

## ONLINE FIRST

# Long-term Outcome of Brain Structure in Premature Infants

## *Effects of Liberal vs Restricted Red Blood Cell Transfusions*

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**Objective:** To assess the long-term outcome of brain structure in preterm infants, at an average age of 12 years, who received a red blood cell transfusion for anemia of prematurity.

**Design:** As neonates, this cohort of infants participated in a clinical trial in which they received red blood cell transfusions based on a high pretransfusion hematocrit threshold (liberal group) or a low hematocrit threshold (restricted group). These 2 preterm groups were compared with a group of full-term healthy control children.

**Setting:** Tertiary care hospital.

**Participants:** Magnetic resonance imaging scans for 44 of the original 100 subjects were obtained.

**Intervention:** Liberal vs restricted transfusion.

**Main Outcome Measures:** Intracranial volume, total brain tissue, total cerebrospinal fluid, cerebral cortex and cerebral white matter volume, subcortical nuclei volume, and cerebellum volume.

**Results:** Intracranial volume was substantially smaller in the liberal group compared with controls. Intracranial volume in the restricted group was not different from controls. Whole-cortex volume was not different in either preterm group compared with controls. Cerebral white matter was substantially reduced in both preterm groups, more so for the liberal group. The subcortical nuclei were substantially decreased in volume, equally so for both preterm groups compared with controls. When sex effects were evaluated, the girls in the liberal group had the most significant abnormalities.

**Conclusion:** Red blood cell transfusions affected the long-term outcome of premature infants as indicated by reduced brain volumes at 12 years of age for neonates who received transfusions using liberal guidelines.

*Arch Pediatr Adolesc Med.* 2011;165(5):443-450.

Published online January 3, 2011.

doi:10.1001/archpediatrics.2010.269

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**A**DVANCES IN PRENATAL medicine and neonatal intensive care have resulted in improved survival for preterm infants, in particular for those infants with extremely low birth weight (<1000 g) and those born at the limits of viability (22-25 weeks' gestation). Despite improvements in survival, the incidence of disability in this population has not diminished accordingly.<sup>1</sup> A major morbidity for this patient group is neurodevelopmental and behavioral abnormalities.<sup>2-8</sup>

Understanding the risk factors for abnormal neurodevelopmental outcomes is critical for implementing intervention strategies to improve the outcomes of premature infants. Some of the key risk factors for adverse outcome are biologic fac-

tors that are not modifiable following preterm birth: gestational age, birth weight, male sex, and multiple birth.<sup>9</sup> However, there are factors with potential impact on developmental outcome that can be targeted for improvement. One important factor is management of the anemia of prematurity, particularly, optimal red blood cell (RBC) transfusion practices. Transfusion of packed red blood cells is a major component of neonatal care of the preterm infant. As many as 95% of extremely low-birth-weight infants will receive at least 1 RBC transfusion, as will up to 80% of preterm infants with birth weights less than 1500 g (very low birth weight), during the first few weeks of life.<sup>10-12</sup> Red blood cell transfusions can be prescribed according to liberal or restricted guidelines (ie, with relatively high or low pretransfu-

**Table 1. Neonatal and Demographic Characteristics of Participants and Nonparticipants**

	Mean (SD)		<i>t</i> Test	<i>P</i> Value
	Participants (n=55)	Nonparticipants (n=45)		
Birth weight, g	945.6 (196.2)	968.7 (188.7)	0.60	.55
Gestational age, wk	27.81 (2.00)	27.70 (1.86)	0.27	.79
Days on ventilator	22.72 (23.72)	22.93 (28.50)	0.04	.97
Apnea episodes	59.90 (72.93)	46.48 (40.35)	1.10	.27
SNAP, first day of life	14.05 (8.32)	14.22 (7.41)	0.10	.92
SNAP, first week	9.94 (6.39)	10.24 (6.67)	0.22	.82

Abbreviation: SNAP, Score for Neonatal Acute Physiology.

sion hematocrit values, respectively) with the possibility of undertransfusion or overtransfusion, either of which may have adverse effects.<sup>13</sup>

Investigation of differential transfusion practices as a potential mechanism of critical importance in neurodevelopmental outcome is an innovative area of research in which important findings are just beginning to emerge. To date, there have been 3 randomized clinical trials that have evaluated the neurodevelopmental impact in preterm infants of differential transfusion practices through a randomized clinical trial. The first study, the Iowa study, randomly allocated preterm infants to a “liberal” or “restrictive” program for RBC transfusion.<sup>14</sup> A short-term outcome measure (ultrasonography obtained after study enrollment, during hospitalization) suggested that severe grades of intraventricular hemorrhage and periventricular leukomalacia were confined to the restricted group. A similar randomized multinational trial, the Premature Infants in Need of Transfusion (PINT) trial, found no differences in primary or secondary outcome measures between premature infants randomized to liberal and restricted RBC transfusion guidelines.<sup>15</sup> However, when the PINT infants were evaluated for developmental outcomes at 18 to 21 months, cognitive delay was more prevalent in the restricted group.<sup>16</sup> Finally, a recent study from Taiwan indicated no differences in clinical outcomes between preterm babies administered either restrictive or liberal transfusions.<sup>17</sup> Although the recent study was negative, the Iowa and PINT studies suggest that liberal RBC transfusions may be neuroprotective (or that restricted RBC transfusions may be harmful).

In regard to outcomes of preterm birth, one consistent finding is that boys tend to fare more poorly in regard to both short- and long-term outcomes. Studies indicate that boys compared with girls have poorer survival, greater incidence of cerebral palsy, and lower measures of mental ability early in life.<sup>18,19</sup>

The current study reports a long-term outcome of brain structure (using magnetic resonance imaging) of the infants from the original Iowa study. Based on the published findings from the Iowa study<sup>14</sup> and the PINT study,<sup>16</sup> our hypothesis was that brain structure and function would be most abnormal in the restricted RBC transfusion group and that boys would fare worse than girls. Quantitative measures of brain structure were compared across 3 groups: (1) subjects from the original re-

stricted transfusion group, (2) subjects from the original liberal transfusion group, and (3) age-equivalent healthy controls born full-term.

## METHODS

### PARTICIPANTS

This study was approved by the University of Iowa institutional review board. Participants were recruited from the 100 infants who participated in the Iowa transfusion trial. Details regarding the inclusion and exclusion criteria for this study have been previously reported.<sup>14</sup> This follow-up study began in 2005, 13 years after the initial study began. From the original sample of 100 preterm infants, a total of 55 subjects participated in the follow-up study and 45 preterm subjects did not. Reasons for not participating included the following: 3 were deceased, 17 declined to participate, and 25 were unable to be contacted despite multiple attempts. A death index search was conducted on those children who were lost to follow-up. These children did not match any death records through 2007.

To evaluate potential for bias in regard to neonatal characteristics in the participant group (n = 55) compared with the nonparticipant group (n = 45), independent-samples *t* tests were conducted. Measures included the Score for Neonatal Acute Physiology<sup>20</sup> (SNAP), which was recorded on the day of birth and once daily through the first week of life; gestational age; birth weight; total number of days on a ventilator; and total number of apnea episodes. There were no differences between the 2 groups (**Table 1**). In addition, presence of intraventricular hemorrhage was compared across groups. A total of 19 of the 55 participants (34%) had an intraventricular hemorrhage compared with 11 of the 45 nonparticipants (24%). A  $\chi^2$  test was nonsignificant (*P* = .27).

Of the 55 participants, 44 completed the magnetic resonance imaging. Of the remaining 11 subjects with no useable scan, 4 had their scans cancelled (2 subjects from the restricted group and 2 from the liberal group) because of contraindication (metal vessel clamp placed for patent ductus arteriosus) and 7 did not complete their scans because they were too impaired cognitively or behaviorally to stay in the scanner (2 from the restricted group and 5 from the liberal group). Important characteristics of the neonatal hospitalization and demographic information are listed in **Table 2** for the 44 preterm subjects who had a high-quality magnetic resonance imaging scan.

Healthy control children were recruited via advertisements from the surrounding communities. Exclusion criteria from the parent interview included any significant medical or psychiatric disorders or history of traumatic brain injury.

### MEASURES

The SNAP was recorded on the day of birth and once daily through the first week of life. The transfusion threshold level for each treatment group consisted of 3 steps in hematocrit level, which became lower as the subjects advanced through 3 phases of progressively better clinical condition based on their respiratory status.<sup>14</sup> Hematocrit levels were obtained each morning in phase 1, 3 times per week in phase 2, and 2 times per week in phase 3. A measure of average hematocrit level was calculated for each infant, reflecting the mean hematocrit level over the course of his or her inpatient stay.

Because neurodevelopmental outcomes have been associated with infection,<sup>21,22</sup> we also documented the number of times each subject underwent evaluation for sepsis and the total number of days they took antibiotics as a means of quantifying infection burden.

**Table 2. Demographics of Sample**

	Mean (SD)			P Value
	Restricted Group (n=18; Male:Female=16:2)	Liberal Group (n=26; Male:Female=10:16)	Controls (n=40; Male:Female=20:20)	
Current age, y <sup>a</sup>	12.56 (1.49)	12.48 (1.73)	11.74 (2.04)	.17
Parental social class <sup>a</sup>	2.66 (0.514)	2.80 (0.530)	2.32 (0.0460)	<.001
Gestational age, wk <sup>b</sup>	28.06 (1.39)	28.22 (2.39)	NA	.79
Birth weight, g <sup>b</sup>	980.6 (156.4)	952.4 (191.2)	NA	.61
SNAP <sup>b</sup>	8.40 (4.24)	7.86 (4.89)	NA	.71
Average hematocrit level, %	36.46 (2.44)	44.76 (4.53)	NA	<.001
Nadir (lowest-recorded) hematocrit level, %	23.94 (3.03)	31.15 (6.10)	NA	<.001
Total No. of transfusions <sup>c</sup>	2.83 (1.75)	4.23 (3.78)	NA	.15
No. of sepsis workups	3.12 (1.45)	2.56 (1.71)	NA	.29
No. of days on ventilator	16.88 (14.30)	19.03 (21.31)	NA	.71
No. of days taking antibiotics	14.56 (12.62)	18.76 (7.47)	NA	.23

Abbreviations: ANOVA, analysis of variance; NA, not applicable; SNAP, Score for Neonatal Acute Physiology (the average score over the first week of life). SI conversion factor: To convert hematocrit to proportion of 1.0, multiply by 0.01.

<sup>a</sup>Current age and parental social class were compared across all 3 groups using ANOVA. Parental social class was determined by a Hollingshead Scale score of 1 to 5 where the lower the score, the higher the social class. Both restricted and liberal groups were significantly different than controls but not different from each other.

<sup>b</sup>Gestational age, birth weight, and SNAP were compared across the preterm group using ANOVA.

<sup>c</sup>A 15-mL/kg transfusion of red blood cells, concentrated by centrifugation (hematocrit level typically 80%-85%), was given by continuous infusion using a syringe pump over 5 hours. A single-donor transfusion program was in effect during most of the period of this trial.

## PROCEDURE

All magnetic resonance imaging data were acquired on a 3-T Siemens Trio scanner (Siemens, Malvern, Pennsylvania). The protocol acquired a 3-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient-echo sequence in the coronal plane with 1-mm slice thickness. A turbo spin-echo T2-weighted sequence was obtained in the coronal plane with 1-mm slice thickness. Scans were processed through an automated procedure implemented in BRAINS.<sup>23</sup> A discriminant tissue classification was then performed<sup>24</sup> and a brain mask was created using an artificial neural network.<sup>25</sup> Measures of gray matter, white matter, and cerebrospinal fluid (CSF) volumes were then completed using the standard Talairach method.<sup>26</sup> Brain measures included intracranial volume (ICV), total brain tissue, cerebellum tissue volume, and total CSF. The cerebrum measures were further broken down into the 4 cerebral lobes (frontal, parietal, temporal, and occipital) and the subcortical nuclei (caudate, thalamus, and putamen). Cerebral gray matter, further divided into surface gray matter (reflecting the volume of the cortex), was divided into lobes, and cerebral white matter, further divided into the 4 cerebral lobes.

## STATISTICAL ANALYSIS

All analyses were performed by using the SAS language with SAS STAT procedures (SAS Institute Inc, Cary, North Carolina). All general brain measures were analyzed using the general linear models procedure. The analysis for ICV was adjusted for height, sex, and age. Analysis for the remaining brain regions was done on measures adjusted for ICV (by using a brain measure:ICV ratio), age, and sex. This is important given the significant difference in sex distribution across groups and the major difference in brain structure between boys and girls.<sup>27</sup> Small structures (caudate, putamen, thalamus) are listed and labeled as ICV percentages to avoid excessive leading zeros. All possible interaction terms were entered into the model but dropped if not significant. If the overall effect of group was significant, post hoc *t* tests were evaluated to determine the differences between the 3 groups. A 2-tailed  $\alpha$  level of .05 was used for significance tests.

Because of the low number of girls in the restricted group, we were unable to evaluate a sex  $\times$  group interaction. Therefore, sex effects were evaluated only in the liberal group compared with controls. For this analysis, a more liberal *P* value of <.10 was used to decide whether the interaction term of sex  $\times$  group should be included in the model, because it is well known that statistical power to detect interactions is lower than for main effects.

## RESULTS

### DEMOGRAPHICS

Table 2 shows demographic data for the 3 groups. Age at study and parental social class were compared across the 3 groups using analysis of variance. There was no significant difference in age at study. Both preterm groups had significantly lower parental social class compared with the controls. Social class did not differ between the 2 preterm groups.

Other pertinent variables from the neonatal period were compared between the 2 preterm samples using analysis of variance. There was no significant difference between groups in gestational age, birth weight, SNAP, number of sepsis evaluations, or number of days taking antibiotics. Average hematocrit level and nadir hematocrit level (lowest hematocrit level recorded) were significantly lower for the restricted group compared with the liberal group, as expected based on transfusion protocols. Although the mean number of transfusions was greater for the liberal group, this difference did not reach statistical significance.

### GROUP COMPARISONS

**Table 3** shows the general and regional brain measures compared across the 3 groups. Raw means and standard deviations for the volume (in milliliters) of each brain region are listed.

**Table 3. General and Regional Brain Measures Compared Across 3 Groups**

	Volume, mL						F Test	P Value	Contrasts
	Restricted Group (n=18)		Liberal Group (n=26)		Controls (n=40)				
	Mean (SD)	Adjusted Mean <sup>a</sup>	Mean (SD)	Adjusted Mean <sup>a</sup>	Mean (SD)	Adjusted Mean <sup>a</sup>			
ICV	1384.96 (147.22)	1344.68	1256.92 (157.48)	1283.94	1422.73 (106.41)	1410.12	9.69	<.001	Lib<Con; Res=Con; Lib=Res
Total brain tissue	1271.94 (132.05)	0.918	1160.40 (150.49)	0.923	1337.68 (97.14)	0.940	7.05	.002	Res=Lib<Con
CSF	113.02 (37.22)	0.081	96.52 (38.32)	0.076	85.04 (24.66)	0.059	7.05	.002	Res=Lib<Con
Cerebral cortex	657.89 (63.94)	0.479	606.36 (69.48)	0.484	686.79 (52.12)	0.481	0.33	.72	Res=Lib=Con
Cerebral white matter	404.48 (49.71)	0.287	361.19 (66.25)	0.285	429.03 (42.96)	0.303	9.59	<.001	Res=Lib<Con
Cerebellum	132.27 (18.85)	0.095	124.20 (14.12)	0.099	138.23 (12.01)	0.097	1.07	.35	Res=Lib=Con
Caudate	4.67 (0.85)	0.338	4.46 (0.74)	0.354	5.62 (0.74)	0.395	10.82	<.001	Res=Lib<Con
Putamen	12.17 (1.30)	0.898	11.50 (1.49)	0.918	13.58 (1.18)	0.951	3.31	.04	Res<Con; Lib=Con; Lib=Res
Thalamus	11.86 (1.55)	0.871	11.02 (1.46)	0.874	13.02 (0.96)	0.920	3.39	.04	Res=Lib<Con

Abbreviations: Con, controls; CSF, cerebrospinal fluid; ICV, intracranial volume; Lib, liberal group; Res, restricted group.

<sup>a</sup>Statistical analyses computed on volumes adjusted for ICV ratios, age, and sex.

There were 2 brain measures (ICV and putamen) in which 1 of the preterm groups was significantly different than controls and the other preterm group was not, yet both preterm groups were not different from each other. For ICV, the restricted transfusion group was not significantly lower than controls ( $t_{83}=1.91$ ;  $P=.06$ ), but the liberal group showed a robust significance compared with controls ( $t_{83}=4.39$ ;  $P<.001$ ), with the liberal subjects having lower volumes. However, the 2 preterm groups were not significantly different from each other ( $t_{83}=1.74$ ;  $P=.08$ ). For the putamen, the mean volume for the restricted group was lower than controls ( $t_{83}=2.36$ ;  $P=.03$ ), but the mean volume for the liberal group was not significantly lower than controls ( $t_{83}=1.71$ ;  $P=.09$ ); the 2 preterm groups were not significantly different from each other ( $t_{83}=0.832$ ;  $P=.41$ ). This pattern suggests that, descriptively, the volume decrement of ICV appears to be greater in the liberal group than in the restricted group, and the volume decrement of the putamen is greater in the restricted group than in the liberal group. These differences, however, did not reach statistical significance when compared across only the 2 preterm groups.

For several brain measures, there was no significant difference in volume between the 2 preterm groups, but both preterm groups were significantly different from the controls in total brain tissue, total CSF, cerebral white matter, caudate, and thalamus. The cerebral cortex and cerebellum were not different across the 3 groups.

Given the differences between the preterm group and controls in regard to parental social class, the analysis was repeated controlling for social class; the findings remained the same.

#### BREAKDOWN BY SEX

Because the restricted female group was too small to be analyzed independently ( $n=2$ ), this group was dropped

from further analyses. Comparison of brain structure was then made between the liberal boys and the control boys and between the liberal girls and the control girls. Age at study did not differ among groups (**Table 4**). For parental social class, again the preterm groups were both significantly lower in social class than controls, but they were not different from each other. In terms of other pertinent variables from the time of birth, the liberal boys, compared with liberal girls, were significantly younger in gestational age. They also had higher SNAPs compared with the liberal girls, but this did not reach statistical significance. The liberal boys and liberal girls did not differ in birth weight, number of sepsis evaluations, or days taking antibiotics.

#### BRAIN MEASURES

**Table 5** shows the analysis of all the general and regional brain measures across the liberal and control groups, separated by sex. **Figure 1** displays these data visually. For the general measures of ICV, total brain tissue, and total CSF, there was no significant sex  $\times$  group interaction. However, the decrement in ICV and total brain tissue and subsequent increase in CSF volume was descriptively more robust in the girls compared with boys.

For several measures, there was a statistically significant sex  $\times$  group interaction. These measures included cerebral cortex, cerebral white matter, and thalamus. To investigate regional measures, both cerebral cortex and cerebral white matter were further divided into the regions of the 4 cerebral lobes. **Table 5** and **Figure 2** show the regional breakdown of the cortical gray matter volumes across the sexes. The liberal girls had elevations in all 4 regions. This reached statistical significance for the frontal lobe. In contrast, the boys had increases in the frontal lobe but decrements in the remaining 3 lobes compared with controls. This difference reached statistically significant levels for the volume of the occipital gray



**Table 4. Demographics of Sample Broken Down by Sex<sup>a</sup>**

	Mean (SD)				F Test	P Value
	Liberal Group (n=26)		Controls (n=40)			
	Boys (n=10)	Girls (n=16)	Boys (n=20)	Girls (n=20)		
Current age, y	12.33 (2.26)	12.57 (1.38)	11.82 (2.14)	11.67 (2.00)	0.83	.51
Parental social class <sup>b</sup>	2.70 (0.34)	2.87 (0.61)	2.32 (0.46)	2.32 (0.46)	4.05	.005
					<i>t</i> Test	P Value
Gestational age, wk	26.75 (1.86)	29.15 (2.25)	NA	NA	7.91	.009
Birth weight, g	961.20 (199.75)	946.93 (192.07)	NA	NA	0.03	.86
SNAP	10.22 (4.91)	6.53 (4.50)	NA	NA	3.62	.07
Average hematocrit level, %	45.08 (2.61)	44.56 (5.48)	NA	NA	0.08	.78
No. of sepsis workups	3.22 (2.48)	2.19 (1.16)	NA	NA	2.02	.17
No. of days taking antibiotics	19.89 (17.38)	11.56 (8.38)	NA	NA	2.65	.12

Abbreviations: NA, not applicable; SNAP, Score for Neonatal Acute Physiology.

SI conversion factor: To convert hematocrit to proportion of 1.0, multiply by 0.01.

<sup>a</sup>Analysis performed on liberal group compared with controls and with each other across sex using analysis of variance. For the liberal group vs controls, both the male and female liberal groups had significantly lower parental social class compared with controls but were not different from each other.

<sup>b</sup>Parental social class was determined by a Hollingshead Scale score of 1 to 5 where the lower the score, the higher the social class.

**Table 5. General and Regional Brain Measures Compared Across the Liberal Preterm Group and Controls for Effects of Sex<sup>a</sup>**

	Mean		<i>t</i> Test	P Value	Mean		<i>t</i> Test	P Value	Sex × Diagnostic Interaction	
	Liberal Girls (n=16)	Control Girls (n=20)			Liberal Boys (n=10)	Control Boys (n=20)			F Test	P Value
	ICV, mL	1202.79			1358.84	4.43			<.001	1370.91
Total brain tissue	0.919	0.944	3.25	.001	0.928	0.936	1.00	.31	1.95	.16
CSF	0.080	0.055	3.25	.001	0.071	0.063	1.00	.32	1.95	.16
Cerebral cortex	0.491	0.482	1.58	.12	0.475	0.481	0.872	.39	2.94	.09
Frontal	0.197	0.191	2.638	.01	0.194	0.191	1.05	.29	0.91	.34
Temporal	0.123	0.122	0.338	.74	0.122	0.122	0.12	.90	0.02	.89
Parietal	0.112	0.110	1.34	.18	0.105	0.108	1.35	.19	3.69	.05
Occipital	0.058	0.058	0.060	.95	0.052	0.058	2.66	.009	3.92	.05
Cerebral white matter	0.274	0.305	5.86	<.001	0.298	0.300	0.390	.70	12.92	.001
Frontal	0.107	0.115	2.69	.009	0.118	0.117	0.484	.63	5.86	.018
Temporal	0.044	0.050	4.69	<.001	0.050	0.049	1.27	.21	19.99	<.001
Parietal	0.067	0.072	2.57	.01	0.074	0.073	0.070	.48	6.05	.01
Occipital	0.022	0.026	2.88	.005	0.263	0.258	0.499	.62	6.63	.01
Cerebellum	0.101	0.097	1.53	.13	0.096	0.097	0.217	.83	1.40	.24
Caudate	0.355	0.390	2.347	.022	0.351	0.401	2.93	.005	0.45	.51
Putamen	0.920	0.967	2.00	.049	0.923	0.939	0.606	.55	0.76	.38
Thalamus	0.868	0.959	3.58	.001	0.890	0.881	0.314	.75	6.85	.01

Abbreviations: CSF, cerebrospinal fluid; ICV, intracranial volume

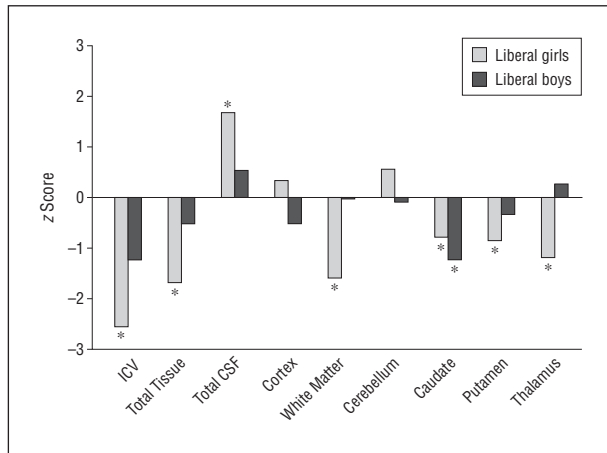
<sup>a</sup>Adjusted means are shown. Intracranial volume was adjusted for height and age. For all other measures, volumes are expressed as a ratio to ICV. For the caudate, putamen, and thalamus, this was multiplied by 100 to decrease excessive zeros.

matter in the liberal boys compared with the male controls. The sex × group interactions were significant for the parietal and occipital regions.

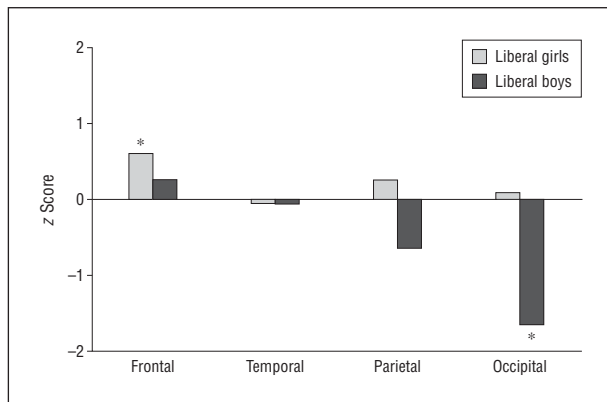
Table 5 and **Figure 3** show the regional breakdown of the cerebral white matter volumes for each sex. The liberal girls had significant decrements in all 4 cerebral lobes, whereas the liberal boys had no significant difference in any region compared with their controls. Therefore, all 4 regions had a statistically significant group × sex interaction. A post hoc analysis of the cerebral lobe white

matter volumes was run on only the liberal girls compared with the female controls to assess if there was a region × group interaction. The lobe × group interaction was significant ( $F=4.04$ ;  $P=.02$ ), indicating that, although all white matter regions in the liberal girls were low, this finding was particularly robust in the temporal lobes.

In regard to the remaining regions of interest, there was no significant sex × group interaction for the cerebellum, caudate, or putamen. However, the thalamus



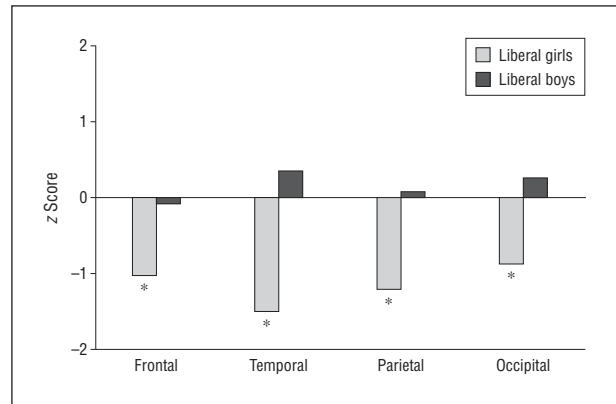
**Figure 1.** The z scores of all brain measures. The 2 preterm groups (liberal girls in gray and liberal boys in black) were compared against their respective sex-matched control group. The control group represents the midline 0 on the y-axis, and therefore, negative scores on the y-axis indicate volumes that are lower than the controls and positive scores on the y-axis indicate volumes that are higher than the controls. \*Measures that are significantly different than controls. CSF indicates cerebrospinal fluid, ICV, intracranial volume.



**Figure 2.** The z scores of cortical gray matter regions. The 2 preterm groups (liberal girls in gray and liberal boys in black) were compared against their respective sex-matched control group. The control group represents the midline 0 on the y-axis, and therefore, negative scores on the y-axis indicate volumes that are lower than the controls and positive scores on the y-axis indicate volumes that are higher than the controls. \*Measures that are significantly different than controls.

was significantly lower in the female liberal group compared with the female controls; the male liberal group was not different compared with the male controls.

Finally, we sought to explore further the relationship between the abnormal brain findings in the liberal female group and measures of transfusion status. The initial analysis used a dichotomous group approach (restricted vs liberal). However, average hematocrit level is a continuous measure that also reflects transfusion status but can be applied to all girls (including the 2 in the restricted group who could not be analyzed separately as a group). Pearson partial correlations were calculated to assess the relationship between the brain measures and average hematocrit level, controlling for current age. To limit the number of tests and minimize the effect of type II error, only those measures that were distinctly abnormal in the female group were assessed: cerebral white mat-



**Figure 3.** The z scores of white matter regions. The 2 preterm groups (liberal girls in gray and liberal boys in black) were compared against their respective sex-matched control group. The control group represents the midline 0 on the y-axis, and therefore, negative scores on the y-axis indicate volumes that are lower than the controls and positive scores on the y-axis indicate volumes that are higher than the controls. \*Measures that are significantly different than controls.

ter and thalamus volume. Both cerebral white matter volume and thalamus volume were significantly inversely correlated with average hematocrit level (18 girls: white matter,  $r = -0.507$ ;  $P = .04$ ; thalamus,  $r = -0.478$ ;  $P = .051$ ). This indicates that the regional brain measures most affected in the girls are directly related to average hematocrit level: those children who had the highest average hematocrit level were the 12-year-olds with the lowest volumes of white matter and thalamus. This finding supports the notion that the abnormalities in the girls are indeed related to hematocrit level (transfusion status).

## COMMENT

### GENERAL GROUP EFFECTS

As a group, premature infants have structural brain abnormalities in almost every measure. These findings are in support of several other studies that have shown widespread abnormalities in long-term outcomes of preterm brain structure.<sup>4,28-35</sup> With respect to the comparisons between premature group effects, we found that, contrary to our original hypothesis, the restricted group did not show the greatest degree of abnormality. In general, there were no significant differences in brain structure between the 2 preterm groups, although descriptively measures tended to be lower in the liberal group than in the restricted group. This pattern is consistent with the results of our assessment of the long-term cognitive outcomes of the original Iowa sample (of which the current study is a subsample) (Thomasin McCoy, PhD, A.L.C., L.C.R., S.D.L., P.C.N., E.F.B., unpublished data, January 2010), which showed that the liberal preterm group performed below that of the restricted group on all cognitive tests that were administered. Most of these effects, as with the brain measures, did not show a significant difference between the 2 preterm groups, but the pattern of the liberal group having overall poorer cognitive outcome is congruent with the structural brain findings of the current subsample.

## EFFECTS OF SEX

Despite the lack of differences in brain volumes between the preterm groups when analyzed as a whole, when the liberal group was analyzed by sex, many significant differences emerged. The results show that the liberal girls had the greatest degree of structural brain abnormality. The liberal girls had more robust abnormalities despite the fact that they were older in gestational age than the liberal boys, a well-documented protective factor for developmental outcomes.<sup>9</sup>

This analysis of sex effects was limited by our inability to assess restricted girls. Therefore, we are left with 2 possibilities: (1) the effects seen in the liberal female group are generalized effects to all premature girls compared with boys; or (2) the sex effect is specific to the girls in the liberal transfusion group. The first possibility is inconsistent with a large body of research supporting the notion that the male brain is more at risk for abnormal neurodevelopmental outcome.<sup>18</sup> The findings from the correlation analysis support the second possibility, that the sex effect is specific to the girls in the liberal group. In that analysis, average hematocrit level was directly related to volume of white matter and thalamus. According to this correlation, female infants with the lowest average hematocrit level have the largest volumes of white matter and thalamus and, therefore, a better long-term outcome in terms of brain structure.

### POTENTIAL DIFFERENCE BETWEEN SHORT- AND LONG-TERM OUTCOMES

The findings of the current long-term study suggest potential neuroprotective benefits of restrictive transfusion status, whereas the short-term outcome studies of this original sample<sup>14</sup> and the PINT study<sup>16</sup> suggested the opposite. One possible explanation for this conundrum is that short-term developmental or cognitive assessments, such as the Bayley Scales of Infant Development (as used in the PINT study), may be relatively poor prognosticators for long-term outcome. One study of 330 preterm infants showed that a subnormal Mental Developmental Index score from the Bayley Scales of Infant Development, at age-corrected age 20 months, was poorly predictive of cognitive function at age 8 years.<sup>36</sup> The same appears to be true for neonatal brain imaging, with studies showing no relationship between neonatal brain ultrasonography abnormality and general cognitive ability at age 9 years<sup>37</sup> or school performance at age 12 years.<sup>38</sup>

### POTENTIAL ETIOLOGY

One possible etiology for our findings is that the restrictive group fared better because endogenous erythropoietin production was suppressed in the liberal transfusion group. Research studies over the past several years have documented in both animal and human studies that erythropoietin has substantial neuroprotective properties.<sup>39-43</sup> Analysis from the original Iowa study showed that erythropoietin levels obtained at 6 weeks into the protocol were significantly higher in the restricted group

compared with the liberal group.<sup>14</sup> Therefore, this suppression of erythropoietin may translate into “loss” of a growth factor known to promote brain growth and recovery from brain injury. Finally, the neuroprotective effect of erythropoietin has been shown to result from selective attenuation of cytokine production and inflammation,<sup>44,45</sup> bringing together inflammation and erythropoietin suppression as 2 potential mechanisms with a common pathway.

In sum, the current study finds that the long-term outcome of brain structure in premature infants may be related to differential transfusion status or difference in mean hematocrit level. Although short-term outcomes suggest that restricted transfusion may bode a poor neurodevelopmental outcome (especially for boys), long-term outcomes may be adversely affected by liberal transfusion (especially for girls). Future studies should be geared toward replication and expansion of long-term outcome studies evaluating brain morphology in the context of anemia and its treatment in the neonatal period. In addition, studies evaluating the roles of inflammation and erythropoietin suppression in response to transfusion are needed.

**Accepted for Publication:** October 20, 2010.

**Published Online:** January 3, 2011. doi:10.1001/archpediatrics.2010.269

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**Author Contributions:** Dr Nopoulos had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Nopoulos, Bell, Strauss, and Lindgren. *Acquisition of data:* Nopoulos, Conrad, Magnotta, and Lindgren. *Analysis and interpretation of data:* Nopoulos, Conrad, Bell, Strauss, Widness, Magnotta, Zimmerman, Georgieff, Lindgren, and Richman. *Drafting of the manuscript:* Nopoulos, Conrad, and Widness. *Critical revision of the manuscript for important intellectual content:* Nopoulos, Conrad, Bell, Strauss, Magnotta, Zimmerman, Georgieff, Lindgren, and Richman. *Statistical analysis:* Zimmerman and Richman. *Obtained funding:* Nopoulos, Bell, Strauss, Widness, Lindgren, and Richman. *Administrative, technical, and material support:* Conrad, Bell, Widness, Magnotta, and Lindgren.

**Financial Disclosure:** None reported.

**Funding/Support:** The current study was funded by grant 2 P01 HL04 6925 from the National Heart, Lung, and Blood Institute (Dr Widness, principal investigator).

**Additional Information:** The trial registration number for the original Iowa study at clinicaltrials.gov is NCT00369005.

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