EFFECTS OF CIS- AND CARBOPLATIN ON DIFFERENT URINARY BIOMARKERS OF KIDNEY INJURY

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INTRODUCTION AND AIMS

Cis- and Carboplatin are frequently part of a cytostatic therapy and have potential nephrotoxic side effects. Early detection of renal damage is of outmost clinical relevance to prevent acute renal failure. However, the standard clinical tests for detection of kidney injury - especially serum creatinine measurements - are insensitive and detect only advanced stages of injury. Therefore, a variety of urinary biomarkers is currently under evaluation to identify biomarkers for early detection of kidney injury. Most studies analyze only changes in one or a few biomarkers, thus information on a direct comparison of a larger number of different markers is limited. This study compares changes a variety of different renal biomarkers measured by clinical chemistry and Luminex-based technology.

METHODS

After approval by the local Ethic Committee, urine samples from 29 patients (M: 11, F: 18; age 64±10.1 years; BMI 29.5± 8.8) were collected at the university hospital of Göttingen before and up to 5 days after administration of Cisplatin (n=11) or Carboplatin (n=18). Patients were suffering from different carcinoma (ovarial 10, cervical 4, endometrium 3, bronchial 1, adeno 1, renal 1) and lymphoma (Non Hodgkin 5, Hodgkin 3, follicular 1).

Demographics of healthy volunteers compared to patients and baseline conditions of patients are provided in **Table 1**. All patients were on a normal mixed diet, 55% of them did some fitness sport.

Measurements of urinary samples for clinical chemistry were performed using a Konelab 60i or a Konelab Prime 60i from Thermo Fisher Scientific (Vantaa, Finland). The following markers were measured: pH, Na+, K+, Cl-, Ca++, Mg++, phosphate, glucose, protein, albumin, creatinine, BUN, ß-NAG, LDH, AST, ALT, GGT, and ALP. Measurements of urinary samples for toxicological markers was based on the Luminex xMAP technology. This technology was used to perform high-content multiplexed and quantitative immunoassaysknown as Multi-Analyte Profiles (MAPs). The assays were performed at Myriad RBM and included the following analytes: Cystatin C, Kidney Injury Molecule-1 (Kim-1), Osteopontin, Neutrophil Gelatinase-Associated Lipocalin (NGAL), ß2-Microglobulin, Tissue Inhibitor of Metalloproteinase-1 (TIMP-1), Vascular Endothelial Growth Factor (VEGF), Albumin, Clusterin, Gluthatione-s-Transferase a (GSTα), a1-Microglobulin (a1-MG), Tamm-Horsfall Protein (THP), Trefoil Factor 3 (TFF3), Connective Tissue Growth Factor (CTGF), and Calbindin. For comparison of different concentrations of urine, all measured markers were normalized to urine creatinine (Crea).

The upper limit of the reference range was defined as mean + 2 SD of biomarker concentration measured in spontaneous urine samples collected at 3 different time-points over the day from 71 healthy volunteers.

Statistical analysis was performed using STATISTICA 12 software. For each parameter, an Omnibus test was used. Afterwards, significance of differences between post-treatment days compared to pre-treatment was analyzed by Wilcoxon-matched pairs test, and the impact of baseline characteristics was analyzed by the Mann-Whitney-U-test.

RESULTS

Most biomarkers were significantly higher in the patients than in healthy volunteers (**Table 2**). Within these mean levels of α1MG, ß2MG, Kim-1, micro-albumin, protein and TIMP were already before therapy even higher than the upper limit of the reference range. Most biomarkers were at baseline signifcantly higher in female patients than in male patients (Table 2). These elevated levels might be in part related to prior treatment with platin-derivates received by 2/3 of all patients and 88% of female patients.

There was no significant worsening of the GFR (calculated by CKD-Epi) after treatment.

Within 1 day after treatment with platin-derivates, ßNAG, α1MG, and ß2MG increased significantly (**Figure 1 A-C**; p<0.02 each biomarker). The fast increase of these markers, all related to the proximal tubule, may reflect early damage induced by increased concentrations of platin-derivates in the proximal tubule following accumulation due to intracellular transport by organic cation-transporters (Ciarimboli et al. Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions, Am J Pathol 176, 1169-1180, 2010). From day 3 onwards, there was a significant increase GSTα (p = 0.02), Kim-1 (p = 0.09) and ostepontin (p = 0.049, **Figure 1 D-F**). Micro-albumin and protein did not change much compared to baseline (**Figure 1 G,H**), but protein peaked at day 4 (p =0.02).

Within the patients, 20.7% suffered from diabetes and 65.5% from arterial hypertension. Patients with hypertension did not show a significant difference to those without hypertension in any of these biomarkers, although Kim-1 levels increased more in patients with hypertension (day 4: 0.49 ± 0.39) than in those without (day 4: 0.25 ± 0.06; p = 0.07). Patients suffering from diabetes showed 3-5 fold higher levels of α1M, Kim1 and osteopontin from day 3 (**Figure 2**, A-C, p=0.03 each) onwards than those without diabetes. There were no significant differences in any biomarker between the two platin-derivates, even though osteopontin levels were more elevated in patients treated with Carboplatin (day 3: 604.09 ± 594.36; day 4: 673.21 ± 684.94) compared to those treated with Cisplatin (day 3: 207.19 ± 95.29; day 4: 298.50 ± 46.56).

CONCLUSIONS

Markers of the proximal tubule (ßNAG, α1MG, ß2MG) reacted fastest to therapy with platin-derivates and may be eligible for early detection of renal damage. Increased levels of proximal (α1M, Kim-1) and glomerular (osteopontin) markers in the urine.of patients with diabetes may reflect a higher susceptibility to kidney damage. Further studies in larger cohorts have to evaluate clinical sensitivity and specificity of these urinary biomarkers.

Table 1:
A) Demographics of healthy volunteers (HV) and patients. None of the patients or HV followed a vegetarian diet.
B) Baseline conditions of patients. eGFR was calculated by CKD-EPI.

A

	Patients	HV
Total N	29	71
Gender Male	11	34
Female	18	37
Age Mean + SD	64 ± 10. 14	39 ± 10.06
Median (range)	65 (35-79)	42 (18-61)
BMI Mean + SD	29.5 ± 8.84	23.4 ± 3.98
Median (range)	27 (21-54)	23 (17-34)
Smoker	2 (6.9%)	6 (8.45%)
Alcohol Yes	17 (58.62%)	43 (60.56%)

B

Total N	29
Serum creatinine Mean + SD	0.79 + 0.19
Median (range)	0.74 (0.61 – 1.56)
Diabetes	6 (20.69%)
Insulin dependent	5 (83.33%)
Hypertension	19 (65.52%)
ACE-I/ARB	6 (31.58%)
Cisplatin	11 (37.93%)
Carboplatin	18 (62.07%)

Table 2: Biomarker values (mean, + SD) in patients at baseline; values highlighted in red were above the upper limit of reference range (ULref, mean +2 SD of healthy volunteers); *comparison vs. healthy volunteers; **patients comparison male vs. female

	ULref	All patients	P-palue*	Male patients	Female patients	P-value**
α1M/crea	0.36 mg/mmol	0,63 ± 1,02	0,00000005	0,48 ± 0,48	0,72 ± 0,79	0.54
ß2M/crea	0.02 mg/mmol	0,05 ± 0,11	0,00000003	0,07 ± 0,16	0,04 ± 0,05	0.07
Kim-1/crea	0.11 µg/mmol	0,17 ± 0,16	0,00000001	0,17 ± 0,21	0,17 ± 0,21	0.54
MiAlb	5.79 mg/mmol	19,13 ± 43,41	0,000001	3,62 ± 1,56	28,61 ± 32,28	0.002
Prot	15,5 mg/mmol	34,1 ± 7,02	0,0000002	11,61 ± 9,11	54,85 ± 87,94	0.0003
K	9.7 mol/mmol	7,36 ± 5,57	0,0004	3,28 ± 0,88	9,85 ± 5,77	0.00002
Mg	0.8 mg/mmol	0,57 ± 0,34	0,62	0,39 ± 0,21	0,68 ± 0,37	0.03
NGAL	60.36 µg/mmol	32,79 ± 51,15	0,000000004	13,22 ± 17,38	44,75 ± 50,05	0.04
THUP	2.73 mg/mmol	2,20 ± 2,73	0,002	1,57 ± 1,11	2,58 ± 2,87	0.46
VEGFA	101,3 ng/mmol	81,59 ± 58,50	0,0004	80,32 ± 66,64	82,36 ± 86,52	0.70
TIMP	0.35 µg/mmol	0,51 ± 0,61	0,0000001	0,31 ± 0,35	0,63 ± 0,70	0.02
GST-α	2.63 µg/mmol	1,18 ± 1,88	0,00003	0,24 ± 0,16	1,76 ± 2,02	0.001
AST	1.01 U/mmol	0,55 ± 0,48	0,0002	0,26 ± 0,15	0,72 ± 0,52	0.0003
ALT	1.04 U/mmol	0,67 ± 0,70	0,00008	0,29 ± 0,14	0,87 ± 0,80	0.007
ALP	5.96 U/mmol	3,88 ± 4,23	0,000003	2,32 ± 2,24	4,83 ± 4,89	0.03
LDH	3.0 U/mmol	1,85 ± 0,77	0,04	2,11 ± 0,40	1,44 ± 1,29	0.03
GGT	4.91 U/mmol	2,85 ± 3,08	0,0002	0,93 ± 0,73	4,03 ± 3,39	0.001

Figure 1: Time course of selected biomarekers; mean + SD; the red line indicates ULref

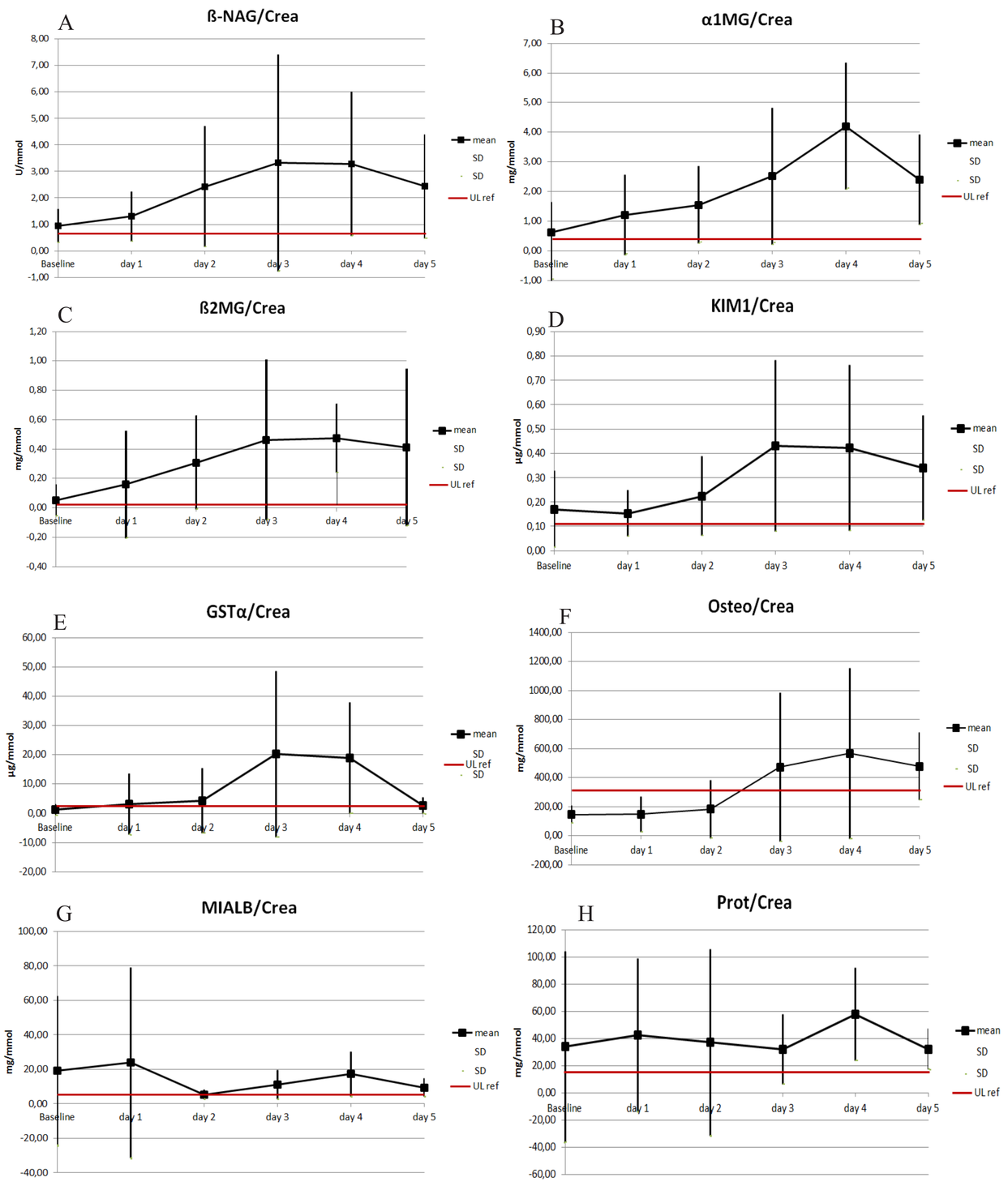
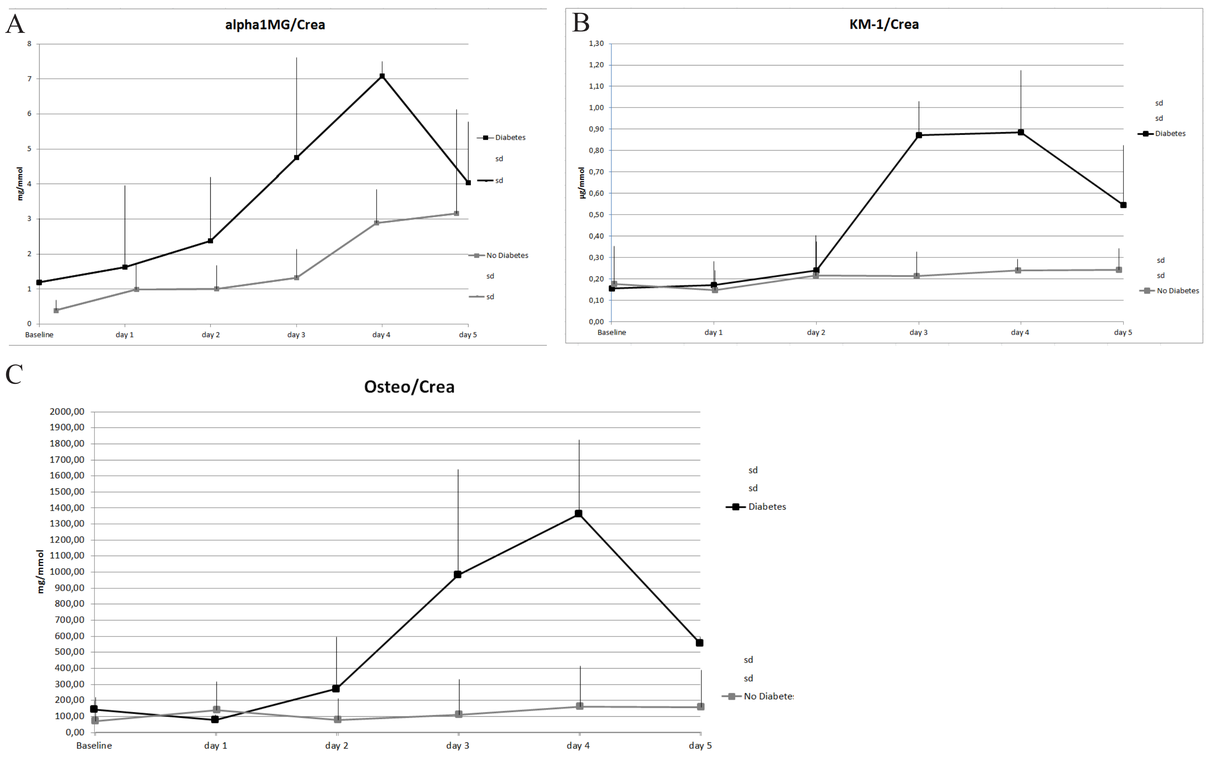


Figure 2: Time course of selected biomarkers, diabetic patients vs. non-diabetic; mean + SD



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