Vascular Health and Cerebral Blood Flow

in Perinatally HIV-Infected Children

C. Blokhuis MD[1], S. Cohen MD PhD[1], H.J. Scherpbier MD PhD[1], H.J.M.M. Mutsaerts MD PhD[2,3], J.C.M. Meijers[4,5], N.A. Kootstra[6], P. Reiss MD PhD[7,8,9], F.W.N.M. Wit MD PhD[7,8,9], C.E. Teunissen PhD[10], D. Pajkrt MD PhD[1]

Corresponding author: Charlotte Blokhuis (c.blokhuis@amc.nl)
Pediatric Infectious Diseases, Emma Children's Hospital, Amsterdam

MAARTEN KAPELLE STICHTING
DR. C.J. VAILLANTFONDS

KEY POINTS:

- Perinatally HIV-infected children showed higher plasma CRP and sVCAM-1 levels, and higher subcortical and white matter cerebral blood flow as compared to controls.
- Higher CRP levels and lower gray matter perfusion were associated with white matter injury, and higher sVCAM-1 with poorer visuomotor integration.
- These results suggest that vascular disease may play a role in cerebral injury and cognitive impairment in pediatric HIV.

BACKGROUND

Despite effective virological suppression with cART, children perinatally infected with HIV show neuropsychological dysfunctioning with underlying macro- and microstructural brain injury.[1,2] Chronic pediatric HIV infection and several components of cART have been associated with vasculopathy, coagulopathy, metabolic comorbidities, and an increased risk of cerebrovascular events.[3,4] However, the interplay between these factors and cerebral perfusion, structure and function is unknown.

This study aimed to assess vascular disease biomarkers and cerebral blood flow (CBF) in HIV-infected children, and to explore potential associations with cerebral and cognitive deficits.

METHODS

This cross-sectional study included cART-treated perinatally infected children aged 8-18 years from the Academic Medical Center in Amsterdam, and age-, sex-, ethnicity- and socio-economic status-matched uninfected controls. Markers of inflammation, endothelial activation (using MesoScale Discovery), and coagulation (using enzyme-linked immunosorbent assays) were measured in blood samples from all participants and in cerebrospinal fluid (CSF) from HIV-infected participants. CBF was determined using arterial spin labeling on a 3-Tesla MRI (Figure 1).

We then explored whether CBF and vascular health markers were associated with MRI abnormalities (gray matter volume, white matter lesion volume, and white matter diffusivity[2]), and cognitive performance (including intelligence, processing speed, working memory, and visuomotor integration[1]) using linear and ordered logistic regression analyses.

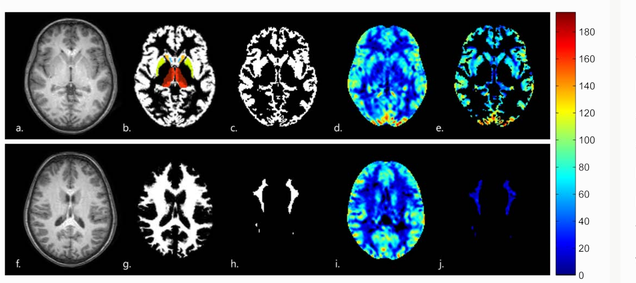

Figure 1. Using arterial spin labeling to measure regional cerebral blood flow.

First row: obtaining ASL-measured CBF in gray matter (GM): (a) 3D T1-scan; (b) GM probability map; (c) GM mask, obtained by WM thresholding the probability map at p>0.8; (d) CBF map; (e) masked GM CBF map. The basal ganglia and thalamus regions, as defined using the Harvard Oxford atlas, are displayed in panel (b): caudate nucleus (orange), putamen (green), nucleus accumbens (blue), and thalamus (red).

Second row: the same process for white matter (WM) CBF: (f) 3D T1-scan; (g) WM probability map; (h) WM mask, obtained by thresholding the WM probability map at p>0.8 and eroding with a 7.5mm disk to avoid GM contamination; (i) CBF map; (j) masked WM CBF map.

RESULTS

STUDY PARTICIPANTS

This study included 36 HIV-infected children and 37 uninfected controls (Table 1). The large majority of HIV-infected children were virologically suppressed on cART at time of inclusion.

37 healthy controls	36 HIV-infected children
median age of 12.1 years	median age of 13.7 years
49% male	50% male
	25% CDC-C diagnosis
	nadir CD4+ T-cell count 445 *10⁶/L (Z-score -0.7)
	86% on cART since median age 2.6 years
	83% virologically suppressed
	CD4+ T-cell count 765 *10⁶/L (Z-score -0.1)

Table 1. Characteristics of study participants. Data are shown as medians or percentages. The CD4 T-cell count Z-score corresponds to standard deviations from the age-appropriate mean.

VASCULAR MARKERS IN PLASMA AND CSF

HIV-infected children showed higher systemic levels of CRP and sVCAM-1, as well as high concordance between blood and CSF levels of CRP, sVCAM-1, and sICAM-1 (Table 2).

	healthy controls	HIV-infected children			
	plasma	*plasma*[1]	*P-value*[a]	*CSF*[1]	*W (P-value)*[b]
CRP (mg/l)	0.28 (0.16-0.81)	0.72 (0.30-2.48)	*.004**	.002 (.001-.007)	*0.98 (.004*)*
IL-6 (pg/ml)	0.25 (0.08-0.38)	0.34 (0.08-0.52)	*.33*	0.7 (0.5-0.8)	*0.51 (.42)*
sVCAM-1 (ng/ml)	591 (437-668)	642 (525-838)	*.026**	6.91 (5.58-11.58)	*0.78 (.041*)*
sICAM-1 (ng/ml)	438 (390-503)	469 (400-538)	*.26*	2.64 (2.03-3.65)	*0.78 (.045*)*
D-dimer (µg/ml)	0.21 (0.16-0.33)	0.27 (0.16-0.46)	*.30*		
F1+2 (pmol/l)	115 (101-151)	128 (108-153)	*.36*		
TAT (µg/ml)	3.5 (3.1-4.2)	3.5 (3-4.3)	*.95*		
vWF-ag (%)	107 (87-138)	110 (86-148)	*.60*		
vWF-pp (%)	101 (89-115)	96 (84-115)	*.65*		

Table 2. Markers are median (IQR). Abbreviations: CRP=C-reactive protein; IL=interleukin; sVCAM=soluble vascular cell adhesion molecule; sICAM=soluble intercellular cell adhesion molecule; F1+2=prothrombin fragment 1 + 2; TAT=thrombin-antithrombin-III complex; vWF (ag)=von Willebrand factor antigen; vWF (pro)= von Willebrand factor (propeptide). [1] n=35 (inflammation and endothelial activation data was missing for one participant, coagulation data for another). [1] n=25 for CRP and IL-6, n=24 for sVCAM-1 and sICAM-1. [a] Group differences in plasma levels (Mann-Whitney-U test). [b] Concordance between blood and CSF levels (Kendall's *W* and Friedman's test); *P-value <.05

CEREBRAL BLOOD FLOW

HIV-infected children had higher CBF in white matter (+10.2%), caudate nucleus (+4.8%), putamen (+3.6%), nucleus accumbens (+3.9%) and thalamus (+5.5%), as shown in Figure 2.

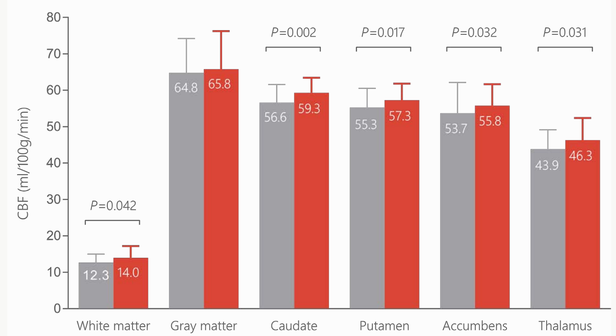

Figure 2. HIV-infected children have higher cerebral blood flow in white matter, basal ganglia and thalamus.

Data was available for 28 HIV-infected children and 34 controls; exclusions were due to motion- or labeling artefacts or lack of MRI consent. We compared CBF between groups using linear regression analysis adjusted for age (years above 16 years), sex and hematocrit. Prior to analysis, CBF values for caudate nucleus, putamen, nucleus accumbens, and thalamus were normalized subject-wise using the overall mean gray matter CBF. The error bars represent standard deviations.

ASSOCIATIONS WITH CEREBRAL INJURY AND COGNITIVE PERFORMANCE IN HIV-INFECTED CHILDREN

Higher levels of CRP were associated with higher white matter mean diffusivity (blood: coef=2.09; P=.029; CSF: coef=2.27; P=.029). Higher systemic sVCAM-1 was strongly associated with poorer visuomotor integration (coef=-17.6; P=<.001; Figure 3). Lower gray matter CBF was associated with higher white matter lesion volume in HIV-infected children (coef=-0.05; P=.039). No further associations with HIV-related cognitive impairment or cerebral injury were found.

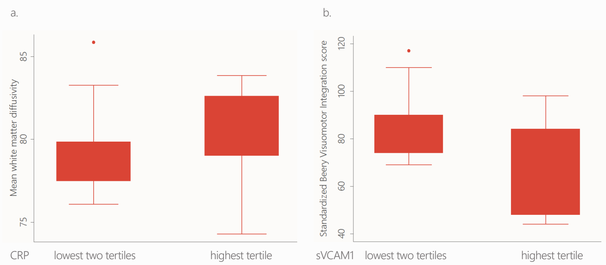

Figure 3. Correlations of inflammatory and endothelial activation markers with cerebral injury and cognitive dysfunction.

a. Higher CRP was associated with mean white matter diffusivity, as measured with diffusion tensor imaging. Increased white matter diffusivity is suggestive of microstructural white matter damage.

b. Higher sVCAM-1 was associated with poorer visuomotor integration, as measured with the Beery visuomotor score.

CONCLUSIONS

In a well-treated cohort of perinatally HIV-infected children, ongoing inflammation and endothelial activation were indicated by elevated systemic CRP and sVCAM-1, which showed strong concordance with CSF levels. Further, they had higher CBF in white matter, basal ganglia, and thalamus, potentially indicating increased metabolic demand or low-grade inflammation in these brain regions.

Lower gray matter CBF and increased inflammation (CRP) were associated with white matter injury, and endothelial activation (sVCAM-1) with poorer visuomotor integration. Vascular disease may thus play a role in pediatric HIV-associated cerebral and cognitive deficits. Longitudinal evaluation is warranted to assess whether CBF changes, inflammation and endothelial activation negatively affect white matter health and cognitive performance in this population over time.

AUTHOR AFFILIATIONS: [1] Department of Pediatric Hematology, Immunology and Infectious Diseases, Emma Children's Hospital AMC, Amsterdam, the Netherlands. [2] Department of Radiology, Academic Medical Center, University of Amsterdam, The Netherlands. [3] Cognitive Neurology Research Unit, Sunnybrook Health Sciences Centre, Toronto, Canada [4] Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. [5] Department of Plasma Proteins, Sanquin Research, Amsterdam, The Netherlands. [6] Department of Experimental Immunology, Academic Medical Center, Amsterdam, the Netherlands. [7] Department of Global Health and Amsterdam Institute of Global Health and Development, Academic Medical Center, Amsterdam, the Netherlands [8] HIV Monitoring Foundation, Amsterdam, the Netherlands. [9] Department of Internal Medicine, div. of Infectious Diseases, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Amsterdam, the Netherlands. [10] Neurochemistry Laboratory and Biobank, Department of Clinical Chemistry, VU University Medical Center and Neurocampus Amsterdam, the Netherlands

REFERENCES: [1] Cohen et al, Clin Infect Dis 2015;60(7):1111-1119. [2] Cohen et al, Neurology 2016;86(1):19-27. [3] Hammond et al, Dev Med Child Neurol 2016;58(5):452-460. [4] Barlow-Mosha et al, J Int AIDS Soc 2013;16(1):18600.