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

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1 **TITLE PAGE**

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

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18

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21

22 Running Title: Blood urea in preterm infants on PN

23

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

28

29 **ABSTRACT**

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

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

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

39 **Results:** We studied 674 patients and 1863 blood urea determinations. Blood urea concentration
40 was positively associated with blood creatinine concentration, intravenous amino acid intake,
41 patent ductus arteriosus and respiratory distress syndrome, and negatively associated with
42 intravenous non-protein energy intakes, daily weight change, gestational age, being small for
43 gestational age, antenatal steroids therapy and reverse flow in the umbilical artery ($p < 0.001$;
44 $R = 0.7$).

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

50
51 **Keywords:** amino acid, blood urea, parenteral nutrition, preterm infants.

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58 INTRODUCTION:

59 In preterm infants on routine parenteral nutrition (PN), blood urea (^BUrea) is often considered a
60 marker of protein intake adequacy and amino acid (AA) oxidation [1]. However, ^BUrea
61 concentration in unstable preterm infants on PN during the first days of life (DOLs) is affected by
62 several clinical factors [2] such as renal function, hydration status, severity of illness and others [3,
63 4]. For these reasons, studies on the association between ^BUrea and intravenous (IV) AA intakes
64 showed conflicting results. Some cohort studies showed positive associations [5, 6], while others
65 [1, 2, 7, 8] were unable to find significant correlations between AA intake and ^BUrea, leading to the
66 interpretations that the preterm infants had higher requirement for AA and decreasing IV AA intake
67 based on ^BUrea concentrations was not necessary. The majority of randomized clinical trials,
68 however, demonstrated that ^BUrea was significantly higher in the groups of infants receiving the
69 highest AA intake [9-16]. To date, the relation between ^BUrea concentration and IV AA intake
70 remains still unclear.

71 We studied the association between ^BUrea and both nutrition and clinical data, in a large cohort of
72 preterm infants with a birth weight (BW) of less than 1250 g on routine PN.

73

74 SUBJECTS AND METHODS:**75 Study design and patients**

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

81 Nutrition protocols and biochemical monitoring policies were established in 2002, and they are still
82 applied. Accordingly, all study infants with a BW less than 1250 g received routine PN as all in one
83 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
84 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

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85 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,
86 and then kept constant until DOL 7. Infants from day 0 to day 7 were on minimal enteral feeding
87 with human milk, when available, or infant milk formula at a maximum intake of 8 mL/kg/d, from
88 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
89 and stopped at a median age of 18 day of life while oral feeding was gradually increased to reach
90 full feed and keep a maximum total fluid intake of 160 mL/kg/d. Elevated ^BUrea was arbitrary
91 defined as a ^BUrea concentration higher than 100 mg/dL. In case of elevated ^BUrea, after ruling out
92 excessive postnatal weight loss, the IV AA intake was reduced by at least 1.0 g/kg/d and checked
93 again after 24 hours.

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

99 Asphyxia was defined as cord blood pH less than 7.0 and standard base excess less than -12.0
100 mmol/L. Cholestasis was defined as plasma direct bilirubin concentration above 1.0 mg/dL.
101 Non-protein energy (NPE) intakes were obtained from the actual IV lipid and carbohydrates intakes
102 (9 and 4 kcal/g, respectively). ^BUrea and blood creatinine were measured as part of routine
103 monitoring on the 3rd, 5th and 7th DOL, by using micro-methods (Reflotron® Plus, Sprint system,
104 Roche Diagnostics s.p.a, Monza – Italy).

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

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

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

115

116 **Statistical analysis**

117 Descriptive statistics was used to describe the study patients. Infants were divided arbitrarily into
118 two groups depending whether ^BUrea value was > 100 at least in one occasion (elevated ^BUrea) or
119 ≤ 100 mg/dL in all the determinations (normal ^BUrea).

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

127

128 **RESULTS:**

129 Eight hundred and fifty-one infants with a BW lower than 1250 g and a GA of less than 32 weeks
130 postmenstrual age were admitted to the NICU of "G. Salesi" Children's Hospital from January 2004
131 to June 2018. One hundred and thirty-five infants were excluded: 31 had congenital malformations;
132 29 were admitted to the NICU after 24 hours of life, 64 died or were transferred to another unit
133 before 7 DOL and 11 had missing data.

134 A total of 1863 ^BUrea determinations were available for 674 infants. ^BUrea concentrations
135 (mean \pm SD) were 54.5 \pm 26.8, 61.2 \pm 31.8 and 54.8 \pm 31.4 mg/dL on day 3, 5 and 7, respectively.

136 ^BUrea concentration >100 mg/dL was found in 116 infants (17%) during the first 7 DOLs with a
137 total of 183 episodes (85% of them occurred between day 5 and day 7). Infants with elevated

138 ^BUrea were smaller than patients with normal ^BUrea (**Table 1**).

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139 The incidence of major complications of prematurity from birth to discharge were: neonatal
 140 asphyxia 8% vs 4% (p=0.1), RDS 96% vs 85% (p=0.001), PDA 73% vs 55% (p<0.001), cholestasis
 141 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)
 142 and late onset sepsis 27% vs 19% (p=0.048) in elevated and normal ^BUrea group, respectively.

143

144

145 **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

146

147 Data are presented as mean ± SD, median |25th 75th perc. | or no. (%). Independent t-test, Mann

148 Whitney test or Chi-Square test were performed. *p<0.05. SGA: small for gestational age.

149

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**.

153

154

155 **Multiple linear regression analysis**

156 Pearson correlation test showed that variables significantly associated with ^BUrea 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 ^BUrea concentration is shown in **Table 2**. ^BUrea
 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, ^BUrea 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. ^BUrea 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.

170

171

172 **Table 2.** Multiple linear regression model predicting ^BUrea 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

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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

173

174

175 Multiple linear regression analysis, R=0.7, R²= 0.5, p<0.001. AA: amino acids; DOL: day of life; GA:
 176 gestational age; IV: intravenous; IVH: intraventricular haemorrhage; NPE: non protein energy; PDA: patent
 177 ductus arteriosus; RDS: respiratory distress syndrome; SGA: small for gestational age.

178

179 **DISCUSSION:**

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

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

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

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191 the previous cohort studies could not demonstrate a significant correlation between ^BUrea and AA
192 intake because of the small sample size or because of limitations in correcting for other important
193 “covariates” as they used “simple univariate” rather than multivariate analyses [1, 2, 7, 8, 22].

194 These studies in our view led to the unsupported interpretation that the preterm infants had higher
195 requirement for IV AA and/or that a reduction of IV AA intake based on ^BUrea concentrations was
196 not justified.

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

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

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

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

224

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

236

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

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245 possible, of the factors associated with elevated ^BUrea may improve the health status of preterm
246 infants.

247

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252 No relevant conflict of interest to declare.

253

254 Authors' Contributions Were as Follows:

255 Virgilio P. Carnielli: Conceptualization and Project Administration;

256 Ilaria Giretti, Alessio Correani, Chiara Monachesi, Ilaria Burattini, Maria Paola Bellagamba, Chiara

257 Biagetti, Rita D'Ascenzo: Investigation, Methodology and Resources;

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

259 Ilaria Giretti: Writing - Original Draft;

260 All Authors: Writing - Review & Editing.

261

262

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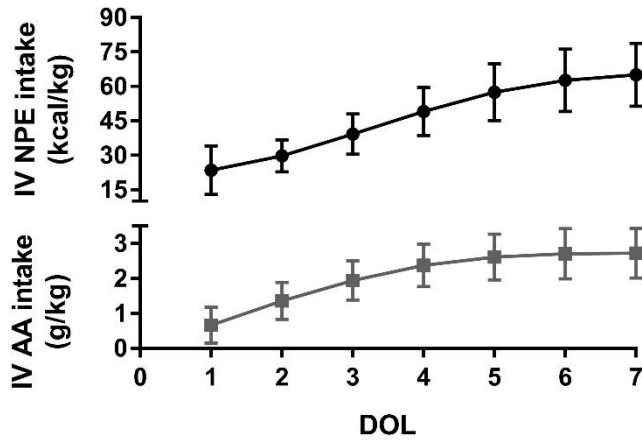
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352 **Figure legends:**

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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