Evaluation of the cisplatin nephrotoxicity using the urinary neutrophil gelatinase-associated lipocalin (NGAL) in patients with head and neck cancer

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Abstract

Introduction: Acute kidney injury (AKI) in patients receiving cisplatin is common, therefore the evaluation of renal function in patients on use of nephrotoxic drugs is fundamental. **Objective:** To evaluate the incidence of AKI and the role of lipocalin associated to neutrophil gelatinase (NGAL) in the monitoring of renal function in patients with head and neck cancer (HNC) who received cisplatin. **Methods:** We prospectively studied 50 patients with HNC treated with three sessions of cisplatin. Blood and urine were collected 24 hours before cisplatin, 24 hours after infusion, 48 hours after each application and 35 days after the end of treatment (urine NGAL, C-reactive protein, creatinine, glomerular filtration rate, plasma lactate dehydrogenase and magnesium). **Results:** AKI was observed in 78% of patients. There was increase in creatinine, and decrease in GFR after each cycle of cisplatin, and increased urine NGAL. Positive association was observed between the levels of NGAL, creatinine and C-reactive protein. It was observed an increase in creatinine, NGAL, C-reactive protein and decreased GFR in AKI patients compared to patients without AKI. **Conclusion:** AKI was noted in 78% of patients with HNC treated with cisplatin and showed the correlation of NGAL with creatinine and GFR in demonstrating renal injury. NGAL levels may be elevated compared to baseline levels, even before the use of cisplatin.

Keywords: acute kidney injury; cisplatin; lipocalins.

Introduction

Complications such as impaired renal function and acute kidney injury (AKI) are caused in individuals with cancer by the disease itself or as a consequence of the medications used in oncologic treatment, particularly chemotherapy. Renal function assessment is carried out to allow safe administration of medication and monitor the effects of treatment on the patient. Serum creatinine may not suffice as an indicator of kidney function, given the error it may introduce in the estimation of the glomerular filtration rate (GFR).[1,2]

Cisplatin predominantly accumulates and is excreted through the kidneys. Non-toxic blood levels of the drug may be toxic in the kidneys, as concentrations in the tubule epithelial cells are five times higher than in blood. The limited possibility of increasing drug dosage posed by dose-dependent renal toxicity may compromise the effectiveness of treatment. Toxic effects occur primarily in the proximal tubule, particularly in the tubule epithelium cells on segment S-3. Distal tubules and glomeruli are involved at a later stage. Chronic nephrotoxicity is rare, as patients usually recover from acute drug toxicity. The main renal

complications arising from toxicity are AKI and hypomagnesemia.[3-5]

Several AKI biomarkers have been studied and shown their relevance in finding kidney injury, especially in septic patients, subjects in critical condition, heart surgery patients, and in individuals with contrast-induced nephropathy. The most commonly studied markers are neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), fatty-acid-binding proteins (FABPs), and cystatin C,[4,6,7] all of which associated with improvements in early kidney injury detection when compared to traditional tests based on creatinine and GFR.

The literature lacks studies on patients with head and neck cancer treated with cisplatin, and few clinical trials have analyzed the role of NGAL as a biomarker of AKI in cancer patients. Enrolled cohorts usually include patients with various tumor types, on different cisplatin protocols, showing mixed results regarding the risk of nephrotoxicity. This is the first study to enroll a homogeneous population with cancer to look into NGAL.[4,8-10]

Murine models of ischemic acute kidney injury have revealed that NGAL is one of the faster proteins to be synthesized by gene expression, with levels detected in the first urine sample within two hours of ischemia. Studies with rodents on cisplatin have also widely reported the presence of urinary NGAL. NGAL levels, despite scarce data on cancer patients, have been correlated with endpoints such as need for dialysis and death, particularly in critical patients.[11-17]

The main purpose of this study was to assess the incidence of AKI and the role of NGAL in the renal function evaluation of patients with head and neck cancer treated with cisplatin, and its potential use in the early diagnosis of acute kidney injury.

Materials and methods

This prospective observational study was approved by the UNIOESTE Research Ethics Committee and given permit 272/2012-CEP. Data collection took place between October of 2012 and November of 2013. Patients deemed able to understand the treatment and its complications were asked to give their informed consent, and allowed the authors to report their information in the study.

Fifty patients aged 18 years and over diagnosed with head and neck cancer treated at the Cascavel Cancer Hospital with three cycles of cisplatin-based chemotherapy at a dosage of 100 mg/m^2 associated with radiotherapy (7000 cGy) were enrolled in the study.

Individuals previously treated with cisplatin, patients with a GFR < 60 ml/min/1.73 m^2, subjects with uncontrolled hyperthyroidism or hypothyroidism, and those who refused to have blood or urine samples taken as per the study protocol were excluded.

Blood and urine samples were collected 24 hours before chemotherapy (D0, D21, D42), 24 hours after chemotherapy (D1, D22, D43), 48 hours after chemotherapy (D3, D24, D45) and 35 days after the last session with cisplatin (D78). Workup included urinary NGAL, C-reactive protein (CRP), creatinine, GFR, lactate dehydrogenase (LDH), and plasma magnesium. Complete blood count, urea, sodium, potassium, calcium, albumin, blood glucose, alkaline phosphatase, glutamic-pyruvic transaminase, and urine I tests were run 24 hours before chemotherapy sessions.

The Du Bois body surface area (BSA) formula was used to calculate the dose of chemotherapy.[18] The Jaffee reaction without deproteinization was used to determine serum creatinine levels in mg/dL. Subsequently, the GFR in ml/min/1.73 m^2 was calculated

using the aMDRD.[1,2] Other lab tests used the following methods: for LDH (U/L), the enzyme method; for urea (mg/dl), the enzyme/automated method; for magnesium (mg/dL), the colorimetric method; for calcium (mg/dL), the colorimetric/automated method; for sodium (mEq/L) and potassium (mEq/L), the selective electrode method; for CRP (mg/dl), the immunoturbidimetric method; for albumin (g/dL), nephelometry; urine was analyzed by sediment qualitative and quantitative analysis; and NGAL (mg/l) chemiluminescence (Abbott Diagnostics) was used to quantify the levels present in urine after storage at -80 °C.

The AKIN criteria was used to define and stage AKI[19] in all observation cycles of the study, as follows: AKIN 1: increases in creatinine greater than 0.3 mg/dL; AKIN 2: increases ≥ 100 % and < 200%; and AKIN 3: increases ≥ 200% from baseline creatinine levels 48 hours into follow-up. Another finding used in the comparative analysis between patients with and without AKI was an increase of 0.3 mg/dl in the baseline creatinine level (D0). Residual kidney disease was defined for subjects with a GFR < 60 ml/min/1.73 m^2 on D78. Serum CRP concentrations ranging from zero to 1.0 mg/DL[20] and magnesium levels from 1.6 to 2.3 mg/dL were deemed normal.[21]

In statistical analysis, quantitative variables were expressed as mean ± standard deviation, for normal and non-normal data. Categorical variables were compared using Fisher's exact test or the chi-square test. In comparisons between two independent groups, Student's *t*-test was used in normal data and the Mann-Whitney U test when the hypothesis of data normality had been rejected. Nonparametric analysis of variance (Dunn's post test following the Kruskal-Wallis test) was employed to compare between three or more groups. Workup results were assessed for each treatment cycle (three days each) and throughout the study. Spearman's rank correlation coefficient was used to evaluate the correlation between the levels of NGAL and other continuous variables with non-normal distributions. Groups with and without AKI were compared against each other. A significance level of 5% ($p < 0.05$) was adopted. Data sets were stored in a Microsoft Excel spreadsheet and treated using software program R (SPSS version 13.0).

Results

Clinical characteristics

Fifty patients with head and neck cancer were considered eligible for the study. Their mean age was 58.5 years, and 80% were males. In terms of histopathology, 94% of the patients had squamous cell carcinomas. Forty-four percent had primary tumors in the oropharynx, 20% in the larynx, and 14% in the oral cavity. Seventy percent of the patients had stage IV disease. Seven patients had high blood pressure and four had diabetes, and none had been diagnosed with chronic kidney disease previously. Table 1 shows the clinical characteristics and the baseline workup of enrolled patients.

Workup test results throughout the study

Patient GRF decreased, while serum creatinine and urea levels increased in the samples collected 48 hours after cisplatin administration (D3- GRF: 70.75 ml/min/1.73 m^2; creatinine: 1.11 mg/dL; urea: 46 mg/dL), D24- GFR: 52.84 ml/min/1.73 m^2; creatinine 1.4 mg/dL; urea: 54.05 mg/dL) and D45- GFR: 54.2 ml/min/1.73 m^2; creatinine: 1.21 mg/dL; urea: 60.25 mg/dL) and D78 (GFR: 71.02 ml/min/1.73 m^2, creatinine: 1.11 mg/dL; urea: 40.95 mg/dL) when compared to D0 (GFR: 100.7 ml/min/1.73 m^2, creatinine: 0.81 mg/dL; urea: 30.05 mg/dL), as when compared to the days before cisplatin administration (D0, D21- GFR: 76.10 ml/min/1.73 m^2, creatinine: 1.01 mg/dL; urea: 37.7 mg/dL) and D42- GFR: 75.24 ml/min/1.73 m^2; creatinine: 1.03 mg/dL; urea: 54.05 mg/dL) and 24 hours after the administration of cisplatin (D1- GRF: 90.01 ml/min/1.73 m^2, creatinine: 0.84 mg/dL; urea: 26.0 mg/dL), D22- GFR: 80.6 ml/min/1.73 m^2; creatinine: 0.99 mg/dL; urea: 30.9 mg/dL) and D43- GFR: 85.7 ml/min/1.73 m^2; creatinine: 0.95 mg/dL; urea: 34.7 mg/dL) ($p < 0.05$).

Table 1 Baseline clinical and workup characteristics (D0) of patients with head and neck cancer treated with cisplatin and radiotherapy

Variable		n (%)
Age (years - mean ± SD)	58.5 ± 9.1	50 (100%)
Gender	Male	40 (80%)
	Female	10 (20%)
Histopathology	Adenocarcinoma	2 (4%)
	Adenoid cystic carcinoma	1 (2%)
	Squamous cell carcinoma	47 (94%)
Tumor site	Oral cavity	7 (14%)
	Oropharynx	22 (44%)
	Larynx	10 (20%)
	Other	11 (22%)
Staging	III	15 (30%)
	IV	35 (70%)
	Mean ± SD	
Creatinine (mg/dL)	0.83 ± 0.23	
GFR (ml/min/1.73 m^2)	108.3 ± 35.5	
Urea (mg/dL)	30.1 ± 8.9	
NGAL (µg/L)	25.0 ± 22.3	
Magnesium (mg/dL)	2.0 ± 0.2	
Sodium (mEq/L)	136.4 ± 3.7	
Potassium (mEq/L)	4.4 ± 0.4	
Calcium (mg/dL)	9.2 ± 0.6	
CRP (mg/dL)	2.3 ± 4.0	
LDH (U/L)	199.8 ± 70.4	
Hemoglobin (g/dL)	13.0 ± 1.6	
Segmented (cells/mm^3)	6151.0 ± 2953.2	
Lymphocytes (cells/mm^3)	1898.1 ± 691.1	
Albumin (g/dL)	5.2 ± 6.2	
GPT (U/L)	84.8 ± 32.4	
Alkaline phosphatase (U/L)	9.2 ± 0.5	

GFR: Glomerular filtration rate; LDH: Lactate dehydrogenase; NGAL: Neutrophil gelatinase-associated lipocalin; CRP: C-reactive protein; GPT: Glutamic-pyruvic transaminase; SD: Standard deviation.

Median NGAL levels were higher on D21 (55.6 µg/L), D24 (69.90 µg/L), D42 (57.5 µg/L), D45 (45.0 µg/L) and D78 (37.40 µg/L) when compared to D0, as well as on D48 after the administration of cisplatin (D3: 37.95 µg/L, D24: 69.60 µg/L and D45: 45.0 µg/L) than 24 hours after cisplatin administration (D1: 10 µg/L, D22: 16.85 µg/L and D43: 16.55 µg/L) ($p < 0.05$). Serum magnesium levels decreased in relation to baseline levels (D0), but statistically significant differences were seen only from D22 onwards ($p < 0.05$). CRP increased on D22, D42 and D43 in relation to D3 ($p < 0.05$). Table 2 shows the results.

Incidence of AKI

Seventy eight percent of the enrolled patients (n = 50) were diagnosed with AKI during the study. According to the AKIN classification, in the first cycle 26% of the patients had AKI, with 17% on AKIN 1 and 9% on AKIN 2; in the second cycle 42% had AKI, with 21% on AKIN 1, 19% on AKIN 2, and 2% on AKIN 3; and in the third cycle 29% had AKI, with 20% on AKIN 1, 6% on AKIN 2, and 3% on AKIN 3 (data not shown). Drug dosage had to be reduced for nine patients (18%) and treatment had to be suspended for another two subjects (4%) on D43 due to febrile

Table 2 Median workup results observed in patients with head and neck cancer treated with cisplatin on specific days

Variables	D0	D1	D3	D21	D22	D24	D42	D43	D45	D78
Mg (mg/dl)	1.99	1.84	1.92	1.77	1.64†	1.72†	1.49†	1.44†	1.49†	1.43†
Cr (mg/dl)	0.81	0.84	1.11•	1.01	0.99	1.40•	1.03	0.95	1.21•	1.11†
GRF (ml/min/1.73 m²)	100.7	90.01	70.75•	76.10	80.6	52.84•	75.24	85.07	54.20•	71.02†
Urea (mg/dL)	30.05	26.00	46.00•	37.70	30.9	54.05•	40.60	34.70	60.25•	40.95†
NGAL (µg/L)	20.10	10.00ǁ	37.95ǁ	55.60†	16.85ǁ	69.90‡	57.50‡	16.55ǁ	45.00‡	37.40ǁ
CRP (mg/dl)	0.98	0.93	1.08	1.91	3.00	1.68	4.74†	4.55†	2.64	2.37

ǁ $p < 0.05$ (Dunn's post test following the Kruskal-Wallis test) on D1; † $p < 0.05$ (Dunn's post test following the Kruskal-Wallis test): relative to D0 and D1; ‡ $p < 0.05$ (Dunn's post test following the Kruskal-Wallis test) relative to D0 and D1 and days within 24 hours of cisplatin infusion (D22, D43); • $p < 0.05$ (Dunn's post test following the Kruskal-Wallis test) relative to values 24 hours before each cycle (D0, D21, D42) and within 24 hours of cisplatin infusion (D1, D22, D43); ǀ $p < 0.05$ (Dunn's post test following the Kruskal-Wallis test) relative 48 hours after cisplatin infusion (D3, D24, D45) and 24 hours before cisplatin infusion (D21, D42). Cr: Creatinine; GFR: Glomerular filtration rate; LDH: Lactate dehydrogenase; NGAL: Neutrophil gelatinase-associated lipocalin; CRP: C-reactive protein; Mg: Magnesium.

neutropenia and for another three (6%) due to AKI, as per the institution's protocol.

Comparison between groups with and without AKI

No statistically significant differences were observed ($p > 0.05$) between the clinical characteristics and baseline workup of patients with and without AKI (see Table 3). When the workup results of patients with and without AKI in the three cycles of treatment were compared, increases were seen in NGAL, CRP and creatinine levels and decreases observed in the GFR. Statistical differences were seen only in the creatinine levels and GFR of patients with AKI against individuals without AKI starting on D21 ($p < 0.05$), while CRP was significantly increased only on D42 ($p < 0.05$); serum magnesium was not significantly different between the two groups throughout the study.

The analysis of Spearman's rank correlation coefficients between urinary NGAL and creatinine, GFR, CRP, LDH, and magnesium revealed the existence of a positive correlation between NGAL and creatinine and a negative correlation between creatinine and GFR in all cycles ($p = 0.000$). A negative correlation between NGAL and magnesium was observed on cycle 1 ($p = 0.037$) and a positive correlation between creatinine and magnesium on cycle 2 ($p = 0.020$) 48 hours after cisplatin administration.

Positive correlations between urinary NGAL and CRP ($p = 0.000$) and creatinine and urinary NGAL ($p = 0.015$) were observed when all values obtained in a single correlation analysis throughout the treatment were included. Graph 1 shows the curve with median levels of NGAL, creatinine and CRP over the course of the study.

Residual disease after treatment

At the end of the study (D78), 32% of the patients (n = 16) still had a GFR below 60 ml/min, a statistically significant difference in relation to the number of individuals without kidney disease (Table 3). The other variables failed to show statistically significant differences between groups with and without residual renal disease. The mean NGAL in patients with residual disease was 97.34 g/L (Table 3), while the mean NGAL for the samples collected at the end of the study was 63.3 mg/l.

Discussion

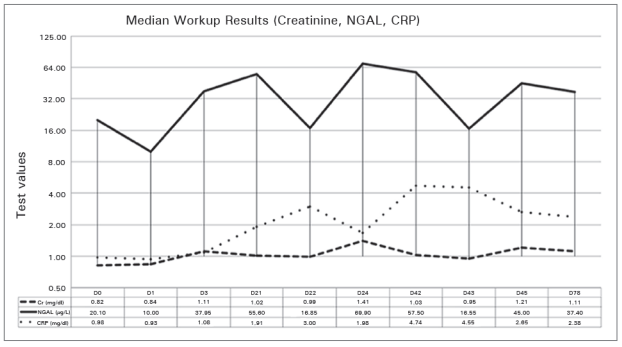
The combination of chemotherapy and radiotherapy increases the possibility of acute toxicity when compared to radiotherapy alone.[22-24] Seventy eight percent of the patients had increases greater than 0.3 mg/dl from their baseline creatinine levels. Based on the AKIN classification, 62% had AKI during the study, 26% had AKI in the first cycle of treatment, 42% on the second cycle, and 29% on the third cycle. The reported incidence of AKI in individuals treated with cisplatin based on creatinine levels and GFR revolves around 20%-40%.[3] Although reports in the literature have described renal function recovery for most patients treated with cisplatin,[25] at the end of our study (D78) 32% of the patients (n = 16) still had a GFR below 60 ml/min and were at risk of developing chronic kidney disease.

Table 3 Baseline and D78 clinical and workup data of head and neck cancer patients treated with cisplatin according to occurrence of AKI

Variable	AKI (n = 39)	No AKI (n = 11)	*p*-value	Residual kidney disease (n = 16)	No residual kidney disease (n = 11)	*p*-value
Age (years)	58.1 ± 8.9	60.3 ± 9.5	0.725†	59.3 ± 9.6	60.8 ± 9.5	0.342†
Males (n)	31 (79%)	10 (90%)	0.807*	11 (68%)	10 (90%)	0.771*
Body surface area (m^2)	1.6 ± 0.3	1.56 ± 0.16	0.325‡	1.71 ± 0.23	1.5 ± 0.16	0.126‡
Creatinine (mg/dL)	0.80 ± 0.18	0.88 ± 0.35	0.914†	1.60 ± 0.48	0.8 ± 0.2	< 0.0001‡
GFR (ml/min/1.73 m^2)	109.2 ± 34.5	103.7 ± 38.9	0.350‡	46.38 ± 10.28	104.6 ± 40.1	0.0010†
Urea (mg/dL)	29.93 ± 9.1	30.4 ± 7.9	0.476†	65.6 ± 35.0	50.48 ± 38.03	0.135‡
NGAL (µg/L)	24.5 ± 23.93	27.5 ± 9.4	0.209‡	97.34 ± 93.67	56.0 ± 54.01	0.1228‡
Magnesium (mg/dL)	1.96 ± 0.25	2.02 ± 0.20	0.085†	1.63 ± 0.31	1.5 ± 0.2	0.316‡
Sodium (mEq/L)	136 ± 3.4	136.88 ± 4.7	0.865‡	132.7 ± 4.8	133.2 ± 3.9	0.891‡
Potassium (mEq/L)	4.3 ± 0.37	4.76 ± 0.37	0.848†	4.8 ± 0.9	4.4 ± 0.38	0.640‡
Calcium (mg/dL)	9.1 ± 0.52	9.6 ± 0.72	0.200‡	9.0 ± 0.7	8.7 ± 0.4	0.427†
CRP (mg/dL)	2.23 ± 3.92	2.6 ± 4.4	0.982‡	5.2 ± 5.0	2.5 ± 1.8	0.192‡
LDH (U/L)	202.7 ± 76.1	196.6 ± 35.5	0.742‡	188.4 ± 43.1	195 ± 85.7	0.841‡
Hemoglobin (g/dL)	13.1 ± 1.6	12.3 ± 1.2	0.326†	10.19 ± 1.46	10.8 ± 0.68	0.420†
Segmented (cells/mm^3)	5974.02 ± 3003.1	6859.1 ± 2793.6	0.147‡	5013.5 ± 3727	2957 ± 813	0.183†
Lymphocytes (cells/mm^3)	1819.8 ± 733.5	1821.4 ± 521.37	0.370†	852.1 ± 359.3	745 ± 438	0.438†
Albumin (g/dL)	4.2 ± 0.39	3.9 ± 0.32	0.400†	3.7 ± 0.4	3.6 ± 0.4	0.828†
GPT (U/L)	26.9 ± 15.8	22.27 ± 16.7	0.394‡	23.9 ± 18.0	17.1 ± 9.0	0.473†
Alkaline phosphatase (U/L)	87.8 ± 33.5	66.51 ± 15.3	0.063‡	100.4 ± 30.8	85.6 ± 28.0	0.150†

* *p*-value from Fisher's exact test; † *p*-value from Student's *t*-test; ‡ *p*-value from Mann-Whitney U test.

Graph 1. Main median workup results throughout the duration of the study. Cr: Creatinine; NGAL: Neutrophil gelatinase-associated lipocalin; CRP: C-reactive protein. Note: See the *p*-value of each test on Table 2.



Many studies have shown that elevation of serum creatinine alone does not correlate with kidney injury and may delay clinical diagnosis. The AKIN[19] and RIFLE[26] classifications have been used to redefine and prevent the occurrence of AKI with parameters based on serum creatinine and urine output. The AKIN classification indicates that elevations of 0.3 mg/dL from baseline creatinine levels within 48 hours are suggestive of subclinical kidney injury (AKIN 1), a finding often overlooked by general practitioners and oncologists, as this scale is more commonly used in critically ill patients and could lead to higher incidences of AKI related to medication, cisplatin in particular, than when creatinine or GFR is used alone.

Our patients had decreases in GRF and increases in serum creatinine and urea 48 hours after each treatment session with cisplatin, thus validating the use of these tests to verify altered kidney function only after and never before exposure to the drug (second and third cycles).

Elevations in mean NGAL levels were observed 48 hours after the first, second, and third cycles of cisplatin administration. NGAL levels were elevated prior to the days of subsequent cycles (before the second and third cycles) and remained high at the end of treatment, possibly indicating the early and persistent increases in NGAL levels before the second and third dose of cisplatin (after the initial insult with the first dose). NGAL levels were high even before creatinine levels had increased 48 hours after cisplatin administration. This may suggest kidney injury occurred before the creatinine levels of patients with head and neck cancer had increased,

as they were given high dosages of a potentially nephrotoxic drug with cumulative toxicity.

Preclinical studies have shown that increased urinary NGAL levels were correlated with increased drug dosage three hours after cisplatin infusion in murine models of drug-induced nephrotoxicity.[14] Moreover, NGAL was easily detected in urine within three hours of drug administration, against the 96 hours it took for serum creatinine levels to change.[27]

NGAL has been used to identify cisplatin-induced acute kidney injury in humans, despite the limitations related to the varied selection of patients and treatment protocols. A study[8] was carried out in 2010 with 24 patients given cisplatin for various tumor types. Twelve subjects with AKI were compared to individuals with stable creatinine levels. Urinary NGAL levels were significantly higher in patients with AKI than in controls on days 1, 2, 3 and 15 after cisplatin infusion. Among patients with AKI, increased urinary NGAL levels appeared to predict residual kidney disease within 15 days.[4,8] In a more recent study with 34 patients with various tumor types and treated with different doses of cisplatin (50 mg/m^2 to 80 mg/m^2), the authors showed that serum NGAL levels could not predict drug-induced nephrotoxicity.[10]

Our study showed patients had increased NGAL levels in the days preceding the administration of cisplatin in the second and third cycles (D21 and D42) before their creatinine levels had increased, despite the lack of a difference when patients with and without AKI were compared. Moreover, a positive correlation was observed between the levels of NGAL and CRP, suggesting that higher NGAL levels in patients with head and neck tumors may also be seen as markers of inflammation, in addition to early nephrotoxicity. Evidence strongly suggests the involvement of inflammatory mechanisms in the pathogenesis of cisplatin nephrotoxicity.[3,5,28,29]

The positive correlation observed between NGAL and CRP at end of treatment (D78) and for sample totals probably relates to exposure to high dosages of cisplatin and cumulative dose effects in the third treatment cycle. Considering renal injury as an inflammatory mechanism, NGAL and CRP could be seen as inflammation markers of kidney injury. However, the disease itself and treatments such as radiotherapy and chemotherapy may be involved in the inflammatory process and in the increased levels of CRP.[25,30]

Our study also showed that the mean NGAL of patients with a GFR below 60 ml/min at the end of the study was higher (97.34 g/L), although not statistically different from patients without renal injury (56.0 mg/L). However, the mean NGAL value of the samples collected in D78 (63.3 mg/L) was statistically different from baseline values (D0). Most studies reporting NGAL levels used ELISA assays, which are not practical in a clinical environment.[31]

Chemiluminescence magnetic microparticle immunoassay (CMIA), commercially available and performed through automated platform ARCHITECT (Abbott Diagnostics), was used in our study to measure urinary NGAL. Research has indicated that this method produces linear regressions quite close to the ELISA kit (*AntibodyShop* NGAL *Rapid* ELISA *Kit, BioPorto, Denmark*) across the entire tested NGAL concentration range, from 2 µg/L to 1500 µg/L.[31-33] Different cutoff levels of urinary NGAL have been described (over 10 g/L, over 60 mg/L, over 100 g/L) to identify patients at a higher risk of developing AKI.[34,35]

Saleena *et al.*[36] (2012) designed a study to assess the efficacy of urinary enzymes α-glutathione-S-transferase (α-GST) and γ-GT as predictors of kidney injury in patients with head and neck cancer treated with cisplatin. The mean urinary levels of α-GST increased along different time intervals, especially within two hours of cisplatin administration, suggesting that urinary levels of proximal tubular enzymes α-GST and γ-GT may be useful in predicting early cisplatin-induced kidney injury.

Hypomagnesemia is one of the side effects seen in patients given cisplatin-based chemotherapy. However, the correlation between cisplatin-induced hypomagnesemia and nephrotoxicity has not been completely elucidated.[37,38] Our study showed decreased levels of serum magnesium even in patients without renal

injury, although without statistically significant differences between AKIN groups, showing that hypomagnesemia is an important effect of cisplatin even in patients without renal injury.[3,25]

Alves *et al.*[39] (2013) have recently described increased presence of hypomagnesemia in patients who did not recover renal function (70% *vs.* 31%), and multivariate analysis identified hypomagnesemia as an independent risk factor for non-recovery of renal function. Rat model studies have suggested that hypomagnesemia could cause dehydration and up-regulation of the cisplatin receptor, the organic cation transporter (OCT2), increasing the renal accumulation of cisplatin and worsening AKI.[40]

Acute kidney injury biomarkers have been extensively studied in the description of AKI,[41] particularly ischemic AKI, both experimentally and in clinical settings in which ischemia is common (e.g.: sepsis and cardiopulmonary bypass). Although few clinical studies have looked into cancer patients, several authors have shed light on the cellular mechanism of cisplatin nephrotoxicity and a considerable number of biomarkers of AKI secondary to nephrotoxicity, particularly in preclinical studies in which cisplatin nephrotoxicity has been included.[42]

Few publications in the literature[36] have assessed the clinical toxicity of cisplatin and the objective measurement of toxicity using biomarkers to detect early kidney injury in patients with head and neck cancer, in whom the use of high doses of cisplatin significantly increases the incidence of AKI and nephrotoxicity, which combined represent a major limitation to the use of cisplatin in the treatment of solid tumors, including tumors of the head and neck.

To our knowledge, there are no studies in the literature describing NGAL as a marker of cisplatin-induced kidney injury in patients with head and neck cancer.[7,8-10,15,36,43] Therefore, new randomized studies are needed to determine the true predictive and prognostic value of NGAL for patients with head and neck cancer treated with cisplatin and the cutoff points for each NGAL level in this specific disease and each clinical outcome.

Conclusions

Seventy-eight percent of the patients with head and neck cancer enrolled in our study treated with three cycles of cisplatin had acute kidney injury verified by NGAL, creatinine, and GFR within 48 hours of cisplatin administration. Their NGAL levels might also be higher than in baseline conditions even before chemotherapy with cisplatin, which may indicate the presence of kidney injury before increases in serum creatinine were observed. These findings suggest that the identification of patients at risk for acute kidney injury induced by cisplatin could stimulate the development of strategies for the treatment and prevention of nephrotoxicity or lead to a drug ban. The findings described herein have to be replicated and validated in prospective randomized trials.

References

1. Aapro M, Launay-Vacher V. Importance of monitoring renal function in patients with cancer. Cancer Treat Rev 2012;38:235-40. DOI: http://dx.doi.org/10.1016/j.ctrv.2011.05.001
2. Launay-Vacher V, Oudard S, Janus N, Gligorov J, Pourrat X, Rixe O, et al.; Renal Insufficiency and Cancer Medications (IRMA) Study Group. Prevalence of Renal Insufficiency in cancer patients and implications for anticancer drug management: the renal insufficiency and anticancer medications (IRMA) study. Cancer 2007;110:1376-84. DOI: http://dx.doi.org/10.1002/cncr.22904
3. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of Cisplatin nephrotoxicity. Toxins (Basel) 2010;2:2490-518. DOI: http://dx.doi.org/10.3390/toxins2112490
4. Sirota JC, Klawitter J, Edelstein CL. Biomarkers of acute kidney injury. J Toxicol 2011;2011:328120. PMID: 22131986 DOI: http://dx.doi.org/10.1155/2011/328120
5. Goldstein RS, Mayor GH. Minireview. The nephrotoxicity of cisplatin. Life Sci 1983;32:685-90. PMID: 6338333 DOI: http://dx.doi.org/10.1016/0024-3205(83)90299-0
6. Tesch GH. Review: Serum and urine biomarkers of kidney disease: A pathophysiological perspective. Nephrology (Carlton) 2010;15:609-16. DOI: http://dx.doi.org/10.1111/j.1440-1797.2010.01361.x
7. Slocum JL, Heung M, Pennathur S. Marking renal injury: can we move beyond serum creatinine? Transl Res 2012;159:277-89.
8. Gaspari F, Cravedi P, Mandalà M, Perico N, de Leon FR, Stucchi N, et al. Predicting cisplatin-induced acute kidney injury by urinary neutrophil gelatinase-associated lipocalin excretion: a pilot prospective case-control study. Nephron Clin Pract 2010;115:c154-60. PMID: 20407275 DOI: http://dx.doi.org/10.1159/000312879
9. Soni SS, Pophale R, Ronco C. New biomarkers for acute renal injury. Clin Chem Lab Med 2011;49:1257-63. DOI: http://dx.doi.org/10.1515/CCLM.2011.664
10. Kos FT, Sendur MA, Aksoy S, Celik HT, Sezer S, Civelek B, et al. Evaluation of renal function using the level of neutrophil gelatinase-associated lipocalin is not predictive of nephrotoxicity associated with cisplatin-based chemotherapy. Asian Pac J Cancer Prev 2013;14:1111-4. PMID: 23621196 DOI: http://dx.doi.org/10.7314/APJCP.2013.14.2.1111

11. Le Cabec V, Calafat J, Borregaard N. Sorting of the specific granule protein, NGAL, during granulocytic maturation of HL-60 cells. Blood 1997;89:2113-21. PMID: 9058734
12. Flower DR, North AC, Sansom CE. The lipocalin protein family: structural and sequence overview. Biochim Biophys Acta 2000;1482:9-24. PMID: 11058743 DOI: http://dx.doi.org/10.1016/S0167-4838(00)00148-5
13. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534-43. DOI: http://dx.doi.org/10.1097/01.ASN.0000088027.54400.C6
14. Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 2004;24:307-15. DOI: http://dx.doi.org/10.1159/000078452
15. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009;54:1012-24. PMID: 19850388 DOI: http://dx.doi.org/10.1053/j.ajkd.2009.07.020
16. Urbschat A, Obermüller N, Haferkamp A. Biomarkers of kidney injury. Biomarkers 2011;16:S22-30. DOI: http://dx.doi.org/10.3109/1354750X.2011.587129
17. Barrera-Chimal J, Bobadilla NA. Are recently reported biomarkers helpful for early and accurate diagnosis of acute kidney injury? Biomarkers 2012;17:385-93.
18. Gehan EA, George SL. Estimation of human body surface area from height and weight. Cancer Chemother Rep 1970;54:225-35. PMID: 5527019
19. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al.; Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31. DOI: http://dx.doi.org/10.1186/cc5713
20. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-12. PMID: 12813013 DOI: http://dx.doi.org/10.1172/JCI200318921
21. Kaplinsky C, Alon US. Magnesium homeostasis and hypomagnesemia in children with malignancy. Pediatr Blood Cancer 2013;60:734-40. DOI: http://dx.doi.org/10.1002/pbc.24460
22. Winquist E, Oliver T, Gilbert R. Postoperative chemoradiotherapy for advanced squamous cell carcinoma of the head and neck: a systematic review with meta-analysis. Head Neck 2007;29:38-46. DOI: http://dx.doi.org/10.1002/hed.20465
23. Brana I, Siu LL. Locally advanced head and neck squamous cell cancer: treatment choice based on risk factors and optimizing drug prescription. Ann Oncol 2012;23:x178-85. DOI: http://dx.doi.org/10.1093/annonc/mds322
24. Pfister DG, Ang KK, Brizel DM, Burtness BA, Cmelak AJ, Colevas AD, et al.; National Comprehensive Concer Network. Head and neck cancers. J Natl Compr Canc Netw 2011;9:596-650.
25. Sánchez-González PD, López-Hernández FJ, López-Novoa JM, Morales AI. An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. Crit Rev Toxicol 2011;41:803-21. DOI: http://dx.doi.org/10.3109/10408444.2011.602662
26. Lopes JA, Fernandes P, Jorge S, Gonçalves S, Alvarez A, Costa e Silva Z, et al. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. Crit Care 2008;12:R110. DOI: http://dx.doi.org/10.1186/cc6997
27. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365:1231-8. PMID: 15811456 DOI: http://dx.doi.org/10.1016/S0140-6736(05)74811-X
28. Yavuz S, Anarat A, Acartürk S, Dalay AC, Kesiktaş E, Yavuz M, et al. Neutrophil gelatinase associated lipocalin as an indicator of acute kidney injury and inflammation in burned children. Burns 2014;40:648-54. DOI: http://dx.doi.org/10.1016/j.burns.2013.09.004
29. Petrovic S, Bogavac-Stanojevic N, Peco-Antic A, Ivanisevic I, Kotur-Stevuljevic J, Paripovic D, et al. Clinical application neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 as indicators of inflammation persistence and acute kidney injury in children with urinary tract infection. Biomed Res Int 2013;2013:947157. DOI: http://dx.doi.org/10.1155/2013/947157
30. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. Biochim Biophys Acta 2012;1826:129-69. PMID: 22513004
31. Clerico A, Galli C, Fortunato A, Ronco C. Neutrophil gelatinase-associated lipocalin (NGAL) as biomarker of acute kidney injury: a review of the laboratory characteristics and clinical evidences. Clin Chem Lab Med 2012;50:1505-17. DOI: http://dx.doi.org/10.1515/cclm-2011-0814
32. Cavalier E, Bekaert AC, Carlisi A, Legrand D, Krzesinski JM, Delanaye P. Neutrophil gelatinase-associated lipocalin (NGAL) determined in urine with the Abbott Architect or in plasma with the Biosite Triage? The laboratory's point of view. Clin Chem Lab Med 2011;49:339-41. PMID: 21091234 DOI: http://dx.doi.org/10.1515/CCLM.2011.044
33. Vanmassenhove J, Vanholder R, Nagler E, Van Biesen W. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. Nephrol Dial Transplant 2013;28:254-73. DOI: http://dx.doi.org/10.1093/ndt/gfs380
34. Schiffl H, Lang SM. Update on biomarkers of acute kidney injury: moving closer to clinical impact? Mol Diagn Ther 2012;16:199-207.
35. Cullen MR, Murray PT, Fitzgibbon MC. Establishment of a reference interval for urinary neutrophil gelatinase-associated lipocalin. Ann Clin Biochem 2012;49:190-3. DOI: http://dx.doi.org/10.1258/acb.2011.011105
36. Saleena UV, Athiyaman MS, Donald F, Vadhiraja BM, Prabhu R, Nalini K. Evaluation of urinary tubular enzymes for the detection of early kidney injury due to cisplatin chemotherapy. Int J Biol Med Res 2012;3:2241-6.
37. Hirai S, Kaida S, Ito T, Hasebe S, Ueno M, Udagawa H, et al. Magnesium premedication prevents Cisplatin-induced nephrotoxicity in patients with esophageal and hypopharyngeal cancer. Gan To Kagaku Ryoho 2013;40:743-7.
38. Lameire N, Van Biesen W, Vanholder R. Electrolyte disturbances and acute kidney injury in patients with cancer. Semin Nephrol 2010;30:534-47. DOI: http://dx.doi.org/10.1016/j.semnephrol.2010.09.002
39. Alves SC, Tomasi CD, Constantino L, Giombelli V, Candal R, Bristot Mde L, et al. Hypomagnesemia as a risk factor for the non-recovery of the renal function in critically ill patients with acute kidney injury. Nephrol Dial Transplant 2013;28:910-6. DOI: http://dx.doi.org/10.1093/ndt/gfs268
40. Yokoo K, Murakami R, Matsuzaki T, Yoshitome K, Hamada A, Saito H. Enhanced renal accumulation of cisplatin via renal organic cation transporter deteriorates acute kidney injury in hypomagnesemic rats. Clin Exp Nephrol 2009;13:578-84. DOI: http://dx.doi.org/10.1007/s10157-009-0215-1
41. Peres LA, Cunha Júnior AD, Schäfer AJ, Silva AL, Gaspar AD, Scarpari DF, et al. Biomarkers of acute kidney injury. J Bras Nefrol 2013;35:229-36. DOI: http://dx.doi.org/10.5935/0101-2800.20130036
42. Peres LA, da Cunha AD Jr. Acute nephrotoxicity of cisplatin: molecular mechanisms. J Bras Nefrol 2013;35:332-40. DOI: http://dx.doi.org/10.5935/0101-2800.20130052
43. Kos FT, Sendur MA, Aksoy S, Sezer S, Civelek B, Yazici O, et al. Evaluation of the renal function using cystatin C level in the patients receiving cisplatin-based chemotherapy. Ren Fail 2013;35:705-10. DOI: http://dx.doi.org/10.3109/0886022X.2013.777929