

# High Plasma Soluble CD163 During Infancy Is a Marker for Neurocognitive Outcomes in Early-Treated HIV-Infected Children

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**Background:** Monocyte activation may contribute to neuronal injury in aviremic HIV-infected adults; data are lacking in children. We examined the relation between monocyte activation markers and early and long-term neurodevelopmental outcomes in early-treated HIV-infected children.

**Setting:** Prospective study of infant and child neurodevelopmental outcomes nested within a randomized clinical trial (NCT00428116) and extended cohort study in Kenya.

**Methods:** HIV-infected infants (N = 67) initiated antiretroviral therapy (ART) at age <5 months. Plasma soluble (s) CD163 (sCD163), sCD14, and neopterin were measured before ART (entry) and 6 months later. Milestone attainment was ascertained monthly during 24 months, and neuropsychological tests were performed at 5.8–8.2 years after initiation of ART (N = 27). The relationship between neurodevelopment and sCD163, sCD14, and neopterin at entry and 6 months after ART was assessed using Cox proportional hazards models and linear regression.

**Results:** Infants with high entry sCD163 had unexpected earlier attainment of supported sitting (5 vs 6 months;  $P = 0.006$ ) and supported walking (10 vs 12 months;  $P = 0.02$ ) with trends in adjusted analysis. Infants with high 6-month post-ART sCD163 attained speech later (17 vs 15 months;  $P = 0.006$ ; adjusted hazard ratio, 0.47;  $P = 0.02$ ), threw toys later (18 vs 17 months;  $P = 0.01$ ; adjusted hazard ratio, 0.53;  $P = 0.04$ ), and at median 6.8 years after ART, had worse neuropsychological test scores (adj. mean Z-score differences, cognition,  $-0.42$ ;  $P = 0.07$ ; short-term memory,  $-0.52$ ;  $P = 0.08$ ; nonverbal test performance,  $-0.39$ ,  $P = 0.05$ ).

**Conclusions:** Before ART, monocyte activation may reflect transient neuroprotective mechanisms in infants. After ART and viral suppression, monocyte activation may predict worse short- and long-term neurodevelopment outcomes.

**Key Words:** monocyte, neurocognitive, antiretroviral, perinatal HIV, CD163, neurodevelopment

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## BACKGROUND

In 2018, there were an estimated 1.6 million HIV-infected children aged younger than 15 years, most of whom

were in sub-Saharan Africa.<sup>1</sup> HIV-infected children have higher risk of neurodevelopmental delay<sup>2</sup> and neurocognitive deficits.<sup>3,4</sup> Neurocognitive deficits may not be reversible by antiretroviral therapy (ART)<sup>5,6</sup> and may persist even in infants who begin treatment as early as 5 months of age.<sup>7</sup>

The precise mechanisms of HIV-related neuropathogenesis are not well defined. However, HIV-infected CD4<sup>+</sup> T lymphocytes or monocytes likely cross the blood–brain barrier into the central nervous system (CNS).<sup>8</sup> HIV replication triggers local inflammation and neuronal damage. HIV-mediated local inflammation increases the permeability of the blood–brain barrier<sup>9</sup> and promotes recruitment of additional target cells from the periphery.<sup>8</sup> Monocytes and their cellular derivatives are important for CNS HIV entry and replication and subsequent neuronal injury.<sup>8</sup> HIV can be detected in perivascular macrophages and microglia in postmortem brain tissue.<sup>10,11</sup> Brain viral isolates replicate well in cultured macrophages,<sup>8</sup> and HIV-infected monocyte-derived macrophages produce neurotoxic factors *in vitro*.<sup>12,13</sup> Adults with high cerebrospinal fluid (CSF) levels of monocyte and macrophage activation markers such as neopterin, sCD14, and sCD163 have higher concentrations of CSF HIV RNA,<sup>14</sup> neurofilament light chain, a soluble marker of neuronal injury,<sup>15–17</sup> and lower neuropsychological test (NPT) performance.<sup>18</sup>

Monocyte expansion and activation in the periphery may contribute to the CNS viral reservoir and exacerbate local CNS inflammation. Adults with HIV-associated dementia had higher circulating concentrations of mature CD14<sup>+</sup>CD16<sup>+</sup> monocytes than those with no dementia, and these cells were neurotoxic *in vitro*.<sup>19</sup> Separately, CD14<sup>+</sup>CD16<sup>+</sup> cells accumulated in brain perivascular spaces and colocalized with p24 antigen in adults with HIV encephalopathy.<sup>20</sup> Adults with higher HIV DNA concentrations in peripheral blood CD14<sup>+</sup> cells have worse cognitive outcomes, including dementia, and higher levels of markers for neuronal injury and CSF inflammation.<sup>21–23</sup> Data for these processes in HIV-infected children are more limited and contradictory; one study noted higher peripheral monocyte concentration and percentages in children (mean age of 2.7 years) with HIV encephalopathy.<sup>24</sup> By contrast, treated and untreated children (median age of 6 years) with higher percentage of activated (CD14<sup>+</sup>/CD16<sup>+</sup>/HLA-DR<sup>+</sup>) monocytes and nonclassical (CD14<sup>low</sup>/CD16<sup>+</sup>) monocytes had higher NPT performance.<sup>25</sup>

CD163 is a hemoglobin scavenger receptor expressed only by monocytes and macrophages, and it is shed in response to innate immune activation.<sup>26,27</sup> The CD14<sup>bright</sup>CD16<sup>+</sup> monocyte subpopulation comprises a more mature phenotype and expresses particularly high levels of CD163.<sup>28</sup> Similarly, CD14 is a receptor for lipopolysaccharide, and it is also shed during innate immune activation.<sup>29</sup> Adults with high plasma soluble CD14 (sCD14) and soluble CD163 (sCD163) concentrations have worse neurocognitive outcomes,<sup>18,30–33</sup> cerebral atrophy and neuronal injury,<sup>34</sup> lower synaptophysin and microtubule associated protein 2 (suggesting synaptodendritic damage), and microglial activation.<sup>35</sup> Individuals with high plasma sCD14 and high sCD163 also have worse NPT performance, despite viral

suppression on ART.<sup>33,36</sup> Neopterin is produced by activated macrophages and is a CSF marker for CNS macrophage activation.<sup>37</sup> Adults with elevated CSF neopterin have higher CSF HIV RNA<sup>14</sup> and poorer NPT performance.<sup>37</sup> Adults with higher CSF neopterin and sCD14 concentrations have higher plasma neopterin and sCD14, respectively.<sup>16,18,37</sup>

In this study of HIV-infected Kenyan children who began ART before 5 months of age, we evaluated the relationship between measures of monocyte and macrophage activation in plasma, before ART initiation and after 6 months of ART, and infant neurodevelopmental and school-age neurocognition. We hypothesized that infants with higher plasma concentration of markers of monocyte and macrophage activation would attain neurodevelopmental milestones later and would have worse neurocognitive function at school age, despite low plasma virus level.

## METHODS

### Study Population

This study included children enrolled as infants in a randomized trial involving early ART initiation and treatment for 24 months in both arms, then randomization to continued versus interrupted treatment (NCT00428116).<sup>38</sup> Briefly, HIV-infected infants were enrolled after routine HIV screening (2007–2009). Entry criteria were confirmation of HIV infection, age <5 months, and no previous ART except for prophylaxis for prevention of mother-to-child transmission (PMTCT). Infants started ART within 2 weeks after study entry. Infants received ritonavir-boosted lopinavir (if previously exposed to nevirapine) or nevirapine combined with lamivudine and zidovudine; infants enrolling later received abacavir instead of zidovudine. Each study visit included ascertainment of growth parameters. Blood samples were collected at entry, then every 3 months after ART initiation. Infants remained in monthly follow-up for the randomized trial for up to 42 months and then were invited to participate in extended quarterly follow-up for 60 additional months. Of 140 infants in the original trial, 41 were excluded from this analysis because they enrolled at an older age, limiting milestone ascertainment. Thirty-two infants had no available specimen for biomarker assays at entry and 6 months (29 were lost, died or withdrew before 6 months and 3 remained on study but had no specimen at 6 months) (see Figure 1, Supplemental Digital Content, <http://links.lww.com/QAI/B280>). This report includes data from 67 infants with biomarker data for at least 1 visit during the first 6 months of ART (entry or 6 months or both). Caregivers of study participants provided written informed consent and the University of Washington and the University of Nairobi/Kenyatta National Hospital Institutional Review Boards each provided ethical approval for this study.

### Laboratory

CD4<sup>+</sup> T-cell count and percentage and plasma HIV RNA were ascertained as described.<sup>38</sup> Plasma concentrations of sCD14, sCD163, and neopterin were assessed using

**TABLE 1.** Entry Characteristics of HIV-Infected Infants Initiating ART

Characteristic	N	Median (IQR) or N (%)
Infant		
Age (mo)	67	3.8 (3.1, 4.0)
Male	67	31 (46.3)
Clinical and growth		
WHO stage 3 or 4 diagnosis	67	30 (44.8)
CD4 percentage (%)	67	19 (14, 25)
Plasma HIV RNA log <sub>10</sub> copies/mL	63	6.6 (6.0, 7.0)
Previously hospitalized	67	38 (56.7)
Received antiretrovirals for PMTCT	58	32 (55.2)
Growth		
WAZ	67	-2.29 (-3.59, -1.03)
HAZ	67	-2.01 (-3.05, -0.94)
WHZ	67	-0.58 (-1.71, 0.75)
Primary caregiver characteristics		
Age (yr)	65	25 (22, 30)
Married	67	53 (79.1)
Education (yr)	59	8 (8, 11)
Maternal CD4 count (cells/ $\mu$ L)	64	344 (190, 481)

HAZ, height-for-age Z-score; WHZ, weight-for-height Z-score; PMTCT, prevention of mother-to-child transmission of HIV.

commercially available kits (sCD14 and sCD163, R&D Systems, Inc., Minneapolis, MN; neopterin, GenWay Biotech Inc., San Diego, CA).

### Neurodevelopmental Milestone Attainment

Neurodevelopmental milestone attainment was assessed as described<sup>39</sup> at entry and monthly visits by caregiver self-report and clinician observation for 24 months. Milestones were selected and adapted based on items in the Denver Developmental Screening Test.<sup>40</sup> Briefly, these included sitting with support (defined as sitting with a straight back on the caregiver's lap or on the floor between the caregiver's legs), sitting unsupported (defined as sitting on a flat surface or on the caregiver's lap without needing support), walking with support (defined as walking well with the help of someone holding one or both hands or using furniture or a wall), walking unsupported (walking at least a few steps unsupported) monosyllabic speech (defined as saying 1-syllable words or sounds, referring to a specific person or object), throwing toys (defined as throwing a toy such as a ball while playing), and naming objects defined as pointing to and naming common household items). For missing milestone data, the age at attainment was calculated by subtracting the date of birth from the date of the visit at which the milestone was first observed by study clinicians.

### Neuropsychological Assessments

All children in follow-up through age 6.1–8.5 years underwent the same NPT testing battery. The battery included the Kaufman Assessment Battery for Children 2nd edition

(KABC) (global cognitive ability, visual-spatial processing, short-term memory, learning, delayed memory, and nonverbal test performance), the Behavior Rating Inventory of Executive Functioning (BRIEF) (executive function), the Visual Test of Variables of Attention (attention), and Bruininks-Oseretsky Test of Motor Proficiency 2nd edition Brief Form (motor) (see Table 2, Supplemental Digital Content, <http://links.lww.com/QAI/B280>). Raw scores for each domain or scale were scaled and standardized as percentiles using US norms.

Assessments were conducted by study staff with either bachelor's degree or graduate level training in psychology. Test administration instructions were translated to Kiswahili and back-translated to ensure accuracy. Tests were administered in the child's preferred language (Kiswahili or English). All testers were fluent in both languages. Testers received one-on-one training from a doctoral level neuropsychologist. Testers did not begin to test for the study until their performance of a full battery tests was deemed proficient by a trainer. Testers participated in periodic internal group, and external review of video recorded assessments for quality assurance.

### Statistical Methods

#### Cutoff Points for Dichotomous Variables

Dichotomous variables for biomarkers were prespecified based on overall median levels (for the entry and 6-month timepoints) for sCD163 and sCD14 (sCD163, >1100 ng/mL; sCD14, >3700 ng/mL). Two cutoffs were required for neopterin to allow sufficient numbers within groups (median neopterin at entry, >35 nmol/L and median neopterin at follow-up, >20 nmol/L). Lower HIV RNA was defined as plasma HIV RNA <1000 copies per milliliter.

#### Cross-sectional Analysis of Cofactors for Biomarker Levels at Entry

Two-sample t tests and  $\chi^2$  tests, as appropriate, were performed to evaluate the relationship between entry plasma HIV RNA levels, CD4 T-cell percentage (CD4%), WHO disease stage, receipt of PMTCT, age at initiation of ART, and growth parameters [Z-scores for weight-for-age (WAZ), height-for-age, and weight-for-height] and dichotomous variables for each biomarker. Similar analyses were performed for biomarker concentrations as continuous variables.

#### Prospective Analysis of Biomarker Levels and Neurodevelopmental Outcomes

The primary analysis was designed to determine the association of activation markers after initiation of ART with subsequent neurodevelopmental outcomes. In addition to biomarkers after initiation of ART, biomarkers at entry (pre-ART) were assessed for associations with neurodevelopmental outcomes. Kaplan–Meier survival methods were used to calculate median age at milestone attainment for groups of infants defined by low vs high biomarker concentrations (as defined above) overall. Models stratified by low vs high plasma HIV RNA at 6 months after initiation of ART were also evaluated, based on our hypothesis that the relation

**TABLE 2.** Kaplan–Meier Analyses Comparing Age at Attainment of Milestones Between Infants With Low vs High sCD163, sCD14, and Neopterin at Entry and 6 Months After ART Initiation

Milestone	sCD163 (ng/mL)		sCD14 (ng/mL)		Neopterin (nM)	
	≤1100 at Entry, N = 16 [7]	>1100 at Entry, N = 26 [2]	≤3700 at Entry, N = 27 [6]	>3700 at Entry, N = 15 [3]	≤35 at Entry, N = 21 [4]	>35 at Entry, N = 21 [5]
Sitting supported	<b>6 (6, 8)</b>	<b>5 (4, 6)***</b>	5 (4, 6)	5 (5, 6)	4 (5, 6)	5 (5, 6)
Sitting unsupported	7 (7, 9)	6 (6, 7) [4]	7 (6, 7) [8]	6 (6, 8)	6 (6, 7)	7 (6, 8) [7]
Walking supported	<b>12 (12, 15) [8]</b>	<b>10 (9, 12) [5]*</b>	10 (9, 12) [8]	10 (9, 12) [5]	10 (9, 12)	11 (10, 13) [9]
Walking unsupported	14 (13, 17) [8]	15 (14, 17) [6]	14 (13, 17) [9]	15 (14, 17) [5]	14 (13, 16) [5]	17 (14, 17) [9]
	≤1100 at 6 mo, N = 29 [0]	>1100 at 6 mo, N = 22 [1]	≤3700 at 6 mo, N = 24 [1]	>3700 at 6 mo, N = 27 [0]	≤20 at 6 mo, N = 25 [0]	>20 at 6 mo, N = 25 [0]
Walking unsupported	15 (13, 16)	17 (13, 19) [2]	15 (14, 17)	15 (13, 19) [1]	15 (13, 20)	15 (13, 17) [1]
Monosyllabic speech	<b>15 (13, 16)</b>	<b>17 (15, 21) [3]***</b>	15 (13, 19) [2]	15 (14, 17) [1]	15 (13, 17)	15 (14, 17) [2]
Throwing toys	<b>17 (15, 18)</b>	<b>18 (17, 19)**</b>	16 (15, 18)	18 (17, 19)	17 (16, 19)	18 (16, 19)
Naming objects	21 (19, 24)	23 (20, 24) [4]	22 (19, 23) [2]	23 (20, 24) [2]	22 (19, 24)	22 (20, 24) [3]

Shown here is the median age (mos), (IQR). N, number at risk (number censored, except where noted). Bold indicates  $P \leq 0.05$ . \* $P = 0.02$ ; \*\* $P = 0.01$ ; \*\*\* $P = 0.006$  (all  $P$  values noted with asterisks retained significance using Benjamini–Hochberg procedure).

between monocyte activation and neurodevelopmental outcomes might differ by virological status. The relationship between age at milestone attainment and biomarker levels as dichotomous variables was also examined using Cox proportional hazards models, adjusted for 6-month plasma HIV RNA level, CD4%, and WAZ (each as continuous variables). Models including HIV RNA levels were evaluated first, and then models including CD4% were evaluated secondarily. Models including both HIV RNA level and CD4% together were not evaluated due to collinearity. Finally, the relationships between biomarkers as continuous variables and age at milestone attainment were evaluated using Cox proportional hazards models.

Univariable and multivariable linear regression models were used to evaluate the relationship between high vs low sCD163 levels at 6 months and neurocognitive domain-specific Z-scores at school age. Separate models adjusted for entry or 6-month HIV RNA level, CD4%, and WAZ, respectively, were evaluated, as described above.  $P$  values  $\leq 0.05$  were considered significant. To account for multiple comparisons, we used the Benjamini–Hochberg procedure and a false discovery rate of 0.25 as described.<sup>41,42</sup>

## RESULTS

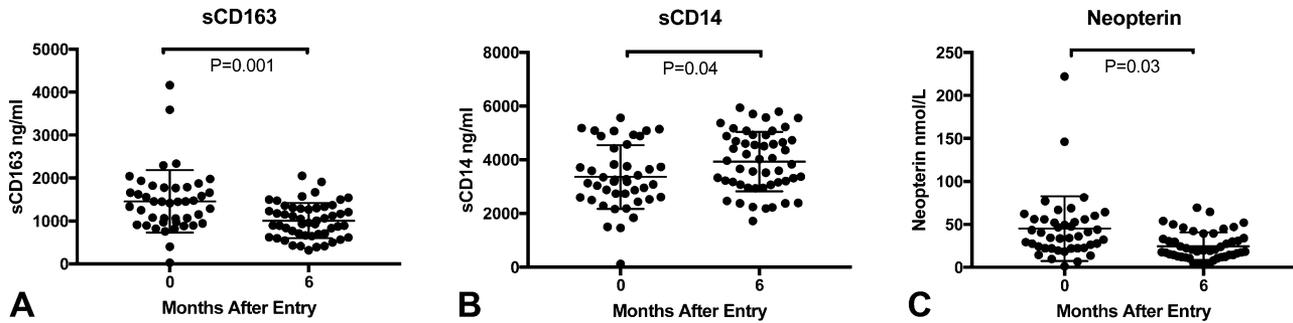
At entry, the median age of the infants was 3.8 months [interquartile range (IQR), 3.1 to 4.0], and 46.3% were male (Table 1). The median birth weight was 3.0 kg (IQR, 2.7 to 3.4). Most infants (87.8%) were ever breastfed. Nearly all infants (97.1%) were cared for by their biological mothers. Infants were generally undernourished with a median WAZ of  $-2.29$  (IQR,  $-3.59$  to  $-1.03$ ). Most were immunosuppressed, with a median CD4% of 19% (IQR, 14 to 25). More than half (55.2%) received antiretrovirals for PMTCT, administered to infants directly, to their mothers, or both.

Infants with high entry sCD14 concentration had higher entry plasma HIV RNA level (mean values, 6.95 vs 6.33

log10 copies per milliliter;  $P = 0.004$ ), and those with high entry neopterin had lower entry CD4% (means 17% vs 22%;  $P = 0.03$ ) than those with low entry sCD14 and low entry neopterin, respectively (see Table 3, Supplemental Digital Content, <http://links.lww.com/QAI/B280>). In Kaplan–Meier analyses, infants with high entry plasma sCD163 concentration had earlier age at attainment of sitting supported and walking supported (median differences in age, sitting supported, 1 month;  $P = 0.006$ , and walking supported, 2 months;  $P = 0.02$ ) (Table 2). Trends for these association persisted in multivariable models adjusted for plasma HIV RNA level and WAZ at entry (see Table 4, Supplemental Digital Content, <http://links.lww.com/QAI/B280>).

Twenty-six infants had biomarker data at entry and 6 months after starting ART. Monocyte activation levels changed significantly from entry to 6 months after ART initiation. Soluble CD163 and neopterin decreased (mean differences,  $-530$  ng/mL,  $P = 0.001$  and  $-11.26$  ng/mL,  $P = 0.03$ , respectively), whereas sCD14 increased (mean difference, 707 ng/mL,  $P = 0.04$ ; Fig. 1).

We hypothesized that infants with high monocyte activation despite lower plasma virus would have poor neurodevelopmental outcomes. Of 67 infants with biomarker data, 51 had biomarker data at 6 months after initiation of ART. Overall, infants with high sCD163 at 6 months after initiating ART were older at achievement of milestones compared to those with low sCD163 (median differences in age, speech, 2 months;  $P = 0.006$  and throwing toys, 1 month;  $P = 0.01$ ) (Table 2). In stratified analyses, these differences were significant only for the subset of infants with 6-month HIV RNA  $< 1000$  copies per milliliter (median differences in age, speech, 2 months;  $P = 0.003$ ; throwing toys, 2 months;  $P = 0.02$ ) (see Table 5, Supplemental Digital Content, <http://links.lww.com/QAI/B280>). The subset lacking 6-month HIV RNA  $< 1000$  copies per milliliter had similar ages at attainment of milestones ( $P = 0.3$  and  $P = 0.5$ , respectively). In Cox proportional hazards models adjusted for 6-month plasma virus



P-values shown are for paired t-tests for 0 vs 6 months.

**FIGURE 1.** Concentration of plasma (A) sCD163, (B) sCD14, and (C) neopterin at entry and 6 months in HIV-infected infants initiating ART. Bars indicate mean values and SDs.

concentration and WAZ, infants with high 6-month sCD163 attained speech later (adjusted hazard ratio, 0.47;  $P = 0.02$ ) and threw toys later (adjusted hazard ratio, 0.53;  $P = 0.04$ ) (see Table 4, Supplemental Digital Content, <http://links.lww.com/QAI/B280>). Infants with high vs low sCD14 and neopterin had similar age at milestone attainment (Table 2). In analyses of soluble biomarker levels as continuous variables, there were no associations between biomarker levels and age at milestone attainment.

Twenty-seven of 67 children (40.2%) were followed for 5.8–8.2 years and underwent a detailed NPT between the ages of 6.1–8.5 years (see Figure 1, Supplemental Digital Content, <http://links.lww.com/QAI/B280>). Children who had NPTs had similar ages at attainment of neurodevelopmental milestones compared with children who did not have an assessment (all  $P$ -values  $>0.2$ ) (data not shown). For this subset, median age at initiation of ART was 3.9 (IQR, 3.4 to 4.3) months, and median entry CD4% was 18% (IQR, 14 to 23). At the time of NPT assessment, median age was 6.8 years (IQR, 6.3 to 7.4), and median CD4% and plasma virus level were 36% and 2.18  $\log_{10}$  copies per milliliter. All

children were diagnosed as having WHO Stage 1 disease, and their median WAZ was  $-0.79$ . Nine of 27 children had elevated sCD163 at 6 months after initiation of ART (during infancy). Compared to children with lower sCD163 at 6 months, these 9 children had worse global cognitive ability (unadj. mean Z-score differences,  $-0.45$ ;  $P = 0.05$ ), short-term memory ( $-0.63$ ;  $P = 0.06$ ), and nonverbal test performance ( $-0.41$ ;  $P = 0.04$ ) (Table 3 and Fig. 2). Results were similar in analyses adjusted for 6-month plasma virus level and WAZ (adj. mean differences, global cognitive ability,  $-0.42$ ;  $P = 0.07$ ; short-term memory,  $-0.52$ ;  $P = 0.08$ ; nonverbal test performance,  $-0.39$ ,  $P = 0.05$ ). Results for separate models adjusted for CD4% and WAZ were also similar (adj. mean differences, global cognitive ability,  $-0.47$ ,  $P = 0.04$ ; short-term memory,  $-0.63$ ,  $P = 0.06$ ; learning,  $-0.73$ ,  $P = 0.02$ ; nonverbal test performance,  $-0.37$ ,  $P = 0.07$ ; delayed recall,  $-0.77$ ,  $P = 0.03$ ).

## DISCUSSION

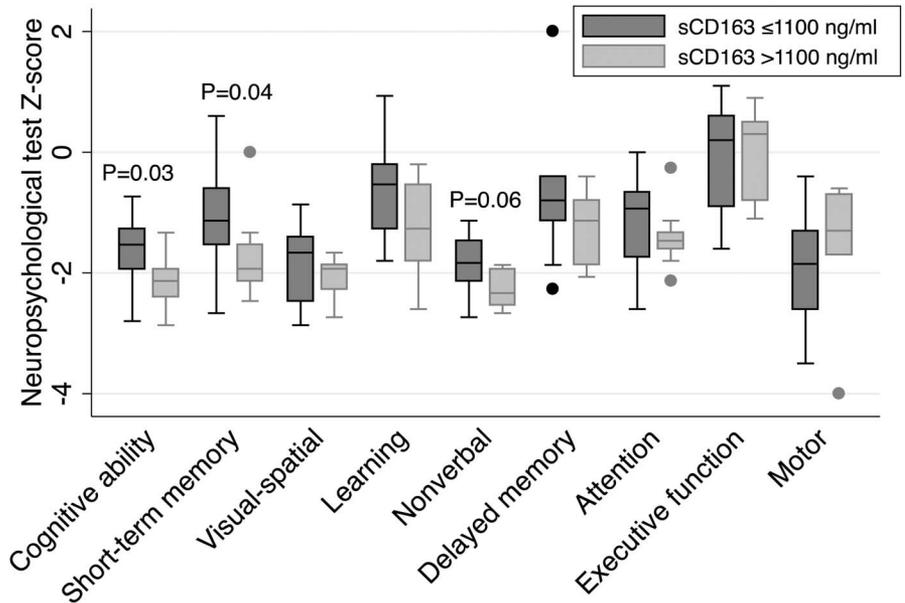
We examined plasma markers of peripheral monocyte and macrophage activation in relation to infant and school-age

**TABLE 3.** Linear Regression Analyses of Low vs High sCD163 at 6 Months After ART Initiation (Infancy) and School-Age Neurocognitive and Motor Scores

Domain	6 mo sCD163 >1100 ng/mL vs ≤1100 ng/mL					
	Unadjusted			Adjusted*		
	N	Mean Difference (95% CI)	P	N	Mean Difference (95% CI)	P
Global cognitive ability	27	-0.45 (-0.90 to -0.00)	0.05†	26	-0.42 (-0.86 to 0.027)	0.07†
Visual-spatial processing	27	-0.20 (-0.65 to 0.26)	0.4	26	-0.17 (-0.63 to 0.29)	0.5
Short-term memory	27	-0.63 (-1.31 to 0.04)	0.06†	26	-0.52 (-1.12 to 0.08)	0.08†
Learning	27	-0.56 (-1.22 to 0.11)	0.1†	26	-0.57 (-1.26 to 0.12)	0.1†
Delayed memory	21	-0.46 (-1.29 to 0.38)	0.3	21	-0.43 (-1.29 to 0.43)	0.3
Nonverbal test performance	27	-0.41 (-0.80 to -0.02)	0.04†	26	-0.39 (-0.78 to -0.00)	0.05†
Executive function	27	-0.24 (-1.00 to 0.51)	0.5	26	-0.19 (-1.02 to 0.62)	0.6
Attention	24	-0.28 (-0.89 to 0.34)	0.4	23	-0.31 (-0.89 to 0.26)	0.3
Motor	26	0.40 (-0.38 to 1.18)	0.3	25	0.42 (-0.37 to 1.20)	0.3

\*Adjusted for 6-month WAZ and plasma HIV RNA level.

†Retained significance using Benjamini–Hochberg procedure.  
CI, confidence interval.



**FIGURE 2.** Box plots comparing neurocognitive ability in HIV-infected children by high vs low sCD163 at 6 months after initiation of ART. Dark gray, low sCD163; light gray, high sCD163.

P values shown are for rank-sum tests. Only P values <0.1 are shown.

neurodevelopmental outcomes in HIV-infected children who initiated ART in infancy. Infants with elevated sCD163 6 months after initiating ART had later age at attainment of milestones. In stratified analyses, these associations persisted in the subset of infants with lower virus levels. Consistent with these findings, ART-treated adults with elevated sCD163 had neurocognitive impairment despite viral suppression.<sup>32,33</sup> Among the subset of infants who remained in follow-up through school age, those with elevated sCD163 at 6 months after ART initiation also had lower scores for neurocognitive ability and nonverbal skills, and trends for worse short-term memory and learning, suggesting long-lasting impacts of early monocyte activation in pediatric HIV.

The role of immune activation markers may be less clear in untreated infants. We observed that pretreatment immune activation was associated with better neurocognitive outcomes. This may reflect the need for a balance between effective immune activation necessary to mount immune responses before ART and damaging activation that is residual after initiation of ART. Soluble CD163 may be shed by peripheral CD14<sup>+</sup>CD16<sup>+</sup> monocytes<sup>43</sup> or monocyte subsets that have recently migrated from bone marrow<sup>44</sup> as part of response to reduce T-lymphocyte activation in the periphery<sup>43</sup> or an anti-inflammatory response in the brain.<sup>44</sup> Thus, elevated sCD163 in the periphery may reflect some protective mechanisms in addition to deleterious expansion of monocyte subsets, which may cause brain injury, as has been demonstrated in untreated simian immunodeficiency virus (SIV)-infected macaques.<sup>36,44</sup> Intriguingly, in uninfected neonate and infant macaques, peripheral monocyte turnover was significantly higher than in adult animals and then lowered to adult levels at approximately 3–4 months of age.<sup>45</sup> After SIV infection, monocyte turnover remained aberrantly high in both

neonate and infant animals,<sup>45</sup> suggesting HIV/SIV infection disrupts normal monocyte activity, which may otherwise be protective. In our study, age at HIV acquisition is unknown, since infants were <5 months of age at entry and HIV acquisition could have occurred late in utero, peripartum or during the postnatal period. Our findings of evidence of both protective and deleterious monocyte activation also mirror recent findings in HIV-infected adults during acute infection.<sup>46</sup> Adults with higher plasma sCD163 during early acute infection (Fiebig stages I and II) had better CNS outcomes (NPT scores), whereas adults with higher plasma sCD163 later in acute infection (Fiebig stage III) had worse CNS outcomes [lower markers of neuronal health (N-acetylaspartate) on brain imaging and lower NPT scores].<sup>46</sup>

Our data contrast with a previous study of late-diagnosed and treated school-aged children, which found that those with elevated subsets of activated monocytes after initiation of ART also had higher neurocognitive scores.<sup>25</sup> The authors of this previous study speculated that in long-term untreated HIV-infected children, immune activation may reflect protective mechanisms.<sup>25</sup> Altogether, these studies of adults, older children, and infants suggest that timing of infection and ART with respect to individual immune maturation may both influence the balance between damaging and protective immune function.

We did not find associations between either plasma concentrations of neopterin or sCD14 before or after ART initiation and neurodevelopmental outcomes. Soluble CD14 was higher in adults with neurocognitive impairment, cerebral atrophy and neuronal injury among subsets with advanced or untreated HIV,<sup>30,34</sup> and adults with viral suppression in 1 study<sup>33</sup> but not others.<sup>18,30,34</sup> In a previous analysis of infants in our cohort, infants had robust immune recovery after ART.<sup>47</sup> Plasma neopterin may reflect macrophage activation in tissues other than the CNS and thus may lack specificity for

neurodevelopmental outcomes. Expression of neopterin and sCD163 are also regulated by distinct cytokine pathways<sup>27,28,37</sup> and thus may exhibit differential predictive value for neurocognitive impairment across cohorts or compartments.

Before ART, infants in our cohort who had high sCD14 or neopterin had more severe HIV disease as characterized by low CD4% and high plasma HIV RNA level. These findings are consistent with a South African study of early-treated children, in whom higher sCD14 correlated with high viremia.<sup>48</sup> Infants in our study had significant declines in sCD163 and neopterin after ART consistent with previous studies in adults<sup>36,49</sup> and Thai/Cambodian HIV-infected children.<sup>25</sup> However, sCD14 levels did not decline after initiation of ART in our study, unlike previous studies of older children.<sup>50</sup> Upward trajectories of sCD14 after initiation of ART have been described in adults.<sup>51</sup>

Our study has several strengths. There have been few prospective studies of immune activation biomarkers in relation to neurodevelopmental outcomes in HIV-infected infants initiating ART, and to the best of our knowledge, only one other study evaluated biomarkers of monocyte and macrophage activation.<sup>24</sup> We evaluated 3 plausible markers of poor neurodevelopment in HIV, had frequent assessment of developmental milestones, and used detailed assessments for school-age neurocognitive functioning. Our study focused on early-treated infants followed for several years and thus enabled evaluation of school-age neurocognitive ability. This study was limited by small sample size and assessment of infant milestones relied partially on caregiver report. Data on gestational age of infants were not available, limiting ability to interpret age at milestone attainment and relationship between preterm birth or small for gestational age and monocyte/macrophage activation markers. The cutoffs used to define low vs high levels for each biomarker reflected medians for this cohort; however, these were prespecified and similar to levels in relevant cohorts.<sup>25,50,52</sup> Analyses of school-age outcomes were exploratory, and interpretation is limited by cohort attrition. In addition, we used a US norm to scale and standardize raw scores, limiting interpretation of Z-scores due to cultural differences. Given small numbers and the exploratory nature of these analyses, it will be important to further evaluate the associations presented here in other cohorts. Our findings do align with previous studies, suggesting sCD163 is an important marker for cognitive differences in HIV-infected individuals on ART and with well-controlled HIV.<sup>33,36</sup>

Based on our findings and previous work in adults and macaques,<sup>43,44</sup> we hypothesize that peripheral blood monocyte activation may signal future brain inflammation and injury in early treated HIV-infected children. High soluble monocyte activation markers may reflect expansion or maturation of specific subsets of monocytes that may traffic to the brain, potentially as part of an initial protective response, which eventually results in deleterious CNS inflammation. Therapeutics that may specifically dampen monocyte activation might offer an avenue for adjunctive treatment that may benefit cognitive outcomes in HIV-infected children.

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