


The Association of Cytokine Levels With Cognitive Function in Children With Sickle Cell Disease and Normal MRI Studies of the Brain

Journal of Child Neurology
1-5
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DOI: 10.1177/0883073814563140
jcn.sagepub.com


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Abstract

Children with sickle cell disease, including those without evidence for cerebral infarcts, are at increased risk for cognitive deficits that can contribute to difficulties in academic and social functioning. Chronic inflammatory processes are endemic to sickle cell disease and are apparent in common comorbidities including asthma. Cytokines mediating inflammatory processes can influence cognition. The authors examined the relationship between plasma levels of cytokines commonly associated with asthma and cognitive functioning using standardized neuropsychological measures in 25 children with sickle cell disease with normal magnetic resonance imaging studies of the brain. Children with sickle cell disease performed significantly below the normative mean on tests of cognitive function. Pearson correlations indicated significant negative relations between cytokines (IL-4, IL-5, IL-8, and IL-13) and standardized tests of executive function ($r = -.54$ to $-.74$). Preliminary evidence suggests an association between cytokine levels and executive function in children with sickle cell disease, indicating a potential role for inflammatory processes in cognitive outcomes in these children.

Keywords

sickle cell disease, executive function, cytokines, asthma, inflammation

Received July 11, 2014. Received revised October 6, 2014. Accepted for publication November 11, 2014.

Sickle cell disease is a progressive genetic disorder, occurring in approximately 1 in 2400 American births and 1 in 400 African American births.¹ All major organ systems, including the central nervous system are affected.²

Related to impairment in central nervous system development, children with sickle cell disease are at increased risk for cognitive deficits that are associated with difficulties in academic and social functioning.³ For example, Hijmans et al⁴ found significant impairments in overall IQ and domains of executive functioning in children with sickle cell disease compared to matched controls. While this neuropsychological profile is related to overt and silent strokes, children with sickle cell disease with normal magnetic resonance imaging (MRI) study of the brain often display similar deficits.⁵ Despite evidence that cognitive deficits are present with and without cerebral infarcts, little is known about the biological pathways that impact cognitive functioning in children with sickle cell disease.

Chronic inflammatory processes are endemic to sickle cell disease⁶ and are apparent in common comorbidities including asthma. These inflammatory processes are 1 possible pathway underlying cognitive deficits associated with sickle cell

disease, particularly for those without evidence of cerebral infarcts. Cytokines mediating inflammatory processes play an important role in cognition through effects on synaptic

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plasticity, neurogenesis, and neuromodulation.⁷ Direct cytokine administration negatively impacts cognition,⁸ suggesting that immunologic responses to inflammation can be an additional mechanism for cognitive impairments in children with sickle cell disease.

Given the well-established observation that individuals with sickle cell disease have an increased level of plasma cytokines compared to individuals without sickle cell disease,⁹ using a convenience sample from 2 studies with this population, the authors performed analyses to determine if there is preliminary support for the hypothesis that cognitive functioning in children with sickle cell disease and normal MRI examinations of the brain would be associated with cytokine levels commonly elevated in asthma. These cytokines (IL-4, 5, 8, and 13) mediate T helper type 2 response known to be associated with vascular inflammation that extends to neural tissues, promotes brain aging, and negatively impacts cognition.^{10,11} This study thus represents the first examination, to our knowledge, of a possible link between inflammatory markers and cognitive abilities and suggests a potential mechanism for deficits that have remained unexplained in this population.

Methods

Protocol

The Washington University School of Medicine Human Research Protection Office approved the protocol for this study. Written informed consent was obtained from all participating children and parents. Neurocognitive testing was conducted by a trained examiner, and tests were administered in a fixed order to all participants. Participants in the study consented to participate in Sleep and Asthma Cohort Study at Washington University designed to assess the influence of asthma and sleep disordered breathing on sickle-cell-disease-related morbidity. Participants were also enrolled in Cognition of Children with Sickle Cell Anemia, a study at Washington University School of Medicine designed to assess the cognitive functioning of individuals with a normal MRI study of the brain. Thus, the participants represent a convenience sample of individuals that participated in both studies simultaneously.

Ethylenediaminetetraacetic acid (EDTA) plasma containing Sigma P-8340 protease inhibitor (10 uL P-8340 per ml of plasma) was analyzed for interleukins-4, -5, -8, and -13 (IL-4, -5, -8, -13). Cytokine analyses were performed using Luminex xMAP technology (Luminex Corporation, Austin, TX, USA). Plasma concentrations of IL-8 were determined using the Milliplex MAP Human Cardiovascular Disease 3 panel (Millipore, Billerica, MA, USA). IL-4, IL-5, and IL-13 were measured using the Milliplex MAP High Sensitivity Human Cytokine panel (Millipore). All analyses were performed according to the manufacturer's recommendations. These cytokines were selected because they have been implicated in inflammatory processes in children with sickle cell disease and other comorbidities, including asthma,¹² and mediate a T helper 2 response involved in neurovascular inflammation and cognitive decline.^{10,11} MRI studies of the brains were performed as part of routine care prior to cognitive testing.

Measures

Full-scale, verbal, and nonverbal IQ were obtained using the Wechsler Abbreviated Scale of Intelligence,¹³ a well-validated measure of

verbal, performance, and full-scale IQ for individuals age 6 to 89 years. Verbal IQ was estimated using the Vocabulary and Similarities subtests, and nonverbal IQ was estimated using the Block Design and Matrix Reasoning subtests. These 4 subtests have adequate reliability for estimates of full-scale IQ ($\alpha = .98$).

Four tests of the Delis-Kaplan Executive Function System¹⁴ were used to assess several aspects of executive function, including mental flexibility, inhibition, and fluency. In the Trailmaking Letter-Number Sequencing Test, a child must manually connect letters and numbers printed on a page in order while switching between numbers and letters (eg, A-1-B-2-C, etc). In the Color Word Interference Test, a child must state the ink color of words printed in incongruously colored ink. The Card Sorting Test requires a child to sort a set of objects into as many different categories as possible in a given period. The Verbal Fluency Test requires a child to verbally state as many words either beginning with a particular letter or from a particular category in a limited time.

Sample scores on neurocognitive tests were compared to published normative data. For the Wechsler Abbreviated Scale of Intelligence, the standardization sample includes 1100 children age 6 to 16 years proportionate to the US census population by geographic region, gender, educational level, and race/ethnicity.¹³ For the Delis-Kaplan Executive Function System, the standardization sample includes 875 children age 8 to 19 years demographically and regionally matched to the US census population.¹⁴

Results

Data from 25 children (13 males; age mean = 11.4 years, SD = 2.7 years) with sickle cell disease were analyzed. All were African American. Mean yearly family household income was 39 100 USD (SD = 30 290 USD), and mean level of formal education attained by the head of the household was 14.8 years (SD = 2.4 years). Sixteen participants (64%) had a diagnosis of asthma. All participants' sickle cell disease condition was stable at the time all neurocognitive assessments were conducted. Participants with asthma did not differ significantly from participants without asthma diagnoses on any demographic variable measured.

As expected in individuals with sickle cell disease, levels indicate mild to moderate increases in plasma cytokine levels when compared to normal controls (see Table 1). Analyses revealed no significant differences between participants with and without asthma diagnoses in plasma cytokine levels measured (see Figure 1). As such, use of the total sample of children with sickle cell disease ($n = 25$) was justified for all further analyses. Wechsler Abbreviated Scale of Intelligence scores indicated that the sample was significantly below the normative population mean and exhibited adequate variability on this cognitive measure (see Table 2). Scores on tests of executive function indicated that the sample was significantly below the normative population mean on select tasks, but exhibited adequate variability on all cognitive measures (see Table 2). These results are consistent with those of previous studies indicating deficits in overall cognitive and executive function abilities in children with sickle cell disease.⁴

An overall pattern of negative associations was observed between performance on cognitive measures and plasma

Table 1. Summary of Plasma Cytokine Levels in pg/mL (n = 25).

Cytokine	Mean (SD)	Median	Range	Normative levels
IL-4	10.2 (12.5)	6.41	0.13-52.4	<1.2 ^a
IL-5	0.3 (0.5)	0.11	0-2.4	<3.0 ^b
IL-8	2.1 (2.1)	1.92	0-8.6	<5.8 ^b
IL-13	2.3 (2.5)	1.44	0-8.2	<8.0 ^a

^aReference for normative values for IL-4, IL-5, IL-13.¹⁵

^bReference for normative values for IL-8.¹⁶

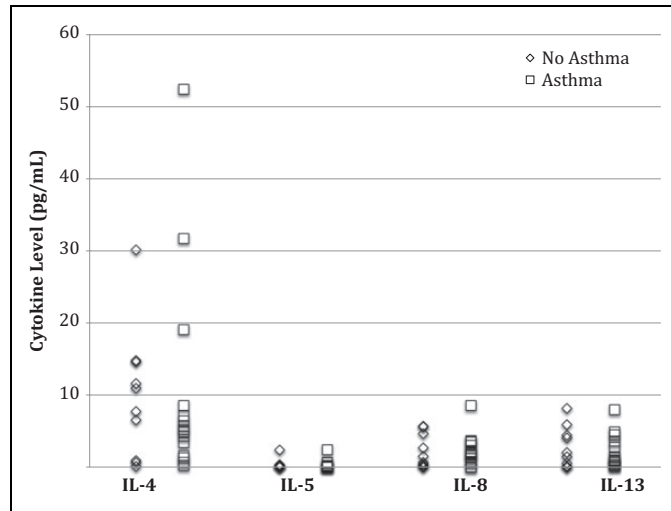


Figure 1. Cytokine levels (pg/mL) by asthma diagnosis.

Table 2. Summary of Cognitive Results Compared to Population Normative Data (n = 25).

Measure	Mean (SD)	Cohen's d
WASI full-scale IQ	91.2 (12.2)*	.59
WASI verbal IQ	93.4 (12.9)*	.44
WASI performance IQ	90.7 (12.6)*	.62
D-KEFS Trailmaking Number-Letter Sequencing	39.9 (13.8)*	.67
D-KEFS Color Word Interference	44.6 (11.3)*	.36
D-KEFS Card Sorting—Total Correct Sorts	48.6 (10.2)	.09
D-KEFS Verbal Fluency—Total Correct	51.8 (10.3)	.12

Abbreviations: D-KEFS, Delis-Kaplan Executive Function System, results presented as T scores; WASI, Wechsler Abbreviated Scale of Intelligence, results presented as standard scores.

*Significantly below normative sample mean with Bonferroni correction for multiple (ie, 7) comparisons, $P < .05$.

cytokine levels (see Table 3). Significant ($P < .05$) associations were found between plasma cytokine levels and measures of executive function (see Figure 2). IL-8 was significantly negatively correlated with performance on the Color Word Interference Test ($r = -.59, P = .027$) and confirmed correct sorts on the Card Sorting Test ($r = -.54, P = .045$). IL-5 was significantly negatively correlated with the confirmed correct sorts on the Card Sorting Test ($r = -.74, P = .002$). In addition, IL-4 ($r = -.61, P = .02$) and IL-13 ($r = -.59, P = .028$) were

Table 3. Pearson Correlations Between Plasma Cytokine Levels and Performance on Cognitive Measures.

	FSIQ	VIQ	PIQ	Trails	Color-Word	Sorting	Fluency
IL-4	-0.30	-0.35 [†]	-0.14	-0.07	-0.07	-0.14	-0.61*
IL-5	-0.32	-0.38 [†]	-0.14	-0.47 [†]	-0.47 [†]	-0.74**	-0.28
IL-8	-0.28	-0.33 [†]	-0.13	-0.1	-0.59*	-0.54*	-0.21
IL-13	-0.29	-0.35 [†]	-0.12	0.03	0.03	-0.12	-0.59*

Abbreviations: Color-Word, Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference Test; Fluency, D-KEFS Verbal Fluency Test; FSIQ, Wechsler Abbreviated Scale of Intelligence (WASI) full-scale IQ; PIQ, WASI performance IQ; Sorting, D-KEFS Card Sorting Test Total Correct Sorts; Trails, D-KEFS Trailmaking Letter-Number Sequencing Test; VIQ, WASI verbal IQ.

[†] $P < .10$. * $P < .05$. ** $P < .01$ (remains significant with Bonferroni correction).

significantly negatively correlated with performance on the Verbal Fluency Test. No other significant correlations between cytokine levels and scores on measures of executive function were obtained.

Discussion

Despite having no evidence of cerebral infarcts, many children with sickle cell disease have lower global IQ and cognitive function when compared to sibling controls without sickle cell disease, which can negatively impact school performance and psychosocial function.¹⁷ Several explanations can be offered to explain these deficits, such as injury below the resolution of the MRI scanner or chronic anemia;¹³ however, no study has explored the possibility that chronic inflammatory processes related to sickle cell disease and common comorbidities such as asthma can be associated with poor cognitive performance. Behavioral measures of cognitive and executive function, including verbal and nonverbal abilities, mental flexibility, inhibition, and verbal fluency, were examined in relation to plasma levels of IL-4, 5, 8, and 13. As hypothesized, significant negative correlations were found between levels of cytokines measured in this study and several indices of executive function abilities.

While this study was limited by a relatively small sample size, it provides initial evidence for 1 potential mechanism for the diminished cognitive abilities that have been noted in children with sickle cell disease who have not experienced overt or silent infarcts. That is, neurovascular inflammation related to interleukin-derived T helper type 2 activation can negatively impact cognition.^{10,11} Although the observed trends suggest an association, several cytokines measured (ie, IL-5, IL-8, and IL-13) were not significantly elevated with respect to the normative reference range for these markers. While IL-4 was more clearly elevated, it is of note that this study depended on published normative reference ranges, and future work should ideally employ cytokine control ranges from childhood disease populations to optimize contextualization of findings. Additional research is necessary to determine the biological importance of the IL-4 elevation observed and its unique relation to cytokine status. While this elevation is potentially causally

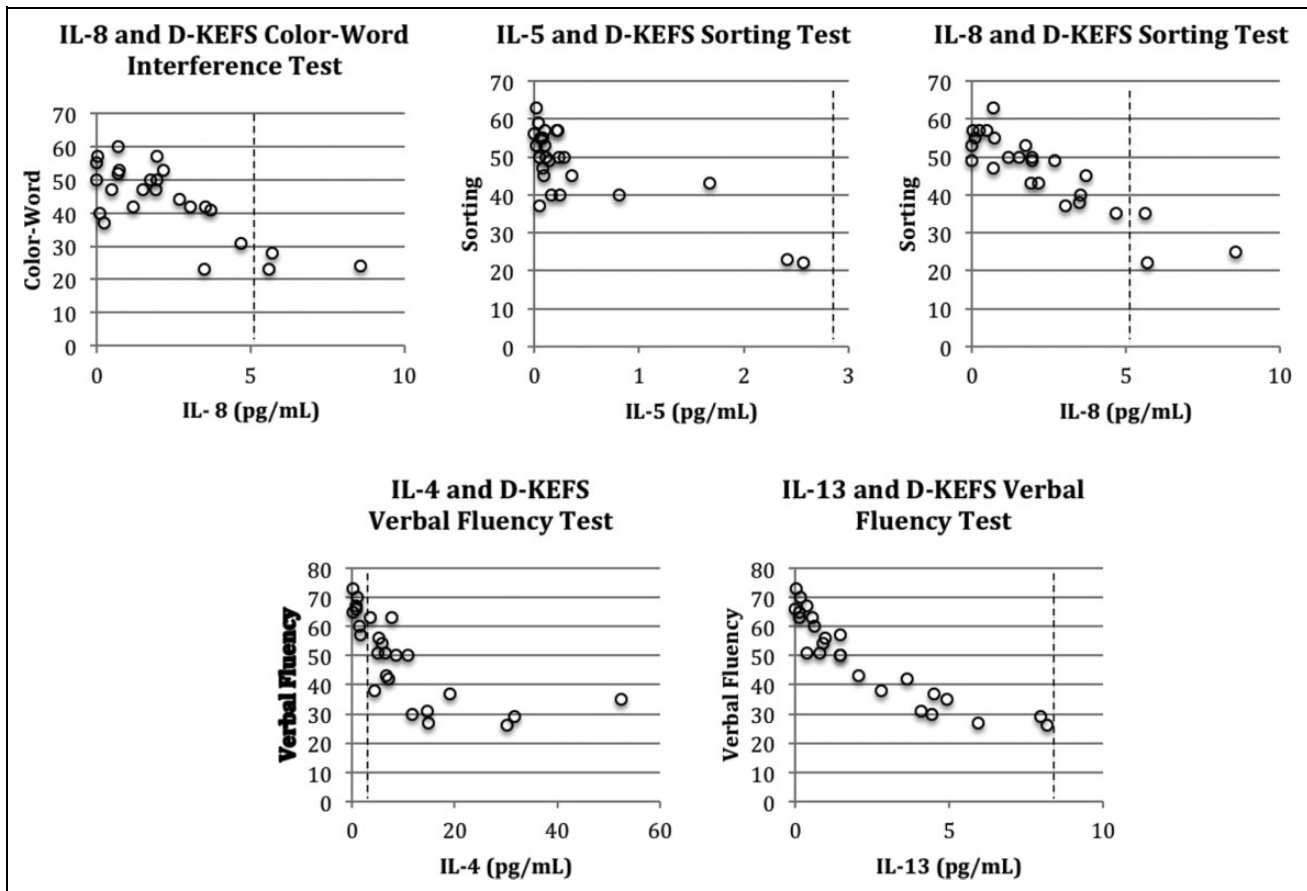


Figure 2. Scatterplots of cytokine levels against cognitive test scores. Dashed lines indicate upper limit of normative reference ranges.

involved in the noted cognitive alterations, it is also therapeutically conceivable that cytokine elevations could be secondary to neuronal damage or vascular damage or even involved in neural repair. Additional work that more clearly elucidates the function of these inflammatory markers will be required to fully understand the associations observed in this study. As such, clear conclusions cannot be made, but the trends of results are unique and hypothesis-generating for future studies, which can examine other inflammatory markers more strongly linked to cognition (eg, IL-6).

The specific cytokine levels signifying inflammatory responses measured in this study have been associated with asthma, a frequent comorbid condition in children with sickle cell disease. Although a significant relation between asthma diagnosis and cytokine levels was not found in the current study, the limited sample size might have led to a lack of the required power to detect such an association given expected overall elevations in cytokine levels in sickle cell disease. In addition, cytokine levels were measured in plasma, not CSF, and might thus have been affected by comorbid medical issues endemic to the population studied (eg, systemic disease, bacterial infection). However, the consistent pattern of negative associations between cytokine levels and cognitive performance observed despite limited power underscores the potential role of inflammatory processes endemic to sickle cell disease and

its comorbidities in deleterious neurocognitive sequelae. Medical approaches currently in use can be further developed to prevent vaso-occlusive episodes and other physiological events directly related to inflammation. In addition, mental health interventions can also be indicated to improve patients' abilities to cope with the stress of chronic illness and psychosocial difficulties common to this population.

Acknowledgements

Data collection performed as part of the Sleep and Asthma Cohort Study at Washington University and the Cognition of Children with Sickle Cell Anemia at Washington University School of Medicine, St. Louis, MO.

Author Contributions

CA performed all data analyses and prepared the first draft of the manuscript. AAK assisted with design of parent studies and collected data. EM collected and entered data and oversaw study operations. BEC performed data analyses and edited the manuscript. MRD designed the parent studies, oversaw data collection of parent studies, and edited the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by funding from the Burroughs Wellcome Foundation, the Doris Duke Charitable Foundation, and grant R01-HL079937 from the National Institutes of Health.

Ethical Approval

The Washington University School of Medicine Human Research Protection Office approved the protocol for this study. Written informed consent was obtained from all participating children and parents.

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