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Oxidative Stress, Motor Abilities, and Behavioral Adjustment in Children Treated for Acute Lymphoblastic Leukemia

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Abstract

Purpose/Objectives—To examine associations among oxidative stress, fine and visual motor abilities, and behavioral adjustment in children receiving chemotherapy for acute lymphoblastic leukemia (ALL).

Design—A prospective repeated measures design was used. Serial cerebrospinal fluid (CSF) samples were collected during scheduled lumbar punctures.

Setting—Two pediatric oncology settings in the southwestern United States.

Sample—Eighty-nine children with ALL were followed from diagnosis to end of chemotherapy.

Methods—CSF was analyzed for oxidative stress biomarkers. Children completed fine motor dexterity, visual processing speed and visual-motor integration measures at three time points. Parents completed child behavior ratings at the same times.

Main Research Variables—Main research variables were oxidative stress, fine motor dexterity, visual processing, visual motor integration, and behavioral adjustment.

Findings—Children with ALL had below average fine motor dexterity, visual processing speed, and visual-motor integration following the induction phase of ALL therapy. By end of therapy, visual processing speed normalized, while fine motor dexterity and visual-motor integration remained below average. Oxidative stress measures correlated with fine motor dexterity and visual-motor integration. Decreased motor functioning was associated with increased hyperactivity and anxiety.

Conclusions—Oxidative stress occurs following chemotherapy for childhood ALL and is related to impaired fine motor and visual symptoms.

Implications for Nursing—Early intervention should be considered to prevent fine motor and visual spatial deficits and behavioral problems.

Knowledge Translation—Basic science findings that chemotherapy induces oxidative stress were validated in a clinical study of children with ALL. Oxidative stress increases risk for fine motor and visual spatial problems.

Keywords

childhood leukemia; fine motor dexterity; visual-motor integration; oxidative stress; cerebrospinal fluid

Introduction

Advances in primary therapy for childhood acute lymphoblastic leukemia (ALL) have resulted in increased five-year survival, which currently approaches 90% (Hunger et al., 2012). Increased overall survival has led to better appreciation of therapy-related morbidity and impaired quality of life (Barr, Feeny, Furlong, Weitzman, & Torrance, 1995; Krull et al., 2008). In contrast to the recognition of childhood cancer-related neurocognitive complications, there is limited research investigating the trajectory of fine motor and visual-motor difficulties and its impact on behavior and emotional function among children with cancer. There is even less understanding of physiologic risk profiles for neurobehavioral problems in children with leukemia. This study investigated the influence of the oxidative

stress pathway on fine and visual motor skills, and behavioral adjustment in children treated for ALL. Oxidative stress results from an imbalance in the production of reactive oxygen species (ROS) and antioxidant defense systems. ROS are formed as byproducts of cellular metabolism, which in excess can result in damage to cellular structures, and anti-oxidant systems are the body's first line defense against cellular injury (Roberts et al., 2010; Stenzel et al., 2010). Brain tissue is particularly vulnerable to oxidative stress due to limited antioxidant capacity, higher energy requirements, and higher concentration of lipids (Floyd, 1999).

A child's motor system experiences rapid development during the first two to five years of life, the time when ALL most commonly occurs in children. Childhood ALL treatment increases the risk for long-term fine motor problems that include peripheral neuropathy, sensory loss, and reduced deep tendon reflexes and motor function changes. Vainonpaa and colleagues (1995) were among the first to describe problems in the peripheral nervous system during childhood ALL treatment; significant prolongation of the median nerve peripheral conduct time caused by vincristine therapy was discovered in a group of 38 children receiving ALL therapy. Today it is evident that motor changes are not restricted to the peripheral nervous system but may cause deficits throughout the motor circuitry, including the central motor system (Harila-Saari, Huuskonen, Tolonen, Vainionpaa, & Lanning, 2001). Reduced motor competence is demonstrated by greater handwriting difficulties as well as deficits in manual dexterity and strength.

Motor and sensory-perceptual function is an important indicator in determining severity of a neurologic insult and extension of impairment across specific regions of the brain. Although motor and sensory perceptual signs are often deemed "soft signs," they can be important indicators of a compromised central nervous system (CNS). Examination of fine motor function can assist in understanding the effects of ALL treatment on the brain and the long-term impact of therapy on development. While most studies demonstrated some evidence of fine motor problems associated with manual dexterity problems during treatment (Dowell Jr, Copeland, & Judd, 1989; Green, Knight, McCarthy, & De Luca, 2013; N. C. Jansen et al., 2005; Kingma et al., 2001; Reinders-Messelink et al., 1999; H. Reinders-Messelink et al., 2001; H. A. Reinders-Messelink et al., 1996; van Brussel et al., 2006), there was inconsistency in findings following completion of ALL treatment. While many of the neurotoxic treatment-related problems disappear over time, findings from several studies demonstrate that gross and fine motor deficits continue to be evident five years after completing therapy (Galea, Wright, & Barr, 2004; Hartman, van den Bos, Stijnen, & Pieters, 2006; N. C. A. J. Jansen et al., 2008; Kingma et al., 2001; Lehtinen et al., 2002; Marchese & Chiarello, 2004; Reinders-Messelink et al., 1999; H. A. Reinders-Messelink et al., 1996; Marilyn J Wright, Galea, & Barr, 2005; M. J. Wright, Halton, Martin, & Barr, 1998). Behavior difficulties including attention problems, anxiety, depression and social problems among childhood cancer survivors have been reported (Brown et al., 1992; Buizer, de Sonnevill, van den Heuvel-Eibrink, & Veerman, 2005, 2006). However, there are inter-individual differences in neurobehavioral outcomes that could be associated with physiologic and/or psychologic factors. A previous study by this research team found that in children with ALL, symptoms of hyperactivity decreased over time for participants with low

oxidative stress levels, but remained stable over time for participants with high oxidative stress levels (Stenzel et al., 2010).

Emerging evidence supports oxidative stress as an underlying biologic process responsible for progressive neurologic injury following chemotherapy. There is growing evidence that alkylating agents and antimetabolites increase ROS production (Illingworth & Glover, 1971; Roberts et al., 2010; Stenzel et al., 2010). Methotrexate-induced oxidative stress has been observed in tumor tissue, cell cultures, rat brain, and children with ALL (Babiak, Campello, Carnieri, & Oliveira, 1998; Cetinkaya, Bulbuloglu, Kurutas, & Kantarceken, 2006; Hockenberry et al., 2013; Jahovic, Cevik, Sehirli, Yegen, & Sener, 2003; Mazor, Abucoider, Meyerstein, & Kapelushnik, 2008; Oktem et al., 2006; Rouse, Nwokedi, Woodliff, Epstein, & Klimberg, 1995; Sener et al., 2006). Oxidative stress may also be responsible for fine motor dexterity problems observed in children with ALL.

This study assessed fine motor dexterity, visual processing, and visual motor integration in children over the course of chemotherapy and examined associations with behavioral outcomes and cerebrospinal fluid (CSF) markers of oxidative stress. Further, we expected oxidative stress within the CNS to be associated with fine motor problems.

Methods

A prospective longitudinal design was used to investigate a model for examining associations among CSF biomarkers of oxidative stress, fine motor, visual motor, and behavioral adjustment in children treated for ALL. The study was approved by local institutional review boards and parent consent and child assent (for children 7 years of age or older) was obtained.

Patients

Eligibility requirements included children diagnosed with pre-B or pre-T cell ALL and being treated on or according to an existing Children's Oncology Group (COG) protocol. For continuity and validity of longitudinal neurocognitive assessments, patients had to be between 2 years 9 months and 15 years of age at the time of diagnosis, and English language dominant. Exclusion criteria included children with CNS leukemia, a pre-existing neurological disorder (i.e. seizures), subsequent brain injury associated with an alteration of consciousness, or a neurodevelopmental disability (e.g. Down Syndrome).

The ALL therapeutic protocols included the following treatments: induction therapy involved weekly treatment with vincristine and daunomycin (for high-risk ALL), a corticosteroid and a dose of PEG-asparaginase, and two intrathecal methotrexate (IT MTX) treatments (Days 8 and 29); post-induction therapy (6–8 months) involved several courses of asparaginase, high- or intermediate-dose intravenous methotrexate (IV MTX) depending on ALL protocol assignment, vincristine, doxorubicin, corticosteroid, cytarabine, mercaptopurine, and IT MTX; continuation therapy consisted of daily mercaptopurine and weekly oral MTX, with monthly pulses of vincristine and a corticosteroid, and an IT MTX treatment every 12 weeks for 2 ½ years (female) to 3 years (male).

Procedures

Baseline measures were collected during post-induction therapy, and during two follow-up sessions (Follow-up 1, during continuation, approximately one year from date of baseline evaluation; and Follow-up 2, at the end of continuation therapy, approximately 2 years from baseline). The test battery included measures of fine motor dexterity (Tiffin, 1968), visual-motor integration (Beery, 1997), visual processing speed (Woodcock, McGrew, & Mather, 2001), and visual spatial perception and construction (Korkman, Kirk, & Kemp, 2007), as well as parental ratings of behavioral functioning (Reynolds & Kamphaus, 2004).

CSF was obtained during scheduled therapeutic lumbar punctures (LPs), with the first sample collected during the diagnostic LP. Subsequent CSF samples were collected during the LP for each IT MTX treatment but prior to injection of any chemotherapy. Samples were obtained during induction, post-induction, and the continuation phases of therapy. Biomarkers of oxidative stress in the CSF consisted of oxidized phosphatidylinositol (PI), and F2-isoprostanes (F2-I). Unoxidized PI, a biomarker of cell membrane disruption, was also measured (Chauhan & Chauhan, 2006; Folch, Lees, & Sloane Stanley, 1957).

CSF samples were placed on ice immediately after collection, centrifuged for 10 min at 1750x g to remove any cellular debris, and then stored at -80°C to limit degradation. PI was extracted from CSF samples using a modified method developed by Folch and others (1957), and separated by high performance liquid chromatography (HPLC) with a normal-phase gradient. Lipids were extracted from the aqueous CSF with 4 ml of chloroform: methanol (2:1, v/v), vortexed for 1 minute and centrifuged at 10,000 rpm for 20 minutes. The organic phase was collected and stored on ice. A second extraction of the aqueous phase with chloroform-methanol (9:1, v/v) was done in order to insure complete extraction of the less polar phospholipids. The organic fractions were combined, evaporated to dryness under nitrogen, and resuspended in hexane-isopropanol (1:1, v/v) prior to HPLC separation. This method recovers 98% of phospholipids.

Mawatari and Murakami (1998) developed a HPLC method for detecting peroxidation of phospholipids in a single chromatic elution. We modified Mawatari and Murakami's method to optimize detection using normal phase HPLC for separation of phospholipid classes. Details of this method have been previously reported (Hockenberry et al., 2013; Moore et al., 2014).

F2-I is a sensitive index of oxidative stress *in vivo*, and was measured by a competitive enzyme-linked immunoassay (ELISA) kit (Cayman Chemical). The immunoassay was validated in human urine samples with gas chromatography/mass spectrometry following solid phase extraction, with an intra-assay correlation of $r > 0.8$. Detailed methods for F2-isoprostanes have been previously reported (Hockenberry et al., 2013).

Statistical Analysis

Descriptive statistics were calculated for patient demographics and treatment variables. Scores on measures of fine motor dexterity, visual motor integration, visual processing speed, and visual spatial abilities were converted to age-adjusted z-scores (mean = 0, standard deviation = 1.0) using national population norms. For behavioral adjustment, T-

scores (mean = 50, standard deviation = 10) were used, as this is the format given by the computerized scoring program. We calculated 95% confidence intervals around each mean z-score and T-score, and those intervals that did not contain the population mean (0 or 50, respectively) were interpreted as being significantly different from the normal population. Pearson correlation was used to test for associations among measures of motor abilities and CSF biomarkers. Some motor and visual motor problems recovered after the post-induction phase of therapy. Therefore, Pearson correlation was used to determine associations between performance on motor measures at Follow-up 1 and behavioral measures at the end of therapy (Follow-up 2). All analyses were conducted using SPSS version 20.

Results

Table 1 presents demographic and clinical characteristics of the 89 participants. Subjects were an average of 7.1 years old at diagnosis and 55% were female. Patients received an average cumulative IV methotrexate dose of 9.4 gm/M², and number of IT methotrexate injections ranged from 13 to 24.

Participants' scores were significantly lower than the population means for fine motor dexterity symptoms following induction therapy (Baseline; Table 2). Performance improved over the course of therapy, such that scores for non-dominant (mean = -0.20, 95% CI = -0.51, 0.10) and both hands (mean = -0.25, 95% CI = -0.58, 0.09) were within the average range by the end of therapy (Follow-up 2). Performance for the dominant hand remained significantly below expectations by the end of therapy (mean = -0.39, 95% CI = -0.70, -0.09). Visual processing speed demonstrated a pattern of low performance following induction (mean = -0.24, 95% CI = -0.45, -0.03) and post-induction therapy (mean = -0.26, 95% CI = -0.50, -0.03), but performance was within the expected range by the end of therapy (mean = -0.03, 95% CI = -0.26, 0.21). Visual-motor integration was also below expectations following induction therapy (mean = -0.22, 95% CI = -0.42, -0.02), and remained so to the end of therapy (mean = -0.37, 95% CI = -0.57, -0.18). No difficulties were observed in spatial perception or construction over the course of treatment.

Following induction, parental rating of hyperactivity was below the norm (mean = 47.95, 95% CI = 46.02, 49.88) and rating of somatic complaints was elevated (mean = 59.90, 95% CI = 57.18, 62.62). Following post-induction therapy, rating of hyperactivity (mean = 47.64, 95% CI = 45.39, 49.89) and aggressive behavior (mean = 47.52, 95% CI = 45.20, 49.83) were below norms and rating of somatic complaints (mean = 57.84, 95% CI = 55.04, 60.65) remained elevated above norms. This pattern continued to the end of therapy. No differences were noted in ratings of emotional or social behaviors.

F2-I, oxidized PI and unoxidized PI levels in the diagnostic CSF samples were not associated with motor dexterity, visual processing speed or visual-motor integration at any of the three assessment points. CSF concentration of F2-I during induction was negatively correlated with motor dexterity symptoms ($r = -0.29, p < 0.05$) and visual-motor integration problems ($r = -0.23, p < 0.05$) at the post-induction assessment. CSF concentration of F2-I during post-induction was significantly associated with motor dexterity symptoms at the end of therapy (Follow-up 2; $r = -0.339, p < 0.05$). As shown in Table 3, oxidized and

unoxidized PI concentrations during induction, post-induction and continuation were negatively correlated with motor dexterity problems at the end of therapy. The number of IT methotrexate injections received was not associated with performance on the motor dexterity, visual processing speed or visual-motor integration measures. However greater total dose of IV methotrexate received was significantly and negatively associated with visual motor integration abilities at the Follow-up 1 ($r = -0.213$; $p < 0.05$) and Follow-up 2 ($r = -0.240$; $p < 0.05$) assessments.

As summarized in Table 4, fine motor dexterity problems at the Follow-up 1 assessment were associated with a range of behavioral problems, including anxiety, somatic complaints, atypicality, and attention problems, at the end of therapy (Follow-up 2 assessment). Poor visual processing speed at Year 1 was associated with increased attention problems ($r = -0.29$, $p < 0.05$) at the end of therapy, and poor visual-motor integration was associated with higher hyperactivity ($r = -0.23$, $p < 0.05$) and lower functional communication ($r = 0.29$, $p < 0.05$) at the end of therapy.

Discussion

Results of the current study demonstrate that children undergoing chemotherapy for childhood ALL experience fine motor dexterity problems within months of diagnosis which persist throughout the course of therapy. Visual processing speed was also initially lower than expected, but improved and normalized by the end of therapy. Visual motor integration, a relatively complex task dependent on visual spatial perception and fine motor dexterity, followed a similar pattern as motor dexterity, and actually worsened with time. Since spatial perception and construction tasks did not show impairment at any time during treatment, visual motor integration problems are likely directly related to poor motor dexterity. Symptoms of impairment in fine motor dexterity appear to be related to oxidative stress and associated disruption in cell membrane integrity in the CNS. F2-I are prostaglandin-like compounds formed by free radical peroxidation of arachidonic acid, and are established markers of oxidative injury (Frere, Chang-Ileto, & Di Paolo, 2012). Although F2-I is a “gold standard” measure of OS, in this study, phospholipids were more strongly associated with fine motor problems.

F2-I levels in CSF increased from the diagnostic LP to the induction sample, and further increased following induction and post-induction. Although F2-I concentration at diagnosis was not associated with motor dexterity, levels in CSF during and following induction significantly correlated with subsequent motor dexterity problems. Methotrexate has been observed to induce oxidative stress and given the timing between increased F2-I and treatment phase in the current study, may be responsible for oxidative stress and subsequent impaired motor dexterity.

Arachidonic acid is a polyunsaturated fatty acid found in phosphatidylinositol (PI) and other phospholipids that comprise cellular membranes in the brain, muscle and liver. In this study we found an increase in CSF levels of oxidized and unoxidized PI over time compared to the diagnostic CSF sample. This pattern is suggestive of oxidative injury resulting in disruption of cellular membranes within the CNS, likely due to the mediating effect of F2-I (Milne,

Musiek, & Morrow, 2005; Montuschi, Barnes, & Roberts, 2004). Although phosphoinositides are quantitatively a minor class of lipids, they have a crucial role in synaptic function. Further, dysregulation of PI metabolism has been implicated in synaptic malfunction associated with a variety of brain disorders. Oxidized and unoxidized PI following induction were predictive of impaired motor dexterity at the end of therapy, further supporting the notion that CNS-based injury is responsible for the fine motor dexterity problems, which could potentially involve dysregulation of neurotransmission (Frere et al., 2012).

Motor functioning following post-induction was related to behavioral adjustment at the end of therapy. Fine motor dexterity was significantly correlated with anxiety and somatic complaints at the end of therapy, while visual-motor integration was associated with hyperactivity and poor functional communication. Visual motor integration is a complex task that involves perception of visual spatial relations combined with fine motor dexterity required to reproduce designs and patterns. In this study, impaired visual motor integration following post-induction therapy was associated with symptoms of increased hyperactivity and reduced functional communication at the end of therapy. Hyperactivity is often viewed as reduced control over motor systems. Functional communication includes not only expressive language and speech but also written communication. Thus, it is not surprising to find that early deficits in complex motor control impact functional behavior as children continue to develop. This association suggests that behavioral problems that emerge over time are related to neurocognitive limitations and not necessarily social or environmental interactions. Further research is needed to evaluate this connection between neurocognitive deficits and behavioral problems.

Slowed visual processing speed following post-induction was associated with increased attention problems at the end of therapy. Attention is a cognitive construct that is heavily dependent on processing speed, as slower rates of information processing require more sustained attentional effort. In the current study visual processing speed appears to normalize by the end of therapy, though the impact on the broader domain of attention remains, which is consistent with recent work by Krull and colleagues (2014) demonstrating that many children treated with chemotherapy for ALL have sustained attention problems on direct assessment at the end of therapy.

This study is not without limitations. The sample size is relatively small compared to other recently reported ALL outcome studies, though the vast majority of those studies are cross-sectional and do not include longitudinal data beginning shortly after diagnosis. All children received vincristine, a known neurotoxic agent, and the contributions of this agent to changes in dexterity were not evaluated. These children also received oral corticosteroids which can cause behavioral problems. However, corticosteroid therapy was most intense during the induction and post-induction phases of treatment and consisted of only monthly pulses during continuation when Follow-up 1 and 2 sessions were completed. The battery of assessment measures is also brief and more focused than other reports in the literature, a consequence of limited choices and time for assessment of young children during treatment of a major medical condition.

Discussion and Implications for Nursing

This study demonstrates that children undergoing chemotherapy for childhood ALL are at risk for early onset and persistence of fine motor dexterity and visual-motor problems that appear to be related to CNS treatment-related neurotoxicity. Early identification of problems with fine motor dexterity and visual-motor integration appear to impact development of functional behavior. Early interventions designed to improve fine motor dexterity and visual motor integration are lacking. Findings from this study support the need to test the efficacy of early interventions designed to improve fine motor dexterity and prevent future problems with visual motor integration and behavior. The association between early motor symptoms and CNS biomarkers of oxidative stress suggest the potential role of antioxidants in limiting the cascade of reactive oxygen species and peroxidation injury of lipid membranes. However, prior to any clinical trial, preclinical studies are necessary to ensure the antioxidants do not limit the efficacy of the primary cancer therapy.

The number of IT methotrexate doses was not associated with any measures of motor function, and total dose of IV methotrexate received was associated only with visual motor integration at Follow-up 1 and 2. These findings suggest that there may be individual differences in susceptibility to chemotherapy-related CNS injury. Differences in susceptibility could be due to epigenetic changes in regulation of gene activity and expression involved in oxidative stress/oxidant defense that could be linked to diminished motor abilities among children with ALL. Future studies are also needed to characterize epigenetic factors that could identify children with ALL who are at greatest risk for fine and visual motor skills, as well as behavioral adjustment problems.

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New Knowledge Gained

- Children with leukemia are at risk for early onset and persistence of fine motor dexterity and visual-motor integration problems that appear to be related to CNS neurotoxicity.
- Early identification of problems with fine motor dexterity and visual-motor integration may impact development of functional behavior
- Early motor symptoms and their relationships with CNS biomarkers of oxidative stress merit further investigation of the role of antioxidants in limiting the cascade of reactive oxygen species and peroxidation injury of lipid membranes.

Table 1

Participant demographic and clinical characteristics

	N	%	
Gender			
Male	40	44.9	
Female	49	55.1	
Race			
White	39	42.9	
non-White	50	57.1	
Ethnicity			
Hispanic	41	45.1	
Non-Hispanic	48	54.9	
Treatment Risk Stratum			
Standard	70	76.9	
High	19	20.9	
	M (SD)	Min	Max
Age at diagnosis	7.1 (3.6)	2.3	14.7
Age at Baseline evaluation (Post-Induction)	7.2 (3.5)	3.1	15.2
Age at Follow-up 1 evaluation (Continuation)	8.0 (3.3)	4.0	16.3
Age at Follow-up 2 evaluation (End of Therapy)	9.1 (3.1)	5.5	17.0
Maternal education (yrs)	13.4 (2.4)	7	20
Paternal education (yrs)	12.8 (2.9)	5	20
IV Methotrexate (g/M ²)	9.4 (10.9)	0.39	39.3
Number of IT injections	17.7 (2.8)	13	24

Table 2
Group Performance On Fine and Visual Motor Performance and Behavioral Outcome Measures.

Performance Measures ^a	Baseline evaluation (Post-Induction)		Follow-up 1 evaluation (Continuation)		Follow-up 2 evaluation (End of Therapy)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Fine Motor Dexterity						
Dominant hand	-1.19	-1.47, -0.91	-0.44	-0.68, -0.19	-0.39	-0.70, -0.09
non-Dominant hand	-1.16	-1.46, -0.86	-0.42	-0.67, -0.18	-0.20	-0.51, 0.10
Both Hands	-1.15	-1.45, -0.86	-0.57	-0.85, -0.29	-0.25	-0.58, 0.09
Visual Processing Speed	-0.24	-0.45, -0.03	-0.26	-0.50, -0.03	-0.03	-0.26, 0.21
Visual Motor Integration	-0.22	-0.42, -0.02	-0.40	-0.66, -0.13	-0.37	-0.57, -0.18
Spatial Construction	-0.16	-0.37, 0.05	-0.04	-0.32, 0.24	0.26	-0.05, 0.57
Spatial Perception	0.10	-0.16, 0.35	0.44	0.21, 0.67	0.21	0.01, 0.41
Behavioral Measures^b						
Hyperactivity	47.95	46.02, 49.88	47.64	45.39, 49.89	47.05	44.60, 49.51
Aggression	47.74	45.28, 50.21	47.52	45.20, 49.83	47.07	44.91, 49.24
Anxiety	52.53	49.88, 55.19	51.94	49.45, 54.43	51.36	48.31, 54.42
Depression	50.92	48.45, 53.39	50.09	47.90, 52.29	49.76	47.69, 51.84
Somatic Complaints	59.90	57.18, 62.62	57.84	55.04, 60.65	57.09	53.35, 60.84
Atypicality	48.26	46.11, 50.40	48.38	46.00, 50.75	48.00	45.80, 50.20
Withdrawal	51.71	49.05, 54.36	51.63	48.79, 54.46	50.95	47.90, 53.99
Attention Problems	47.97	45.79, 50.16	48.61	46.13, 51.09	47.91	45.44, 50.38
Social Skills	51.42	49.27, 53.56	51.81	49.22, 54.40	51.25	48.20, 54.31
Activities of Daily Living	51.39	48.92, 53.87	51.03	48.67, 53.40	50.31	47.53, 53.09
Functional Communication	50.42	48.32, 52.52	49.66	47.35, 51.96	50.36	47.94, 52.79

^a Fine motor dexterity and visual-motor performance scores presented in age-adjusted standard scores (population mean = 0, standard deviation = 1.0), with 95% confidence intervals (CI) for the participants. Lower scores reflect worse performance.

^b Behavioral ratings presented in age-adjusted standard scores (population mean = 50, standard deviation = 10), with 95% confidence intervals (CI) for the participants. Higher scores reflect more problematic behavior.

Table 3

Correlations among CSF Phosphatidylcholine Biomarkers and Fine Motor Dexterity

Peak Area				
Treatment Phase	Mean ^a	SD ^a	Follow-Up 1 (Continuation)	Follow-up 2 (End of Therapy)
Induction				
Oxidized PI	8.23	9.57	NS	− 0.24 [*]
Unoxidized PI	48.39	57.11	NS	−0.27 [*]
Post-Induction				
Oxidized PI	18.04	17.06	NS	−0.30 [*]
Unoxidized PI	112.96	103.69	NS	−0.32 [*]
Continuation				
Oxidized PI	8.88	8.25	−0.31 [*]	−0.34 [*]
Unoxidized PI	57.67	60.25	−0.32 [*]	NS

^aMean and SD/100,000^{*}Significant at $p < 0.05$

NS= Not significant

Table 4

Correlations between fine motor and visual- motor performance at Follow-up 1 and behavior ratings at the end-of-therapy.

Behavior Rating	Neurocognitive Performance		
	Dexterity	Visual Processing	Visual-Motor Integration
Hyperactivity	-0.35**	NS	-0.23*
Aggression	NS	NS	NS
Anxiety	-0.42**	NS	NS
Depression	NS	-NS	NS
Somatic Complaints	-0.34**	NS	NS
Atypicality	-0.54**	-0.23*	NS
Withdrawal	-0.26*	NS	-0.23*
Attention Problems	-0.349**	-0.29*	NS
Social Skills	NS	NS	NS
Activities of Daily Living	NS	0.22	NS
Functional Communication	0.24*	0.25*	0.30*

* significant at $p < .05$

** significant at $p < .01$