Blood urea in preterm infants on routine parenteral nutrition: A multiple linear regression analysis

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TITLE PAGE

Blood Urea in Preterm Infants on Routine Parenteral Nutrition: A Multiple Linear Regression Analysis

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ABBREVIATIONS: AA: amino acid; [B]Urea: blood urea; BW: birth weight; DOL: day of life; GA: gestational age; IV: intravenous; IVH: intraventricular hemorrhage; NEC: necrotizing enterocolitis; NPE: non-protein energy; PDA: patent ductus arteriosus; PN: parenteral nutrition; RDS: respiratory distress syndrome; SGA: small for gestational age.

ABSTRACT

Background: Blood urea is considered a marker of amino acid utilization in preterm infants on routine parenteral nutrition. However, the association between blood urea and intravenous amino acid intake remains debated.

Aims: To evaluate the association between blood urea and both nutrition and clinical data, in a large cohort of preterm infants.

Subjects and methods: Consecutively admitted preterm infants with a gestational age of less than 32 weeks and a birth weight lower than 1250 g on routine parenteral nutrition from the first hour of life were studied. Clinical and nutrition data collected hourly during the hospitalization were used in multiple linear regression analysis.

Results: We studied 674 patients and 1863 blood urea determinations. Blood urea concentration was positively associated with blood creatinine concentration, intravenous amino acid intake, patent ductus arteriosus and respiratory distress syndrome, and negatively associated with intravenous non-protein energy intakes, daily weight change, gestational age, being small for gestational age, antenatal steroids therapy and reverse flow in the umbilical artery ($p<0.001$; $R=0.7$).

Conclusions: From a nutrition perspective, in our large cohort of small preterm infants blood urea was positively correlated with intravenous amino acid intake and negatively correlated with intravenous non-protein energy intake. This is in line with current knowledge in human physiology and suggest that a reduction of intravenous amino acid intake based on blood urea concentrations was justified.

Keywords: amino acid, blood urea, parenteral nutrition, preterm infants.

INTRODUCTION:

In preterm infants on routine parenteral nutrition (PN), blood urea (^{B}Urea) is often considered a marker of protein intake adequacy and amino acid (AA) oxidation [1]. However, ^{B}Urea concentration in unstable preterm infants on PN during the first days of life (DOLs) is affected by several clinical factors [2] such as renal function, hydration status, severity of illness and others [3, 4]. For these reasons, studies on the association between ^{B}Urea and intravenous (IV) AA intakes showed conflicting results. Some cohort studies showed positive associations [5, 6], while others [1, 2, 7, 8] were unable to find significant correlations between AA intake and ^{B}Urea, leading to the interpretations that the preterm infants had higher requirement for AA and decreasing IV AA intake based on ^{B}Urea concentrations was not necessary. The majority of randomized clinical trials, however, demonstrated that ^{B}Urea was significantly higher in the groups of infants receiving the highest AA intake [9-16]. To date, the relation between ^{B}Urea concentration and IV AA intake remains still unclear.

We studied the association between ^{B}Urea and both nutrition and clinical data, in a large cohort of preterm infants with a birth weight (BW) of less than1250 g on routine PN.

SUBJECTS AND METHODS:

Study design and patients

In this retrospective cohort study, all infants admitted to NICU of the “G. Salesi” Children’s University Hospital between January 2004 and June 2018 were assessed for eligibility. Inclusion criteria were a BW lower than 1250 g and a gestational age (GA) of less than 32 weeks. Exclusion criteria were: major congenital abnormalities, admission to the NICU after 24 hours of life, death or transferred to another unit before 7 DOL and missing clinical information.

Nutrition protocols and biochemical monitoring policies were established in 2002, and they are still applied. Accordingly, all study infants with a BW less than 1250 g received routine PN as all in one mixture from the first hours of life. IV AA were infused at dose of 1.5 g/kg/d on the DOL 1, and up to 3.5 g/kg/d on the DOL 5. From DOL 1 to 7, glucose was increased from 6.0 to 14.0 g/kg/d while

IV lipids were administered at dose of 1.0-1.5 g/kg/d on the DOL 1 up to 3.5 g/kg/d on the DOL 5, and then kept constant until DOL 7. Infants from day 0 to day 7 were on minimal enteral feeding with human milk, when available, or infant milk formula at a maximum intake of 8 mL/kg/d, from day 1 to day 4 and 16 mL/kg/d from day 5 to day 7. For each infant after day 7, PN was tapered and stopped at a median age of 18 day of life while oral feeding was gradually increased to reach full feed and keep a maximum total fluid intake of 160 mL/kg/d. Elevated ^{B}Urea was arbitrary defined as a ^{B}Urea concentration higher than 100 mg/dL. In case of elevated ^{B}Urea, after ruling out excessive postnatal weight loss, the IV AA intake was reduced by at least 1.0 g/kg/d and checked again after 24 hours.

Diagnoses and complication of prematurity were prospectively defined according to the Vermont-Oxford and prospectively recorded. BW standard deviation score (SDS) was electronically estimated by using Italian growth charts [17]. Small for GA (SGA) was defined as SDS-BW less than 10th centile. Daily weight change was defined as the weight lost or gained expressed as a percentage of the BW.

Asphyxia was defined as cord blood pH less than 7.0 and standard base excess less than -12.0 mmol/L. Cholestasis was defined as plasma direct bilirubin concentration above 1.0 mg/dL. Non-protein energy (NPE) intakes were obtained from the actual IV lipid and carbohydrates intakes (9 and 4 kcal/g, respectively). ^{B}Urea and blood creatinine were measured as part of routine monitoring on the 3rd, 5th and 7th DOL, by using micro-methods (Reflotron® Plus, Sprint system, Roche Diagnostics s.p.a, Monza – Italy).

Clinical data included in the analysis were prospectively recorded from birth to 7th DOL and were: GA at birth, BW, SDS-BW, Apgar at 5 minutes, hypertension during pregnancy, reverse flow in the umbilical artery, antenatal steroids therapy, daily weight change vs BW, ibuprofen and indomethacin therapy, hemodynamically significant patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH), cholestasis, sepsis, Necrotizing Enterocolitis (NEC), perinatal asphyxia, respiratory distress syndrome (RDS), ^{B}Urea concentration, creatinine concentration, administered IV AA (g/kg/d) and IV NPE (kcal/kg/d). All PN intakes were collected on hourly basis throughout the

study. IV AA and NPE intakes were calculated for the 24 hours before sampling by averaging the hourly values (g/kg/d).

The study was approved by the local ethics committee (Prot. 2018117).

Statistical analysis

Descriptive statistics was used to describe the study patients. Infants were divided arbitrarily into two groups depending whether ^{B}Urea value was > 100 at least in one occasion (elevated ^{B}Urea) or ≤ 100 mg/dL in all the determinations (normal ^{B}Urea).

Multiple linear regression analysis was performed in the all study patients to find predictors for ^{B}Urea concentrations in preterm infant on routine PN. Pearson's correlation test was performed to identify variables significantly associated to ^{B}Urea concentration at univariate analysis. Results of univariate analysis were considered statistically significant if p –value was less than 0.1. Multiple linear regression analysis was developed by using enter method and p-value <0.05 was considered significant. Missing data were handled by using pairwise deletion. IBM SPSS software was used for the statistical analysis (version 23, SPSS Inc, Chicago, Illinois).

RESULTS:

Eight hundred and fifty-one infants with a BW lower than 1250 g and a GA of less than 32 weeks postmenstrual age were admitted to the NICU of "G. Salesi" Children's Hospital from January 2004 to June 2018. One hundred and thirty-five infants were excluded: 31 had congenital malformations; 29 were admitted to the NICU after 24 hours of life, 64 died or were transferred to another unit before 7 DOL and 11 had missing data.

A total of 1863 ^{B}Urea determinations were available for 674 infants. ^{B}Urea concentrations (mean±SD) were 54.5±26.8, 61.2±31.8 and 54.8±31.4 mg/dL on day 3, 5 and 7, respectively. ^{B}Urea concentration >100 mg/dL was found in 116 infants (17%) during the first 7 DOLs with a total of 183 episodes (85% of them occurred between day 5 and day 7). Infants with elevated ^{B}Urea were smaller than patients with normal ^{B}Urea (**Table 1**).

The incidence of major complications of prematurity from birth to discharge were: neonatal asphyxia 8% vs 4% (p=0.1), RDS 96% vs 85% (p=0.001), PDA 73% vs 55% (p<0.001), cholestasis 7% vs 11% (p=0.2), NEC grade II - III 6% vs 5% (p=0.5), IVH grade III – IV 16% vs 7% (p<0.001) and late onset sepsis 27% vs 19% (p=0.048) in elevated and normal ^{B}Urea group, respectively.

Table 1. Demographic data and early diagnosis of the study preterm infants

	Elevated ^{B}Urea (N=116)	**Normal ^{B}Urea (N=558)**	**p**
Gestation Age (days)	185±13	199±14	<0.001
Birth Weight (g)	864±202	934±200	0.001
SDS Birth Weight	-0.02±0.96	-0.76±0.95	<0.001
Total Length (cm)	34.7±3.0	35.3±3.0	0.031
SDS Total Length	0.08±0.82	-0.62±0.95	<0.001
Head Circumference (cm)	24.1±1.8	25.1±1.9	<0.001
SDS Head Circumference	0.08±0.92	-0.52±0.98	<0.001
Gender (male)	57 (48)	272 (49)	0.8
SGA^{10centile}	13 (11)	178 (32)	<0.001
Apgar at 5 minutes	8 |7 8|	8 |7 9|	<0.001
Antenatal Steroids	90 (81)	482 (91)	0.001
Hypertension during pregnancy	22 (4)	0 (0)	0.028
Reverse Flow in umbilical artery	43 (8)	1 (1)	0.006

Data are presented as mean ± SD, median \|25th 75th perc. \| or no. (%). Independent t-test, Mann Whitney test or Chi-Square test were performed. *p<0.05. SGA: small for gestational age.

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150 The mean IV AA and NPE administered intakes during the first 7 DOLs were 2.1±0.9 g/kg/d and
151 46.7±18.8 kcal/kg/d, respectively. Daily intravenous amino acid and non-protein energy intakes in
152 all patients is shown in **Figure1**.

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154

155 **Multiple linear regression analysis**

156 Pearson correlation test showed that variables significantly associated with ^{B}Urea concentrations
157 at univariate analysis were: GA (weeks), being SGA^{10centile}, Apgar at 5 minutes, DOL, daily weight
158 change vs BW, hypertension during pregnancy, reverse flow in umbilical artery, antenatal steroids
159 therapy, IV AA intakes, IV NPE intake, creatinine, RDS, sepsis, PDA, IVH grade 3 and 4 and
160 ibuprofen therapy.
161 Multiple linear regression analysis for ^{B}Urea concentration is shown in **Table 2**. ^{B}Urea
162 concentration was significantly positively associated with creatinine, IV AA intake, RDS and PDA
163 disease, whereas it was negatively associated with the use of IV NPE intake, daily weight change
164 vs BW, older GA, the use of antenatal steroids, reverse flow in umbilical artery and being
165 SGA^{10centile} ($p<0.001$; $R=0.7$; $R^2=0.5$). Briefly, ^{B}Urea increased by 32.9 mg/dL for each mg/dL of
166 creatinine, by 11.5 mg/dL for each g/kg of IV AA intakes and by 5.8 and 4.5 if infants had PDA or
167 RDS, respectively. ^{B}Urea decreased by 11 mg/dL for 10% daily weight change vs BW, 5.5 mg/dL
168 for each 10 kcal/kg of IV NPE, 3.5 mg/dL for week's gestation and about by 7 mg/dL if infants were
169 SGA or had reverse flow in umbilical artery or in case of maternal antenatal steroids therapy.

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172 **Table 2.** Multiple linear regression model predicting ^{B}Urea concentration

PARAMETER	B (95% C.I.)	**p**
Creatinine (mg/dL)	32.9 (28.8 - 37)	<0.001
IV AA Intake (g/kg/d)	11.5 (9.2 - 13.8)	<0.001
PDA	5.8 (2.6 - 9)	<0.001
IVH III-IV	4.5 (-1.8 - 10.8)	0.2

RDS	4.5 (0.7 - 8.2)	0.020
DOL	1.2 (0.0 - 2.5)	0.1
Apgar at 5 minutes	-0.8 (-1.8 - 0.1)	0.1
Daily weight change vs BW (%)	-1.1 (-1.3 - -0.9)	<0.001
Sepsis	-1.8 (-7.1 - 3.5)	0.5
GA (Weeks)	-3.5 (-4.2 - -2.8)	<0.001
Hypertension during pregnancy	-5.2 (-12.7 - 2.3)	0.2
IV NPE intake (10 kcal/kg/d)	-5.5 (-6.9 - -4.1)	<0.001
Antenatal Steroids therapy	-6.8 (-10.7 - -2.9)	0.001
Reverse Flow in umbilical artery	-7.0 (-12.3 - -1.8)	0.009
SGA^{10centile}	-7.3 (-10.4 - -4.3)	<0.001
Constant	122.1 (101.2 - 142.9)	<0.001

Multiple linear regression analysis, R=0.7, $R^{2=}$ 0.5, p<0.001. AA: amino acids; DOL: day of life; GA: gestational age; IV: intravenous; IVH: intraventricular haemorrhage; NPE: non protein energy; PDA: patent ductus arteriosus; RDS: respiratory distress syndrome; SGA: small for gestational age.

DISCUSSION:

In our large cohort study ^{B}Urea was positively correlated with IV AA intake, renal failure and weight loss, and negatively correlated with IV NPE. This is in line with current knowledge in human physiology. In addition, we found significant correlations with other clinical variables that we will discuss below.

According to data previously reported by other authors [5, 9-11, 13, 15, 16, 18, 19], high IV AA intake leads to increased ^{B}Urea concentration due to AA oxidation [20, 21]. Our data suggest that this was also true in our cohort of small preterm on PN. In our model, administered IV AA intake was positively and significantly correlated with ^{B}Urea (odd ratio +11.5; 95% CI, +9.2 to +13.8; p<0.001).

Other cohort studies failed to demonstrate this association, leading to the concept that preterm infants could benefit from higher AA intake before increasing oxidation. We believe that some of

the previous cohort studies could not demonstrate a significant correlation between ^{B}Urea and AA intake because of the small sample size or because of limitations in correcting for other important "covariates" as they used "simple univariate" rather than multivariate analyses [1, 2, 7, 8, 22]. These studies in our view led to the unsupported interpretation that the preterm infants had higher requirement for IV AA and/or that a reduction of IV AA intake based on ^{B}Urea concentrations was not justified.

We also found that NPE intakes and ^{B}Urea concentrations were negatively correlated, suggesting that AA oxidation is reduced by energy intake favoring protein synthesis [16]. In this respect we comment that there is limited information on the most efficient protein/energy ratio in small preterm infants on PN.

According to previous studies [6, 23], we found a strong association between ^{B}Urea and blood creatinine concentration, as a marker of reduced glomerular filtration. We did not find a significant correlation with acute kidney injury. This could be explained by the limited number of patients with acute kidney injury in our cohort or because, in fact, we believe that the major determinant of elevated ^{B}Urea in preterm infants is a reduced glomerular filtration without significant kidney injury. Elevated ^{B}Urea was strongly and significantly associated with postnatal weight loss possibly due to reduce glomerular filtration rate and haemoconcentration [24]. In our unit, ^{B}Urea is considered a useful marker of excessive weight loss and it prompts us to check for dehydration and kidney function. In our clinical practice we consider elevated ^{B}Urea a marker of excessive AA intake or insufficient NPE only when significant weight loss and glomerular filtration rate have been ruled out.

We found an interesting negative association between ^{B}Urea and antenatal steroids therapy which to the best of our knowledge has not been reported before. We speculate that a lower ^{B}Urea concentration in patients exposed to antenatal steroids may reflect a better overall metabolic maturation of the preterm infant including better kidney function [25]. A positive effect of steroids on glomerular filtration rate and renal cells differentiation, in human and animal models, have been reported [26, 27].

We do not have a clear interpretation for the association between ^{B}Urea and RDS. Of note these diagnoses and their related therapies were reported to be associated with renal failure, increase in protein catabolism and uraemia [28]. Regarding the association between hemodynamically significant PDA and elevated ^{B}Urea this could be explained by kidney hypoperfusion caused by blood-stealing through the open PDA and in addition by the effect of ibuprofen on glomerular filtration rate [29].

This study has limitations. As all retrospective studies, this study carries the risk of unknown confounders. Our model was able to predict about 50% of the ^{B}Urea variability. ^{B}Urea was not measured at all time points (8% of missing determinations). This often happens in clinical care and it could be applicable to several biochemical determinations that are performed in preterm infants during the first DOLs. We believe that missing data did not alter the main study findings as there were no differences in clinical characteristics between the patients with the complete ^{B}Urea determination and those with missing points. The present study was performed over a rather large period (about 14 years) however, all the data were prospectively recorded according to predefine criteria and nutrition protocols were unchanged during the study period. Furthermore, we did not find any differences in the incidence of elevated ^{B}Urea per year during the study period (data not shown).

In conclusion, we studied the association between ^{B}Urea and both nutrition and clinical data in a rather large cohort of preterm infants on routine PN. From a nutrition perspective, we found a strong positive association between ^{B}Urea and IV AA and a negative one between ^{B}Urea and IV NPE intakes. This is in line with current knowledge of human physiology and it may be useful to improve clinical management of infants with elevated ^{B}Urea concentrations. We believe that our work could help clinicians in identifying factors associated with ^{B}Urea in small preterm infants on PN. Our regression is helping us in our daily clinical work to increase the awareness of factors associated with elevated ^{B}Urea. We speculate that the early recognition and correction, whenever

possible, of the factors associated with elevated ^{B}Urea may improve the health status of preterm infants.

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No relevant conflict of interest to declare.

Authors' Contributions Were as Follows:

Virgilio P. Carnielli: Conceptualization and Project Administration;

Ilaria Giretti, Alessio Correani, Chiara Monachesi, Ilaria Burattini, Maria Paola Bellagamba, Chiara Biagetti, Rita D'Ascenzo: Investigation, Methodology and Resources;

Luca Antognoli, Paolo Marchionni, Paola Cogo: Data Curation and Formal Analysis;

Ilaria Giretti: Writing - Original Draft;

All Authors: Writing - Review & Editing.

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352 Figure legends:

353 Figure 1. Daily intravenous amino acid and non-protein energy intakes in all study patients. Data

354 are shown as mean±SE. AA: amino acid; IV: intravenous; NPE: non protein energy.

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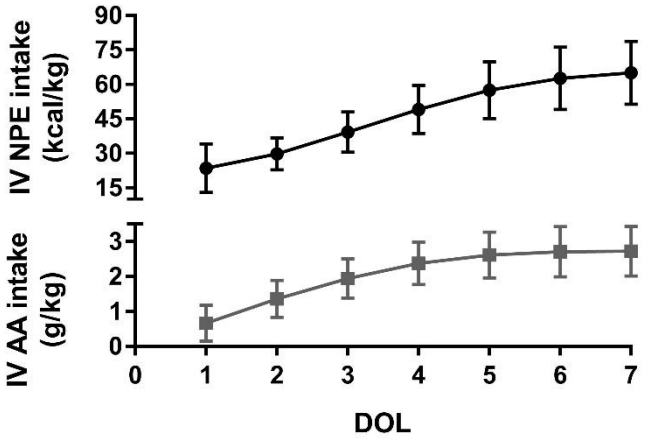

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