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Postnatal *N*-acetylcysteine does not provide neuroprotection in extremely low birth weight infants: A follow-up of a randomized controlled trial

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1. Introduction

Advances in neonatal intensive care have dramatically improved the survival rate for extremely low birth weight (ELBW, < 1000 g) infants, but long-term follow-up studies have shown a high risk of neurodevelopmental disabilities, cognitive dysfunction and emotional/behavioural problems [1–4]. Even in children without obvious neurological deficits, subtle abnormalities, such as lower cognitive test scores and poorer academic performance, have been reported compared to normal birth weight controls [5,6]. Areas of cognitive functioning that are particularly affected by ELBW include attention and executive functions [7–9], perceptual-motor abilities and visuospatial processing [10,11]. The underlying pathology is termed encephalopathy of prematurity, a combination of white matter damage and neuronal/axonal disease that results in the modification of key developmental pathways in the developing human brain [12]. Suggested mechanism for the common neonatal morbidities, including perinatal brain injury, bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP), is a combination of hypoxia, inflammation, infection and oxidative stress [13,14].

In a multicenter randomized controlled trial, we set out to investigate whether *N*-acetylcysteine (NAC), an antioxidant, precursor of glutathione and a free radical scavenger [15], decreases these morbidities. The primary outcome measure was BPD defined as oxygen dependence at an age corresponding to 36 gestational weeks [16]. NAC is available as a registered drug to treat acetaminophen poisoning, but it has multiple other uses as well, supported by varying levels of evidence [17]. Studies of the effects of NAC on various neurological disorders or brain functions have mostly been carried out in animal models with only a few studies completed in humans [18]. In rats, NAC has been demonstrated to improve hippocampal neuronal survival after transient forebrain ischemia [19] and reduce the infarct area and volume in a model of experimental stroke [20]. Moreover, it has been shown to provide substantial neuroprotection against perinatal brain injury in newborn rats [21]. In human clinical trials, NAC has been associated with reduced cognitive deficits in patients with probable Alzheimer's disease [22] and mild traumatic brain injury [23]. In addition, there is a growing body of literature of potential benefits of NAC in the treatment of several neuropsychiatric disorders, but many of these studies require replication and are methodologically preliminary [24].

The present study is a one-center long-term follow-up investigation of a Nordic multicenter randomized controlled trial including 391 ELBW infants. The dosage used (16–32 mg/kg/d continuous intravenous NAC infusion over 6 days starting before 36 h of age) was based on a pharmacokinetic study [25]. The control group received a corresponding placebo infusion with 0.9% sodium chloride. The sets of vials (10 per infant) to be diluted in glucose and infused were similar for NAC and placebo and the personnel and researchers were blinded to the content. There was no benefit of NAC on the primary combined outcome measure, BPD and death (51 vs 49% in placebo group) [16]. However, there was a slight, but non-significant difference in cystic periventricular leukomalacia (PVL, 8 vs 11%). This motivated us to assess whether NAC would decrease more common, less severe

Abbreviations: ATTEX, The Attention and Executive Function Rating Inventory; BPD, bronchopulmonary dysplasia; BW, birth weight; ELBW, extremely low birth weight; FSIQ, full-scale intelligence quotient; FTF, Five to Fifteen; GA, gestational age; IQ, intelligence quotient; IVH, intraventricular hemorrhage; NAC, *N*-acetylcysteine; ROP, retinopathy of prematurity; NEC, necrotizing enterocolitis; SGA, small for gestational age; WISC-III, Wechsler Intelligence Scale for Children – third edition

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neurocognitive sequelae and perform a follow-up study in pre-adolescence. We hypothesized that the postnatal NAC infusion would decrease the risk of neurocognitive dysfunction.

2. Materials and methods

2.1. Study groups

The randomized, placebo-controlled multicenter trial of ELBW infants on ventilator or nasal continuous positive airway pressure was conducted in 10 academic intensive care units in Denmark, Finland, Norway, Sweden and Iceland between 1997 and 2001. In total, 194 infants received NAC and 197 placebo for 6 days [16].

The present study is a follow-up of the infants enrolled in the trial at the Helsinki University Children's Hospital in Finland (n = 123). Of these, 103 were alive at the time of the follow-up. Parents of 12 children could not be reached; however, for six of these children cognitive status was known from previous cognitive assessments. One family declined participation. Thus, the study group in this follow-up assessment consisted of 96 children (Fig. 1).

2.2. Follow-up

2.2.1. Assessment of neurocognition

The children were assessed by a psychologist of the Helsinki University Children's Hospital or a trained psychology undergraduate, who were unaware of the patients' treatment assignments in the trial. General cognitive ability was assessed with a short form of the Finnish Wechsler Intelligence Scale for Children – third edition (WISC-III) [26]. Three scales were employed: estimated verbal intelligence quotient (IQ; subtests information, similarities, vocabulary), performance IQ (picture completion, coding, block design), and the combined full-scale IQ (FSIQ). Using the original test standardization norms, the scales have a mean of 100 (SD 15).

The NEPSY-II neuropsychological test battery evaluates cognitive functioning in six domains [27]. A total of 14 age-appropriate subtests from five different domains were administered: the attention and executive functions domain (subtests animal sorting, auditory attention and response set, inhibition); language (comprehension of instructions, phonological processing, word generation); memory and learning (memory for designs, narrative memory, word list interference); sensorimotor (imitating hand positions, visuomotor precision); visuospatial processing (arrows, design copying, geometric puzzles). Domain scores were used in statistical analyses.

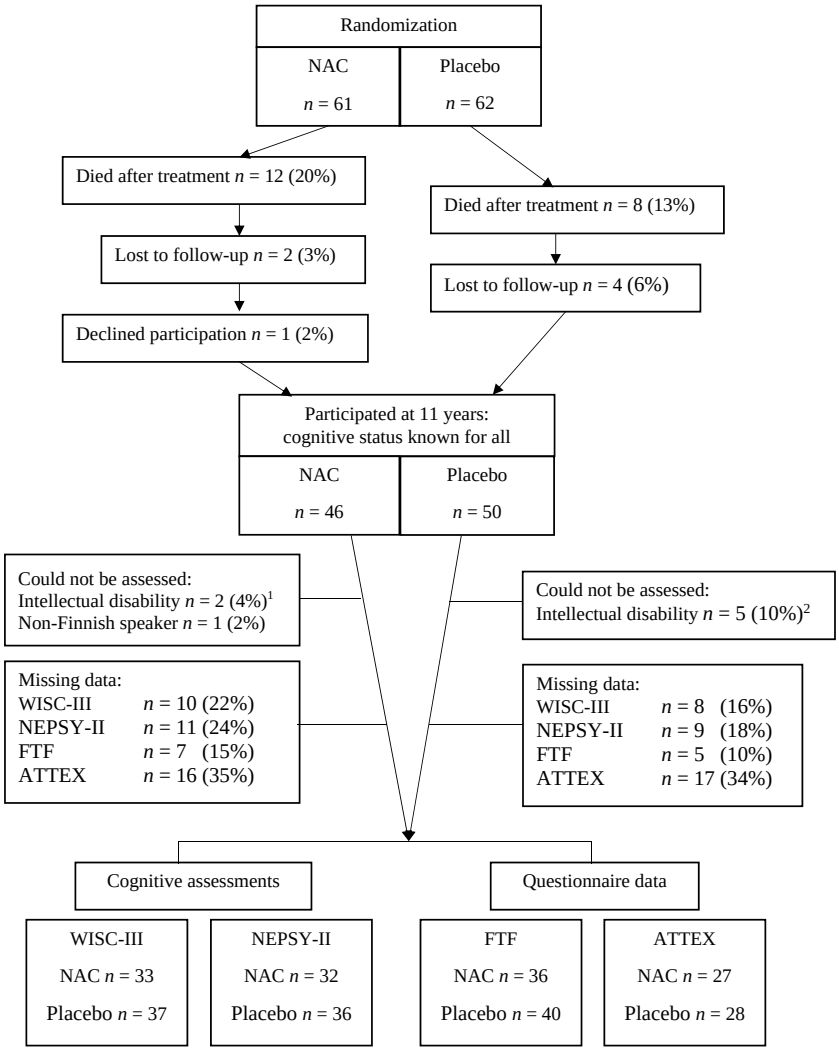

Fig. 1. Flow chart of study participation and non-participating children.
[1]One child additionally had severe cerebral palsy and PVL. [2]Two children additionally had cerebral palsy and one had autism.

Table 1
Background characteristics of children included in the follow-up.

	NAC (n = 46)	Placebo (n = 50)	p
At birth			
Birthweight (g)	770.6 (118.2)	771.1 (137.7)	.984
Gestational age (wk)	26.8 (1.8)	26.9 (2.0)	.724
Maternal age (y)[a]	30.4 (5.7)	31.2 (4.5)	.444
Boys	21 (46%)	24 (48%)	.840
At follow-up			
Age at study (y)[b]	11.4 (1.2)	11.1 (0.9)	.203
Maternal education[b]			.700
Primary school	6 (15%)	4 (9%)	
Vocational school	11 (28%)	9 (21%)	
Upper secondary school or vocational college	11 (28%)	14 (33%)	
University or university of applied sciences	12 (30%)	16 (37%)	
Risk factors			
Small for gestational age	23 (50%)	24 (48%)	1.00
Periventricular leukomalacia	3 (7%)	4 (8%)	1.00
Intraventricular hemorrhage, grade ≥ 2	8 (17%)	8 (16%)	1.00
Necrotizing enterocolitis, grade ≥ 3	7 (15%)	7 (14%)	1.00
Retinopathy of prematurity, grade ≥ 2	19 (41%)	23 (46%)	.684
Oxygen dependency at the age corresponding to 36 gestational weeks	20 (44%)	17 (34%)	.403

Values are mean (SD) or n (%).
[a] Data missing for one child in the NAC group.
[b] Data not known for 6 children in the NAC and 7 in the control group whose cognitive status was based on hospital records.

2.2.2. Questionnaires

A standardized parental questionnaire (Five to Fifteen, FTF [28,29]) was sent to the families to be completed before the formal assessment. The questions have been designed to evaluate developmental problems in children aged five to fifteen years. In this study, 25 items of the executive functions domain were used, which were compiled into four subscales according to manual instructions. Lower scores indicate better outcome.

Another questionnaire was sent to the teachers (The Attention and Executive Function Rating Inventory, ATTEX [30]). ATTEX is a teacher rating scale with 55 items in 10 domains describing difficulties of inhibition, attention, and executive functions in the school setting. Lower scores indicate better outcome.

2.2.3. Background data collection and ethics

The perinatal and neonatal data (including grading of the medical risk factors) was obtained from the initial trial database. In the original study, intraventricular hemorrhage (IVH) was classified according to the criteria of Papile et al. [31], PVL according to the criteria of Trounce et al. [32], necrotizing enterocolitis (NEC) grade III or higher (NEC needing surgical treatment) according to Bell et al. [33], and ROP classified as the stage of disease in the more severely affected eye, based on the International Classification of Retinopathy of Prematurity [34]. Neurological assessment was not carried out in this follow-up study. The current demographic data was collected from the parents. For the children who did not participate in the formal neurocognitive assessments, follow-up data was collected from hospital records with parental permission. Approval to conduct this study was granted by the ethical committee of the Helsinki University Children's Hospital and the National Medical Products Agency. Written informed consent was obtained from the children and their parents.

2.3. Statistical analyses

Background variables were compared using the independent samples t-test for continuous variables and the exact χ^2-test for categorical data. Group differences in outcome variables were compared using general linear model. The presented analyses were controlled for sex and maternal education (divided into 4 classes). An additional analysis controlled for gestational age (GA), small for gestational age (SGA), IVH grades 2–4, ROP grades 2–4, NEC grades 3–4 and oxygen dependency at the age corresponding to 36 gestational weeks (indicator of BPD) was performed to analyse the effect of these risk factors.

In the neurocognitive data, seven children had 5.0 to 10.0% missing subtest results; these were estimated with the expectation maximisation algorithm [35]. The same procedure was used for FTF and ATTEX (1 child with 4.0% and 6 children with 1.8 to 9.1% missing values, respectively). Statistical analyses were performed using IBM SPSS Statistics 24.0 software (SPSS Inc., Chicago, Illinois, USA). All significance tests were two-tailed, and $p < .05$ was considered significant. Partial eta-squared (η_p^2) served as an indicator of effect size.

3. Results

3.1. Study population

The number of deaths in the NAC and placebo groups was 12 (20%) and 8 (13%) children (Fig. 1), respectively (exact $\chi^2(1) = 1.035$, $p = .309$). Of the 103 survivors, some cognitive outcome data was available for 96 (93%) children (46 (94%) in the NAC and 50 (93%) in the placebo group, exact $\chi^2(1) = 0.067$, $p = 1.00$). The participants and non-participants were compared with respect to medical risk factors, but no differences emerged.

There were no statistically significant differences in the background characteristics or neonatal complications between the participating children in the treatment groups (Table 1). Of the 96 children, seven were severely impaired and one did not speak Finnish and could not be assessed. Families of 16 children declined to participate in the formal neurocognitive assessment but completed questionnaire data or gave their permission to collect data from hospital records. In addition, two children participating in the formal assessment were excluded due to incomplete testing. Thus, a complete assessment of neurocognition was obtained in 70 children. Parental and teacher questionnaires were available for 76 and 55 children, respectively (Fig. 1).

3.2. Cognitive status

We allocated the 96 children with some outcome data into three outcome groups based on the formal assessment (complete $n = 70$, incomplete $n = 2$) or hospital data (previous neurocognitive assessment, $n = 20$). If both were missing, the allocation was based on the FTF questionnaire (whole version, $n = 4$). All four children allocated based on the FTF had very few parent-reported developmental problems, thus their outcome was defined as normal.

1) Normal outcome: FSIQ ≥85, no specific impairments (both verbal and performance IQ ≥ 70).
2) Mild to moderate dysfunction: FSIQ 70–84, or a specific impairment (verbal or performance IQ < 70 despite average FSIQ).
3) Severe dysfunction: FSIQ < 70 or a diagnosed severe impairment (e.g. intellectual disability).

There were no statistically significant differences in cognitive status between the treatment groups (exact $\chi^2(2) = 0.364$, $p = .851$; Table 2).

3.3. Neurocognitive test performance

Of the 70 children who underwent neurocognitive assessment, there were no significant differences in general cognitive ability (WISC-III) between the groups, $F(3, 63) = 0.44$, $p = .726$; Wilk's $\Lambda = 0.980$, $\eta_p^2 = 0.020$. Similarly, in specific neurocognitive skills (NEPSY-II), no significant group differences were observed, $F(5, 58) = 0.60$, $p = .699$;

Table 2
Numbers (%) of children with normal or impaired cognitive status in the study groups.

Cognitive status	NAC (n = 46)	Placebo (n = 50)
Normal: FSIQ ≥ 85, no specific impairments	33 (72%)	36 (72%)
Mild to moderate dysfunction: FSIQ < 85 or a specific impairment (verbal or performance IQ < 70)	8 (17%)	7 (14%)
Severe dysfunction: FSIQ < 70	5 (11%)	7 (14%)

Table 3
Mean values (SD) between study groups for all outcome variables from Bonferroni corrected post hoc-tests.

Test	NAC	Placebo	p
Index/subdomain			
General cognitive ability	(n = 33)	(n = 37)	
Verbal IQ	100.1 (17.6)	100.2 (18.8)	.985
Performance IQ	80.3 (20.1)	84.0 (21.4)	.426
Full-scale IQ	90.0 (15.8)	92.0 (16.8)	.601
Specific neurocognitive skills	(n = 32)	(n = 36)	
Executive functions	7.5 (2.1)	8.0 (2.3)	.277
Language	8.4 (1.9)	8.6 (2.1)	.696
Memory	8.2 (2.4)	7.9 (2.6)	.614
Sensorimotor functions	7.6 (2.8)	7.6 (3.0)	.939
Visuospatial functions	7.1 (2.4)	7.6 (2.5)	.368
Parent report	(n = 36)	(n = 40)	
Attention/concentration	6.8 (5.0)	6.3 (5.3)	.690
Overactivity/impulsivity	3.6 (4.1)	3.2 (4.4)	.678
Passivity/inactivity	2.3 (1.9)	1.7 (2.0)	.134
Planning/organizing	1.8 (1.7)	1.6 (1.8)	.579
Teacher report	(n = 27)	(n = 28)	
Distractibility	2.1 (1.8)	1.4 (1.9)	.164
Impulsivity	4.3 (4.2)	2.4 (4.5)	.113
Motor hyperactivity	1.8 (1.9)	0.8 (2.1)	.063
Directing attention	3.0 (2.8)	2.7 (2.9)	.717
Sustaining attention	2.6 (3.2)	2.3 (3.4)	.694
Shifting attention	1.9 (2.1)	1.8 (2.2)	.949
Initiative	2.8 (2.7)	2.3 (2.9)	.484
Planning	2.1 (2.0)	1.3 (2.1)	.151
Execution of action	3.7 (3.7)	3.1 (3.9)	.555
Evaluation	1.5 (1.4)	1.0 (1.5)	.211

Wilk's $\Lambda = 0.951$, $\eta_p^2 = 0.049$. For Bonferroni corrected post hoc-analyses, see Table 3. These results remained after controlling for GA, SGA, IVH, ROP, NEC and oxygen dependency. None of the risk factors had a significant impact on the test results.

When comparing the background characteristics of the children with a complete assessment to the children with data on cognitive status but who did not participate in the formal assessment, the proportion of children with severe dysfunction in cognitive status and the prevalence of PVL was significantly higher among the 13 children in the placebo group who did not participate in the formal assessment than those 37 who participated (exact $\chi^2(2) = 8.81$, $p = .020$ and exact $\chi^2(1) = 5.43$, $p = .049$). No differences emerged in the NAC group.

3.4. Parental and teacher evaluations

In parental questionnaires (FTF), there were no significant group differences in executive functions, $F(4, 67) = 0.79$, $p = .538$; Wilk's $\Lambda = 0.955$, $\eta_p^2 = 0.045$, when controlling for sex and maternal education. Similarly, in teacher reports (ATTEX), no significant group differences were observed, $F(10, 40) = 0.98$, $p = .476$; Wilk's $\Lambda = 0.803$, $\eta_p^2 = 0.197$. For Bonferroni corrected post hoc-analyses, see Table 3. However, when controlling for GA, SGA, IVH, ROP, NEC and oxygen dependency, a main effect of group emerged in ATTEX, $F(10, 32) = 2.17$, $p = .047$; Wilk's $\Lambda = 0.596$, $\eta_p^2 = 0.404$, with less symptoms of attention and executive dysfunction in the placebo group. In Bonferroni corrected post hoc tests, the difference was significant in only one of 10 domains: distractibility ($p = .025$). Of the risk factors, ROP had a significant impact on overall test results of the FTF, $F(4, 59) = 2.86$, $p = .031$; Wilk's $\Lambda = 0.838$, $\eta_p^2 = 0.162$.

4. Discussion

With the aim to decrease oxidative stress in very preterm infants, we performed a randomized controlled trial of continuous intravenous infusion of NAC or placebo. In this study of a secondary outcome measure, neurocognition at 11 years of age, there were no statistically significant differences between the treatment arms, except for slightly less teacher-reported symptoms of distractibility in the placebo group. The clinical importance of this finding is, however, minimal. This study is the first trial to assess the long-term benefit of postnatal NAC administration in ELBW infants.

In experimental animal studies and human clinical trials, potential neuroprotective effects of NAC have been observed [19–23]. There are several possible explanations for the contradictory results of this study. First, the number of studies of the effects of intravenous NAC on children is small and, thus, the evidence is limited. Therefore, it is not known how the immature brain reacts to NAC. In the initial trial, the change in plasma total cysteine concentration during the NAC infusion did not differ from that in the placebo infants, which might indicate that intravenous NAC is poorly deacetylated in preterm infants and so is unable to act as a glutathione precursor and as an antioxidant [16]. In addition, the effects of factors in design and implementation of the initial trial (dosage, timing and duration of intervention) remain unclear.

Second, methodological issues in the present study may influence the results. A possible confounder is linked to the representativeness of our study groups. In studies of preterm infants, it is common that more families with low socioeconomic background but also patients with lower cognitive abilities drop out of studies [36]. In this study, the cognitive status was not known for the 7 children lost to follow-up or whose families declined participation. However, among the children with data on cognitive status but who did not participate in the formal neurocognitive assessment, there was an over-representation of children with adverse cognitive outcome and PVL in the placebo group among those children who did not participate in formal assessments. Nevertheless, the cognitive status of the 96 children did not differ between the NAC and the placebo groups. Hence, we can expect that this selection effect did not obscure the actual differences in neurocognitive performance between the study groups.

The strengths of the present study include its randomized, double-blind, placebo-controlled design. The participation rates among survivors remained high (94% for NAC and 93% for placebo groups) despite the extended follow-up time. The neurocognitive assessments were comprehensive and utilised tests that are frequently used in clinical work. Parental and teacher evaluations of executive functions in home and school settings provide additional information on possible cognitive deficits. Further, the data records concerning neonatal variables were systematic and the analyses were adjusted for several confounding factors.

A limitation of this study is that the sample size was relatively small, which might not yield enough statistical power; hence, only large effect sizes would be expected to yield statistically significant differences between the groups. Since this is the first study to assess the influence of NAC treatment on cognitive outcome in ELBW children, this will set a benchmark that possible future studies can use when doing power analysis and determining sample size. Only children from one participating center were included in this study, but they represent one third of all included infants and the standardized assessments could be designed to strictly adhere to the one-center protocol, without problems arising of regional and language differences. It is unlikely that there would be a statistically significant difference in neurocognitive test

performance if all survivors in the different countries were assessed. Moreover, a small difference in a large sample would not be clinically relevant for recommending a prophylactic NAC infusion over the first week of life to all ELBW infants.

5. Conclusion

This is the first randomized controlled trial of postnatal administration of NAC in ELBW infants. NAC treatment did not improve neurocognitive functioning in preadolescence. The use of NAC in the neonatal care of ELBW children is not justifiable. However, this study sets a benchmark for future studies in larger populations.

Declarations of interest

None.

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Contributorship statement

VF and TA designed the data collection instruments, VF and PK coordinated and supervised data collection, VT, TA and PK participated in the acquisition of data, AK, AH, VF and LK conceptualized and designed the study, AH carried out the analysis of the data, AK, AH, LK, AL and VF interpreted the data, AK drafted the initial manuscript and all authors reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflict of interest statement

None declared.

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