

# Correlation of Markers of Vascular Dysfunction with Neurodevelopmental Outcomes in Children and Adolescents with Perinatally Acquired HIV Infection

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## ABSTRACT

**Background:** Vascular dysfunction has been associated with Central Nervous System (CNS) disease in HIV-infected (HIV+) adults, but its role in pediatric HIV is unclear. Better understanding of this role is needed for development of neuroprotective treatments.

**Methods:** Neurodevelopmental status was evaluated in relation to nine selected markers of vascular dysfunction ("biomarkers") in 89 children with perinatally-acquired HIV infection enrolled in the Adolescent Master Protocol (AMP), a prospective cohort study conducted at 15 US sites. Serum levels of the following biomarkers were assessed: P-selectin, fibrinogen, adiponectin, vascular cell adhesion molecule, monocyte chemoattractant protein, interleukin-6, C-reactive protein, E-selectin and intracellular adhesion molecule. Children enrolled in AMP were administered the Wechsler Intelligence Scale for Children-IV (WISC-IV) at entry, yielding index scores for Verbal Comprehension, Perceptual Reasoning, Working Memory and Processing Speed, as well as an overall composite score (FSIQ). Linear regression models were used to evaluate the neurodevelopmental outcomes (measured with WISC-IV) as a function of each biomarker while adjusting for demographics, disease severity and receipt of Highly Active Antiretroviral Therapy (HAART). Biomarker levels were evaluated as high versus low, as continuous outcomes, and in quartiles to evaluate dose-response trends.

**Results:** 89 children in AMP had the biomarkers and neurodevelopmental outcomes measured (median age=12 years, 56% female, 71% Black, 16% Hispanic). Log(P-selectin) was significantly associated with all four index scores and FSIQ; adjusted slopes indicated a 6 to 11 point average decrease in scores for each one log unit increase in P-selectin. Final adjusted linear regression models for log(fibrinogen) also indicated a negative association with all scores; estimated decreases were about 13 to 30 points for each one log unit increase in fibrinogen. These decreases were significant in the Verbal Comprehension, Perceptual Reasoning, and FSIQ scores. The associations persisted after adjustment for significant sociodemographic and HIV disease characteristics.

**Conclusions:** Pro-coagulant and inflammatory mechanisms may have a significant role of in the pathophysiology of CNS disease in children and adolescents with perinatally acquired HIV infection.

## BACKGROUND

Youth with perinatal HIV-1 infection are at risk for various forms of central nervous system (CNS) disease, ranging from frank encephalopathy to neurodevelopmental delays.<sup>1</sup>

Evidence suggests an important role of micro-vascular processes in the peripheral circulation, such as inflammation, leukocyte pre-activation, and transendothelial migration, resulting in infiltration of HIV-infected (HIV+) leukocytes into CNS and ultimately leading to synaptodendritic injury.<sup>2,5</sup>

These processes might be facilitated by chemokines and other markers of vascular dysfunction ("biomarkers"). Understanding role(s) of specific biomarkers in development of CNS disease may inform development of intervention strategies.

## OBJECTIVES

To examine relationships between nine selected biomarkers\* and neurodevelopmental outcomes in a cohort of children and adolescents with perinatally acquired HIV infection:

- **E-selectin**
- **P-selectin**
- **fibrinogen**
- **adiponectin**
- **C-reactive protein [CRP]**
- **interleukin [IL]-6**
- **monocyte chemoattractant protein [MCP-1]**
- **vascular cell adhesion molecule [VCAM]-1**
- **intracellular adhesion molecule [ICAM]-1**

## METHODS\*

### Study Population

The Adolescent Master Protocol (AMP), a prospective cohort study, opened to enrollment in March 2007 at 15 sites in the United States and Puerto Rico and is part of the Pediatric HIV/AIDS Cohort Study. Children 7-16 years-old, born to HIV+ mothers, were eligible for enrollment. The present study included AMP participants with available biomarker levels obtained for a previously conducted hyperlipidemia study<sup>5</sup> and neurodevelopmental measures.

### Definitions and Statistical Methods

#### Biomarkers.

- **Fibrinogen** and **CRP** were measured by nephelometry on the Dade-Behring (Deerfield, Illinois) auto-analyzer using manufacturer's reagents and instructions. Intra- and inter assay CVs were 2.6% and 2.7%, respectively for fibrinogen and 4.4% and 5.7%, respectively for CRP.
- **IL-6, MCP-1, and soluble VCAM-1, ICAM-1, E-selectin and P-selectin** were measured by ELISA using reagents manufactured by R&D Systems (Minneapolis, MN). Intra- and interassay CVs were, respectively: IL-6, 6.8% and 9.4%; MCP-1 4.0% and < 7.5%; soluble VCAM-1, 5.9% and 10.2%; soluble ICAM-1, 4.8% and 10.1%; E-selectin, 5.0% and 8.8%; P-selectin, 4.2% and 9.8%
- **Adiponectin** was measured by a double antibody radioimmunoassay (Linco Research, St. Charles, Missouri); intra- and inter-assay CVs were < 5%.

#### Neurodevelopmental outcomes.

Wechsler Intelligence Scale for Children-4<sup>th</sup> edition (WISC-IV)<sup>6</sup> was administered at entry. The mean and standard deviation of WISC-IV composite scores and full scale IQ are 100 and 15, respectively. The outcomes were WISC-IV Full Scale IQ (FSIQ), and index scores for:

1. **Verbal Comprehension**
2. **Perceptual Reasoning**
3. **Working Memory**
4. **Processing Speed**

#### Statistical methods.

- The analysis was based on 89 AMP participants with WISC-IV results who had biomarker levels measured within 6 months.
- For each biomarker,
  - WISC-IV FSIQ and index scores were compared between those with high vs. low levels of the biomarker.
  - All biomarkers except ICAM-1 and E-selectin had right-skewed distributions and were log-transformed for statistical analysis.
  - Correlation coefficients were calculated between biomarker levels and the WISC-IV scores.
  - General linear regression models were fit adjusting for selected covariates (those with p<0.10. in final models).
  - Biomarker levels were also grouped in quartiles to explore trends in response.
  - Analyses were conducted using SAS Version 9.1.3, and statistical significance was based on p-values<0.05.

\*Data presented in poster based on data available by December 16<sup>th</sup>, 2008

## RESULTS

**Participants.** Among 319 perinatally HIV+ AMP participants as of December 2008, 100 had biomarker measurements and 92 of these had WISC-IV assessments done within 6 months. 3 were excluded due to CRP > 10 mg/L indicating possible acute inflammation. Characteristics of the remaining 89 participants are shown in the Table 1 and Figure 1.

**Neurodevelopmental outcomes.** Mean WISC-IV scores ranged from 87.2 to 94.1; (SD of 14.3-16.6), minimum scores: 45 - 52; maximum scores:125-135. (Figure 2)

#### Relationship between the neurodevelopmental outcomes and biomarkers.

All four indices showed negative correlations with some biomarkers:

- **Verbal Comprehension** scores with log(fibrinogen) and log(P-selectin) (p<0.01);
- **Perceptual Reasoning** scores with log(adiponectin) (p<0.1) and log(fibrinogen) and log(P-selectin) (p<0.001);
- **Working Memory** scores with log(fibrinogen) and log(P-selectin) (p<0.1);
- **Processing Speed** scores with log(adiponectin), log(CRP), log(P-selectin) and ICAM-1 levels (p<0.1).

Table 1: Demographic and health characteristics of the 89 participants

Characteristic	Total (N=89)
Age (years)	12.30 (10.50-14.10)*
Female	50 (56%)
Race	
Black or African American	63 (71%)
White	19 (21%)
Other	7 (7%)
Hispanic or Latino	14 (16%)
English Primary Language	79 (89%)
Primary Caregiver is Biological Parent	36 (40%)
Low Annual Household Income: \$ 20K or less	36 (43%)
Low Caregiver Education (High School or less)	51 (57%)
CDC Class C	24 (27%)
Nadir CD4 Count (cells/mm <sup>3</sup> )	366 (184-595)
Nadir CD4 %	20 (12-26)
Log(10) Peak Viral Load	5.39 (4.97-5.78)

\*Continuous variables are described as Median (Q1-Q3)

Figure 1: ARV treatment characteristics of the 89 participants

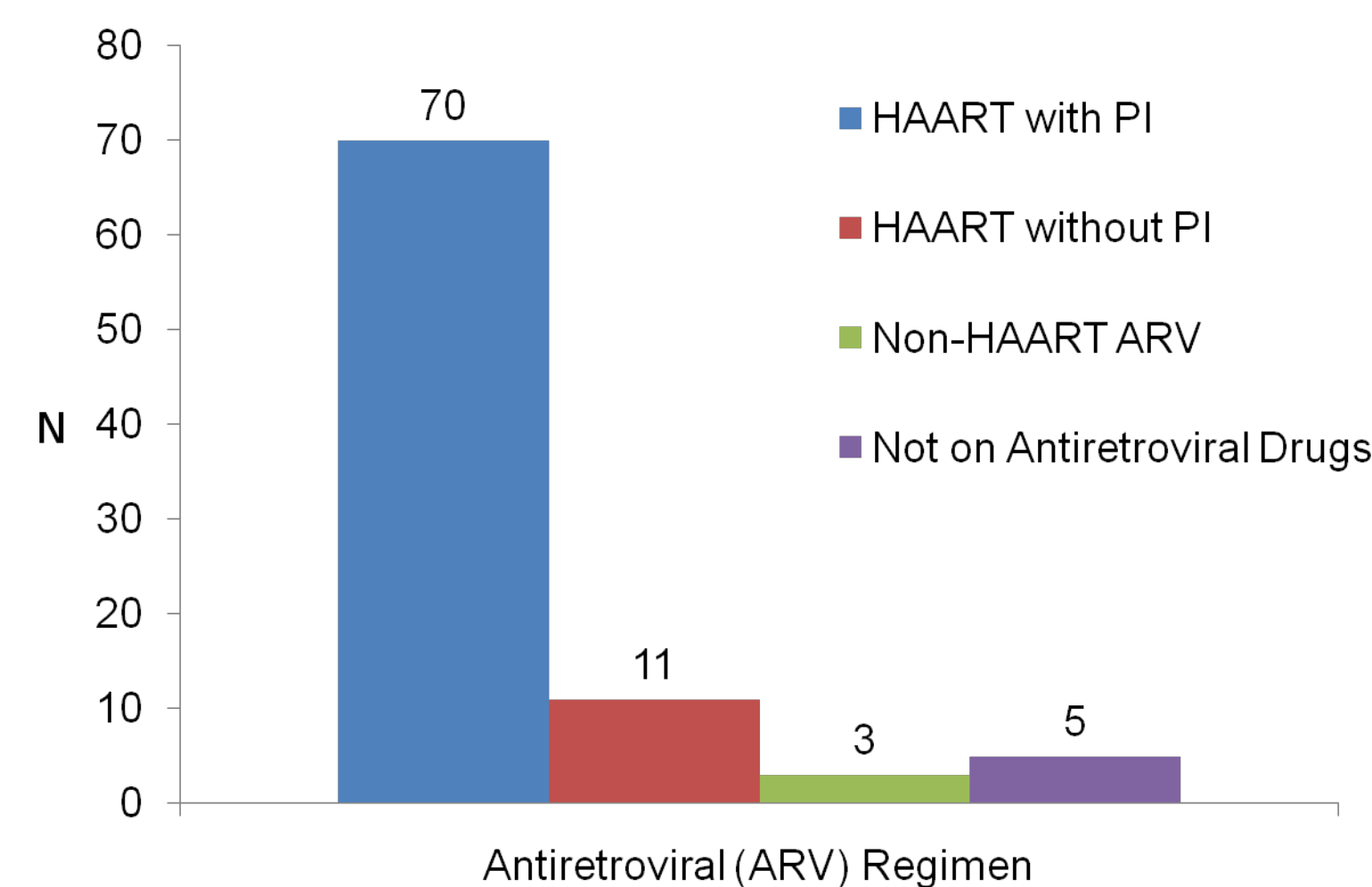


Figure 2: Distribution of WISC-IV index scores and the full-scale IQ in 89 perinatally HIV-infected youth included in the analysis

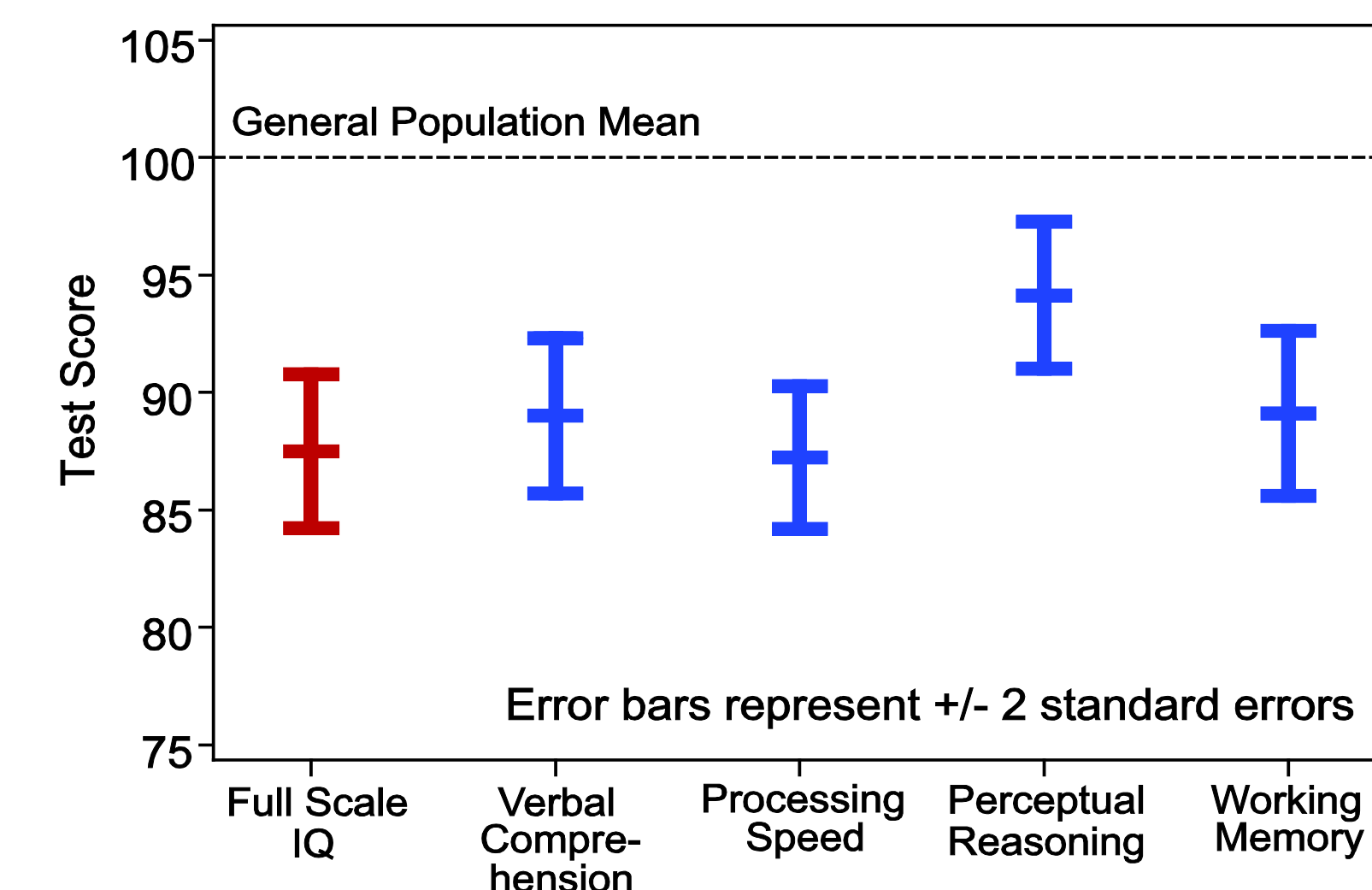


Table 2: Final linear regression models for effects of log(P-selectin) and log(fibrinogen) on neurodevelopmental outcomes, adjusting for potential confounders.

Outcome	Full Model Results*				Reduced Model Results**			
	N	Estimate	SE	P-value	N	Estimate	SE	P-value
<b>BIOMARKER: P-Selectin</b>								
Full Scale IQ	87	-9.70	2.94	0.001	87	-10.49	2.79	<.001
Verbal Comprehension	88	-8.13	2.98	0.008	88	-8.14	2.81	0.005
Processing Speed	88	-5.83	2.96	0.05	89	-5.62	2.76	0.05
Perceptual Reasoning	88	-7.81	2.69	0.005	88	-8.14	2.63	0.003
Working Memory	88	-7.56	3.35	0.03	88	-7.96	3.23	0.02
<b>BIOMARKER: Fibrinogen</b>								
Full Scale IQ	87	-24.98	9.13	0.008	88	-25.90	8.73	0.004
Verbal Comprehension	88	-23.58	9.13	0.01	88	-21.20	8.73	0.02
Processing Speed	88	-11.05	9.18	0.23	89	-13.44	8.30	0.11
Perceptual Reasoning	88	-24.23	8.19	0.004	89	-29.91	7.86	<.001
Working Memory	88	-16.49	10.40	0.12	88	-18.84	9.82	0.06

\*Adjusted for age, sex, Hispanic ethnicity, Non-English language, relationship to caregiver, caregiver education, receipt of HAART, nadir CD4%, and log peak HIV-1 RNA viral load; \*\*Adjusted for covariates significant at p<0.10 in a reduced multivariate model.

#### In linear regression models, the following results were observed:

- Participants with higher P-selectin levels had significantly lower scores on all four WISC-IV indices and FSIQ (p<0.1), with an adjusted decrease of 6-11 points for each 1 log unit increase in P-selectin.
- Participants with higher fibrinogen levels had significantly lower scores on multiple WISC-IV indices (Verbal Comprehension, Perceptual Reasoning and FSIQ), with adjusted decreases of 13-30 points for each one log unit increase in fibrinogen.
- The effects of P-selectin and fibrinogen persisted after adjustment for potential covariates in linear regression models (Table 2 and Figure 3).
- Participants with higher peak viral load levels had significantly lower FSIQ, Verbal Comprehension, Working Memory, and Perceptual Reasoning scores. Current viral load was not associated with the WISC-IV outcomes.

## SUMMARY

This exploratory analysis evaluated peripheral blood levels of nine selected biomarkers in relation to WISC-IV scores in 89 children and adolescents with perinatally acquired HIV. The cohort was representative of the US youth with perinatally acquired HIV.

#### Key findings:

- All four primary WISC-IV index scores were negatively correlated with P-selectin levels.
- FSIQ, perceptual reasoning, and verbal comprehension were negatively correlated with fibrinogen levels.
- CDC disease severity class was not associated with WISC-IV index scores.
- Current VL was not correlated with WISC-IV scores, but the peak VL showed significant associations with FSIQ scores

#### Study limitations:

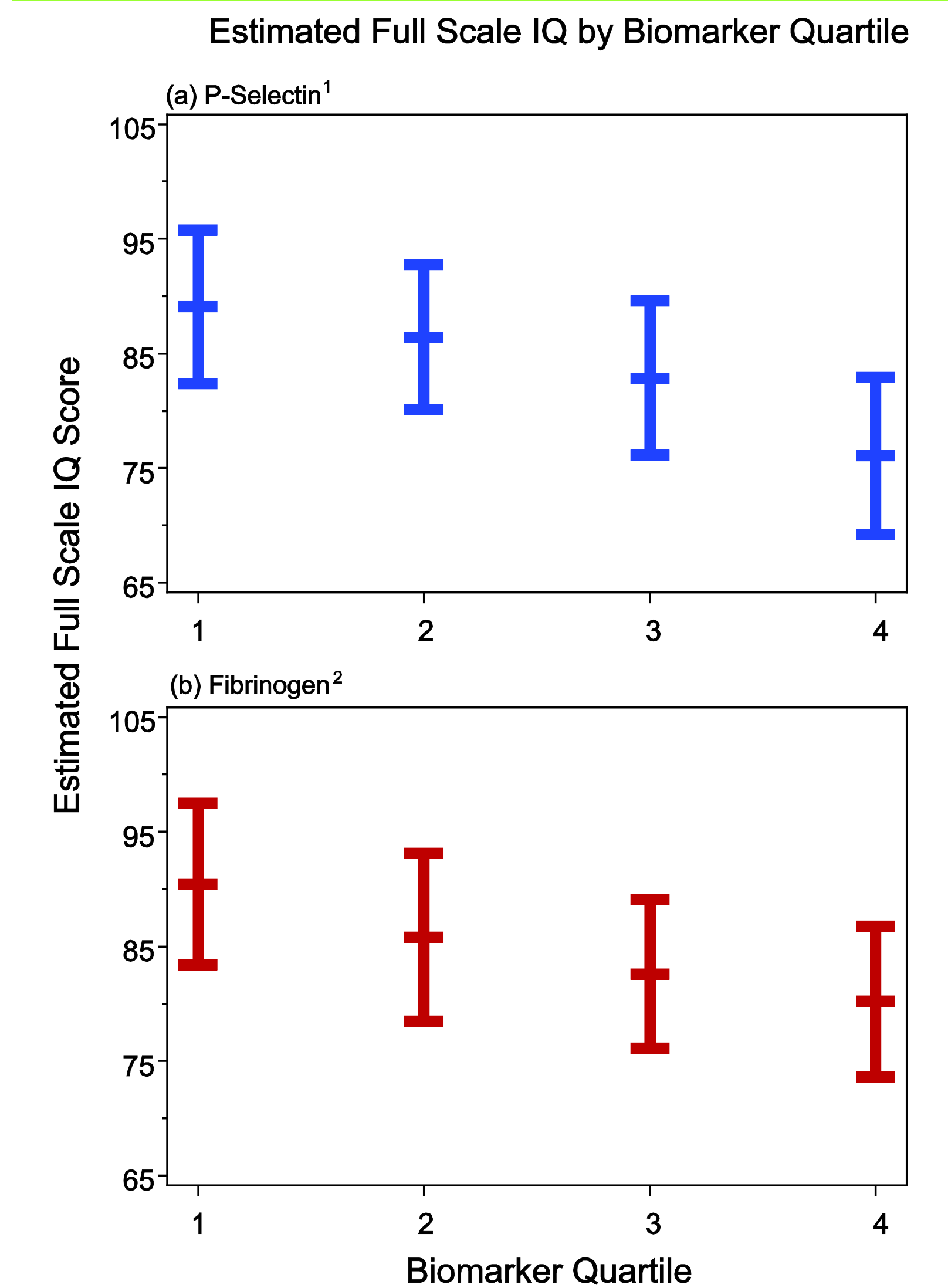
- **Cross-sectional design**
- **No HIV-uninfected control group**

## CONCLUSIONS

#### In children and adolescents with perinatally acquired HIV infection:

- **P-selectin and fibrinogen may play an important role in the pathophysiology of adverse neurodevelopmental outcomes.**
- **Factors other than peripheral viremia and symptom control may be responsible for the vulnerability of the developing CNS, including ones that disrupt normal neuroimmune communication and perpetuate a pro-inflammatory milieu (or environment)**
- **Future research should seek to further elucidate these mechanisms and possible treatment implications.**
- **In the HAART era, HIV disease severity criteria may need to be revised to include more subtle, but clinically significant neurodevelopmental outcomes.**

Figure 3: Estimated Full Scale IQ by Biomarker Quartile



<sup>1</sup>Adjusted for peak viral load and caregiver education  
<sup>2</sup>Adjusted for caregiver education

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