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CLINICAL RESEARCH ARTICLE

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Impact of nephrotoxic drugs on urinary biomarkers of renal function in very preterm infants

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BACKGROUND: Following preterm birth, the immature kidney is exposed to several harmful conditions, with an increased risk of renal impairment. We aimed to assess urinary biomarkers of renal function in very preterm infants during early nephrotoxic treatments.

METHODS: Infants ≤32 weeks' gestation and ≤1500 g were enrolled in this observational prospective study. Urine samples were collected on day 1(T1), 2–4(T2), 5–7(T3), 8–10(T4), 11–13(T5). The following urinary biomarkers were determined: osteopontin (uOPN), epidermal growth factor (uEGF), neutrophil gelatinase-associated lipocalin (uNGAL), cystatin C (uCysC). The infants were grouped according to their exposure to amikacin or ibuprofen during the study period and a between-group comparison of urinary biomarkers at each time point was performed.

RESULTS: Thirty-six infants were included. Urinary CysC, uOPN, and uNGAL rose significantly during ibuprofen or amikacin treatment, while no difference was observed for uEGF. After adjustment for possible influencing factors, amikacin administration was associated with higher uCysC at T1 ($p = 0.007$) and T2 ($p = 0.016$), whereas ibuprofen increased uOPN ($p = 0.001$) and uNGAL concentration ($p = 0.009$) at T3.

CONCLUSION: Nephrotoxic therapies induce molecule-specific change patterns of renal function biomarkers in treated preterm infants. Serial assessments of these biomarkers may aid to identify neonates at risk of renal impairment and to develop tailored therapeutic approaches.

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IMPACT:

- Despite the wide use of nephrotoxic therapies in neonatal settings, little is known on their effect on renal function biomarkers in preterm infants.
- This study describes molecule-specific change patterns of urinary biomarkers during ibuprofen and amikacin administration, suggesting underlying pathophysiological effects on renal function.
- Given their low analytical costs and non-invasive collection, the urinary biomarkers investigated in this study represent a promising strategy for serial monitoring of renal function in at-risk neonates and may aid the early detection of renal function impairment at different kidney levels during nephrotoxic treatments.

BACKGROUND

Despite the advances in neonatal care have significantly improved the survival of the most preterm infants over the past decades, premature birth is still burdened by several short-and long-term sequelae, with significant implications on health care resources.[1,2]

The extra-uterine renal maturation after preterm birth has been associated with a higher prevalence of morphologically abnormal glomeruli and an enlarged renal corpuscle cross-sectional area, which may ultimately result in a nephron deficit and increase preterm infants' susceptibility to renal function impairment.[3] In addition to this altered kidney development, multiple factors contribute to challenge renal function in this delicate population during the early postnatal period. The haemodynamic disturbances associated with a haemodynamically significant patent ductus arteriosus (hsPDA) or with septic shock due to perinatal or nosocomial infections may profoundly affect renal perfusion.[4,5] Besides, these conditions often require the use of medications with potential nephrotoxic effects (e.g., cox-inhibitors for pharmacological hsPDA closure or antibiotics), which can further harm the immature kidney.[6,7]

Due to the small and heterogeneous cohorts included in clinical trials and observational studies, as well as to the lack of comparative studies evaluating possible therapeutic alternatives, the risk-benefit profile of drug therapies in preterm infants has not

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been well-documented; nevertheless, potentially nephrotoxic drugs are widely used in this population.[8] Hence, the development of acute kidney injury (AKI) is not uncommon, especially at lower gestational ages (GAs)[9] and has been associated with increased mortality rates.[10] Recent evidence from a large cohort of hospitalized preterm neonates reported an estimated AKI incidence of 12%, whereas either functional or intrinsic renal failure was detected in half of the study population during hospital stay.[11] Infants born preterm or with a low birth weight (BW) are also at increased risk of developing glomerulosclerosis, chronic kidney disease and hypertension from childhood into adult life.[12–14]

Routine monitoring of renal function in preterm infants would aid not only to identify neonates at higher risk of impairment, but also to develop tailored strategies aimed at safeguarding the immature kidney from multifactorial insults, including nephrotoxic exposures. Although serum creatinine (sCreat) is widely used for the assessment of neonatal renal function, its reliability is scarce, as it is influenced by several factors (e.g., maternal levels, GA and postnatal age) and, during AKI, its changes occur later than those of glomerular filtration rate.[15] Over the past decade, novel urinary biomarkers have been translated to neonatal settings, showing encouraging results on the early detection of renal function impairment.[16–18] To date, however, little is known on the effects of nephrotoxic drugs on these biomarkers in the preterm population.[19,20]

This study aimed to assess the patterns of urinary biomarkers of renal function in very-low-birth-weight (VLBW) preterm neonates undergone nephrotoxic treatments over the first 2 weeks of life.

MATERIAL AND METHODS

Study population and ethics

Preterm infants ≤32 weeks' gestation and with a BW ≤ 1500 g admitted to the Neonatal Intensive Care Unit (NICU) of S. Orsola-Malpighi University Hospital between September 2016 and September 2017 were consecutively enrolled in this monocentric observational prospective study. Major congenital abnormalities, including congenital heart defects, metabolic diseases and perinatal asphyxia (defined by the evidence of an arterial pH ≤7.0 or base excess ≤−12 mMol/l on arterial cord blood and/or Apgar ≤5 or need for resuscitation at 10 min) were exclusion criteria. Infants deceased within the first 48 h after birth or undergone major surgical interventions during the study period were also ruled out.

The study protocol adhered to the Declaration of Helsinki and was approved by the S. Orsola University Hospital Ethics Committee, Bologna, Italy (protocol no. 154/2015/U/Oss). Written informed consent for study participation was obtained from the infants' parents/legal guardians.

Measurements

For each enrolled infant, the following data were collected: BW, GA, maternal diseases and antenatal drug exposure, mode of delivery, twin pregnancy, intrauterine growth restriction (IUGR, defined as a BW < 10° percentile for GA), Apgar scores at 1 and 5 min. During the study period, the following conditions, which have an impact on the clinical status or on the renal function of preterm infants[21–23] were also noted: hsPDA (defined by echocardiographic evidence of left-atrial-to-aortic-root ratio >1.5 and/or pulsatile left-to-right transductal shunt and/or mean velocity in the left pulmonary artery >0.6 m/s and/or evidence of diastolic reflow in the descending aorta or in cerebral arteries),[24] respiratory distress,[25] sepsis (defined as relevant symptoms with positive blood culture and/or C-reactive protein >25 mg/l and >5 days of antibiotic treatment),[26] necrotizing enterocolitis Bell stage ≥II.[27] Data on the drug exposure during the first 2 weeks after birth were also collected; in particular, the exposure to amikacin (ATC code: J01GB06),[28] ibuprofen (M01AE01)[28] and other nephrotoxic drugs was noted. Based on their pharmacological exposure during the study period, the enrolled infants were classified into the following groups: amikacin exposure (exposed vs. non-exposed); ibuprofen exposure (exposed vs. non-exposed).

Blood and urine specimens were collected twice per week over the first 2 weeks of life at the following time points: day 1 (T1), days 2–4 (T2), 5–7 (T3), 8–10 (T4), 11–13 (T5). Blood samples were collected in Vacutainer tubes with clot activator and gel for serum separation. Serum was separated by centrifugation at 2500 rpm for 15 min and creatinine was assayed immediately. Serum and urine creatinine (uCreat) were assayed using the Jaffe's method. The obtained values were used to calculate the estimated glomerular filtration rate (eGFR) by applying Schwartz's formula, using the reference constant for preterm infant ($K = 0.33$).[29]

Urine samples were collected by placing an absorbing gauze in the infants' diapers. The obtained samples were centrifuged at 1500 rpm for 10 min. The supernatant was then stored at −20 °C and subsequently thawed for the determination of the following biomarkers: osteopontin (uOPN), epidermal growth factor (uEGF), neutrophil gelatinase-associated lipocalin (uNGAL), cystatin C (uCysC). The Milliplex kit MAP Human kidney injury magnetic based (panel 2 and 3) was used the determination of these renal biomarkers.

The occurrence of AKI, defined as an increase in sCreat ≥0.3 mg/dl by 48 h or ≥1.5 times baseline or urine output below 0.5 ml/kg/h for at least 6 h,[30] was also evaluated during the study period.

Statistical analysis

Data distribution was evaluated using the Shapiro–Wilk test for normality. Depending on their distribution, continuous variables were expressed as median (interquartile range [IQR]) or mean (standard deviation) as appropriate, whereas categorical variables were summarized as frequencies and percentages. Chi-square test and Fisher's exact test were applied to compare categorical variables. Since the data did not follow a normal distribution, Mann–Whitney U test was used to compare continuous variables between the study groups at any time point and between males and females. To account for repeated measures on each subject, generalized linear mixed-effect models (GLMMs) were built to evaluate the adjusted effects of amikacin and ibuprofen exposure at each time point (T1–T5) on each renal biomarker (model terms: time × amikacin exposure and time × ibuprofen exposure, respectively). Clinical variables that differed significantly between the study groups or that may influence both renal function and pharmacokinetics (e.g., GA) were also included in the model. Intercepts at the patient level constituted the random part of model, assuming equal variances and autoregressive covariances. Fixed-effects hypothesis was tested using a robust estimation method. Bonferroni adjustment was applied for multiple comparisons. Since GLMMs allow for unbalanced repeated measures, no imputation of missing data was performed.

Statistical analysis was performed using IBM SPSS, version 26 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, version 26.0, Armonk, NY: IBM Corp). The level of statistical significance was set at $p < 0.05$.

RESULTS

Thirty-eight preterm infants were enrolled. One infant died within the first 48 h after birth, and one underwent intestinal resection on day 5 due meconium obstruction; these two infants were therefore excluded. Antenatal, perinatal and postnatal clinical characteristics of the 36 infants included in the study analysis are illustrated in Table 1.

Maternal drug exposure during pregnancy was reviewed. Thirty mothers received prophylactic corticosteroids for preterm delivery, while 12 required acute or chronic therapies for the treatment of hypertension (methyldopa and nifedipine) or of preterm labor (progesterone derivatives, atosiban). None of the mothers received nephrotoxic treatments during pregnancy.

In the presence of specific risk factors for early-onset sepsis (i.e., preterm labor, premature rupture of membranes, chorioamnionitis or intraamniotic infection, and/or acute and otherwise unexplained onset of non-reassuring fetal status), amikacin was commenced within the first 24 of life at a dosage of 18 mg/kg (36-hourly if ≥30 weeks' gestation or 48-hourly if <30 weeks). Out of 36 infants, 27 (75%) received amikacin treatment and 9 (25%) did not. Clinical characteristics of these two groups, together with the results of between-group comparisons, are shown in Table 2. Amikacin treatment was discontinued if two normal CRP values were detected over a 2-day period and blood cultures turned out negative. The median duration of amikacin treatment was 3 (IQR 2–4) days.

The persistence of a hsPDA for >48 h represented an indication for pharmacological closure with ibuprofen according to the following dosages: 10 mg/kg/day iv (loading dose) followed by 5 mg/kg/day iv (maintenance dose) for overall 3 days. If PDA closure was not achieved, in the absence of concomitant contraindications, the maintenance dose was continued for additional 24–48 h. Ibuprofen was commenced in 11 (30.6%) infants at a median age of 3 (IQR 2–5) days; the median treatment duration of ibuprofen treatment was 3 (IQR 2–3) days. Clinical characteristics of treated and untreated infants and the results of between-group comparison are shown in Table 3.

All the study infants received prophylactic ampicillin since their first day of life, for a median period of 5 (IQR 3–6) days. Of the three infants who developed sepsis during the study period, two received piperacillin and tazobactam ($n = 1$ from day 9; $n = 1$ from day 11) and 2 vancomycin ($n = 1$ from day 9; $n = 1$ from day 12).

At the univariate analysis, significantly higher values of uCysC at T1 (median 111.2 [IQR 40.9–309] vs. 43.1 [29.1–62] mg/dl, $p = 0.042$) and T2 (median 59.9 [IQR 28.3–154.1] vs. 6.1 [3.8–15.5] mg/dl, $p = 0.013$), of uOPN at T2 (median 110.4 [IQR 91.7–194.9] vs. 42.2 [0.4–84.7] ng/ml, $p = 0.011$) and of uNGAL at T1 (median 110.4 [IQR 28.6–248.9] vs. 15 [12.2–39.3] ng/ml, $p = 0.008$) and T3 (median 135.9 [IQR 49–287.7] vs. 41 [19.4–81.6] ng/ml, $p = 0.034$) were documented in infants who received amikacin compared to those who did not, whereas no between-group difference was observed for uEGF at any time point.

Compared to controls, infants treated with ibuprofen showed significantly increased levels of uCysC at T3 (median 166.7 [IQR 68.7–255] vs. 30 [23–39.3] mg/dl, $p = 0.002$) and T4 (median 105.5 [IQR 65.6–165.1] vs. 16.4 [11.9–26.9] mg/dl, $p = 0.047$), of uOPN at T3 (median 265.6 [IQR 192–454.6] vs. 60.7 [33.4–97.7] ng/ml, $p = 0.004$) and of uNGAL at T2 (median 231.7 [IQR 174.1–690.8] vs. 105.8 [22.9–261.2] ng/ml, $p = 0.045$) and T3 (median 339.8 [IQR 191.5–608.6] vs. 59.3 [26.4–117.4] ng/ml, $p < 0.001$), while uEGF did not differ significantly between the two groups.

The effect of sex on the biomarker concentration was also investigated; uNGAL levels during the first 2 weeks of life were significantly higher in females compared to males (median 151 [IQR 130–191] vs. 64 [IQR 33–169] ng/ml, $p = 0.034$), while no difference was observed for the other biomarkers.

In order to investigate the independent changes of urinary biomarkers in relation to ibuprofen and amikacin administration and to adjust the observed results for GA, which differed significantly between the treatment groups, multiple GLMMs were built for each biomarker as previously described. According to the GLMM results, amikacin administration was associated with significantly higher levels of uCysC at T1 ($\beta = 91.499$ [95% CI 25.690; 157.309], $p = 0.007$) and T2 ($\beta = 103.065$ [95% CI 19.763; 186.368], $p = 0.016$) (Fig. 1), whereas infants treated with ibuprofen had significantly higher levels of uOPN ($\beta = 257.36$ [95% CI 103.05; 411.67], $p = 0.001$) and of uNGAL ($\beta = 238.90$ [95% CI 60.014; 417.79], $p = 0.009$) at T3 compared to untreated ones (Fig. 2). The multivariate analysis also confirmed a significant, independent association of GA with uCysC ($\beta = -9.510$ [95% CI −17.232; −1.788], $p = 0.016$) and uNGAL ($\beta = -46.405$ [95% CI −77.508; −15.301], $p = 0.004$).

Since urine output displays a maturational increase over the first days of life and may be affected by ibuprofen administration, a sensitivity analysis was performed adjusting the concentration of the renal biomarkers for uCreat, available in 28 out of 36 infants. The GLMMs, repeated using the adjusted values, confirmed significantly higher uCysC values at T1 ($\beta = 12.603$, $p = 0.048$) in association with amikacin administration, and a significant increase of uOPN ($\beta = 17.955$, $p = 0.033$) and uNGAL ($\beta = 29.952$, $p = 0.036$) at T2 during ibuprofen treatment. The inverse

Table 1. Clinical characteristics of the study population.

Clinical characteristics	$n = 36$
Gestational age (weeks), median (interquartile range [IQR])	29 (26–30)
Birth weight (g), median (IQR)	1092 (829–1362)
Apgar score at 5 min, median (IQR)	9 (9–9)
Maternal hypertension, *n* (%)	8 (22.2)
Intrauterine growth restriction, *n* (%)	5 (13.9)
Sex (males), *n* (%)	28 (77.8)
Twinhood, *n* (%)	13 (36.1)
C-section delivery, *n* (%)	30 (83.3)
Respiratory distress, *n* (%)	33 (91.7)
hsPDA, *n* (%)	13 (36.1)
Sepsis, *n* (%)	3 (8.3)
Necrotizing enterocolitis, *n* (%)	0 (0)
Acute kidney injury, *n* (%)	4 (11.1)

Table 2. Clinical characteristics of the study groups in relation to amikacin administration and results of between-group comparison.

Clinical characteristics	Amikacin		*p* value
	Given ($n = 27$)	Not given ($n = 9$)	
Gestational age (weeks), median (IQR)	28 (36–30)	30 (29–31)	**0.036**
Birth weight (g), median (IQR)	1010 (794–1295)	1232 (1105–1367)	0.127
Apgar score at 5 min, median (IQR)	9 (9–9)	9 (9–10)	0.160
Maternal hypertension, *n* (%)	7 (25.9)	1 (11.1)	0.649
Intrauterine growth restriction, *n* (%)	5 (18.5)	0 (0)	0.312
Sex (males), *n* (%)	19 (70.4)	7 (77.8)	1.000
Twinhood, *n* (%)	8 (29.6)	5 (55.6)	0.235
C-section delivery, *n* (%)	22 (81.5)	8 (88.9)	1.000
Respiratory distress, *n* (%)	25 (92.6)	8 (88.9)	1.000
hsPDA, *n* (%)	9 (33.3)	4 (44.4)	0.693
Sepsis, *n* (%)	3 (11.1)	0 (0)	0.558
Acute kidney injury, *n* (%)	4 (14.8)	0 (0)	0.553
Concomitant ibuprofen treatment, *n* (%)	9 (33.3)	2 (22.2)	0.690

Statistically significant *p*-values are in bold.

Table 3. Clinical characteristics of the study groups in relation to ibuprofen administration and results of between-group comparison.

Clinical characteristics	Ibuprofen		*p* value
	Given ($n = 11$)	Not given ($n = 25$)	
Gestational age (weeks), median (IQR)	26 (25–29)	29 (28–30)	**0.006**
Birth weight (g), median (IQR)	804 (759–1160)	1227 (930–1392)	**0.013**
Apgar score at 5 min, median (IQR)	9 (9–9)	9 (9–9)	0.788
Maternal hypertension, *n* (%)	4 (36.4)	4 (16)	0.195
Intrauterine growth restriction, *n* (%)	1 (9.1)	4 (16)	1.000
Sex (males), *n* (%)	7 (63.6)	19 (76)	0.454
Twinhood, *n* (%)	4 (36.4)	9 (36)	1.000
C-section delivery, *n* (%)	3 (27.2)	3 (12)	0.343
Respiratory distress, *n* (%)	11 (100)	22 (88)	0.538
hsPDA, *n* (%)	11 (100)	2 (8)	**<0.001**
Sepsis, *n* (%)	2 (18.2)	1 (4)	0.216
Acute kidney injury, *n* (%)	1 (9.1)	3 (12)	1.000
Concomitant amikacin treatment, *n* (%)	9 (81.8)	20 (74.1)	0.690

Statistically significant *p*-values are in bold.

correlation between GA, uCysC ($\beta = -2.603$, $p = 0.030$) and uNGAL ($\beta = -6.511$, $p = 0.009$) was also confirmed.

DISCUSSION

According to the present results, the administration of such nephrotoxic drugs as amikacin and ibuprofen during the first 2 weeks of life in VLBW preterm infants is associated with molecule-specific fluctuations of uCysC, uOPN and uNGAL.

During the early postnatal period, the immature kidney is exposed to several potentially harmful conditions, including prematurity-related complications and their pharmacological treatments, whose effects on renal function and development have not been extensively investigated. Together with the assessment of urine output, sCreat is the current gold standard for AKI diagnosis.[30] The multiple pathophysiological mechanisms underlying AKI development, however, can involve different structural and functional areas of the kidney. In this regard, both sCreat and urine output reflect the status of renal function, but do not provide specific information on the establishment of structural changes within the nephron, or on the occurrence early tubular damage.

Several urinary molecules discovered over the past decades have contributed to the understanding of AKI pathogenesis, effectively identifying different AKI phenotypes. In response to harmful stimuli, these substances are either filtered or released from different parts of the nephron, providing important information on the occurrence of renal damage even at a subclinical level.[31] Differently from sCreat, these biomarkers can be collected non-invasively even in preterm infants using such practical methods as the one adopted in the present study, and this, with the additional benefit of their low cost, allows to perform serial assessments over short time periods.

Cystatin C is a cysteine protease inhibitor produced by nucleated cells. Due to its low molecular weight, it is freely filtered by the glomeruli, whereas at the level of proximal tubule is reabsorbed and metabolized; hence, increased concentrations can be observed in association with a glomerular or proximal tubular injury.[32] NGAL belongs to the lipocalin superfamily and is secreted by the thick ascending limb of loop of Henle and collecting ducts; because of its small molecular size, NGAL is freely filtered and can be easily detected in urine. High uNGAL levels reflect an enhanced production and release from proximal tubular cells after various kinds of harmful stimuli and, compared to sCreat, have demonstrated an earlier sensitivity for the detection of AKI.[33] OPN is a cytokine involved in the pathophysiology of experimental AKI and can be increased in case of tubular damage.[7] While uNGAL has been shown to effectively predict renal function impairment even in premature infants,[17,33–35] evidence on uCysC and uOPN in this population is still limited.[36,37]

The validation of urinary biomarkers for renal damage and function in preterm infants would bring several advantages, especially when nephrotoxic treatments are required.

Aminoglycosides are widely prescribed in this population for the prevention and treatment of early-onset sepsis.[38] By binding to acidic phospholipids in the brush-border membrane of epithelial cells, these molecules are taken up in proximal renal tubules, residing in a poorly exchangeable pool; the resulting accumulation within the renal cortex thus contributes to their nephrotoxic effects.[39] Megalin is a multi-ligand receptor abundantly expressed on the apical membrane of proximal tubule cells and has been shown to play a key role in the endocytosis of aminoglycosides, explaining the cell- and tissue-specificity of their toxicity.[40] Megalin is also involved in the reuptake of uCysC in the proximal tubule;[41] hence, the increased uCysC levels observed during amikacin treatment may be consistent with the nephrotoxic mechanisms associated with this molecule.

While uCysC fluctuations during aminoglycosides administration has not been investigated yet, the impact of this treatment on uNGAL levels has been previously evaluated in both term and preterm infants.[19,20] A significantly increased uNGAL excretion during gentamicin treatment has been described by Jansen et al. in neonates with a median GA of 37 weeks; of note, uNGAL excretion peak preceded the increase in sCreat, thus supporting the greater sensitivity of this urinary biomarker.[19] Higher uNGAL levels have also been reported in preterm neonates treated with multiple courses of gentamicin;[20] however, after the adjustment for potential confounders, the effect of this aminoglycoside on uNGAL was not confirmed, similarly to our present findings. Increased sCr has been recently reported in association with amikacin exposure during the first week of life in extremely low BW infants; this finding, which is likely due to their limited clearance capacities, further highlights the renal toxicity of amikacin during early postnatal phases.[42]

Although the pathophysiology of kidney damage related the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is believed

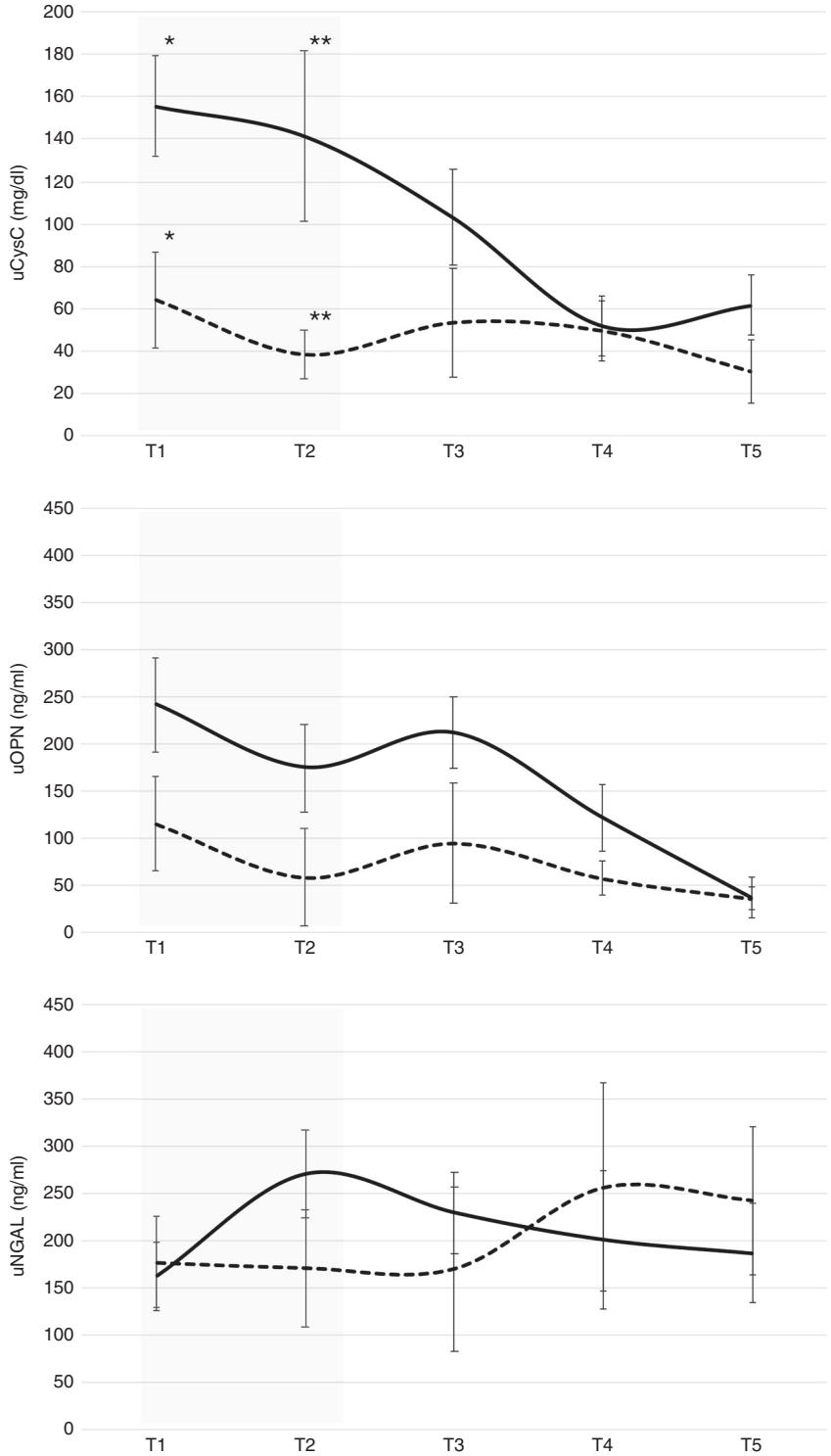

Fig. 1 Time patterns of urinary cystatin C (uCysC), osteopontin (uOPN) and neutrophil gelatinase-associated lipocalin (uNGAL) in infants who received amikacin (full line) vs. those who did not (dotted line) at T1 (day 1), T2 (days 2–4), T3 (days 5–7), T4 (days 8–10), T5 (days 11–13). Asterisks indicate significant contrasts at $p < 0.05$ (*) and $p < 0.01$ (**). The gray shade indicates the median period of exposure.

to be mainly due to their vasoconstrictive effects on renal arterioles and on the cyclooxygenase-mediated inhibition of the tubuloglomerular feedback, several aspects need to be fully clarified. Ibuprofen is widely used for pharmacological closure of a hsPDA.[24] Despite early ibuprofen treatment has not been associated with decreased renal function in former preterm neonates during young adolescence,[43] short-term effects of its administration in early neonatal life include a transient reduction of renal perfusion and GFR secondary to prostaglandin inhibition, with subsequently decreased urine output and increased serum creatinine.[44,45] On the other hand, evidence from animal models has described significant tubular changes after early postnatal treatment[46] and has shown that ibuprofen administration in the presence of an asymptomatic renal failure can trigger an acute

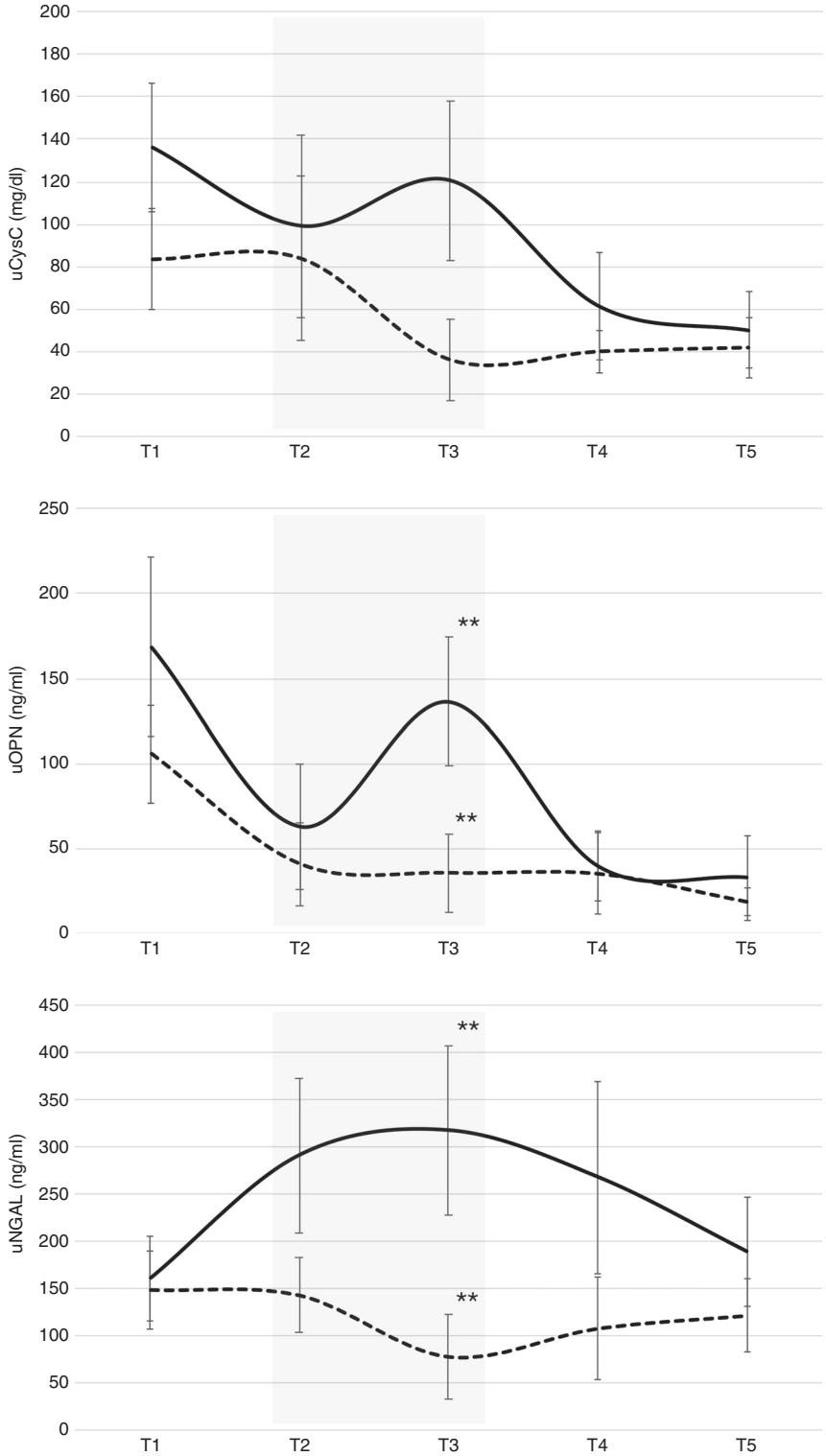

Fig. 2 Time patterns of urinary cystatin C (uCysC), osteopontin (uOPN) and neutrophil gelatinase-associated lipocalin (uNGAL) in infants who received ibuprofen (full line) vs. those who did not (dotted line) at T1 (day 1), T2 (days 2–4), T3 (days 5–7), T4 (days 8–10), T5 (days 11–13). Asterisks indicate significant contrasts at $p < 0.05$ (*) and $p < 0.01$ (**). The gray shade indicates the median period of exposure.

tubulointerstitial toxicity.[47] We observed increased uNGAL levels at 2–4 and 5–7 days of life in infants treated with ibuprofen compared to untreated controls; however, when adjusted for GA and concomitant amikacin administration, the impact of ibuprofen was confirmed significant only at T3, which coincides with final treatment phases, gradually decreasing afterwards. Significantly higher levels of uNGAL in infants with a hsPDA before, during and after ibuprofen treatment have been reported by Tosse et al., suggesting a concomitant noxious effect of both hsPDA and ibuprofen on renal function;[48] nevertheless, infants in the hsPDA group had a significantly lower GA compared to controls, and these results were not adjusted for this potential confounder.

Increased concentrations of uNGAL have also been documented by Waldherr et al. in VLBW infants treated with indomethacin who were not fulfilling AKI criteria, thus further supporting the role of this biomarker in the detection of subclinical kidney injury.[49]

To the best of our knowledge, this is the first study documenting the independent effects of such nephrotoxic drugs as amikacin and ibuprofen on uNGAL, uOPN and uCysC in VLBW preterm neonates during the first 2 weeks of life. Despite the urinary biomarkers investigated in this study showed significant fluctuations in association with amikacin and ibuprofen treatment, the prevalence of AKI did not differ between treated and untreated infants; hence, these biomarkers may have been able to detect drug-related effects at a subclinical level.

Concomitant ibuprofen administration has been previously shown to significantly increase the serum half-life of amikacin in treated preterm infants <31 weeks' gestation.[50] Although the time interval between consecutive amikacin doses in our cohort was 12-h longer compared to this previous evidence, this further supports the importance of monitoring nephrotoxic effects when these drugs are co-administered. On a similar note, evidence from a rat model has shown that the renal function impairment caused by ibuprofen can remain subclinical unless combined with other nephrotoxic drugs or unfavorable conditions.[51] During the study period only 3 out of 36 infants received additional nephrotoxic medications (e.g., vancomycin, piperacillin/tazobactam) that could have further affected renal function and the study biomarkers;[52,53] unfortunately, this small number did not allow to perform a targeted analysis on the effects of these drugs.

Our data confirm the previously described correlation between GA, uCys and uNGAL levels,[22,37,54–56] characterized by decreasing range values for increasing GAs. This finding further highlights the importance of this maturational variable in the interpretation of urinary biomarkers and in the attempt of defining their normal ranges in the preterm population.

Our results also confirm the sex-related difference previously described for NGAL, characterized by higher urinary concentrations in female neonates.[55–57] Although the small size of the study cohort did not allow to add sex to the covariates in the uNGAL GLMM, no difference in the distribution of males and females was observed between exposed and non-exposed infants; hence, we believe that the changes in uNGAL concentration observed in response to ibuprofen administration are not biased by this effect.

The following study limitations need to be acknowledged. The small study sample and the monocentric nature of this study are an important limitation to the generalizability of the present findings, which thus need to be further confirmed on larger preterm cohorts, also to better ascertain the effects of both maturational and non-maturational covariates (e.g., PDA, sex) on these biomarker patterns.

Since the persistence of a hsPDA itself is an indication for ibuprofen treatment, it was not possible to clarify the independent effect of the ductal patency on the observed fluctuations of urinary biomarkers. Moreover, the design of this observational study did not allow to investigate potential duration- or dose-related effects on renal biomarkers, which require targeted trials to be assessed.

Eventually, a more general reflection on AKI prevention strategies in preterm neonates should be made. The Baby NINJA trial has recently shown that the implementation of a systematic pharmacological surveillance program combined with serial sCr monitoring effectively reduces the use of nephrotoxic drugs, drug-related AKI occurrence and AKI intensity in hospitalized neonates.[52] Of note, the Baby NINJA results were not yet available during our study design and conduction; however, based on this evidence, the adoption of surveillance measures on highly nephrotoxic medication exposure and a real-time assessment for AKI risk based on widely and easily available biomarkers is an essential step to prevent drug-induced AKI in NICU settings.

This study adds promising evidence on the role of uCysC, uOPN and uNGAL for renal function monitoring during nephrotoxic treatments in the early postnatal period. The change patterns of renal biomarkers emerged during amikacin and ibuprofen administration suggest underlying molecule-specific pathophysiological mechanisms of kidney injury. Serial assessments of these biomarkers may aid to detect a subclinical impairment of renal function in at-risk neonates and, together with pharmacological surveillance programs, may support the development of individualized therapeutic approaches aimed at reducing the renal burden in the preterm population.

Given the ease and non-invasive collection of urine specimens and the low analytical costs of urinary biomarkers, if validated on a larger scale, the present results may support the development of diagnostic panels inclusive of uCysC, uNGAL and uOPN for the early detection of renal function impairment at different kidney levels during nephrotoxic treatments, even more prolonged than in the present study or involving different drug combinations. Whether the observed increase in urine biomarkers may predict the potential development of long-term renal sequelae (e.g., decreased renal function, chronic kidney disease, microalbuminuria, etc.) deserves to be investigated in future follow-up studies.

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AUTHOR CONTRIBUTIONS

E.P., I.C., and S.G. designed the study. F.V. enrolled the patients and acquired the data. C.D. analyzed the study samples. S.M. and E.P. performed the statistical analysis. E.R., V.A., F.D.P., and L.C. contributed to data interpretation. S.M. wrote the first draft of the manuscript; E.P., F.V., and I.C. contributed to the draft writing. All the authors critically revised the manuscript for important intellectual content, approved the final version of the manuscript submitted for publication and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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COMPETING INTERESTS

The authors declare no competing interests.

CONSENT TO PARTICIPATE

The study protocol was approved by the S. Orsola University Hospital Ethics Committee, Bologna, Italy (protocol no. 154/2015/U/Oss) and written informed consent for study participation was obtained from the parents/legal guardians of each patient.

ADDITIONAL INFORMATION

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