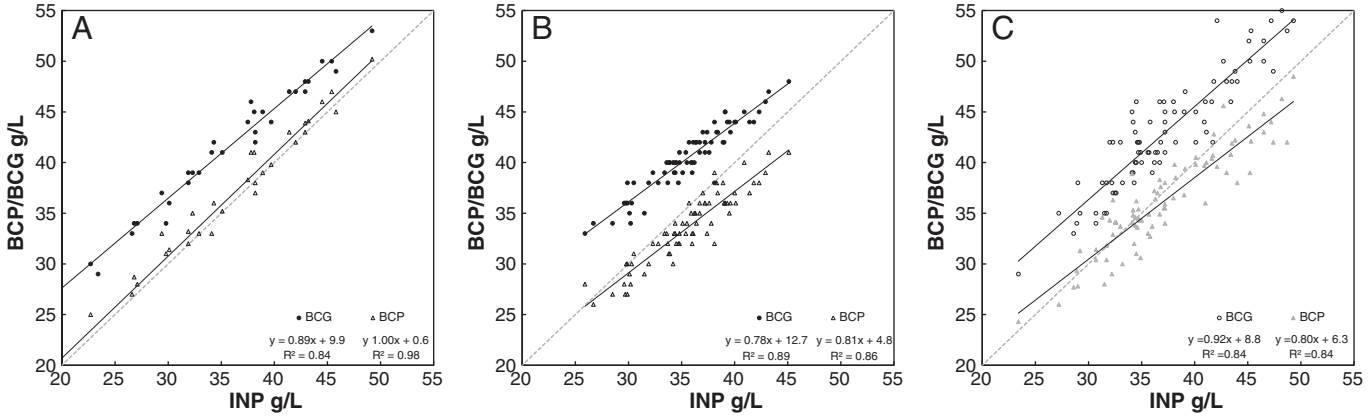

Fig. 1. Comparison between three methods for albumin. Bromocresol green (BCG) and Bromocresol purple (BCP) as a function of INP in plasma of patients with normal kidney function (A) and patients with end-stage kidney failure requiring hemodialysis (B/C). Data shown in B represents samples that were obtained pre-dialysis. Data shown in C represents samples that were obtained post-dialysis. The solid lines represent the equation for BCG/BCP as a function of INP determined by linear regression analysis. The dashed line is the identity line (x = y).