Erythropoietin Improves Neurodevelopmental Outcome of Extremely Preterm Infants

Achim-Peter Neubauer, MD,[1] Wolfgang Voss, MD,[2]
Michael Wachtendorf, DiplPsych,[2] and Tanja Jungmann, PhD[3]

Objective: Erythropoietin has been reported to possess neuroprotective properties in animal studies. No previous studies have investigated the neurodevelopmental outcome of extremely low birth weight (ELBW) infants treated with recombinant human erythropoietin (rEpo) and evaluated it at school age.
Methods: Of 200 ELBW infants treated from 1993 to 1998, 171 (86%) survived, and 148 (87%) were followed up to the age of 10 to 13 years. The neurodevelopmental and school outcome of the ELBW infants receiving rEpo treatment for stimulation of erythropoiesis in the first weeks of life (n = 89) was compared to that of untreated children (n = 57). To test for a neuroprotective effect of erythropoietin therapy, analyses of variance (ANOVAs) were conducted with erythropoietin treatment and intraventricular hemorrhage (IVH) as independent variables and Hamburg-Wechsler Intelligence Test for Children-III (HAWIK-III) intelligence quotient (IQ) scores as dependent variables.
Results: The rEpo group scored significantly better than untreated children in the overall developmental assessment (55% vs 39% normally developed, $p < 0.05$) as well as in the psychological examination (mean composite HAWIK-III IQ score, 90.8 vs 81.3, $p < 0.005$). The results of ANOVAs show that these differences were ascribable to children with IVH. Whereas those children with IVH treated with rEpo scored significantly better than untreated children (52% vs 6% normally developed, composite HAWIK-III IQ score, 90.3 vs 67.0), treated and untreated children without IVH did not differ in their outcome. The treatment and control groups were comparable in perinatal parameters relevant to prognosis.
Interpretation: The results of our observational study confirm the hypothesis of a neuroprotective effect of rEpo in ELBW infants with IVH. This offers a promising preventative therapeutic option for the treatment of these high-risk infants.

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Extremely low birth weight (ELBW) infants show a high risk of neurodevelopmental delay.[1–3] According to recent literature, approximately 5 to 15% percent of ELBW infants will display signs of cerebral palsy,[2,4] and 15 to 20% of mental retardation.[5] As part of our follow-up program for high-risk infants, we prospectively evaluated surviving children up to school age.[6] Infants with high-grade intraventricular hemorrhage (IVH) and/or periventricular hemorrhage were known as particularly high-risk candidates for persistent impairments. Thus, therapies that could protect the developing brain from injury or facilitate the regeneration of damaged cerebral tissues are warranted to reduce the proportion of children with disabilities.

Erythropoietin has been reported to possess neuroprotective properties in animal studies. Clinical trials with recombinant human erythropoietin (rEpo) have shown promising results in adult patients with nervous system diseases, such as a stroke, multiple sclerosis, or schizophrenia.[7]

In preterm infants, rEpo therapy stimulates erythropoiesis and reduces transfusion requirements.

Three reports on the neurodevelopmental outcome of ELBW infants after rEpo treatment have been pub-

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Address correspondence to Dr Neubauer, St. Bernward Krankenhaus, Treibestrasse 9, 31134 Hildesheim, Germany.
E-mail: neubauer-hannover@t-online.de

From the [1]Children's Hospital "Auf der Bult", Hannover, Germany; [2]Sozialpädiatrisches Zentrum, Hannover; and [3]Leibniz Universität Hannover, Germany.

lished.[7–9] Definite evidence of neuroprotective effects could not be produced in these studies.

For this report, we evaluated the long-term outcome of ELBW infants at the age of 10 to 13 years. Because clinical trials to stimulate erythropoiesis with rEpo had been carried out at our institution during the study period, we were able to compare infant groups with and without rEpo treatment retrospectively. To focus on high-risk preterm infants, children both with and without intraventricular hemorrhage were analyzed separately. We hypothesized that rEpo treatment in infancy may reduce the proportion of children with neurodevelopmental impairment by age 10 to 13 years, and that the benefit may be particularly strong in high-risk infants with IVH.

Patients and Methods

Study Population

The study participants were survivors of a cohort of 200 consecutive, live-born infants with a birth weight <1,000g. They were treated at our level III neonatal intensive care unit (NICU) at the Children's Hospital "Auf der Bult." Of 200 ELBW infants born from January 1993 to December 1998, 171 (86%) survived. Of the 171 surviving children, 23 (13%) children were lost to follow-up. A total of 148 (87%) were prospectively assessed at our outpatient clinic up to 10 to 13 years of age at regular intervals. There were no significant differences in perinatal characteristics between the study group and the ELBW infants who were lost to follow-up.

Neonatal Care

During the neonatal period, high-resolution (7MHz) cranial ultrasonography was performed routinely at the following timepoints: on the first or second day; during follow-up at weeks 1, 2, 4, and 8; and when the child was scheduled to be discharged. Periventricular hemorrhage and IVH were classified among 4 grades of severity according to Papile et al.[10] Periventricular leukomalacia was diagnosed if periventricular echodensities or cysts were observed on cranial ultrasound scans.[11] Other details reporting neonatal care are described elsewhere.[6,12]

Erythropoietin Therapy

A total of 146 ELBW infants were included in this study. Of these, 89 received adequate rEpo therapy to stimulate erythropoiesis, after obtaining parental informed consent. Retrospectively, these children constituted the treatment group for studying rEpo as a neuroprotective factor. Two ELBW infants who had received only sporadic rEpo treatment were excluded from the analysis. Within the treatment group, 77 infants were treated in the first 14 days of life (early treatment), and 12 infants were treated after the 14th day of life (late treatment). The cumulative dosage of erythropoietin averages 8,574U/kg (range, 1,750–21,500U/kg) administered over a 68-day (range, 15–121 days) period. Initial doses were based on birth weight and adjusted weekly on the basis of the actual weight. rEpo was administered as an intravenous infusion or subcutaneously when intravenous access was not available. Fifty-seven infants remained untreated (control group).

The control group consisted either of infants randomized in a double-blind, multicenter trial group[13,14] or of infants born before or between the erythropoietin study phases. Table 1 provides an overview of the different study phases and the dosages of erythropoietin. The control group was composed of children born in study phases where no erythropoietin was applied, children excluded from treatment because no parental consent were available, and children receiving iron treatment in study 2. Infants in the control group did not participate in other treatment studies.

Table 2 summarizes the characteristics of study survivors in the treatment and control groups. Group comparisons (*t* tests) revealed no significant differences in obstetric variables and birth data, in NICU care and complications, or in socioeconomic data.

Follow-up Assessments of Survivors

All surviving children were enrolled in a high-risk infant follow-up program. Assessments were conducted after parental consent at term; at the corrected age of 3, 6, 12, and 18 months; at 2 and 3 years; and at 4, 5, 6, 7, 8, and 10 years of postnatal age. A final assessment was performed at age 10 to 13 years. rEpo-treated infants participated as often in follow-up assessments as control group infants (averaging 9.8 assessments with a standard deviation of 2.6 vs 10 assessments with a standard deviation of 3.2, not reaching statistical significance). Investigators were uninformed concerning the infants' rEpo treatment status. The follow-up visit included an interview with the infant's parents or other primary caregiver as well as an examination, which included measurement of weight, height, and head circumference. Additional information collected at the follow-up visit included the caregiver's education and medical history after initial hospital discharge.

The following neurodevelopmental outcomes were considered.

NEUROLOGIC OUTCOME. An abnormal outcome classification was assigned to infants with cerebral palsy (diplegia, hemiplegia, quadriplegia, ataxia) or significant abnormalities in motor coordination causing functional impairment. Bilateral blindness or hearing deficits requiring hearing aids or other communication aids were classified as abnormal neurosensory outcomes.

COGNITIVE OUTCOME. At the final assessment, the child's intelligence quotient (IQ) was determined using the Hamburg-Wechsler Intelligence Test for Children-III (HAWIK-III).[15] In addition to a composite IQ score, verbal and nonverbal IQ scores were considered separately. All scores were standardized to a mean average of 100 and a standard deviation of 15 (normal range, 85–115). Scores between 1 and 2 standard deviations below the mean were considered borderline (70–84);

TABLE 1: Overview of the Different Study Phases and the Dosages of Erythropoietin

Study Phase	Treatment Group with Follow-up		Control Group	Died	Lost to Follow-up	Total
	Early (1st–14th day of life)	Late (>14th day of life)				
No treatment, January 5, 1993–February 2, 1995	—	—	n = 36 (67.9%)	n = 8 (15.1%)	n = 9 (17.0%)	n = 53
Study 1[a] 750 vs 1,500IU/kg per week, March 3, 1995–October 10, 1995	n = 16 (66.7%)	—	n = 2[b] (8.3%)	n = 4 (16.7%)	n = 2 (8.3%)	n = 24
No treatment, October 11, 1995–January 19, 1996	—	—	n = 10 (83.3%)	n = 1 (8.3%)	n = 1 (8.3%)	n = 12
Routine erythropoietin treatment 750 IU/kg per week, January 20, 1996–June 8, 1998	n = 54 (58.7%)	n = 8 (8.7%)	n = 6[b] (6.5%)	n = 13[c] (14.1%)	n = 11 (12.0%)	n = 92
Study 2,[d] early vs late erythropoietin treatment vs iron treatment, June 9, 1998–December 26, 1998	n = 7 (36.8%)	n = 4 (21.1%)	n = 3 (15.8%)	n = 3 (15.8%)	n = 2 (10.5%)	n = 19
Total	n = 77	n = 12				
		n = 89	n = 57	n = 29	n = 25	n = 200

[a]Randomized, double-blind, multicenter trial. Infants with birth weights between 500 and 999g were treated with either recombinant human erythropoietin (rEpo) 750 or 1,500IU/kg per week from day 3 of life until 37 weeks corrected age.[14] This paper includes 16 of 184 patients from the randomized controlled trial referred to as Study 1.
[b]No parental consent to treatment attendance.
[c]In 5 of 13 infants who died, the rEpo treatment had already started; the other 8 infants died before the start of treatment.
[d]Blinded multicenter trial. Extremely low birth weight infants were randomized on day 3 to 1 of 3 groups: early rEpo group (rEpo from the first week for 9 weeks), late rEpo group (rEpo from the fourth week for 6 weeks), or control group (no rEpo). The rEpo dose was 750IU/kg per week.[13] This paper includes 14 of 219 patients from the randomized controlled trial referred to as Study 2.

scores <70 indicate significant delay. Subtests of HAWIK-III were used to assess the speech development of the child.

Each child was classified as either being normal or having minor or major impairment according to the overall results of these tests, as summarized in Table 3. Children whose intelligence was incapable of measurement because of impairment severity were assigned IQ scores below the lowest standard score (39, because 40 is the lowest HAWIK-III composite IQ score; and 44, because 45 is the lowest HAWIK-III verbal and nonverbal IQ score).

Statistics

SPSS for Windows (version 16.0; SPSS Inc., Chicago, Ill) was used as a database and for the statistical analyses.

t Tests and chi-square tests were conducted to test for treatment effects on anthropometrics, as well as neurodevelopmental and school outcomes. Analyses of variance (ANOVAs) featuring the independent variables erythropoietin treatment and IVH, as well as the dependent variables composite IQ score, verbal IQ score, and nonverbal IQ score, were conducted to test for a neuroprotective effect of erythropoietin therapy. The time epoch effect of being born sooner or later in the study period was controlled within the ANOVAs, as well as effects of gender, gestational age, and birth weight (all as covariates). All tests of significance were 2-sided. Data are presented as means and standard deviations in brackets, if not stated otherwise, and the level of significance was set at $p < 0.05$ for all tests.

Results

Table 4 summarizes the neurodevelopmental outcome at the last assessment between 10 and 13 years of age for the treatment and control groups. Children originally born ELBW treated with erythropoietin early in their lives

TABLE 2: Characteristics of Study Survivors in the Treatment and Control Groups

Characteristic	Treatment Group, n = 89	Control Group, n = 57
Obstetric variables and birth data		
Birth weight, g	804 (118)	790 (131)
Gestational age, wk	27.1 (1.9)	27.2 (2.1)
Head circumference, cm	23.6 (1.5)	23.6 (1.4)
SGA (<10th percentile)	28.1%	31.6%
No RDS-prophylaxis	27.0%	22.8%
Single birth	69.7%	70.2%
Inborn	33.7%	26.3%
Male gender	43.8%	45.6%
APGAR score at 5-10 minutes	6.3 (1.8)/7.2 (1.5)	7.0 (1.7)/7.5 (1.3)
CRIB score	5.5 (3.5)	5.8 (3.5)
Hematocrit at birth (%)	48.2 (8.4)	48.9 (8.3)
NICU care and complications		
Days on ventilation	18.0 (20.6)	16.7 (16.2)
Postnatal steroids	18.0%	19.3%
Intraventricular hemorrhage		
None	66.3%	70.2%
Grades I–II	22.5%	17.6%
Grades III–IV	11.1%	12.3%
Hydrocephalus requiring shunting	4.5%	7.0%
ROP ≥stage III	11.4%	16.1%
Patent ductus arteriosus	41.6%	42.1%
Bowel perforation and/or NEC	10.1%	12.3%
Neonatal seizures	5.6%	8.8%
Septicemia (culture proven)	43.8%	49.1%
Number transfused	48.3%[a]	71.9%[a]

[a]Significant differences between the treatment and control group ($p < 0.05$).
SGA = small for gestational age; RDS = respiratory distress syndrome; APGAR = appearance, pulse, grimace, activity, respiration; CRIB = Clinical Risk Index for Babies; NICU = neonatal intensive care unit; ROP = retinopathy of prematurity; NEC = necrotizing enterocolitis.

achieved significantly higher IQ scores in the HAWIK-III in comparison to untreated children. Considering the distribution of overall outcomes, normal development occurred significantly more often in the treatment than in the control group (55% vs 39%). The opposite was true for major impairment (9% in the treatment group vs 23% in the control group). A positive effect of erythropoietin treatment was also significant for the anthropometric measure of head circumference, whereas no significant effects on body weight, body height, or sensoryneural functions such as sight and hearing were found. Severe blindness and hearing loss occurred comparably often in both groups.

The differences between treated and untreated groups were even more significant when a subset of children with IVH was analyzed. Children with IVH treated with rEpo scored significantly better than children with IVH not treated with rEpo with regard to neurodevelopmental and school outcomes (Table 5).

An ANOVA with the composite IQ score in the HAWIK-III as dependent variable and erythropoietin treatment and IVH as independent variables resulted in a neuroprotective effect of erythropoietin in children with IVH. Children with IVH had significantly poorer outcomes in the HAWIK-III composite IQ than children without IVH (main effect IVH, $p < 0.001$). However,

TABLE 3: Classification of Overall Outcome

Normal development	All of the following:
	● Normal neurological evaluation, IQ > 84
	● No neurodevelopmental deficits
Minor impairment	One or more of the following problems:
	● Subnormal cognitive abilities (IQ, 70-84)
	● Gross and fine motor activity deficits
	● Disorders of language development
	● Visual and auditory deficiencies
	● Attention disorders
	● Abnormal socioemotional development
Major impairment	One or more of the following problems:
	● Cerebral palsy
	● Intellectual disability (US mental retardation) with IQ < 70
	● Blindness, deafness
	● Intractable epilepsy

this is the case only in those children without erythropoietin treatment, whereas children with erythropoietin treatment receive composite IQ scores in a range equal to that of children without IVH (cf Table 5, significant interaction effect IVH × treatment, $p < 0.01$). Even after controlling for historical effects (time epochs, year of birth), birth weight, gestational age, and gender, this interaction effect remains unchanged. The same pattern of results

TABLE 4: Neurodevelopmental Outcome at the Last Assessment between the Ages of 10 and 13 Years for the Treatment and Control Groups

Characteristic	rEpo Treated, n = 89	Untreated, n = 57	*p*
Age of follow-up, yr	10.7 (0.6)	10.9 (1.2)	NS
Cognitive outcomes (HAWIK-III)			
Composite IQ scores	90.8 (17.2)	81.3 (21.1)	<0.005
Verbal IQ scores	95.7 (16.2)	88.1 (21.7)	<0.05
Nonverbal IQ scores	86.0 (16.5)	77.4 (17.9)	<0.005
Overall outcomes			<0.05
Normal	49 (55%)	22 (39%)	
Minor impairment	32 (36%)	22 (39%)	
Major impairment	8 (9%)	13 (23%)	
Blindness (severe)	4 (5%)	4 (7%)	NS
Hearing loss (severe)	2 (2%)	0	NS
Growth			
Weight, kg	31.6 (7.5)	31.0 (9.6)	NS
Height, cm	140 (8.3)	138 (10.6)	NS
Head circumference, cm	52.0 (1.8)	51.2 (2.1)	<0.05
Head circumference <10th percentile	30 (34%)	30 (53%)	<0.05

rEpo = recombinant human erythropoietin; NS = not significant; HAWIK-III = Hamburg-Wechsler Intelligence Test for Children-III; IQ = intelligence quotient.

TABLE 5: Neurodevelopmental Outcome at the Last Assessment between the Ages of 10 and 13 Years

Characteristic	IVH, n = 46		*p*	No IVH (n = 100)		*p*
	rEpo Treated, n = 29	Untreated, n = 17		rEpo Treated, n = 60	Untreated, n = 40	
Age at follow-up, yr	10.7 (0.6)	10.4 (0.6)	NS	10.6 (0.7)	11.1 (1.4)	NS
Pediatric variables						
Birth weight, g	798 (120)	757 (128)	NS	807 (117)	803 (132)	NS
Gestational age, wk	26.4 (1.8)	26.1 (1.6)	NS	27.4 (1.9)	27.7 (2.2)	NS
SGA (<10th percentile)	4 (14%)	2 (12%)	NS	21 (35%)	16 (40%)	NS
Male gender	11 (38%)	6 (35%)	NS	28 (47%)	20 (50%)	NS
CRIB score	6.7 (3.7)	6.9 (3.2)	NS	4.9 (3.2)	5.3 (3.6)	NS
Growth						
Weight, kg	31 (9.3)	27 (3.8)	NS	32 (6.5)	33 (10.8)	NS
Height, cm	141 (10.1)	132 (5.9)	<0.005	139 (7.3)	140 (11.5)	NS
Head circumference, cm	52.1 (1.9)	50.1 (1.7)	<0.005	51.9 (1.8)	51.6 (2.1)	NS
Head circumference <10th percentile at						
Birth	5 (17%)	4 (23%	NS	18 (30%)	15 (37%)	NS
Expected date of delivery	21 (72%)	13 (76%)	NS	45 (75%)	29 (73%)	NS
Last assessment	8 (28%)	13 (77%)	<0.005	22 (38%)	17 (42%)	NS
Neurodevelopmental outcomes						
Cognitive outcomes (HAWIK-III)			<0.02			NS
IQ ≥85	18 (62%)	4 (24%)		39 (65%)	24 (60%)	
IQ 70-84	8 (28%)	6 (35%)		18 (30%)	11 (28%)	
IQ <70	3 (10%)	7 (41%)		3 (5%)	5 (12%)	
Composite IQ scores	90.3 (18.2)	67.0 (20.8)	<0.005	91.0 (16.8)	87.4 (18.3)	NS
Verbal IQ scores	94.9 (16.4)	74.9 (21.6)	<0.005	96.1 (16.2)	93.7 (19.4)	NS
Nonverbal IQ scores	85.4 (19.3)	64.3 (15.8)	<0.005	86.3 (15.1)	83.0 (15.8)	NS
Cerebral palsy	4 (14%)	5 (29%)	NS	1 (2%)	0	NS
Fine motor activity deficits	5 (17%)	12 (71%)	<0.001	8 (13%)	4 (10%)	NS
Gross motor activity deficits	6 (21%)	12 (71%)	<0.001	7 (12%)	9 (22%)	NS
Overall outcomes			<0.005			NS
Normal	15 (52%)	1 (6%)		34 (57%)	21 (53%)	
Minor impairment	9 (31%)	8 (47%)		23 (38%)	14 (35%)	
Major impairment	5 (17%)	8 (48%)		3 (5%)	5 (12%)	
School outcomes			<0.01			NS
Regular classes, in time	15 (52%)	1 (6%)		34 (57%)	20 (50%)	
Regular classes, delayed	9 (31%)	8 (47%)		14 (23%)	11 (27%)	
Special school	5 (17%)	8 (47%)		12 (20%)	9 (23%)	

IVH = intraventricular hemorrhage; rEpo = recombinant human erythropoietin; NS = not significant; SGA = small for gestational age; CRIB = Clinical Risk Index for Babies; HAWIK-III = Hamburg-Wechsler Intelligence Test for Children-III; IQ = intelligence quotient.

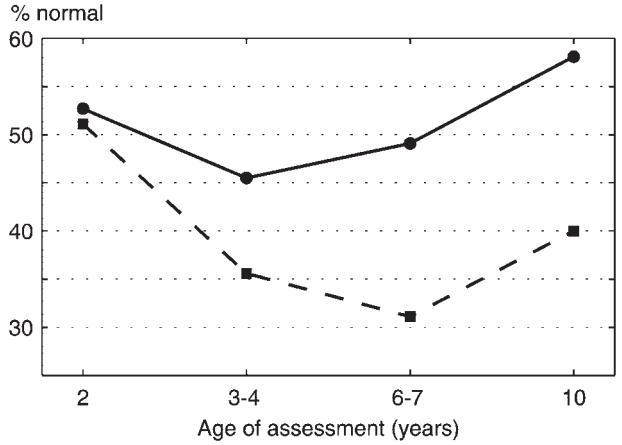

FIGURE: Percentages of children assessed longitudinally as normal (according to the definition in Table 3) at the ages of 2, 3 to 4, 6 to 7, and 10 years (only children with complete data sets are included). Groups of recombinant human erythropoietin-treated *(continuous line)* and untreated infants *(dashed line)* differ in overall outcomes at the ages of 6 to 7 years as well as 10 years ($p = 0.05$).

holds true if the HAWIK-III nonverbal and the verbal IQ scores are entered as dependent variables instead of the composite IQ score. Additionally, no statistical difference is found in outcomes between children receiving early and late initiation of erythropoietin treatment. This is also the case for children receiving different cumulative dosages.

Further longitudinal results from explorative analyses are available for 100 ELBW infants with complete data sets at developmentally important time points (preschool age 2 years, 3–4 years marking the transition to institutional education at kindergarten, 6–7 years marking the transition to formal school education, and 10 years as the last assessment point). As the Figure illustrates, rEpo-treated and untreated infants differ in their overall outcome assessment at the age of 3 to 4 years (46% vs 36% normally developed). With transition to formal education, the numerical group differences almost reach significance (49% vs 31% normally developed at the age of 6–7 years, $p = 0.05$; 58% vs 40% normally developed at the age of 10 years, $p = 0.05$).

Discussion

ELBW infants are at high risk for brain injury and subsequent neurodevelopmental problems. No previous studies have investigated the neurologic and cognitive outcome of preterm infants treated with rEpo and evaluated it at school age. With our observational study, we report the first long-term outcomes that demonstrate improved neurologic and cognitive outcomes in children previously born ELBW treated with rEpo during their initial hospitalization, compared with those not treated with rEpo. The frequency of major impairment in the control group (23%) is in the range previously reported. In contrast, the frequency is only 9% in the treatment group of our study. These findings substantiate the representativeness of the study cohort and emphasize the impact of rEpo.

Most importantly, children previously born ELBW with IVH benefited to the greatest extent from rEpo treatment. About one-third of ELBW infants will have some degree of IVH identified via cranial ultrasound in the early newborn period.[6,16] Major grades of IVH are significant risk factors for impairment at the school age.[17] Normal development and regular school attendance is found in 15 of 29 (52%) children of rEpo-treated ELBW infants with IVH but only in 1 of 17 (6%) in the untreated group with IVH.

The neurologic and neurodevelopmental outcome after erythropoietin therapy of ELBW infants has, so far, been reported in only three studies. Newton et al[9] reported that rEpo, given in clinical trials to reduce transfusions in low birth weight infants <1,250g, did not significantly influence the neurologic or cognitive outcome at 2.5 to 8 years. We hypothesize that there may be three reasons that our data are different from those reported by Newton et al: (1) In the study conducted by Newton et al, infants with grade III or IV IVH were excluded; however, in our study, ELBW infants with IVH benefited particularly from rEpo. (2) Eight of the 20 infants from the treatment group received subtherapeutic rEpo doses (200U/kg per week). (3) The age of ELBW infants at the latest assessment displayed a high variation.

Ohls et al[18] did not find significant differences in Bayley II Mental Developmental Index or other neurologic outcome parameters at 18 to 22 months corrected age in 51 ELBW infants in comparison with the control group. One limitation of their study was the fact that the treatment group included a higher rate of severe IVH compared with controls (6/51 vs 1/51). A possible explanation for missing group differences may be unexpected better scores of rEpo-treated ELBW infants with severe IVH. Furthermore, only 70% of survivors were evaluated. According to results from Ohls et al, we found no difference in overall outcomes at the age of 2 years, but we found differences coinciding with transition to formal education.

Bierer et al[8] reported significantly higher mental developmental scores at 18 to 22 months (corrected age for infants) with serum concentrations of >500mU/ml (n = 6) in comparison to 6 infants with lower rEpo serum concentrations. Their results suggest a neuroprotective effect

of rEpo, but the authors were not able to formulate conclusions from their observations because of the small overall number of children.

In accordance with our results, all of the previous studies demonstrated a significantly higher percentage of infants with head circumference >10th percentile in the rEpo-treated groups. Head size has been found to correlate with neurocognitive outcome.[19,20] This finding suggests that children having undergone rEpo treatment and displaying more promising head growth may catch up in their further development.

This is the first study to investigate the effect of rEpo treatment on school age outcome of children previously born ELBW. The key finding of our study was that ELBW infants with IVH showed a remarkably strong benefit from rEpo treatment, whereas no significant difference in outcome was found in ELBW infants without IVH. Furthermore, we were able to show a positive effect of rEpo treatment even when given days to weeks after the onset of brain damage.

Since the 1990s, several studies have indicated beneficial effects of rEpo on neuroprotection. Konishi et al[21] first demonstrated in their animal study that rEpo acts as a neurotrophic factor, influencing the differentiation and regeneration of neurons. This might be the key to understanding the different paths of neurodevelopment in the rEpo treatment and control groups. Damage to brain tissue through severe IVH unfolds its malevolent effects especially in phases of increasing learning complexity, because it is typical for the period taken into consideration (eg, transition to school and further formal education). Our results of explorative analyses of developmental pathways for a subgroup of 100 ELBW infants with and without rEpo treatment confirm this in displaying a more promising development of rEpo-treated infants in comparison to untreated infants in the time span between ages 3 to 4 years and 10 years.

Currently, understanding the underlying mechanisms by which rEpo is neuroprotective is anything but complete and remains an area of intensive investigation. Brain injury in ELBW infants arises from neuronal losses and as a result of inflammatory processes. Studies suggest that rEpo crosses the blood-brain barrier[22] and attenuates both processes. There are several possible mechanisms underlying a rEpo-mediated protective effect. Hypoxia and ischemia have been recognized as important driving forces of rEpo expression in the brain. It has been suggested that, during ischemia, rEpo protects neurons by decreasing susceptibility to glutamate toxicity,[23] increases the activity of antioxidant enzymes in neurons,[23] modulates angiogenesis in the ischemic brain, improving blood flow and tissue oxygenation in the border zone of the ischemic area,[24] and protects endothelial cells from apoptotic cell death.[25] rEpo may also provide neuroprotection by reducing cerebral vasoconstriction with improved blood flow to a damaged region following injury.[26] In summary, rEpo acts in an antiapoptotic and anti-inflammatory manner during the acute postinjury period and has neurogenic and vasculogenic effects during the recovery period.[27]

Several reports show the therapeutic potential of treatment with erythropoietin for various neurological and cardiovascular diseases in adults.[28–32] Clinical trials in humans with strokes have provided substantial evidence for significant neuroprotective effects of rEpo.[33] Data from Ehrenreich et al[34] allow the conclusion that positive effects of rEpo treatment on cognition are not mediated only by an increase of hemoglobin level. In preterm infants, rEpo therapy stimulates erythropoiesis and reduces transfusion requirements. The drug is usually administered 3 to 5 times per week in weekly doses between 750 and 1,500U per kilogram body weight. The neuroprotective range of erythropoietin concentrations in neonatal cerebrospinal fluid is considered to lie between 20 and 30mU/ml, and during a phase I/II study in ELBW infants, sufficient concentrations for neuroprotection were reached after rEpo dosages of 1,000 and 2,500U/kg.[35]

In adults, rEpo treatment has been associated with some adverse events, for example, hypertension, thrombotic events, seizures, and polycythemia. In ELBW infants, long-term rEpo treatment has not been associated with any of these complications.[36,37] Furthermore, Fauchere et al[38] did not identify significant adverse effects of an early high-dose rEpo treatment in very preterm infants. Animal data and observational studies in humans support a possible association between treatment with rEpo and the development of retinopathy of prematurity. In a Cochrane meta-analysis of early treatment studies, Ohlsson and Aher[39] found an increased frequency of severe retinopathy of prematurity. In accordance with recently published data of Schneider et al,[40] we could not confirm this adverse effect in our own study. In summary, early high-dose recombinant erythropoietin is well tolerated by ELBW infants, causing no excess mortality or other important neonatal complications, such as IVH, necrotizing enterocolitis, chronic lung disease, or septicemia.

This study has several strengths: the relatively high number of children, the high follow-up rate (87 %), the long-term follow-up procedure (until middle school age), and the standardized assessment. Furthermore, the inves-

tigators had no knowledge of the rEpo treatment parameter. A limitation of our observational study is the fact that the study was not initially designed to evaluate neurodevelopmental outcomes after rEpo treatment. Therefore, the assignment of ELBW infants to treatment and control groups was not a random result. By thorough statistical control of important parameters differing between these groups, a bias due to this limitation can be excluded as far as possible.

One major contribution of this study is to encourage other investigators to evaluate the neuroprotective effect of rEpo in ELBW infants. Based on previous experimental observations and our current results, rEpo appears to be a promising candidate for neuroprotection in ELBW infants suffering from IVH. More information is required with regard to the optimal rEpo dose and duration of therapy. Furthermore, it is unclear whether rEpo will be more effective as a prophylactic treatment or as a rescue treatment in this population.

Potential Conflicts of Interest

Nothing to report.

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