INTERFERENCES IN NON-IMMUNOLOGICAL ALBUMINASSAYS BY CARBAMYLATED ALBUMIN

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Background - Aim

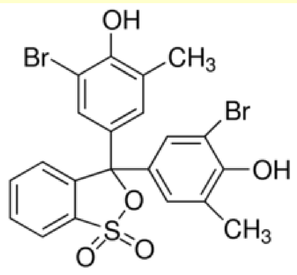
Albumin as an outcome measure in hemodialysis (HD) is an important predictor of morbidity and mortality in HD patients. Albumin as a marker of HD quality has become an important audit measure and therefore the correct analysis of albumin is crucial. Next to different immunological methods there are the two major 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 3- Carboxy-4-methyl-5-propyl-2-furanpropionic acid (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 hemodialysis. Carbamylation describes a non-enzymatic, posttranslational protein modification on multiple lysine side chains including human albumin mediated by cyanate, a dissociation product of urea. The proportion of carbamylated albumin is more than twice as high in ESRD patients than in non-uremic subjects and is a risk factor for mortality in patients with kidney failure.

Methods

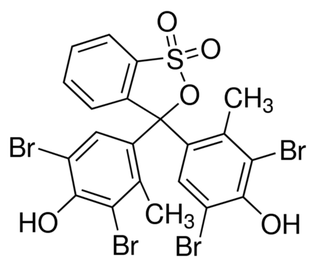
Albumin concentration was measured by three methods, bromocresol green (BCG, **Fig. 1a**) and bromocresol purple (BCP, **Fig.1a**) on the Siemens Advia 1800 and an immunological method on the Siemens BN ProSpec System in 100 non-renal patients and 100 HD patients. Method comparisons were made between both groups and all three methods.

As possible interference 3-carboxy-4-methyl-5-propyl-2-furan-propionic acid **(Fig. 1a**) was added in vitro in different concentrations to serum and albumin was determined by all three methods.

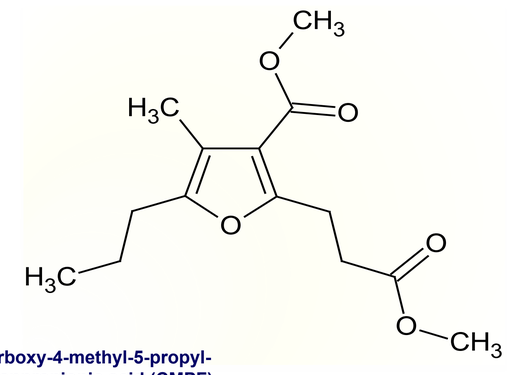
Carbamylated albumin (**Fig. 1b**) was produced by adding 0,5 M Potassium cyanate (KOCN) in phosphate buffer (Na_2HPO_4, KH_2PO_4, pH 7,2) to albumin (5%) or serum. Carbamylation was verified by capillary electrophoresis analysis (Sebia Capillarys System, Buffer pH 9,9 ± 0,5, Detection 200 nm, **Fig. 3**) and mass spectrometry (TOF, LCMS) **(Fig.4)**.



Bromocresol Purple (BCP)



Bromocresol Green



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

Figure 1a Structures

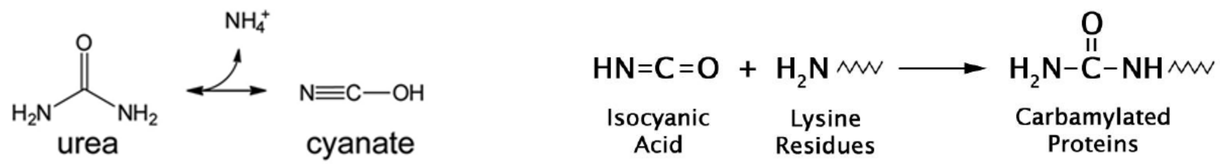


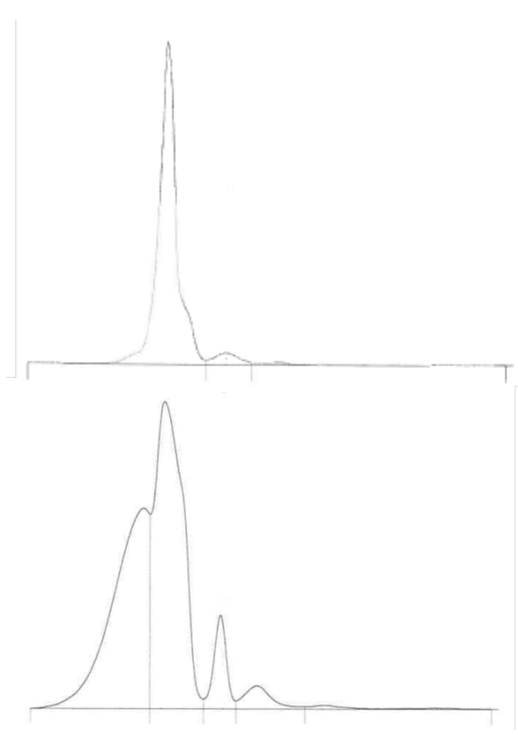
Figure 1b Carbamylation

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. 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.

With increasing carbamylation of an albumin solution (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) (Tab. 1). 12 sites of albumin carbamylation were found with K.KVPQVSTPTLVEVSR.N being the most prominent (Tab. 2).

Carbamylation of ESRD patient samples showed no change of immunological measured albumin but decreased albumin concentrations measured with both the BCP and BCG method (Tab. 1).



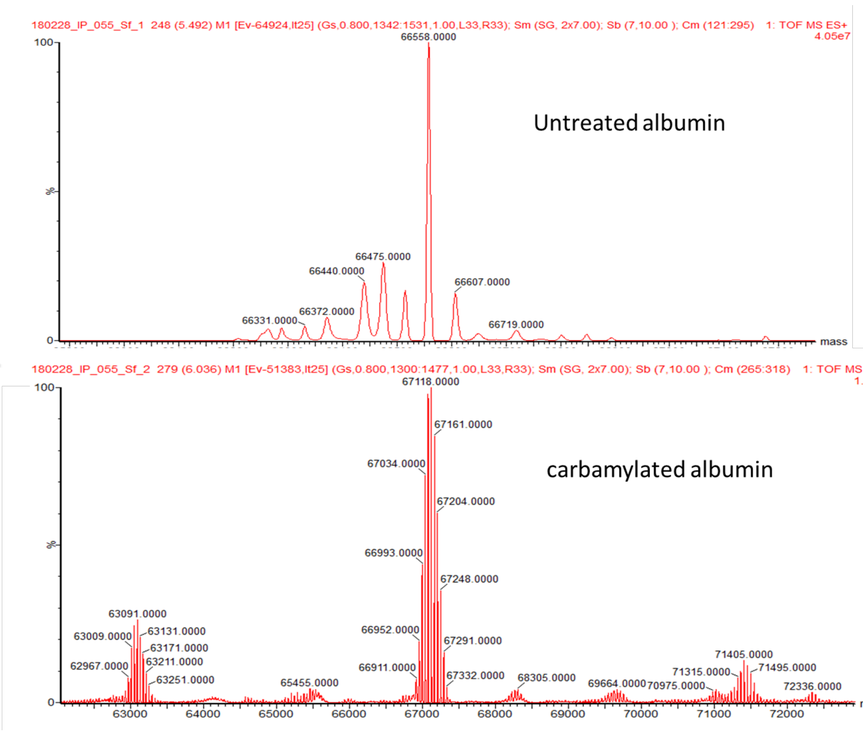


Fig. 3 untreated and carbamylated albumin CE

Figure 4 MS Data deconvolution (TOF)

Carbamylation	BCP	BCG	BN
Albumin	g/L	g/L	g/L
0 hours	50,9	39,9	47,5
24 hours	38,4	32,6	47,2
72 hours	34,5	31,0	47,5
Patient 1			
0 hours	37,9	38,8	41,1
24 hours	32,9	33,4	41,2
72 hours	30,4	31,7	42,2
Patient 2			
0 hours	37,8	38,4	40,6
24 hours	31,4	32,3	39,7
72 hours	29,8	31,4	42,2
Patient 3			
0 hours	35,8	38,5	40,4
24 hours	30,6	31,7	40,3
72 hours	29,8	31,5	41,4

Tab. 1 Albumin measurements of carbamylated albumin / ESRD patient samples

Most prominent carbamylation site

untreated albumin

Confidence	Annotated Sequence	Modification	Modification	Master Pro	Positions in Master Pro	Theo. MH+ [I	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+ [I	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%

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]

Tab 2 Albumin carbamylation sites

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 and posttranslational modifications such as carbamylation. 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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