Underestimation of serum/plasma albumin in hemodialysis patients by changing the bromcresol green method to the bromcresol purple method

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Introduction and Aims

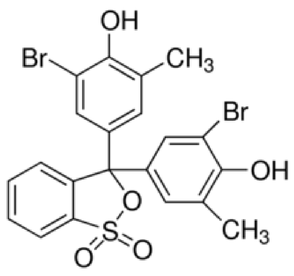
Serum/plasma albumin as an outcome measure in hemodialysis (HD) is an important predictor of morbidity and mortality in HD patients (1). Albumin as a marker of HD quality has become an important audit measure and therefore the correct analysis of albumin is crucial. In 2015 Siemens Healthcare Diagnostics switched its serum albumin method from the Siemens® Advia 1800 bromocresol green (BCG) method to the bromocresol purple (BCP) method. Whereas in most patients the new method showed better correlations to immunological methods we found lower albumin values for HD patients, especially for patients on long term HD. As possible interference 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), a well known uremic toxin, was quantified in all study samples by gas chromatography-mass spectrometry (GC-MS). Experiments were carried out to determine competitive interactions between CMPF, BCG and BCP on albumin binding sites.

Methods

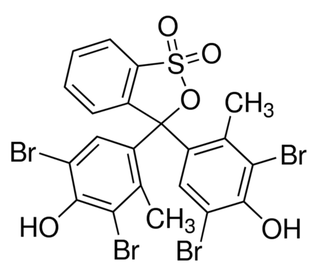
Albumin concentration was measured by three methods, BCG and BCP on the Siemens Advia® 1800 clinical chemistry system and an immunological method on the Siemens BN ProSpec® nephelo-metric system (98 non-renal patients, 124 HD patients). The Albumin BCP and BCG procedures are based on the binding of bromcresol purple respectively bromocresol green specifically with human albumin to produce a colored complex.

Method comparisons were made between both groups and all three methods. In addition, determination of CMPF in all these samples with an adapted GC-MS method (**2**) after solid phase extraction and methylation to the corresponding ester (**Figure 2a and 2b**). 4-Acetyl-3,5-dimethyl-1H-pyrrole-2-carboxylic acid was used as internal standard. The calibration curve is linear up to 20 µg/ml CMPF (R^2 = 0,9993)

Furthermore albumin in CMPF spiked native plasma samples of a healthy probate (fasting and drug free) with different concentrations of CMPF at different .concentrations of albumin were determined.



Bromocresol Purple (BCP)



Bromocresol Green

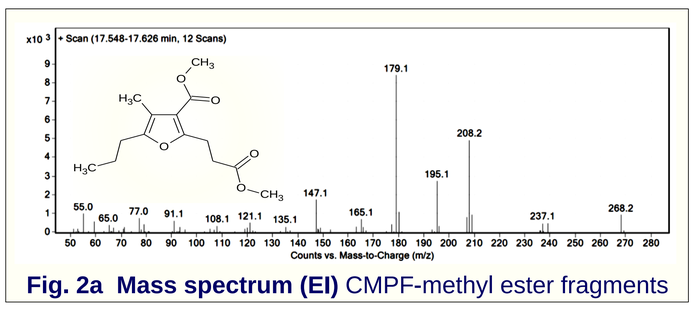
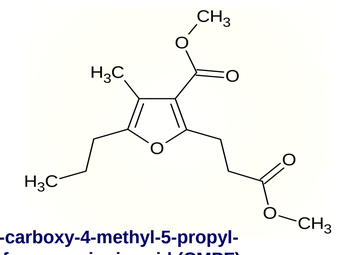


Fig. 2a Mass spectrum (EI) CMPF-methyl ester fragments

Figure 1 Structures



3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF)

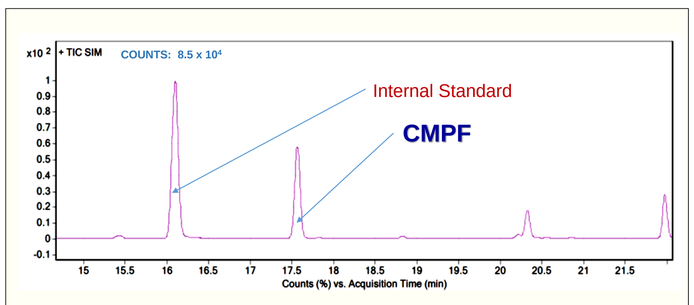


Fig. 2b GC/MS Sample chromatogram

Results

The new Advia BCP method has a negative bias as compared to the previous Advia BCG method. The negative bias is most marked in the hypoalbuminemia range (e.g. a BCG of 25 g/L is equivalent to a BCP 18.3 g/L) and decreases proportionally with higher albumin concentrations (e.g. a BCG of 45 g/L is equivalent to a BCP of 43.0 g/L). The comparison between the two methods is given by the regression equation: Advia BCP = Advia BCG * 1.23 - 12.5 g/L. The underestimation could be shown to be greatest for high concentrations of CMPF in samples of HD Patients. The comparison between the Advia BCP and BN ProSpec in HD patients is given by the regression equation: Advia BCP = BN ProSpec * 0.86 + 1.14 g/L and between the Advia BCG and BN ProSpec by Advia BCG = BN ProSpec * 0.73 + 10.82 g/L. Thus an immunological measured aLbumin of 40 g/L (BN ProSpec) is equivalent to 35 g/L for the BCP method and 40 for the BCG method. The corresponding correlations are shown in **Figure 3**. The mean concentration (+/- SEM) of CMPF was 1.04 +/- 0.10 mg/l for the non-HD and 3.20 +/- 0.25 mg/l for the HD patients.

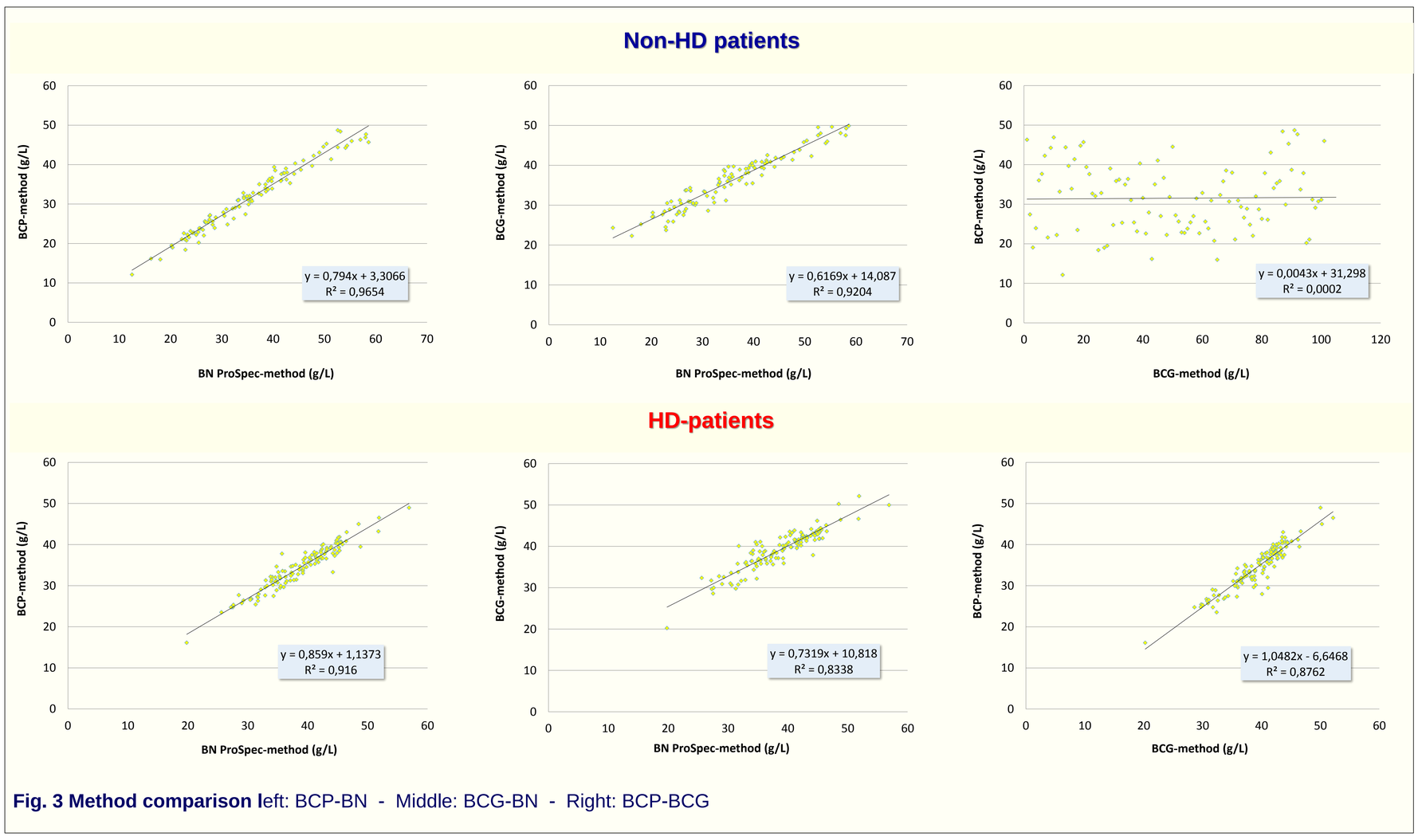


Fig. 3 Method comparison left: BCP-BN - Middle: BCG-BN - Right: BCP-BCG

The experiments with CMPF spiked plasma samples showed an analogous false-negative deviation of the albumin determination for the BCP method. At an immunological determined albumin of 46.6 g/L the BCP determined albumin dropped continuously down with increasing CMPF concentrations whereas there was no significant decrease with the BCG method (**Figure 4**). This effect is best seen at low albumin and medium elevated CMPF concentrations. In contrast, with very high CMPF levels, albumin concentration has only a small influence on the BCP determined albumin concentration.

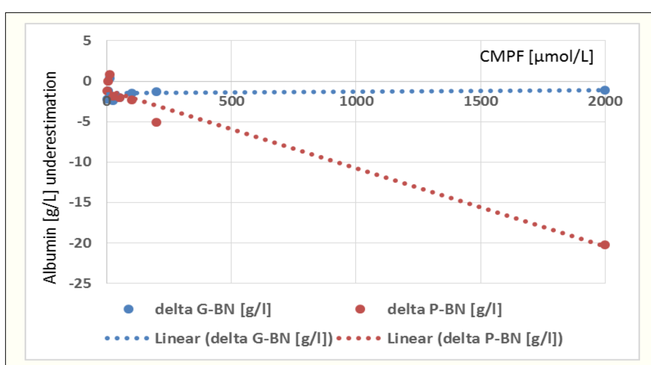


Fig. 4 Effect of CMPF on Albumin determination
Differences between the BCG respectively BCP method and the immunological determination (BN)

Conclusions

CMPF and various drugs compete for the binding sites of the albumin. CMPF accumulates in HD patients over time. CMPF has a very high binding constant to albumin (pK 7.11) (**3**). And so, binding sites occupied by CMPF are possibly no longer available for BCP. As a result, the albumin determination is underestimated in HD patients with the BCP method. Whereas in most patients the new method showed good correlations to immunological methods we found lower albumin values for HD patients, especially for patients on long term HD. The underestimation could be shown to be greatest for high concentrations of CMPF in samples of HD Patients. These results are in good agreement with the results by Mabuchi (4). We therefore switched back our routine analysis of albumin to the BCG method. Although the immunological method is more expensive than the two dye-binding BCP and BCG methods it might be the better -or even only- way of determine such a crucial outcome and quality marker of hemodialysis. The studies with CMPF spiked samples show that the underestimation is not just a function of CMPF concentration but a rather complex interaction of CMPF and albumin concentration. Further investigations including other uremic toxins should demonstrate the possible influence of albumin-bound drugs on albumin determination methods.

References

(**1**) Carfray A, Patel K, Whitaker P, Garrick P, Griffiths GJ, Warwick GL. Albumin as an outcome measure in haemodialysis in patients: the effect of variation in assay method. Nephrol Dial Transplant. (2000) 15:1819-22.
(**2**) Wahl, HG; Dissertation; 1994; Tübingen; Germany
(**3**) Kratochwil NA, Huber W, Müller F, Kansy M, Gerber PR. Predicting plasma protein binding of drugs: a new approach. Biochem Pharmacol. (2002) 64:1355-74.
(4) Mabuchi H, Nakahashi H. Underestimation of serum albumin by the bromcresol purple method and a major endogenous ligand in uremia. Clin Chim Acta (1987)167:89-96.

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