Published in final edited form as:

J Pediatr. 2008 September ; 153(3): 375–378. doi:10.1016/j.jpeds.2008.03.041.

Association between Urinary Lactate to Creatinine Ratio and Neurodevelopmental Outcome in Term Infants with Hypoxic-Ischemic Encephalopathy

William Oh, MD, Rebecca Perritt, MS, Seetha Shankaran, MD, Matthew Merritts, MD, Edward F. Donovan, MD, Richard A. Ehrenkranz, MD, T. Michael O'Shea, MD, Jon E. Tyson, MD, Abbot R. Lupton, MD, Abhik Das, PhD, and Rosemary D. Higgins, MD

Department of Pediatrics (W.O.), Warren Alpert Medical School of Brown University, Providence, RI; Research Triangle International (R.P., A.D.), Research Triangle Park, NC; the Department of Pediatrics (S.S.), Wayne State University, Detroit, MI; the Department of Nuclear Imaging (M.M.), Southwestern University, Dallas, TX; the Department of Pediatrics (E.D.) University of Cincinnati, Cincinnati, OH; the Department of Pediatrics (R.E.), Yale University, New Haven, CT; the Department of Pediatrics (T.O.), Wake Forest University, Wake Forest, NC; the Department of Pediatrics (J.T.), University of Texas at Houston; the Department of Pediatrics (A.L.), Warren Alpert Medical School of Brown University, Providence, RI; and NICHD NIH (R.H.), NICHD Neonatal Research Network, Bethesda, MD

Abstract

Objective—To assess the association between urinary lactate to creatinine ratio (ULCR) and neurodevelopmental outcome in term infants with hypoxic ischemic encephalopathy and examine the effect of hypothermia on the change in ULCR.

Study design—Spot urine samples were collected in 58 term infants (28 hypothermia, 30 control subjects) with hypoxic ischemic encephalopathy. Urinary lactate and creatinine were measured by using ¹H nuclear magnetic resonance spectroscopy and expressed as ULCR. Survivors were examined at 18 months of age.

Results—The ULCR was significantly higher in infants who died or had moderate/severe neurodevelopmental disability. Logistic regression analysis controlling for hypothermia and severity of encephalopathy confirmed the association (adjusted odds ratio, 5.52; 95% CI, 1.36, 22.42; *P* < .02). Considerable overlap in ULCR was observed between infants with normal/mild disability and those who died or survived with moderate/severe disability. ULCR fell significantly between 6 and 24 hours and 48 and 72 hours of age for all infants. The magnitude of decline did not differ between hypothermia and control groups.

Conclusions—High ULCR is associated with death or moderate/severe neurodevelopmental disability. Significant overlap in values between the normal/mild and moderate/severe disability groups limits predictive value of this measure. Whole-body hypothermia did not affect the decline in ULCR.

Hypoxic-ischemic encephalopathy is a serious morbidity in newborn infants with high mortality rate and a high rate of poor neurodevelopmental outcome among survivors.^{1,2} This condition is often accompanied by severe metabolic acidosis caused by fetal hypoxia. The

Reprint requests: Dr. William Oh, Department of Pediatrics, Brown Medical School, Women and Infants' Hospital of Rhode Island, 101 Dudley Street, Suite 1100, Providence, RI 02905. woh@wihri.org.

Authors declare no conflict of interest.

varying degree of severity of fetal hypoxia may account for the varying degrees of neurodevelopmental disability among survivors. Lactate is a byproduct of anaerobic metabolism and accumulates in the circulation during fetal ischemic hypoxic states. The potential relationship between degree of fetal hypoxia, lactic acidosis, and neurodevelopmental outcome prompted several investigators to assess blood lactate as a measure of varying degrees of fetal hypoxia that may predict short- and long-term outcomes.^{3–5} Because lactate is in part excreted through the kidney, measurement of urinary lactate may also reflect the blood lactate level and the degree of metabolic derangement as a result of hypoxia/ischemia. Ma et al⁶ and Zuppi et al,⁷ using (¹ H) proton nuclear magnetic resonance spectroscopy, have shown that urinary lactate and creatinine can be measured and used clinically. Huang et al⁸ further demonstrated a direct correlation between urinary lactate to creatinine ratio and neurodevelopmental outcomes of a group of 16 term infants with hypoxic ischemic encephalopathy. We performed the current study to confirm the association of high urinary lactate to creatinine ratio and poor neurodevelopmental outcome and to evaluate the effect of hypothermia on urinary lactate to creatinine ratio.

METHODS

This study involved a subset of term infants randomly assigned in a controlled trial conducted by the NICHD Neonatal Research Network to evaluate the efficacy and safety of whole-body hypothermia in term infants with hypoxic ischemic encephalopathy. This study was approved by the institutional review board in each site. The subjects and methods of the primary protocol have been published.⁹ A set of predetermined criteria was used by trained investigators to establish the diagnosis and severity of hypoxic ischemic encephalopathy. Two hundred eight infants with hypoxic ischemic encephalopathy were enrolled in this trial. Of these infants, 58 were enrolled in the current study, 28 in the hypothermia group, and 30 in the control group.

Collection of spot urine was initiated on study infants as soon as consents were obtained. The first urine was collected between 6 and 24 hours and repeated at 48 to 72 hours, using a bag placed over the perineum of girls and a test tube placed over the penis for boys. Urine was aspirated from the bag or test tube using a syringe connected to a catheter. Urine spilled on the diaper was also aspirated. Urine samples were stored at –20°C until shipment in dry ice to the central laboratory (Ralph B. Rogers MR Center, University of Texas-Southwestern Medical Center, Dallas, Texas) for assay.

Samples were prepared for nuclear magnetic resonance (NMR) spectroscopy by thawing and removing a 550-μL aliquot of the urine. To provide a lock signal for the spectrometer, 50 μL of 99% D₂O was added to the sample, giving a 600-μL total volume that was pipetted into 5-mm NMR tubes for analysis. The ¹H NMR experiment was carried out at either 400- or 600-MHz frequency, using a Varian INOVA NMR console. The wet1d sequence was used to suppress the large water signal. A 90° pulse was used with a 2-second acquisition time and a 4-second delay time, giving a time to repeat of 6 seconds. The spectra were weighted with a 0.3-Hz exponential line broadening function before Fourier transformation. The ratio of lactate to creatinine was estimated by integration of the relative peak areas, using the Varian software. Results were expressed as a ratio of lactate to creatinine because samples were not time-collected to provide volume per unit of time and quantitatively assess lactate excretion.

All surviving infants were evaluated at 18 months' corrected age. Neurological and developmental testing was performed by trained examiners who were blinded to intervention status and urinary lactate to creatinine ratio values. The procedures and methodology for follow-up assessment including the criteria for classification of severity of disability have been described in detail in the publication of the main trial.⁹ For this report, the moderate/severe

disabilities were combined as one group and normal or mild disability as another for data analysis.

Data Analysis

Categorical and continuous data were analyzed by χ^2 and Wilcoxon tests, respectively. Multiple logistic regression analysis was performed to calculate the adjusted odds ratios for the association between first urinary lactate to creatinine ratio and death or moderate/severe disability using urinary lactate to creatinine ratio of 2.9 (median for the entire cohort) as the cutoff value. Logistic regression models were adjusted for cooling and severity of encephalopathy. To analyze the effect of hypothermia on the change in urinary lactate to creatinine ratio, we used only infants with paired values of 1st and 2nd urine samples. ($n = 27$ and 23 for hypothermia and control groups, respectively). We used median values of the 2 groups (hypothermia and control) to analyze the difference between the first and second urine values because the data were not normally distributed and had substantial outliers. Analyses were performed with the use of SAS at Research Triangle International, Research Triangle Park, North Carolina.

RESULTS

The age of urine collection (in hours) for the first and second samples were similar between the hypothermia and control group (first sample, 8.2 ± 2.4 vs 12.7 ± 6.7 hours; second sample, 65.7 ± 10.9 vs 63.0 ± 13.0 hours, respectively).

There was no difference in maternal and neonatal demographic and clinical characteristics between the 2 groups except for more outborn in the hypothermia group (Table I). These maternal and neonatal variables were also similar when compared with those infants who were enrolled in the primary protocol (data not shown).

The scatter plot in the Figure shows that the urinary lactate to creatinine ratios measured at 6 to 24 hours of age (mean of 8.2 and 12.7 hours for hypothermia and control group, respectively) were significantly higher in those infants who either died or survived with moderate or severe disability when compared with those who were normal or had mild disability (median: 13.4 vs 1.6 , respectively, $P < .001$). However, the data have wide variation with considerable overlap of values between the 2 outcome groups. Because of this wide variation and overlap and relatively small sample size, we did not perform statistical analysis to assess the predictive values of this measure. Table II shows the results of logistic regression analysis incorporating hypothermia and severity of encephalopathy as covariates. After adjustment for hypothermia treatment and severity of encephalopathy, higher urinary lactate to creatinine ratio collected during 6 to 24 hours of age was independently associated with death or moderate/severe neurodevelopmental disability (OR, 5.52 ; 95% CI, 1.36 to 22.42 , $P = .02$).

There was a significant decline in lactate to creatinine ratio between the first and second urine samples (13.2 ± 16 (SD) to 5.0 ± 19 , $P < .004$, and 15.1 ± 26.8 to 1.0 ± 1.2 , $P < .004$ for hypothermia and control groups, respectively). There was no difference in the change of lactate to creatinine ratio in hypothermic and control groups (-92.5% vs -83.2% for hypothermia and control groups, respectively, $P = .40$).

DISCUSSION

The objective of this study was to evaluate the association between urinary lactate to creatinine ratio obtained at 6 to 24 hours of age and neurodevelopmental outcomes of infants with hypoxic ischemic encephalopathy. We found that high urinary lactate to creatinine ratio is independently associated with death or moderate or severe neurodevelopmental disability. The

finding is consistent with the concept that high urinary lactate excretion soon after birth reflects high blood lactate level caused by perinatal ischemic hypoxia.^{3–5}

Several studies have been done in the past regarding the role of blood^{3–5} or urinary^{6,8} lactate level at birth in predicting outcomes of term infants with hypoxic ischemic encephalopathy. Huang's study⁸ showed a good predictive value of urinary lactate to creatinine ratio for the development of hypoxic ischemic encephalopathy. The report also documented a direct relationship between urinary lactate to creatinine ratio and poor neurodevelopmental outcome. Their data showed a clear separation of values between those with and those without neurodevelopmental abnormality. Our finding of significant overlap in the urinary lactate to creatinine ratio differs from those of Huang's study. We did not perform the receiver operating characteristics to assess the predictive values of the urinary lactate to creatinine ratio because the large variability and considerable overlap of the data make it highly unlikely that the calculation would yield significant predictive values. For example, using a cutoff value of ≥ 25 would give a sensitivity of 33% (8/24) and specificity of 88% (30/34), whereas a cutoff value of ≥ 50 gives a sensitivity of 25% (6/24) and specificity of 97% (33/34) (Figure). In comparing the study by Huang and ours, both studies used the same methodology in measuring urinary lactate and creatinine. Huang's study involved a single center whereas ours was multicenter, and the sample size was actually larger in our study (55 vs 16). Our laboratory personnel were blinded to the identity of the infants in regard to outcome, whereas the blinding issue was not stated in Huang's study. The sampling times of urine were similar between the 2 studies. Thus, the reason(s) for the difference in the findings between Huang's study and ours is not entirely clear. Nevertheless, our study shows that the utility of urinary lactate to creatinine ratio in predicting neurodevelopmental outcome is limited.

Shah et al³ showed that blood lactate is an early predictor of hypoxic ischemic encephalopathy but did not address longer-term outcome. Ambalavanan et al¹⁰ showed that a composite scoring system is a good predictor of death or poor neurodevelopmental outcome. The composite score included decerebrate posture, high base deficit (>22 mEq/L), Apgar score at 5 minutes of less than 4, absence of spontaneous activities and absence of chronic hypertension, and pregnancy-induced hypertension. Although base deficit is a reasonable surrogate of blood lactate level, the specific role of lactate level alone in predicting outcome was not addressed. Thus, a reliable measure of predicting neurodevelopmental outcome in infants with hypoxic ischemic encephalopathy warrants future investigation.

Although we only have values at 2 time points, the lower urinary lactate to creatinine ratio at 48 to 72 hours when compared with the value at 6 to 24 hours suggests that there is a decline in blood lactate level as a result of amelioration of hypoxia and ischemia with medical therapy during this period of time independent of hypothermic intervention.

Our study has limitations. First, the sample size was limited because of a late initiation of our study that resulted in a smaller number of subjects enrolled when the primary protocol completed its accrual of subjects. Second, we did not collect data on liver functions of the study infants. Because lactate is primarily metabolized in the liver, its blood as well as urinary level could be affected by liver dysfunction. Third, the outliers in the report may have resulted in large variability in urinary lactate to creatinine ratios that limited the precision of our statistical analysis and affected our ability to predict outcome. Finally, there is limited potential application of the data in parental counseling because of the need of sophisticated technology that may not be readily available in most hospitals settings.

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Acknowledgments

The authors thank the individuals involved in the conduct of this study:

The Hypothermia Study Group

Case Western Reserve University Rainbow Children's Hospital Principal Investigator: Avroy A. Fanaroff, MD; Co-PI: Michele C. Walsh, MD; Study Coordinator: Nancy Newman, BA; RN; Follow up Principal Investigator: DeeAnne Wilson-Costello, MD; Follow up Coordinator: Bonnie Siner, RN. **Brown University** Women & Infant's Hospital Principal Investigator: William Oh, MD; Study Coordinator: Angelita Hensman, BSN, RNC; Follow Up Principal Investigator: Betty Vohr, MD; Follow Up Coordinator: Lucy Noel, RN. **Duke University** Principal Investigator: C. Michael Cotten, MD; Study Coordinator: Kathy Auten, BS; Follow Up Principal Investigator: Ricki Goldstein, MD; Follow Up Coordinator: Melody Lohmeyer, RN. **Emory University** Grady Memorial Hospital and Crawford Long Hospital Principal Investigator: Barbara J. Stoll, MD; Co-PI: Lucky Jain, MD; Study Coordinator: Ellen Hale, RN, BS. **Indiana University** Riley Hospital for Children and Methodist Hospital Principal Investigator: James A. Lemons, MD; Study Coordinators: Diana Dawn Appel, RN BSN, Lucy Miller, RN, BSN; Follow Up Principal Investigator: Anna Dusick, MD; Follow Up Coordinator: Leslie Richard, RN. **Stanford University** Principal Investigator: David K. Stevenson, MD; Co-PI: Krisa VanMeurs, MD; Study Coordinator: M. Bethany Ball, BS, CCRC; Follow Up Principal Investigator: Susan R. Hintz, MD. **University of Alabama at Birmingham** University Hospital-UAB Principal Investigator: Waldemar A. Carlo, MD; Study Coordinator: Monica Collins, RN, BSN, Shirley Cosby, RN, BSN; Follow Up Principal Investigator: Myriam Peralta-Carcelen, MD; Follow Up Coordinator: Vivien Phillips, RN, BSN. **University of Cincinnati** The University Hospital, Cincinnati Children's Hospital Medical Center; Principal Investigator: Edward F. Donovan, MD; Study Coordinators: Cathy Grisby, BSN, Barb Alexander, RN, Jody Shively, RN, Holly Mincey, RN; Follow Up Principal Investigator: Jean Steichen, MD; Follow Up Coordinator: Teresa Gratton, PA. **University of California-San Diego** UCSD Medical Center and Sharp Mary Birch Hospital for Women Principal Investigators: Neil N. Finer, MD; Co-PI: David Kaegi, MD; Study Coordinators: Chris Henderson, CRTT, Wade Rich, RRT-NPS, Kathy Arnell, RN; Follow Up Principal Investigator: Yvonne E. Vaucher, MD, MPH; Follow Up Coordinator: Martha Fuller, RN, MSN. **University of Miami** Principal Investigator: Shahnaz Duara, MD; Study Coordinator: Ruth Everett, BSN; Follow Up Principal Investigator: Charles R. Bauer, MD. **University of Rochester** Golisano Children's Hospital at Strong Principal Investigator: Ronnie Guillet, MD; PhD; Study Coordinator: Linda Reubens, RN; Follow Up Principal Investigator: Gary Myers, MD; Follow Up Coordinator: Diane Hust, RN. **The University of Texas Southwestern Medical Center at Dallas**: Parkland Hospital Principal Investigator: Abbot R. Laptook, MD; Study Coordinators: Susie Madison, RN, Gay Hensley, RN, Nancy Miller, RN; Follow Up Principal Investigator: Roy Heyne, MD, Sue Broyles, MD; Follow Up Coordinator: Jackie Hickman, RN. **University of Texas – Houston** Memorial Hermann Children's Hospital Principal Investigator: Jon E. Tyson, MD, MPH; Study

Coordinator: Georgia McDavid, RN, Esther G. Akpa, RN, BSN, Claudia Y. Franco, RN, BSN, MSN, NNP, Patty A. Cluff, RN, Anna E. Lis, RN, BSN; Follow-Up Principal Investigators: Brenda H. Morris, MD, Pamela J. Bradt, MD, MPH. **Wayne State University** Hutzel Women's Hospital & Children's Hospital of Michigan Principal Investigator: Seetha Shankaran, MD; Study Coordinators: Rebecca Bara, RN, BSN, Geraldine Muran, RN, BSN; Follow Up Principal Investigator: Yvette Johnson, MD; Follow Up Coordinator: Debbie Kennedy, RN. **Yale University** New Haven Children's Hospital Principal Investigator: Richard A. Ehrenkranz, M.D. Study Coordinator: Patricia Gettner, RN; Follow Up Coordinator: Elaine Romano, RN.

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Data Coordinating Center: RTI International

Principal Investigator: W. Kenneth Poole, PhD; Coordinators: Betty Hastings and Carolyn M. Petrie, MS.

National Institute of Child Health and Human Development

Program Scientist: Rosemary D. Higgins, MD, Linda L. Wright, MD; Coordinator: Elizabeth McClure, MEd.

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Children's National Medical Center Gordon Avery, MD; *Columbia University* Mary D'Alton, MD; *RTI International* W. Kenneth Poole, PhD (ex officio); *University of Virginia* John C. Fletcher, Ph.D. (deceased); *University of Washington* Christine A. Gleason, MD; *University of Pittsburgh* Carol Redmond, Ph.D.

Supported by Neonatal Research Network NICHD. The list of grant support for each participating center is available at www.jpeds.com.

Supported in part by grants: U10 HD34216, U10 HD27853, U10 HD27871, U10 HD40461, U10 HD40689, U10 HD27856, U10 HD27904, U10 HD40498, U10 HD40521, U01 HD36790, U10 HD21385, U10 HD27880, U10 HD27851, U10 HD 21373 and GCRCs: M01 RR 08084, M01 RR 00125, M01 RR 00750, M01 RR 00070, M01 RR 0039-43, M01RR 00039, 5 M01 RR00044.

Urinary lactate to creatinine ratio

Figure.

Scattergram of urinary lactate to creatinine ratio collected between 6 to 24 hours of age versus 18-month outcomes. Data for hypothermia and control groups were combined. h and c represent each hypothermic and control infant, respectively. Dotted lines indicate median values.

Table I

Maternal and infant demographic and clinical characteristics of study subjects

	Hypothermia (n = 28)	Control (n = 30)
Maternal data		
Age (year)	26.1 ± 4.3	27.5 ± 6.2
Chronic hypertension	3 (11%)	6 (20%)
Antepartum hemorrhage	3 (11%)	8 (27%)
Diabetes	1 (4%)	5 (17%)
Fetal heart rate deceleration	22 (79%)	23 (77%)
Cord prolapse	5 (18%)	1 (3%)
Maternal fever	4 (14%)	1 (3%)
Shoulder dystocia	4 (14%)	2 (7%)
Labor duration (h)	12.2 ± 8.8	11.7 ± 6.6
Duration of ruptured membrane (h)	6.5 ± 8.2	5.0 ± 5.7
Emergency C section	20 (71%)	24 (80%)
Neonatal data		
Outborn	13 (46%)	5 (17%)
Male	11 (39%)	18 (60%)
Apgar at 5 min <5	22 (79%)	21 (70%)
Apgar at 10 min <5	16 (59%)	15 (60%)
Birth weight (g)	3277 ± 613	3290 ± 788
Moderate encephalopathy	20 (71%)	24 (80%)
Severe encephalopathy	8 (29%)	6 (20%)
Cord blood pH	6.8 ± 0.2	6.9 ± 0.2
Cord blood base deficit (mmol/L)	18.7 ± 6.5	17.5 ± 8.6

There were no differences between the 2 groups except for more infants being outborn in the hypothermia group ($P < .05$). There is also no difference in these characteristics when compared with those enrolled in the main trial.

Table II

Adjusted odds ratio for death or moderate and severe developmental disability

Variable	Odds ratio	95% CI	P value
First urinary lactate to creatinine ratio >2.9*	5.52	1.36–22.42	.02
Severe vs moderate HIE	9.86	1.57–61.76	.01
Hypothermia	0.14	0.03–0.65	.01

HIE, hypoxic ischemic encephalopathy.

* Median value of all samples.