# INDOXYL SULFATE RELATED TO PROTEIN INTAKE AND INTRAHEMODIALYSIS ARTERIAL PRESSURE VARIATION, AND $\beta_2$-MICROGLOBULIN TO ALBUMIN LEVELS AND INFLAMMATION

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## INTRODUCTION AND AIM

- Hipoalbuminemia is a strong factor for morbility and mortality in the general and renal population. Inflammation and dietary protein intake exert competing effects on serum albumin over time.
- The nPCR is considered a valid surrogate for dietary protein intake under steady-state conditions, in the face of inflammation may overestimate protein intake because of endogenous nitrogen breakdown.
- High protein intake is associated with increased levels of albumin-bound uremic solutes in hemodialysis patients and this products show concentrations not determined by hemodialysis clearances.

→The objective of the study is to analyze the patterns of relationship of the prototypic uremic toxins with protein metabolism, inflammation, and other candidate clinical variables

## METHODS 1: SAMPLES AND STATISTICS

- Cross-sectional study of 60 chronic hemodialysis (HD) patients.
- Pre/postHD total serum Indoxyl Sulfate (IS) by high-performance liquid chromatography (HPLC).
- Pre/post (post in hemodiafiltration patients-HDF-) serum β2-microglobulin (β2M) by nephelometry.
- Urea kinetic model calculated by *Solute Solver* in midweek sessions: KD urea (urea dialyzer clearance), K dif urea (urea difusive dialyzer clearance), Eq Kt/V (equilibrated Kt/V), KoA urea (urea mass transfer-area coefficient), G2p urea (urea double pool generation rate), TAC urea, SAN_DstdKt/V (weekly Dialysis Standard Kt/V normalized to body Surface Area), dp nPCR (normalized Protein Catabolic Rate double pool), V2p urea double pool.
- Testing of variables by Kolmogorov-Smirnov for normality (p>0,20).
- T-test or ANOVA for IS and β2M as dependent variables; and as categorical factors: albumin ≥ or < to 38 g/L, dp nPCR categorized by levels, and albumin and dp nPCR profiles. T-test for albumin, dp nPCR and CRP as dependent variables; and as categorical factors: IS higher or lower than mean, and $\beta_2$M higher or lower than mean.
- Bivariate correlations (Pearson) and multiple regression analyses of IS and β2M as dependent variables ; as independent variables: biologic (hemoglobin, lymphocytes, BUN, urate, $HCO3^-$, P, Na, Log CRP, albumin by Bromcresol Green…), kinetic (SAN_DstdKt/V, dp nPCRN, URR, IS reduction rate -RR-, β2M RR…), and other clinical variables (residual diuresis, mean arterial pressure/MAP change, convective volume/CV in HDF, BMI, BSA…).
- MAP change: calculated as the difference between measures at the start and the end of each session. MAP= DAP+1/3(SAP-DAP).

## METHODS 2: PATIENTS AND SESSIONS

- 60 patients of an university regional hospital based chronic haemodialys unit.
- 60±20 (X±SD) years (range 20-84). 32 male (53,3%) / 28 (46,6%) female.
- Height 161,1±1,03 cm. Dry weight 67,5±19,3. BMI 25,5±0,9 kg/m². BSA 1,7±0,03 m².
- Modified Charlson´s Index 7,2±2,85.
- Residual diuresis: 34 p null, 21 p <500 ml/24h, 5 p >500 ml/24 h.
- 21,66% (13 p) of the patients with positivity for hepatitis C/B or HIV: C hepatitis 8, B hepatitis 2, HIV 1, HIV and C hepatitis 1, B and C hepatitis 1.
- Haemodialysis session time 244±19´. $Q_B$ 356,8±6,13 ml/min . $Q_D$ standard HD 500-800 ml/min. $Q_D$ HDF 800 ml/min.
- Dialyzers: Membrane: 56 polynephron, 4 cellulosic. 34 High flux, 26 medium flux. Surface: 11 1.7 m², 33 1.9 m², 16 2.1 m² .
- 29 p (48,3%) on line hemodiafiltration / 31 p (51,6%) standard hemodialysis.
- Ultrafiltration 2,3±0,93 L. Convective volume in HDF 23,8±2,8 L. Infusion volume in HDF 21,4±2,7 L.
- Mean dialysis prevalence months 46,8 (3,9 years) (range 3-299 months): mean 58,3 months HDF patients and 36 months standard HD patients.

## METHODS 3: NORMALITY

**Normality:**

Indoxyl sulfate D=0,09 p=>0,20, IS RR D=0,08 p>0,20, $\beta_2$microglobulin (HDF) D=0,16 p=>0,20, $\beta_2$microglobulin (standard HD) D=0,14 p=>0,20, $\beta_2$microglobulin RR D=0,18 p>0,20, urea D=0,07 p>0,20, hemoglobin D=0,05 p>0,20, hematocrit D=0,06 p>0,20, leucocytes D=0,10 p>0,20, lymphocytes D=0,10 p>0,20, residual diuresis D=0,14 p>0,2, convective volume D=0,13 p>0,20, infusion volume D=0,16 p>0,20, HCO3 D=0,09 p>0,20, pH D=0,09 p>0,20, P D=0,11 p>0,20, Log CRP D=0,05 p>0,20, MAP change D=0,07 p>0,20, urate D=0,12 p>0,20, height D=0,05p>0,20, Charlson index D=0,11 p>0,20, albumin D=0,10 p>0,20, dp nPCR D=0,08 p>0,20, Eq Kt/V urea D=0,06 p>0,20, G2P urea D=0,09 p>0,20, , TAC urea D=0,07 p>0,20, BSA D=0,09 p>0,20

**No normality:**

URR D=0,14 p<0,15, prevalence months D=0,21 p<0,1, time in minutes D=0,35 p<0,01, $Q_B$ D=0,28 p<0,1, PTHi D=0,16 p<0,10, ionic Ca D=0,16 p<0,10, Na D=0,26 p<0,01, CRP D=0,32 p<0,01, BMI D=0,18 p<0,05, KoA urea D=0,25 p<0,01, KD urea D=0,17 p<0,10, K dif urea D=0,15 p<0,15, Kc D=0,19 p<0,05, V2P urea D=0,19 p<0,05, SAN_DstdKt/V D=0,19 p<0,05

## METHODS 4: VARIABLES

**Regression analysis:**

- Indoxyl sulfate
- $\beta_2$microglobulin
- Indoxul sulfate reduction rate
- $\beta_2$microglobulin reduction rate

- BUN preHD 78,6±1,8 mg/dl
- Hemoglobin 11,2±0,2 g/dL
- Hematocrit 34,4±0,5 %
- Lymphocytes 1.490±86,9 cel/mcl
- Albumin 37,8±0,5 g/L
- $HCO3^-$ 23,9±0,3 mmol/L
- pH 7,4±0,01
- P 4,3±0,18mg/dl
- Log CRP
- Urate 5,8±0,18mg/dl
- Eq Kt/V 1,67±0,05
- G2p urea 5,3±0,26 mg/min
- TAC urea 34,3±1,26 mg/dl
- SAN_DstdKt/V: 2,7±0,07
- dp nPCRN: 1±0,04 g/Kg/day
- Mean arterial pressure:
- Initial: 87,3±2,13 mmHg
- Final: 86,5±2,01 mmHg
- Mean arterial pressure change: -0,6 mmHg
- Modified Charlson index 7,2±2,85 points
- Height 161,1±1,03 cm
- Body surface area (BSA) 1,7±0,03 m2.

## METHODS 5: VARIABLES

**Student-t /ANOVA:**

Dependent variables:
- Indoxyl sulfate
- $\beta_2$microglobulin

Categorical factors:
- CRP ≥ 1 mg/dl /< 1 mg/dl
- Albumin ≥ 38 g/L / < 38 g/L
- dp nPCR ≥ 1 /< 1 g/kg/day
- dp nPCR (g/kg/day): (Figure 1a)
  - <0,6: 5 p (8,3%)
  - 0,6-0,79: 11 p (18,3%)
  - 0,8-0,99: 14 p (23,3%)
  - 1-1,19: 17 p (28,3%)
  - ≥1,2: 13 p (21,6%)
- Albumin and dp nPCR profiles(Figure 1b)
  - Catabolic (Albumin< 38 g/L
  - dp nPCR ≥1 g/Kg/day): 12 p (20%)
  - Low intake (Albumin< 38 g/L
  - dp nPCR < 1 g/Kg/day): 21 p (35%)
  - High intake (Albumin≥ 38 g/L
  - dp nPCR ≥ 1 g/Kg/day): 19 p (31,6%)
  - Anabolic (Albumin≥ 38 g/L
  - dp nPCR < 1 g/Kg/day): 8 p (13,3%)

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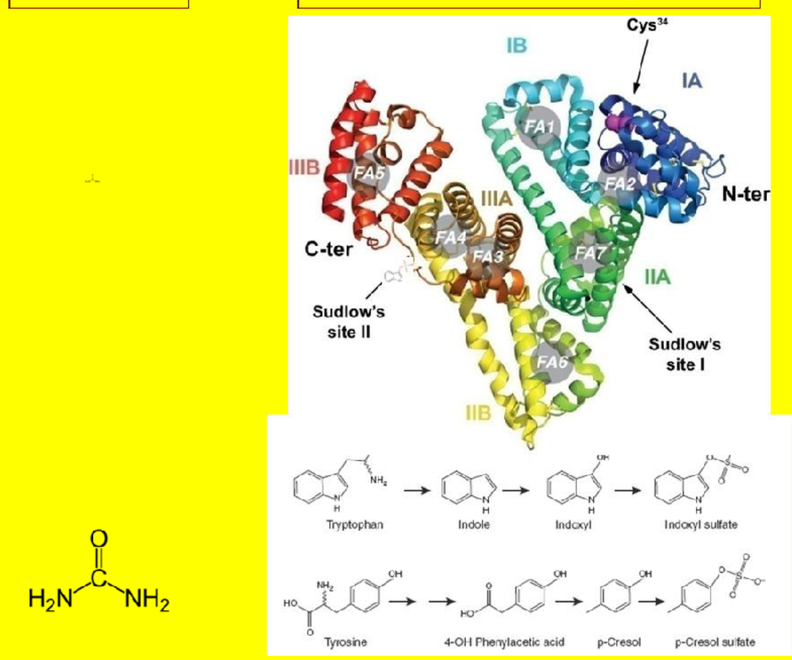
Dependent variables:
- Albumin
- dp nPCR

Categorical factors:
- IS≥ mean /< mean
- $\beta_2$M≥ mean /< mean




| | X±SE (mg/L) | Range (mg/L) | Hemodiafiltration p | Standard HD p | Normal values | Mortality risk augmented |
|---|---|---|---|---|---|---|
| $\beta_2$M | 35,6±1,9 | 11,5-72,1 | 32,8±1,9 mg/L | 38,2±3,1 mg/L | <3,5mg/L | >25mg/L |
| IS | 18,91±1,63 | 0,28-55,12 | 22,4±2,7 mg/L | 16,04±1,6 mg/L | <1,2mg/L | >4mg/L |

**RESULTS 1:**
$\beta_2$M and IS means in the whole population and in the hemodiafiltration and standard hemodialysis patients

| | Total | Hemodiafiltration p | Standard HD p |
|---|---|---|---|
| Urea Reduction Rate | 78,6±1,1 % | 82,04±1,1 % | 75,2±1,6 % |
| $\beta_2$microglobulin Reduction Rate | | 79,8±1,1 % | |
| Indoxyl sulfate Reduction Rate | 47,1±2,1 % | 49,07±2,8 % | 45,2±3,1 % |

**RESULTS 2:**
Urea, $\beta_2$M and IS reduction rates

| | Mean IS (CI 95%) | p | Mean β2M (CI 95%) | p |
|---|---|---|---|---|
| CRP ≥ 1 mg/dl (n=22) | 17,5 (11,7-23,3) | NS | 43,9 (37,3-50,4) | 0.0006 |
| CRP < 1 mg/dl (n=38) | 19,7 (15,6-23,8) | | 30,8 (26,6-34,9) | |
| Albumin ≥ 38 g/L (n=25) | 22,2 (16,5-27,9) | NS | 29,4 (24,7-34,2) | 0.002 |
| Albumin < 38 g/L (n=35) | 16,5 (12,6-20,4) | | 40,9 (35,6-46,1) | |
| dp nPCRN ≥ 1 g/kg/day (n=31) | 22,8 (18-27,6) | 0.01 | 36,3 (35,5-41,1) | NS |
| dp nPCRN < 1 g/Kg/day (n=29) | 14,4 (10,6-18,8) | | 35,9 (29,5-42,4) | |

**RESULTS 3: Student-t Test**
Uremic toxins means as dependent variables vs
CRP, albumin and protein catabolic rate as categorical factors:

| | Mean Albumin (CI 95%) | p | Mean dp nPCR (CI 95%) | p |
|---|---|---|---|---|
| IS 18,9 ≥ mg/L (n=26) | 38.1 (36,5-39,7) | NS | 1,08 (0,98-1,18) | 0,03 |
| IS 18,9 < mg/L (n=34) | 37,4 (35,8-39) | | 0,93 (0,83-1,03) | |
| $\beta_2$M 35,6 ≥ mg/L (n=25) | 35,7(33,8-37,7) | 0,001 | 0,99 (0,85-1,13) | NS |
| $\beta_2$M 35,6 < mg/L (n=35) | 39,1 (38-40,3) | | 1 (0,92-1,08) | |

**RESULTS 4: Student-t Test**
Albumin and protein catabolic rate means as dependent variables vs
uremic toxins as categorical factors:

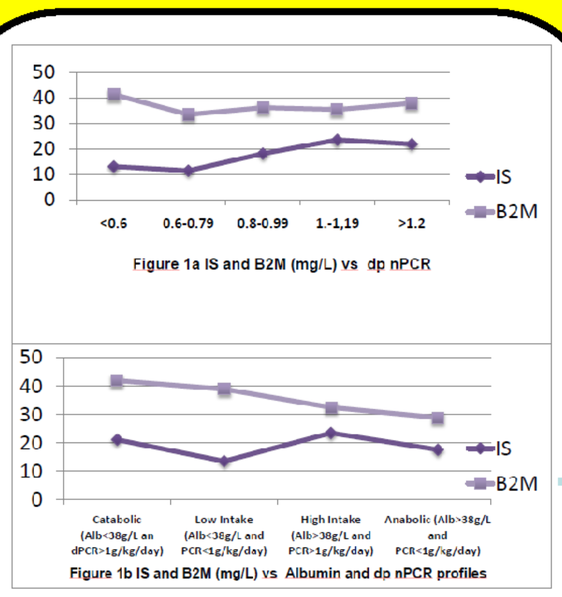



Figure 1a IS and B2M (mg/L) vs dp nPCR

Figure 1b IS and B2M (mg/L) vs Albumin and dp nPCR profiles

**RESULTS 5: ANOVA**
IS and $\beta_2$M as dependent variables
vs dp nPCR categorized by levels (Figure 1a)
and vs albumin/dp nPCR profiles (Figure 1b)

## RESULTS 6: SIGNIFICANT BIVARIATE CORRELATIONS

- Indoxyl sulfate:
  - P: r+ / $r^2$=0,11 / p=0,007
  - BUN: r+ / $r^2$=0,11 / p=0,009
  - Albumin: r+ / $r^2$=0,10 / p=0,01
  - TAC urea: r+ / $r^2$=0,09 / p=0,01
  - PCRN_2P: r+ / $r^2$=0,08 / p=0,02
  - MAP change: r- / $r^2$=0,08 / p=0,02
- Indoxyl sulfate reduction rate:
  - G2p urea: r- / $r^2$=0,17 / p=0,0008
  - Eq Kt/V: r+ / $r^2$=0,15 / p=0,002
  - Height: r- / $r^2$=0,11 / p=0,007
  - MAP change: r+ / $r^2$=0,11 / p=0,008
  - TAC urea: r- / $r^2$=0,11 / p=0,009
  - BSA: r- / $r^2$=0,08 / p=0,02
  - $HCO_3^-$: r+ / $r^2$=0,19 / p=0,01
  - BUN: r- / $r^2$=0,07 / p=0,04
- $\beta_2$microglobulin:
  - Residual diuresis: r- / $r^2$=0,17 / p=0,001
  - Albumin: r- / $r^2$=0,11 / p=0,007
  - Log CRP: r+ / $r^2$=0,07 / p=0,03
- $\beta_2$microglobulin reduction rate:
  - G2p urea: r- / $r^2$=0,32 / p=0,001
  - Log CRP: r- / $r^2$=0,30 / p=0,001
  - Eq Kt/V: r+ / $r^2$=0,27 / p=0,003
  - Infusion volume: r+ / $r^2$=0,21 / p=0,01
  - BSA: r- / $r^2$=0,17 / p=0,02
  - TAC urea: r- / $r^2$=0,16 / p=0,02

## RESULTS 7: MULTIPLE REGRESSION

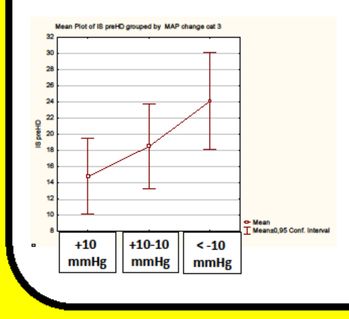
- Indoxyl sulfate: Adjusted $R^2$ =0,24
  - Mean arterial pressure change p=0,01
  - P p=0,01
  - BUN p=0,03
  - TAC urea p=0,04
- Indoxyl sulfate reduction rate: Adjusted $R^2$ =0,34
  - Mean arterial pressure change p=0,007

$\beta_2$microglobulin: Adjusted $R^2$ 0,26
- Residual diuresis p=0,001
- Albumin p=0,01

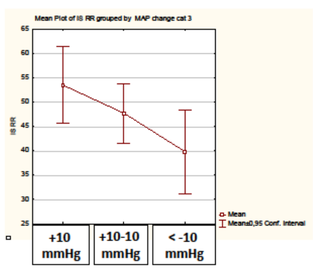
$\beta_2$microglobulin reduction rate: Adjusted $R^2$ 0,65
- Infusion volume p=0,0009
- G2P urea p=0,002
- TAC urea p=0,01

## RESULTS 8: MEAN ARTERIAL PRESSURE CHANGE

MAP vs Indoxyl Sulfate




MAP vs Indoxyl Sulfate Reduction Rate

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## CONCLUSIONS

- ✓Indoxyl sulfate levels had direct correlation with normalized protein catabolic rate, phosphorus, albuminemia, BUN and TAC urea.
- ✓$\beta_2$microglobulin had inverse correlation to albumin and direct correlation with C-reactive protein.
- ✓Residual diuresis was the strongest predictive variable for $\beta_2$microglobulin serum concentration in the multiple regression analysis.
- ✓Indoxyl sulfate highest and lowest levels in high and low protein intake patients.
- ✓Catabolic patients had the highest and anabolic patients the lowest $\beta_2$microglobulin levels.
- ✓Mean arterial pressure change linked to indoxyl sulfate and indoxyl sulfate reduction rate.
- ✓Indoxyl sulfate determined by protein intake.
- ✓$\beta_2$microglobulin determined by malnutrition-inflammation.

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