Proposed Plasma Ammonia Reference Intervals in a Reference Group of Hospitalized Term and Preterm Neonates

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Background: Plasma ammonia is commonly measured in the diagnostic evaluation of hospitalized newborns, but reference values are not well defined.

Methods: We prospectively enrolled newborns admitted to the level III/IV neonatal intensive care unit and level II intermediate special care nursery from January 2017 to January 2018. Infants with inborn errors of metabolism or liver disease were excluded. Plasma ammonia concentrations were measured once within the first week of life and evaluated by sex, gestational age, timing of the draw, blood collection method, and type of nutrition. Reference intervals were calculated.

Results: 127 neonates were included; one third (34%) were term infants born at ≥37 weeks gestation, and two thirds (66%) were born preterm at <37 weeks gestation. Median plasma ammonia concentrations were 32 μmol/L (range <10 to 86 μmol/L). Median ammonia concentrations were higher among preterm compared to term infants (35 vs. 28 μmol/L, $p = 0.0119$), and term female compared to term male infants (34 vs. 26 μmol/L, $p = 0.0228$). There was no difference in median ammonia concentrations between female and male preterm infants, based on gestational age within the preterm group, timing of the blood draw, presence of hyperbilirubinemia, blood collection method, or type of nutritional intake.

Conclusions: Plasma ammonia concentrations among newborns are higher than the expected adult concentrations and may vary by gestational age and sex. Blood collection method, type of nutrition, hyperbilirubinemia, and timing of the draw do not impact concentrations. We propose a reference limit of ≤82 μmol/L for newborns less than one week of age.

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IMPACT STATEMENT

Plasma ammonia concentrations are commonly measured in newborns, as conditions associated with hyperammonemia, such as inborn errors of metabolism, can present similarly to sepsis or other illnesses in the early postnatal period. Expected values for newborns are not well defined, so clinicians must rely on adult reference intervals when interpreting ammonia concentrations in newborns. Our study established plasma ammonia reference intervals among term and preterm neonates without inborn errors of metabolism or underlying liver disease. We also evaluated the impact of sex, gestational age, neonatal nutrition, hyperbilirubinemia, blood collection method, and timing of the draw on ammonia values.

INTRODUCTION

Inborn errors of metabolism presenting in the neonatal period are rare but potentially life-threatening conditions that require a high index of suspicion for neonatal providers, as early diagnosis and treatment is crucial in preventing morbidity and mortality *(1, 2)*. Symptoms in affected neonates are often nonspecific, including poor feeding, vomiting, lethargy, tachypnea or apnea, and are difficult to distinguish from other more common problems, such as sepsis *(2)*.

Hyperammonemia is observed in a number of inborn errors of metabolism, including urea cycle defects, organic acidemias, and disorders of fatty-acid oxidation *(1)*. Measurement of plasma ammonia is part of the basic workup for a neonate with a suspected metabolic condition *(2, 3)*. However, reference intervals for ammonia among neonates, especially premature neonates, are not well defined. Most laboratories, including ours, do not report a reference interval (RI) for ammonia based on age, with RIs generally derived for healthy adults. Prior studies suggest that ammonia concentrations in newborns may be higher than those observed in older infants, children, and adults *(4–8)*, and decline after 1 to 2 weeks following birth *(4, 5, 7, 9)*. Plasma ammonia concentrations also may vary depending upon gestational age *(9)*. We sought to establish RIs for ammonia among term and preterm neonates by prospectively enrolling infants admitted to the level III/IV neonatal intensive care unit (NICU) and level II intermediate special care nursery (ISCN).

METHODS

Reference sample group

All neonates admitted to the NICU or ISCN at Mayo Clinic in Rochester, MN between January 2017 and January 2018 were eligible to be included in this study. Infants whose parent or legal guardian gave informed consent for study participation within 72 h of birth were prospectively enrolled. The medical records of infants and their mothers were reviewed to gather demographic information, delivery information, comorbidities, and clinical findings. Enrolled infants with a positive screen for a metabolic disorder on the Minnesota Newborn Screening (MNS) confirmed in subsequent testing, or those with liver disease diagnosed during their hospitalization were excluded. The MNS includes over 50 disorders including amino acid disorders, fatty acid oxidation disorders, organic acid disorders, endocrine disorders, lysosomal disorders, hemoglobinopathies, and other congenital disorders

(https://www.health.state.mn.us/people/newbornscreening/program/newbornscreeningpanel.html). All neonates were assessed for hyperbilirubinemia, as recommended by the American Academy of Pediatrics *(10)*. Total serum bilirubin was measured with a modified Diazo method using the Roche Total Bilirubin reagent (Roche Diagnostics, Indianapolis, IN) run on Roche Cobas c501 or c701 instruments. Determination regarding the need for phototherapy was made by the treating physicians according to the nomogram of hour-specific serum bilirubin values. This study was approved by Mayo Clinic's Institutional Review Board.

Blood sampling and ammonia measurements

A venous blood sample of at least 0.5 mL was collected in an ethylene diamine tetraacetic acid (EDTA) tube, obtained by venipuncture or aspiration from an umbilical venous catheter (UVC). Specimens were placed on ice immediately after collection, and centrifuged at refrigerated temperature (2°-6°C) to separate out the plasma, which was kept on ice until analyzed. Plasma ammonia concentrations were measured by bromophenol blue photometry (VITROS® 350, Ortho Clinical Diagnostics, Raritan, NJ). This method demonstrates coefficients of variation of 15.5% at 23 μmol/L, 5.1% at 83 μmol/L, and 3.6% at 249 μmol/L. The expected normal range for adults according to the manufacturer's package insert is ≤30 μmol/L and was verified according to CLSI EP28-A3c *(11)*.

Statistical analysis

Reference limits and associated 90% confidence intervals were calculated as 2.5th and 97.5th percentiles to derive the reported central 95% RIs from nonparametric (overall) and log transformed parametric (subgroup) analysis using EP Evaluator® (Data Innovations, Burlington, VT). Confidence ratios represent the ratio of average confidence interval width to RI width to ascertain inclusion of sufficient reference values, with values <0.20 preferred *(11)*. The program's partitioning test was used to determine whether the RI would benefit from being calculated separately for subgroups with Z max >2.2 and SD Ratio >1.5 suggested critical limits. Descriptive summaries were reported as medians (minimums, maximums) for continuous variables, and as frequencies and percentages for categorical variables. Normality assumption for ammonia concentration as an outcome of interest was assessed using the Kolmogorov-Smirnov test of normality. Comparisons of ammonia concentrations within various subgroups of categorical variables were performed using Kruskall-Wallis or Wilcoxon rank sum tests, as appropriate. All tests were two sided; *P* values <0.05 were considered statistically significant. Analysis was performed using SAS software version 9.4 (SAS Inc, Cary, NC).

RESULTS

One hundred and forty-eight neonates were enrolled in the study over one year. Twenty-one infants were excluded, 13 for lack of sample collection after enrollment, and 8 for collection of sample from an arterial source. The remaining 127 infants were included in the study. All had normal MNS results or normal follow up testing. None of the infants were known to have underlying liver disease. One third (34%) of infants were born at term (≥37 weeks gestation), and two-thirds (66%) were born preterm (<37 weeks gestation). The preterm group included infants born at 34-36 weeks (n = 54, 42%), 29-33 weeks (n = 25, 20%), and <29 weeks (n = 5, 4%). Seventy-three (57%) infants were male, and 54 (43%) female. Among female infants, 13 (24%) were born at term, and 41 (76%) preterm. Among male infants, 30 (41%) were born at term, and 43 (59%) preterm.

Blood was drawn between the day of birth and day-of-life six. The median age at blood draw was

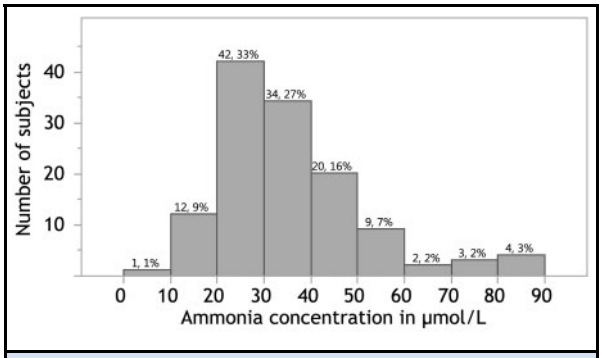

Fig. 1. Distribution of ammonia concentrations in µmol/L among all 127 tested neonates. Histogram displaying the distribution of ammonia concentrations among all tested neonates in 10 µmol/L increments. The numbers above the columns represent the number of infants and percentage of values within each column, respectively.

39.3 h. The distribution of ammonia concentrations is shown in Fig. 1. Although approaching a normal distribution, values were not normally distributed when assessed with the Kolmogorov-Smirnov test of normality. Overall median ammonia concentrations were 32 µmol/L, with a range of <10 to 86 µmol/L and a calculated central 95% RI of 12–82 µmol/L. Table 1 shows ammonia concentrations by subgroups. RI analysis was performed on subgroups that demonstrated statistically significant differences. The median ammonia concentrations were higher among female compared to male infants (35 vs. 29 µmol/L, $P = 0.0079$), and among preterm compared to term infants (35 vs. 28, $P = 0.0119$). When comparing term and preterm infants by sex, there was a difference between median ammonia concentrations of female and male term infants (34 vs. 26 µmol/L, $P = 0.0228$), but not among female and male preterm infants (35 vs. 33 µmol/L, $P = 0.3448$). The calculated RIs for these subgroups were subjected to a partitioning test for gestational age and sex. Notably, they did not reach significance based on both calculated Z max and SD Ratio. There was no statistical difference in median ammonia concentrations based on gestational age within the preterm group, timing of the blood draw, method of blood collection, presence of hyperbilirubinemia requiring phototherapy, or type of nutritional intake (comparing total parenteral nutrition with enteral nutrition or a combination of both, as well as comparing those receiving formula only, breastmilk only, or a combination of formula and breastmilk).

DISCUSSION

Our findings are in accordance with studies that report higher ammonia concentrations among newborns *(4–8)*. Also in keeping with a prior study *(9)*, we found higher median ammonia concentrations among preterm newborns, among whom median ammonia concentrations did not vary based on gestational age. We also noted a difference between median ammonia concentrations of female and male term infants. Usmani et al. noted no difference in ammonia concentrations based on sex, but only evaluated preterm infants <36 weeks, a population in which we also saw no difference between female and male infants *(9)*. Clemmens et al. described no significant difference in ammonia concentrations based on sex, but reported higher values among female compared to male term infants *(8)*. We note that the statistically significant differences we detected in median ammonia concentrations among term and preterm infants, as well as female and male infants, may not be of clinical significance, as supported by the non-significant Z max and SD Ratio for subgroup RI analysis. For this reason, we propose a single reference limit of ≤82 µmol/L for all newborns less than one week of age as the lower reference limit (12 µmol/L) approaches the lowest ammonia concentration that can be detected reliably (10 µmol/L) and distinction of low ammonia concentration is of little clinical value.

Table 1. Median ammonia concentrations among different subgroups of neonates.

	Number of neonates (%)	2.5th and 97.5th reference limits (90% CI) μmol/L	Confidence ratio[a]	Z max[b]	SD Ratio[b]	Median ammonia concentration in μmol/L (minimum, maximum)	*P* value
All neonates	127 (100%)	12 (10-15)	0.14			32 (<10, 86)	-
		82 (72-86)					
Sex				1.6	1.4		0.0079
Male	73 (57%)	11 (9-13)	0.22			29 (<10, 86)	
		77 (66-91)					
Female	54 (43%)	20 (17-22)	0.22			35 (18, 82)	
		65 (57-73)					
Gestational age at birth				1.7	1.0		0.0119
Term (≥37 weeks)	43 (34%)	13 (11-15)	0.27			28 (10, 86)	
		66 (55-79)					
Preterm (<37 weeks)	84 (66%)	14 (12-16)	0.19			35 (<10, 82)	
		79 (69-90)					
Collection method				n/a	n/a		0.2610
Venipuncture	80 (63%)	n/a				33 (<10, 86)	
Umbilical venous catheter	47 (37%)	n/a				31 (12, 74)	
Collection time				n/a	n/a		0.2820
<24 hours of life	7 (6%)	n/a				31 (12, 57)	
24-48 hours of life	67 (53%)					33 (<10, 82)	
48-72 hours of life	38 (30%)					28 (14, 86)	
>72 hours of life	15 (12%)	n/a				38 (14, 61)	
Feeding at the time of ammonia draw				n/a	n/a		0.5789
Total parenteral nutrition only	12 (9%)	n/a				35 (10, 72)	
Enteral nutrition only[c]	80 (63%)	n/a				32 (14, 86)	
Both parenteral and enteral nutrition	35 (28%)	n/a				32 (<10, 55)	
Hyperbilirubinemia requiring phototherapy				n/a	n/a		0.3798
Yes	62 (49%)	n/a				33 (<10, 86)	
No	65 (51%)	n/a				31 (10, 82)	

[a] Confidence ratio <0.20 is desirable.
[b] Either Z max >2.2 or SD Ratio >1.5 suggests a separate reference interval (RI) may be warranted.
[c] Includes formula and breastmilk by oral intake and tube feedings. Some infants in this group were receiving dextrose-containing intravenous fluids, but not parenteral amino acid or lipid formulations.

Previous studies suggested higher ammonia concentrations among infants receiving certain formulas *(12)* and among those receiving total parenteral nutrition *(13, 14)*. We did not find a difference in ammonia concentrations based on these variables. Similarly, elevated concentrations of ammonia were

described in infants with hyperbilirubinemia in one older study *(7)*, an association our study and another small study *(8)* did not confirm.

This study has several limitations. Ammonia RIs were calculated from a reference population of infants admitted to the hospital, which represents the most common clinical scenario where ammonia is measured. Transference of this RI should be done with caution to non-hospitalized neonatal populations, as well as other testing platforms *(15)*. Blood was collected only once from each infant, and therefore trends in ammonia concentrations over time could not be studied. Also, all blood samples were collected within the first week of life, therefore no estimates of the RI for older infants were made.

In summary, ammonia concentrations among neonates within the first week of life were higher than generally accepted for healthy adults. Our study established a 97.5th percentile plasma ammonia reference limit of $\leq$82 μmol/L for hospitalized infants less than one week of age.

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