INVESTIGATION OF INTERFERENCE WITH BINDING PROTEINS IN TWO COMMONLY USED 25OHD ASSAYS

E. Toldy[1,2], E. Virágh[3], D.E. Horváth[3], E. A. Kovács[1], L. Kovács[3], P. Lakatos[4], Z. Lőcsei[3]

[1]Institute of Diagnostics, Faculty of Health Science, University of Pécs, [2]Central Laboratory, [3]Department of General Internal Medicine, Markusovszky University Teaching Hospital, Szombathely [4] Semmelweis University, Faculty of Medicine , Institute of Internal Medicine I, Budapest, Hungary

Background:

Adequate vitamin D supply is necessary for bone metabolism and also for the general maintenance of health. 25OHD is transported primarily bound to vitamin-D binding protein (DBP), but also to albumin. Lower concentration of plasma proteins may cause difficulties in immunoanalytics.

Aims:

To investigate the protein dependence of the two most often used 25OHD chemiluminescence immuno- (CLIA) and elactrochemiluminescence protein binding- (ECLPBA) assays.

Patients:

Exogenous albumin was added by in vitro experiment at five dilution steps to serum pools from patients (N=24) with low albumin while DBP remained permanent. 109 clinical cases with variant DBP were investigated too, 63 patient with subnormal albumin levels (16 cirrhosis, 6 nephrosis, 11 malabsorption syndrome, 30 chronic renal failure) and 46 healthy control with normal albumin levels.

Measured biochemical markers:

t-25OHD (ECLPBA Roche and CLIA DiaSorin), bioPTHi (ECLMA, Cobas, Roche), DBP (Immuno-turbidimetry, Dako), albumin (colorimetry, Modular Roche. The Bio-25OHD values were calculated using a mathematical model (Powe et al, 2011).

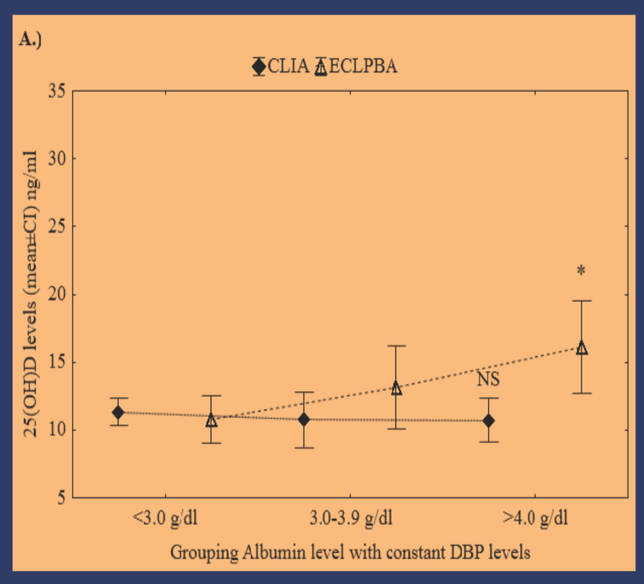


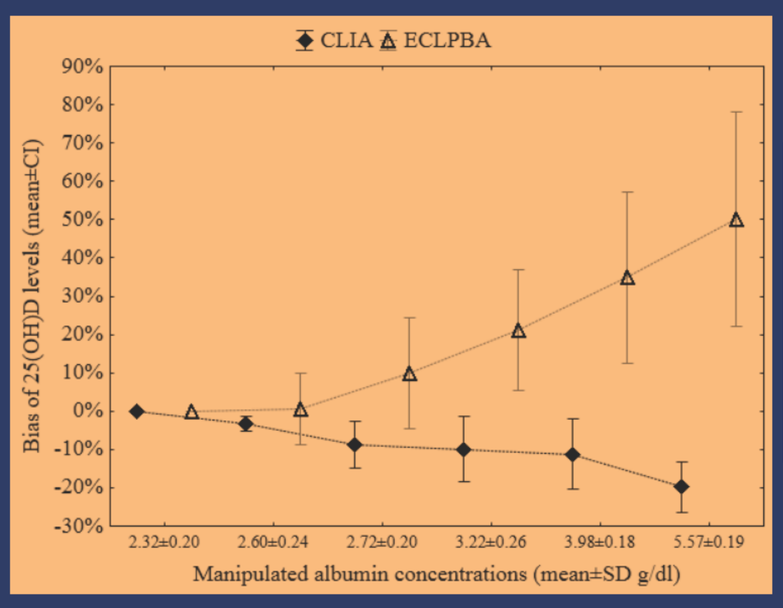
Fig2. **Variations of albumin related changes in measured 25OHD levels:** **A.)** in vitro experience: (DBP remained permanent) **B.)** ex vivo experience in individual samples with variant DBP. Patient's sera with low albumin showed significantly lower 25OHD levels compared to specimens with higher albumin levels by both methods in ex vivo experience.

Correlations between t-25OHD and DBP are : r=0.51 by CLIA and r=0.38 by ECLPBA.

25(OH)D fraction	Control mean±SD CLIA	ECLPBA	Differences between two methods p	Hospitalised patients mean±SD CLIA	ECLPBA	Differences between two methods p	Differences between two groups p
t-25(OH)D (ng/ml)	26.4±8.7	25.6±9.9	NS	12.7±7.1	9.8±6.8	<0.0001	<0.0001
b-25(OH)D (ng/ml)	2.7±1.0	2.6±1.2	NS	1.0±0.6	0.8±0.6	<0.0001	<0.0001
f-25(OH)D (pg/ml)	7.0±2.8	6.7±3.1	NS	4.0±2.0	3.2±2.3	<0.0001	<0.0001

Table1: Analyzing the obtained concentrations of 25OHD between two methods there was no difference in controls, but it was significant difference in hospitalized patients.

Results:



Legends of all figures and tables:
Mean ± 95 % Confidence Interval (CI) of mean (Whisker).
SD; standard deviation.
*p< 0.05; **p<0.001
NS: not significant

Fig1: **Bias of 25OHD estimation by two methods following in vitro addition of albumin to the sera.**

In the in vitro experiment the bias of 25OHD was markedly positive by increasing concentrations of albumin in case of ECLPBA. By the CLIA the bias of 25OHD was mostly slight and negative.

Clinical subgroups Vitamin D fractions	mean±SD CLIA	ECLPBA	Differences between two methods p
Cirrhosis			
t-25(OH)D (ng/ml)	12.7±5.5	11.3±6.0	NS
b-25(OH)D (ng/ml)	1.2±0.5	1.1±0.6	NS
f-25(OH)D (pg/ml)	5.4±2.2	4.9±3.0	NS
Nephrosis/malabsorption syndr.			
t-25(OH)D (ng/ml)	13.1±8.5	9.4±8.2	p=0.004
b-25(OH)D (ng/ml)	0.9±0.6	0.6±0.6	p=0.004
f-25(OH)D (pg/ml)	3.6±2.0	2.6±2.0	p=0.004
Chronic renal failure			
t-25(OH)D (ng/ml)	12.5±7.2	9.2±6.4	p<0.001
b-25(OH)D (ng/ml)	1.0±0.6	0.8±0.5	p<0.001
f-25(OH)D (pg/ml)	3.6±1.7	2.6±1.5	p<0.001

Table2: Among clinical subgroups, the only significant difference between the two methods was in **cirrhosis group**, which didn't show different DBP/Albumin ratio from the **control group**.

Conclusions:

- Our results suggest that CLIA method interact more pronounced with DBP and less with albumin, contrary to the ECLPBA.
- Both 25OHD methods depend somewhat on albumin levels, it is marginal in sera with normal albumin levels, but characteristic in sera with lower albumin levels.
- Our results call attention to the fact , that each method showed a proportionate 25OHD vitamin level change in accordance with the changing albumin levels and DBP/albumin ration.

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