# UNDERESTIMATION OF ALBUMIN IN HAEMODIALYSIS PATIENTS BY BOTH CARBAMYLATED ALBUMIN AND THE UREMIC TOXIN 3-CARBOXY-4-METHYL-5-PROPYL-2-FURANPROPIONIC ACID (CMPF)


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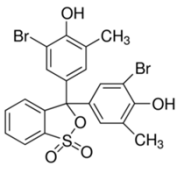
## Introduction and Aims

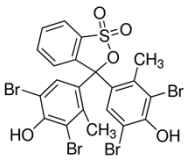
Albumin as an outcome measure in haemodialysis (HD) is an important predictor of morbidity and mortality in HD patients. Albumin as a marker of HD quality has become an important audit measure and therefore the correct analysis of albumin is crucial. Next to different immunological methods there are the two mayor dye methods bromocresol green (BCG) and bromocresol purple (BCP). We recently could show that there is a significant underestimation of albumin in the presence of CMPF by the BCP method. Next to uremic toxins bound to albumin posttranslational modifications such as carbamylation could cause interferences when albumin is determined in patients with chronic kidney disease (CKD) ore on haemodialysis. Carbamylation describes a non-enzymatic, posttranslational protein modification on multiple lysine side chains including human albumin mediated by cyanate, a dissociation product of urea.

## Methods

Albumin concentration was measured by three methods, bromo-cresol green (BCG, **Fig. 1a**) and bromocresol purple (BCP, **Fig.1a**) on the Siemens Advia 1800 and an immunological method on the Siemens BN ProSpec System in 100 non-renal patients and 100 HD patients. Method comparisons were made between both groups and all three methods. As possible interference 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid **(Fig. 1a)** was added in vitro in different concentrations to serum and albumin was determined by all three methods. Determination of CMPF in all these samples with an adapted GC-MS method (**Fig. 2**). Carbamylated albumin (**Fig. 1b**) was produced by adding 0,5 M Potassium cyanate (KOCN) in phosphate buffer ($Na_2HPO_4$, $KH_2PO_4$, pH 7,2) to albumin (5%) or serum. Carbamylation was verified by capillary electrophoresis analysis (Sebia Capillarys System, Buffer pH 9,9 ± 0,5, Detection 200 nm, **Fig. 3**) and mass spectrometry (TOF, LCMS) **(Fig.4)**.

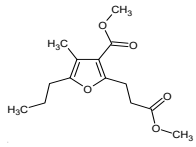



Bromocresol Purple (BCP)




Bromocresol Green

**Figure 1a Structures**




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

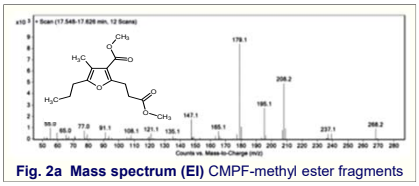


**Fig. 2a Mass spectrum (EI)** CMPF-methyl ester fragments

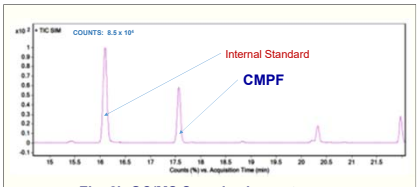



**Fig. 2b GC/MS Sample chromatogram**

## Results

The BCP method has a negative bias as compared to the BCG method. The negative bias is most marked in the hypoalbuminemia range and decreases proportionally with higher albumin concentrations. The underestimation could be shown to be greatest for high concentrations of 3- Carboxy-4-methyl-5-propyl-2-furanpropionic acid in samples of HD Patients. When increasing amounts of CMPF are added to serum or albumin (5%) there are no significant changes with the immunological (BN ProSpec System) or the BCG method but a significant underestimation of albumin concentration by the BCP method. With increasing carbamylation (shown by CE analysis) there was again no significant changes with the immunological method but an underestimation of albumin by 18 to 32 % (BCP method) and 18 to 23 % (BCG method).

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 (**Fig. 5**). 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.

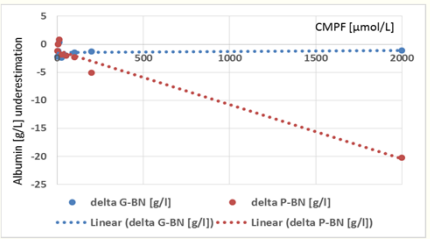



**Fig. 5 Effect of CMPF on Albumin determination**
Differences between the BCG respectively BCP method and the immunological determination (BN)

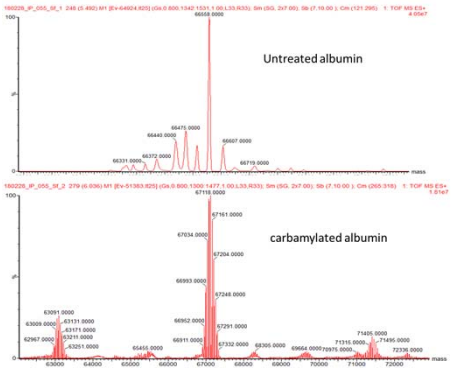



**Figure 4 MS Data deconvolution (TOF)**

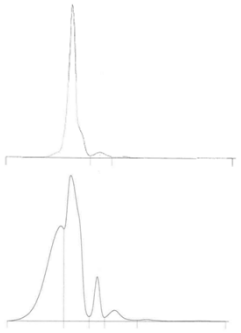


**Fig. 3 untreated and carbamylated albumin CE**

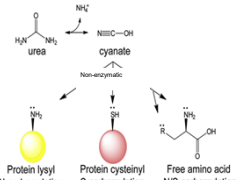



**Figure 1b Carbamylation**

| Carbamylation | BCP | BCG | BN |
|---|---|---|---|
| | g/L | g/L | g/L |
| 0 hours | 50,90 | 39,92 | 47,5 |
| 24 hours | 38,39 | 32,62 | 47,2 |
| 72 hours | 34,47 | 30,97 | 47,5 |

**Tab. 1 Albumin measurements of carbamylated albumin**

| Most prominent carbamylation site | | | | | | | |
|---|---|---|---|---|---|---|---|
| untreated albumin | | | | | | | |
| Confidence | Annotated Sequence | Modification | Modification | Master Pro | Positions in Master Pro | Theo. MH+ [ | Abundance |
| High | [KR].KVPQVSTPTLVEVSR.[NS] | | | P02768-1 | P02768-1 [438-452] | 1639,93775 | 1.787.530.042 |
| High | [KR].KVPQVSTPTLVEVSR.[NS] | 1xCarbamyl | P02768-1 1x( | P02768-1 | P02768-1 [438-452] | 1682,94356 | 28.929.180 |
| | | carbamylated | | | | Delta=43 | 1,6% |
| carbamylated albumin | | | | | | | |
| Confidence | Annotated Sequence | Modification | Modification | Master Pro | Positions in Master Pro | Theo. MH+ [ | Abundance |
| High | [KR].KVPQVSTPTLVEVSR.[NS] | | | P02768-1 | P02768-1 [438-452] | 1639,93775 | 1.273.503.806 |
| High | [KR].KVPQVSTPTLVEVSR.[NS] | 1xCarbamyl | P02768-1 1x( | P02768-1 | P02768-1 [438-452] | 1682,94356 | 868.786.022 |
| | | carbamylated | | | | Delta=43 | 40,6% |

**Tab 2 Albumin carbamylation sites**

| Carbamylation sites (carbamylated albumin) | | | | | |
|---|---|---|---|---|---|
| MH+ [Da] | Abundance | RT [min] | Sequence in Protein | Positions | Modifications in Protein |
| 1583,94792 | 129.881.272 | 39,46 | R.QIKKQTALVELVK.H | [546-558] | 2xCarbamyl [K548; K549] |
| 1931,95296 | 67.306 | 30,16 | K.LDELRDEGKASSAKQR.L | [206-221] | 3xCarbamyl [K214; K219; ] |
| 1779,93881 | 118.517.310 | 35,36 | R.LSQRFPKAEFAEVSK.L | [243-257] | 1xCarbamyl [K249] |
| 1888,94714 | 24.627.522 | 29,5 | K.LDELRDEGKASSAKQR.L | [206-221] | 2xCarbamyl [K214; K219] |
| 2075,14953 | 453.614.592 | 36,04 | R.YTKKVPQVSTPTLVEVSR.N | [435-452] | 1xCarbamyl [K] |
| 2118,15534 | 212.380.264 | 38,9 | R.YTKKVPQVSTPTLVEVSR.N | [435-452] | 2xCarbamyl [K437; K438] |
| 2098,10213 | 14.518.819 | 42,6 | R.RHPYFYAPELLFFAKR.Y | [169-184] | 1xCarbamyl [K] |
| 1561,78164 | 1.762.679 | 28,74 | K.LDELRDEGKASSAK.Q | [206-219] | 1xCarbamyl [K214] |
| 1295,66303 | 201.361.152 | 36,45 | R.FPKAEFAEVSK.L | [247-257] | 1xCarbamyl [K249] |
| 2109,12265 | 266.162 | 50,05 | R.FPKAEFAEVSKLVTDLTK.V | [247-264] | 2xCarbamyl [K249; K257] |
| 1942,00102 | 92.493.556 | 45,66 | R.HPYFYAPELLFFAKR.Y | [170-184] | 1xCarbamyl [K183] |
| 1682,94356 | 868.786.022 | 37,91 | K.KVPQVSTPTLVEVSR.N | [438-452] | 1xCarbamyl [K438] |

## Conclusions

The correct determination of albumin in patients with CKD or on haemodialysis with methods based on different dyes is difficult and hampered by a complex mixture of uremic toxins. Although the immunological method is more expensive than either one of the two dye-binding BCP and BCG methods it might be the better and may be even the only way to determine such a crucial outcome and quality marker of haemodialysis.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