Systemic monocyte activation levels and developmental milestone attainment in HIV-infected infants initiating antiretroviral therapy

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Abstract

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Background: Peripheral monocyte activation has been associated with poor neurocognitive outcomes in HIV-infected adults. We hypothesized that HIV infected infants with persistent systemic monocyte activation following antiretroviral treatment (ART) would have later attainment of developmental milestones.

Materials and Methods: HIV infected infants initiated ART at age <5 months as part of the Optimizing Pediatric HIV-1 Therapy Study (OPH03) (NCT00428116). Plasma soluble CD14 (sCD14), soluble CD163 (sCD163) and neopterin concentrations were measured using enzyme-linked immunosorbent assays pre-ART and at 6 months post-ART. Concentrations were dichotomized based on published studies with thresholds of 3,800ng/ml for sCD14, 1,100 ng/ml for sCD163, and 7.9ng/ml for neopterin. Ages of milestone attainment were reported by caregivers at serial monthly follow-up visits. Paired t-tests and linear regression models were

used to compare age of milestone attainment between infants with high versus low biomarker levels, at entry and at 6-months.

Results: Among 69 infants, median age at enrollment was 3.8 months. Pre-ART median plasma HIV RNA level was 6.55 $\log_{10}$ copies/ml, median CD4% was 18%, and median WAZ was -2.20. Mean sCD14 was 3,421ng/ml, sCD163: 1,385ng/ml and neopterin: 11.7ng/ml. At 6-months post-ART, sCD14 increased (mean, 4,114ng/ml; p=0.08); sCD163 decreased (mean 1,088; p<0.001), and neopterin decreased (mean 7.01; p=0.04). At baseline, high sCD14 was associated with plasma HIV RNA (p=0.02) and high neopterin with lower CD4% (p=0.003).

Infants with higher baseline sCD163 achieved earlier sitting (1.1 months earlier [95% confidence interval (CI) 0.3, 2.0 months); p=0.009], but had no differences in age at walking or speech. Infants with elevated sCD163 following 6 months ART had later speech (2 months later, p=0.03)], and this difference was more pronounced among the subset of infants with viral suppression [3.4 months later; 95% CI, 0.9, 5.9 months; p=0.01]. sCD14 and neopterin levels at baseline or 6-months were not associated with timing of milestone attainment.

Conclusion: sCD163 levels were significantly associated with timing of milestone attainment. Prior to ART, elevated sCD163 was associated with earlier milestones and may reflect potentially beneficial immune activation in the absence of ART. Elevated sCD163 in the setting of viral suppression on ART had the opposite effect on milestone attainment – perhaps reflecting potentially deleterious persistent immune activation.

Background

HIV infected children have higher risk of language impairment[1], learning disabilities and lower than normal cognitive scores.[2,3] There appear to be persistent developmental and neurologic delays in HIV infected children with or without antiretroviral treatment (ART).[4] Most children living with HIV reside in sub-Saharan Africa[5] and in this setting many children are not diagnosed until later in infection.[6] Untreated perinatal HIV can result in neurocognitive deficits that might not be reversible with ART alone.[7]

The precise mechanisms of HIV-related neuropathogenesis are poorly defined. A "Trojan Horse" model has been proposed, in which HIV crosses the blood-brain-barrier (BBB) to the central nervous system (CNS) early in the infection, through infected CD4+ cells monocytes and T cells, which incorporate the genome of the virus.[8] In contrast, in the "late invasion model", monocytes are infected with the virus during later chronic HIV infection and enter the CNS where they continue viral replication and increase inflammation.[9] Viral replication within the brain may not be sufficient to cause detrimental neurological effects.[10] Trafficking of HIV in the brain is associated with the presence of monocytes.[11] High levels of monocyte activation have been associated with poor HIV disease outcomes[12]. CD14+ monocytes are preferentially infected by HIV[13] and CD14 expression in the cerebral spinal fluid (CSF) has been associated with impaired neurocognitive outcomes in HIV infected adults.[14] Plasma sCD14 levels have been associated with risk of learning and attention impairments in adults with HIV.[15] CD163+ monocytes are hypothesized to be related to brain pathology in SIV and HIV[16] and have been associated with plasma HIV RNA and CD4 levels before and after initiation of ART, during the first year of infection.[17] Another marker of interest, neopterin, a macrophage activation marker, has been associated with plasma HIV RNA levels, and its presence in the CSF is related to HIV associated dementia and opportunistic CNS infections. [18,19]

One way to measure neuropathogenesis in children is to assess the attainment of developmental milestones. We conducted a prior study which demonstrated that later age of milestone attainment was associated with poor pre- and post-ART immune status as well as poor growth.[20]

In this study, we assessed associations of soluble CD14 (sCD14), soluble CD163 (sCD163) and neopterin at baseline and the relationship of these markers to children's subsequent age of attainment of the milestones.

Methods

Study population

Specimens were provided by a historical cohort of infants [Optimizing Pediatric HIV-1 Therapy 03 (OPH03)].[21] During the 2007 to 2009 routine HIV screening at Nairobi City Council Maternal

Child Health clinics, HIV infected infants were enrolled in the study. Enrollment criteria were detection and confirmation of HIV DNA in the blood, age <5 months and no previous history of ART (except for prevention of mother to child transmission PMTCT). Infants started receiving ART approximately 2 weeks after enrolment. Plasma samples from the participants were collected at enrolment, and then every 3 or 6 months for 24 months after ART initiation. The study included 69 infants that had biomarker data for at least one of the visits during the first year of ART as well as data on the attainment of at least 1 milestone.

Laboratory assays

Enzyme-linked Immunosorbent Assays (ELISA) were performed to measure the expression levels of sCD14, sCD163 and neopterin in the plasma samples. Commercially available kits were used to perform ELISAs. The kits were purchased from R&D systems for sCD14 and sCD163 and from Genway Biotech Inc for neopterin. The assays were carried out following the protocols provided by the companies. The detection limit for neopterin was 0.177ng/ml, for sCD163 0.613ng/ml and for sCD14 0.125ng/ml.

Milestone Attainment

Milestone attainment was assessed at baseline and monthly after enrolment. They were determined using a protocol adapted from the Denver Developmental Screening Test. The milestones of interest were neck support (infant could fully support their neck), sitting (infant could sit on a flat surface or on a lap without support), walking (child could walk a few steps unsupported) and, speech (child could form monosyllabic words or sounds and refer them to specific people or objects). The age of attainment was self-reported by caregiver at the visit and if this was not possible the age was calculated by subtracting the date of birth from the date of the visit where the milestone was first observed. For infants who had already achieved the earliest milestone (neck control), self-reported ages were used when possible and if not the enrolment date was used as proxy for the age calculation.

Statistical analysis

Median (50^{th} percentile) and interquartile range (IQR 25^{th} and 75^{th} percentiles), or frequency percentage were calculated for the baseline characteristics of interest for infants and their primary caregivers as well as the concentrations of sCD14, sCD163 and neopterin at baseline, 6 and 12 months. Two sample paired t-tests were performed to compare the mean concentration of each biomarker at baseline and 6 months after enrollment. Box and whiskers graphs were plotted to show the range of expression levels of each marker at enrolment, 6 and 12 months after ART initiation.

The expression levels of the biomarkers were converted to dichotomous variables based on cut off points found in the literature. For sCD14 low (≤3,800 ng/ml), high (>3,800ng/ml).[22] for sCD163 low (≤1,100 ng/ml), high (>1,100 ng/ml),[23] for neopterin low (≤7.9ng/ml), high (>7.9ng/ml).[24]

Two-sample t-tests and χ^2 tests were performed to compare the means of HIV disease indicators including HIV plasma RNA levels, CD4 cell percentage, growth measures (WHZ,

WAZ, HAZ) and WHO disease stage between participants with low and high levels of the biomarkers.

Logistic regression was used to determine association between plasma HIV RNA at ART initiation and biomarker concentration at 6 months post-ART. A secondary analysis was performed to assess the association between viral load at 6 months after ART and sCD14, sCD163 and neopterin expression levels at 12 months post-ART.

Univariate linear regression was used to determine the relationship between expression levels of sCD14, sCD163 and neopterin and age at attainment of each neuromotor milestone. 2-sample paired t-tests were also performed to compare the mean age of attainment of each milestone between groups of low and high concurrent expression of the biomarkers.

Data were additionally stratified by plasma HIV RNA (low <1000 copies/ml, high ≥1000 copies/ml) at 6 months after enrollment and 2-sample paired t-tests were performed to compare the differences in mean age at attainment of walking and speaking between low and high concentrations of the biomarkers. Box and whiskers and scatter plots were graphed to illustrate these differences.

Results

Baseline characteristics of HIV-infected infants

This study included 69 infants with biomarker and milestone achievement data (Table 1, Figure 1). The median age of the infants at study enrollment was 3.78 months (IQR 3.22, 4.01) and 33 of the infants (47.8%) were male. The majority of infants (87.7%) were breastfed at least once before enrollment. Thirty infants (43.5%) were diagnosed as WHO Stage III or IV at enrollment. The median CD4 percentage was 18% (IQR 14, 24) and the median plasma HIV RNA level was 6.55 $\log_{10}$ copies/ml (IQR 6.05, 6.97). Thirty-eight (55%) infants had been hospitalized at least once since birth before enrollment and 27 (39%) received prevention of mother-to-child transmission (PTMCT) antiretrovirals. The median birthweight was 3.0 kg (IQR 2.7, 3.4). The median weight for age z-score (WAZ) was -2.20 (IQR -3.51, -0.98). The majority of the primary caregivers, (97.1%) were biological mothers of the infants. The median age of the caregivers was 26 years (IQR 22, 30). The median number of years of education of the caregivers was 8 (IQR 8, 11) and most (79.7%) were married. The median CD4 cell count for the caregivers was 350 cells/ml (IQR 198, 484). 7 (10%) of the 69 mothers were on antiretrovirals at enrollment.

Monocyte/macrophage activation levels in infants prior to and during ART

Biomarker levels changed significantly from enrollment to 6 months after ART initiation. sCD14 expression increased whereas sCD163 and neopterin decreased. At enrollment the mean level of expression of sCD14 was 3,492ng/ml (95% CI 3,027, 3,956) and mean levels increased to 4,113ng/ml (95% CI 3,648, 4,579) at 6 months post-enrollment (p=0.08). Mean sCD163 at baseline was 1,483 ng/ml (95% CI 1,218, 1,750) and decreased to 1088 ng/ml (95% CI 918.8, 1,258) at 6 months after enrollment (p=0.0007). Mean neopterin decreased from 9.948 ng/ml (95% CI 7.714, 12.18) at baseline to 7.008ng/ml (95% CI 5.176, 8.841) (p=0.04) (Table 2,

Figure 2).

Monocyte/macrophage activation levels association with CD4 and HIV viral load, prior to ART

Infants with high baseline sCD14 had a higher plasma HIV RNA level (means, 6.95 vs 6.42 $\log_{10}$ copies/ml; p=0.02) (Table 3). Infants with higher baseline neopterin had lower mean CD4% (CD4% 14% vs 16% in infants with high vs. low neopterin, p=0.003). Levels of sCD163 were not associated with infant virological or immunological status. Neither WHO clinical stage nor indicators of nutritional status (WAZ, HAZ, and WHZ) were associated monocyte/macrophage activation levels.

Monocyte/macrophage activation pre-ART and age at developmental milestone achievement

Infants with high levels of baseline sCD163 had an earlier mean age of sitting unsupported than infants with low baseline sCD163 (6.3 versus. 7.6 months, p=0.006). There were no significant differences in age of attainment of milestones between children with high and low baseline concentrations of sCD14 or neopterin (Table 4).

Monocyte/macrophage activation post-ART and milestone achievement

At 6 months post-ART, infants with high sCD163 had later age at speech than those with low sCD163 (16.7 versus 14.9 months, p=0.003) (Table 5). In the subset of infants with documented viral suppression at the 6-month post-enrollment visit, the age difference of attaining speech was even greater when comparing low sCD163 (13.8 months versus17.3 months, p=0.006). There were no other significant differences in mean ages of achievement of milestones between children with high or low biomarker levels at 6 months after enrollment when stratified by plasma HIV concentration at the same visit (Table 6).

Discussion

In this study among infants with HIV infection diagnosed in the first year of life, baseline levels of immune activation were high. Levels of sCD163 and neopterin were lower and levels of sCD14 were higher than HIV infected adults in previous studies.[25,24] Levels of sCD163 and neopterin decreased significantly following ART between baseline and 6 months with sustained levels thereafter to 12 months. The decline in monocyte activation following ART we observed is consistent with previous studies of adults.[23] In adults, ART results in sCD163 declines to levels similar to HIV uninfected individuals. However, residual monocyte and macrophage activation measured through expression of sCD14 is observed even after ART.[17] Mean sCD14 and sCD163 levels in our cohort were similar to those of virally suppressed adults following ART but slightly higher than uninfected individuals.[25] Mean neopterin levels were lower than those of virally suppressed infected adults.[25]

While levels of neopterin and sCD14 were not associated with any differences in age of milestone attainment, plasma levels of sCD163 were associated with age of attainment of two of the milestones we investigated. High baseline expression of sCD163 was associated with 1 month earlier age of sitting unsupported. At 6 months after ART initiation, high expression of sCD163 was associated with 2 months later age of speaking monosyllable words. This

difference was more pronounced in the subset of virally suppressed participants. In a recent study, Royal et al, demonstrated correlation of plasma sCD163 concentration and neurophysiological test scores in HIV infected adults.[26] Furthermore, high sCD163 expression has been associated with impaired global deficit scores (GDS) in HIV infected adults who were virally suppressed.[25] This is consistent with our findings and suggests that residual monocyte activation after viral suppression on ART is associated with poorer neurological outcomes.

Burdo et al, in their 2011 study showed that prior to ART initiation, high levels of sCD163 expression are associated with the high plasma HIV RNA concentration.[17] This combined with our result that earlier age of sitting was associated with high levels of baseline sCD163, suggest that activation of monocytes and macrophages in the absence of the treatment might reflect functional immune activation in response to untreated viral infection.

Our study has several strengths. The prospective cohort study design enabled us to evaluate impact of biomarkers on subsequent outcomes. We evaluated 3 plausible markers of neuropathogenesis and had frequent assessment of neurodevelopmental outcomes. Our study has limitations. Milestones were assessed on monthly visits reported by caregivers and verified by study staff. More detailed assessments may have enhanced precision of age estimates. The small sample size is a limitation. However, we had over 90% power to detect differences in age of the achievement of the milestones by dichotomized biomarker levels.

In conclusion, this study provides evidence for the relation of monocyte activation and neurological development of HIV infected infants. sCD163 levels were significantly associated with timing of milestone attainment. Elevated sCD163 in the setting of viral suppression on ART was associated with delayed milestones– perhaps suggesting potentially deleterious persistent immune activation after viral suppression. This persistent immune activation and inflammation, as measured by monocyte biomarkers, might be a key component of the mechanism by which HIV infection relates to neuropathogenesis.

Tables

Table 1. Baseline characteristics of HIV-infected infants

Characteristic	N	Median (25^{th} percentile, 75^{th} percentile) or N (%)
Infant		
Age at enrolment (months)	69	3.78 (3.22, 4.01)
Age at attainment of neck control (months)	64	4.33 (3.77, 5.10)
Male	69	33 (47.8)
Ever breastfed	65	57 (87.7)
Clinical		
WHO disease stage	69	
Stage I or II		39 (56.5)
Stage III or IV		30 (43.5)
CD4 percentage (%)	69	18 (14.0, 24.0)
Log_{10} plasma HIV RNA	64	6.6 (6.05, 6.97)
Previously hospitalized	69	38 (55)
Exposed to PMTCT antiretrovirals*	69	27 (39)
Growth		
Birth weight (kg)	65	3.0 (2.7, 3.4)
WAZ*	69	-2.2 (-3.51, -0.98)
HAZ*	67	-1.91 (-2.98, -0.93)
WHZ*	67	-0.58 (-1.57, 0.57)
Primary caregiver characteristics		
Biological mother	69	67 (97.1)
Age (years)	67	25 (22.0, 30.0)
Married (monogamous and polygamous)	69	55 (79.7)
Education (years)	61	8 (8.00, 11.0)
Maternal CD4 count (cells/uL)	66	350 (198, 484)
Mother on ARVs at enrollment	69	7 (10)

* Notes: Z-score for Weight-for-age (WAZ), Z-score height-for-age (HAZ), Z-score for weight for height (WHZ), prevention of mother-to-child transmission (PMTCT)

Table 2. Biomarker expression levels means at enrollment, 6 and 12 months after ART initiation

		sCD14				sCD163				Neopterin		
Time of visit	N	Mean	95% CI	p-value	N	Mean	95% CI	p-value	N	Mean	95% CI	p-value
Baseline	24	3492	3027, 3956		24	1385	1218, 1750		23	9.948	7.714, 12.18	
6 months	24	4113	3648, 4579	0.09	24	1088	918.8, 1258	0.0007	23	7.008	5.176, 8.841	0.04
12 months*	44	4152	3716, 4588	0.7	44	1014	867, 1160	0.6	41	4.464	3.090, 5.836	0.06

*p-value for comparison with 6 months

Table 3. HIV disease indicators and growth compared between infants with low and high levels of biomarker expression

	sCD14					sCD163					Neopterin				
Mean (95% CI) or N (%)	N	sCD14 ≤3800ng/ml	N	sCD14 >3800ng/ml	p-value	N	sCD163 ≤1100 ng/ml	N	sCD163 >1100ng/ml	p-value	N	Neopterin ≤7.90ng/ml	N	Neopterin >7.90ng/ml	p-value
Viral Load (log10)	26	6.42 (6.16, 6.69)	12	6.95 (6.59, 7.31)	0.02	14	6.59 (6.29, 6.89)	24	6.59 (6.27, 6.91)	0.99	15	6.49 (6.19, 6.79)	23	6.65 (6.32, 6.98)	0.5
CD4%	28	19.8 (16.6, 23.0)	12	18.2 (13.7, 22.7)	0.6	16	21.6 (16.3, 26.9)	24	17.8 (15.3, 20.3)	0.1	15	24.0 (20.1, 27.9)	25	16.5 (13.6, 19.4)	0.003
WHZ	28	-0.689 (-1.27, -0.11)	12	-1.46 (-2.49, -0.422)	0.2	16	-1.20 (-2.04, -0.350)	24	-0.737 (-1.39, -0.0852)	0.4	15	-0.625 (-1.38, 0.130)	25	-1.10 (-1.78, 0.411)	0.4
WAZ	28	-2.43 (-3.09, -1.76)	12	-2.76 (-3.83, -1.70)	0.6	16	-2.99 (-3.81, -2.17)	24	-2.22 (-2.96, -1.48)	0.2	15	-2.24 (-3.25, -1.23)	25	-2.70 (-3.37, -2.04)	0.4
HAZ	28	-2.37 (-3.03, -1.71)	12	-2.21 (-3.45, -0.959)	0.8	16	-2.66 (-3.51, -1.82)	24	-2.09 (-2.88, -1.30)	0.3	15	-2.16 (-3.21, -1.11)	25	-2.41 (-3.12, -1.71)	0.7
WHO Stage I or II		16 (84.2)		3 (15.8)			7 (36.8)		12 (63.2)			9 (47.4)		10 (52.6)	
WHO Stage III or IV		12 (57.1)		9 (42.9)	0.06		9 (42.9)		12 (57.1)	0.7		6 (28.6)		15 (71.4)	0.2

Table 4. Mean age of attainment of milestones compared between low and high biomarker levels at enrollment

	sCD14			sCD163			Neopterin		
Mean Age (95%CI) (months)	sCD14 ≤3800ng/ml	sCD14 >3800ng/ml	p-value	sCD163 ≤1100 ng/ml	sCD163 >1100ng/ml	p-value	Neopterin ≤7.90ng/ml	Neopterin >7.90ng/ml	p-value
Sitting	6.63 (6.05, 7.21)	6.78 (5.81, 7.74)	0.8	7.58 (6.53, 8.64)	6.28 (5.84, 6.71)	0.006	6.27 (5.55, 7.00)	6.93 (6.30, 7.57)	0.2
Walking	15.3 (13.7, 16.9)	16.25 (13.1, 19.4)	0.5	15.9 (12.7, 19.1)	15.4 (13.8, 17.1)	0.8	15.6 (12.9, 18.3)	15.6 (13.8, 17.3)	0.9
Speaking	15.4 (13.8, 17.0)	15.25 (12.8, 17.6)	0.9	14.6 (11.6, 17.5)	15.7 (14.2, 17.1)	0.4	15.2 (12.6, 17.8)	15.5 (14.1, 16.8)	0.8

Table 5. Mean Age of Attainment of Walking and Speech Compared Between Low and High Biomarker Levels at 6 Months Post - Enrollment

	sCD14			sCD163			Neopterin		
Mean Age (95%CI) (months)	sCD14 ≤3800ng/ml	sCD14 >3800ng/ml	p-value	sCD163 ≤1100 ng/ml	sCD163 >1100ng/ml	p-value	Neopterin ≤7.90ng/ml	Neopterin >7.90ng/ml	p-value
Walking	16.1 (14.2, 17.9)	15.9 (14.3, 17.5)	0.9	15.5 (14.1, 17.0)	16.7 (14.6, 18.7)	0.3	16.4 (15.0, 17.9)	14.6 (13.3, 15.9)	0.2
Speaking	15.63 (14.0, 17.3)	15.8 (14.7, 16.8)	0.9	14.9 (4.0, 15.9)	16.9 (15.1, 18.7)	0.03	15.9 (14.8, 17.1)	14.9 (13.4, 16.5)	0.3

Table 6. Mean Age of Attainment of Later Milestones Compared Between Low and High Biomarker Levels at 6 months Stratified by Plasma HIV RNA at 6 months

		Low Plasma HIV RNA				High Plasma HIV RNA				
		sCD14					sCD14			
Mean Age (95%CI) (months)	N	sCD14 ≤3800ng/ml	N	sCD14 >3800ng/ml	p-value	N	sCD14 ≤3800ng/ml	N	sCD14 >3800ng/ml	p-value
Walking	11	15.8 (13.7, 17.8)	13	15.7 (13.5, 17.8)	0.9	15	16.4 (12.9, 20.0)	9	16.5 (13.8, 19.2)	0.9
Speaking	12	14.7 (12.31, 17.2)	12	15.5 (13.8, 17.2)	0.6	10	16.7 (14.2, 19.2)	13	16.2 (14.5, 17.8)	0.7
		Low Plasma HIV RNA				High Plasma HIV RNA				
		sCD163					sCD163			
Mean Age (95%CI) (months)		sCD163 ≤1100 ng/ml		sCD163 >1100ng/ml	p-value		sCD163 ≤1100 ng/ml		sCD163 >1100ng/ml	p-value
Walking	13	15.3 (13.7, 16.8)	11	16.4 (13.4, 19.5)	0.4	13	16.1 (13.1, 19.1)	11	16.8 (13.7, 20.0)	0.7
Speaking	15	13.8 (13.0, 14.6)	9	17.3 (14.1, 20.5)	0.006	13	16.3 (14.6, 18.1)	10	16.5 (14.1, 18.9)	0.9
		Low Plasma HIV RNA				High Plasma HIV RNA				
		Neopterin					Neopterin			
Mean Age (95%CI) (months)		neopterin ≤7.90ng/ml		neopterin >7.90ng/ml	p-value		neopterin ≤7.90ng/ml		neopterin >7.90ng/ml	p-value
Walking	19	16.1 (14.4, 17.7)	5	14.4 (12.0, 16.8)	0.3	18	16.9 (14.2, 19.50	6	15.2 (12.9, 17.4)	0.5
Speaking	19	15.3 (13.6, 17.0)	5	14.4 (12.5, 16.3)	0.6	18	16.6 (15.1, 18.1)	5	15.6 (11.7, 19.5)	0.5

Figures

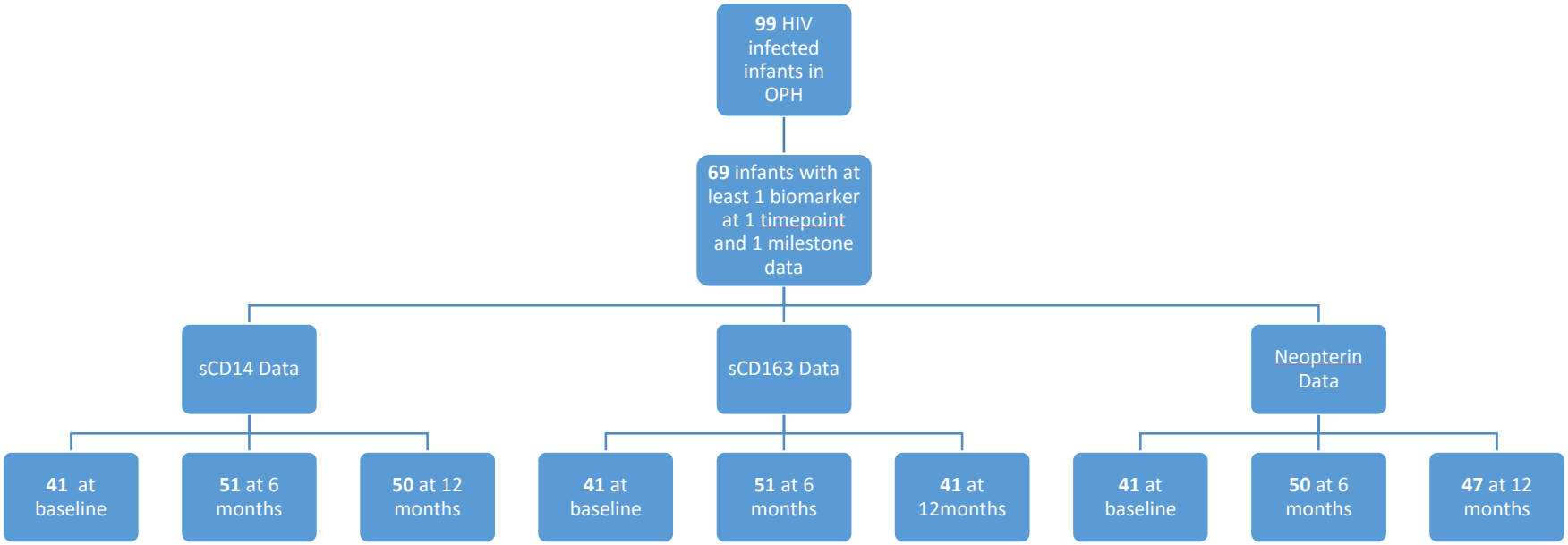

Figure 1 – Flow chart illustrating biomarker data available for study population.

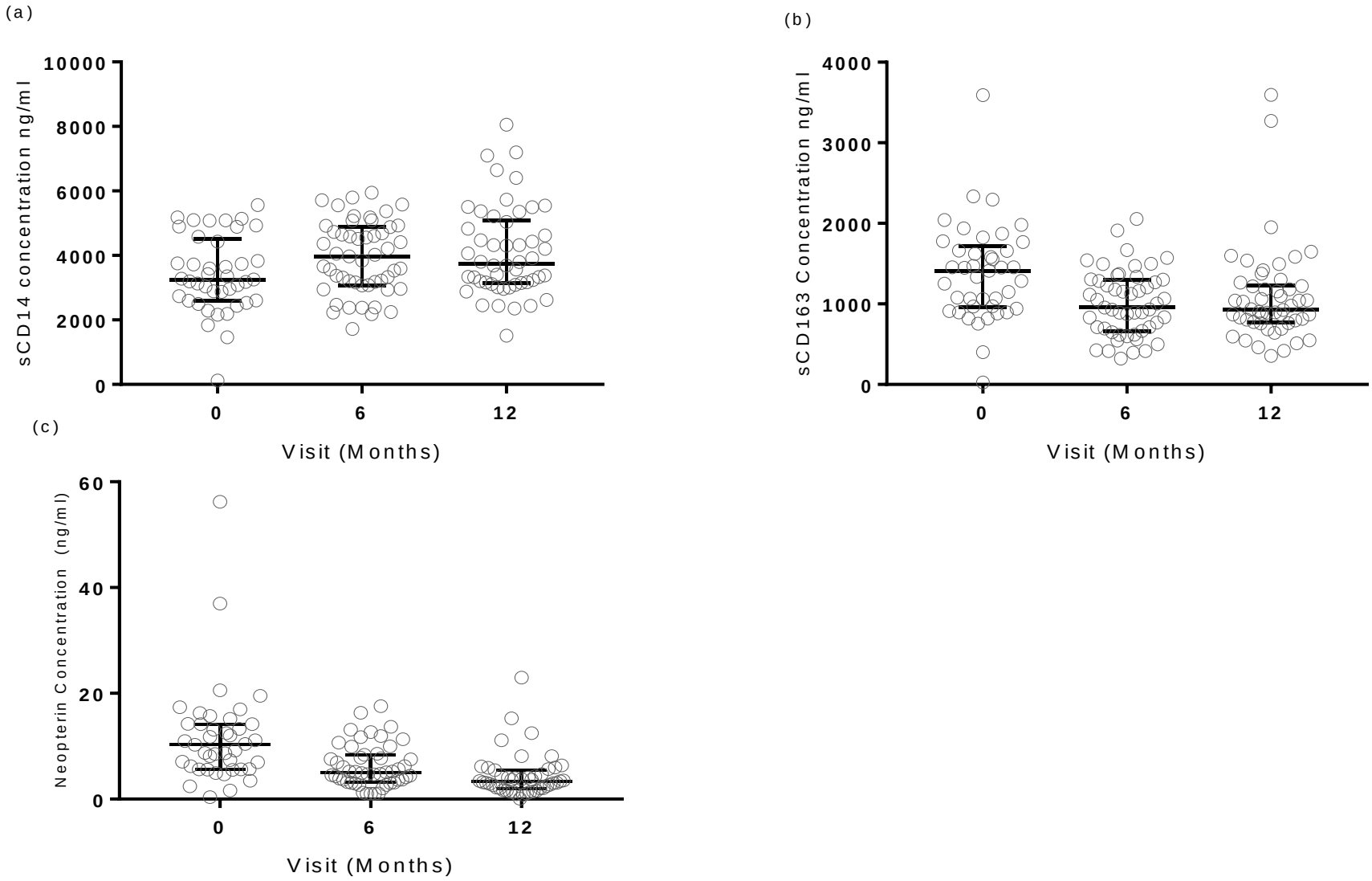

Figure 2 – (a) Concentration of sCD14 measured by ELISA at enrollment, 6 and 12 months. (b) Concentration of sCD163 measured with ELISA at enrollment, 6 and 12 months. (c) Concentration of neopterin measured with ELISA at enrollment, 6 and 12 months.

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