Biomarkers of Inflammation and Endothelial Activation in Vertically HIV-1 Infected Children

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Background: Chronic inflammation, which may predispose to cardiovascular disease (CVD), persists in HIV-1 infected adults despite sustained viral suppression (SVS). There are limited data on chronic inflammation and endothelial activation (EA) in vertically infected children. This study examines markers of inflammation and EA in virally suppressed, vertically HIV infected children enrolled in the Canadian EPIC4 cohort.

Methods: A cross-sectional analysis of pro-inflammatory cytokines (PIC) and measures of EA was performed in HIV-1 infected children with a viral load (VL) < 40 copies/mL while on combination antiretroviral therapy (cART). Selected PIC and biomarkers of EA were measured using commercially available cytometric bead array assay (Beckton-Dickinson) and ELISA (R&D Systems).

Results: 63 vertically HIV-infected children were included with median age of 13 years (range 4-19); 35/63 (55.6%) were girls. Thirty-four subjects had initiated cART in the first year of life with subsequent SVS. The remaining 29 subjects had either initiated cART after one year of age (n=6), or had at least one regimen failure before achieving SVS (n=23). PIC levels (IL-12p70, TNF, IL-10, IL-6, IL-1β, IL-8) were low (< 20pg/mL) in all but two patient. Median (inter-quartile range) levels of the biomarkers of EA markers angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), and soluble endoglin (sEng) were 18 (13-37), 0.93 (0.60-4.1), 77 (68-106), 1.7 (0.44-5.4), and 33 (20-58) ng/mL, respectively. Biomarkers of EA were correlated: Ang-2 with sVEGFR-1, Ang-2 with sEng, sVEGFR-1 with sEng (ρ>0.4, p< 0.01 for all comparisons).

Conclusions: Despite SVS with cART, persistent systemic EA is detected in a proportion of vertically HIV-infected children and may potentially associated with low-level chronic inflammation. Further studies are needed to determine the significance of these findings with respect to future CVD risk and beyond.

Background

Mother-to-child transmission (MTCT) is the primary method of pediatric human immunodeficiency virus 1 (HIV-1) infection. Presently, ~3.2 million children are living with HIV-1 infection and approximately 240 000 infants are newly infected every year [1]. Although there is currently no cure for HIV, the use of combination antiretroviral therapy (cART) has significantly increased the life expectancy of infected individuals and decreased transmission of the virus. However, long term complications are emerging even after successful chronic cART. Higher levels of inflammation and endothelial activation still persist in HIV infected individuals despite fully suppressed viral replication [2]. These factors are thought to increase risk of cardiovascular disease, yet their contribution in HIV infection remains unclear [3,4].

A recent study among HIV infected infants found that immune activation occurred early in perinatally-infected infants, with higher levels of soluble CD14 (sCD14), interleukin 6(IL-6) and C-reactive protein (CRP) by 6 weeks of age compared to HIV-exposed uninfected or HIV-unexposed infants [5]. Elevated levels of proinflammatory cytokines (Tumor necrosis factor (TNF-α), IL-6, IL-1β,)) have been implicated in loss of lean body mass, growth impairment, and poorly controlled HIV viral replication in children [6-8]. Other inflammatory biomarkers that have been documented in its association with chronic HIV-1. Some examples

include the CXC-cytokine 10 kDa interferon gamma-induced protein (IP-10) and the soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) [9-12]. However, IP-10 and sTREM-1 have only been documented in adults and have not yet been investigated in infants with HIV-1.

There has also been increasing evidence to suggest that endothelial activation plays a key role in pathogenesis and disease progression of HIV-1. A pro-inflammatory state and endothelial activation persist in patients with chronic, well-controlled HIV-1 infection for as long as 12 years after successful antiretroviral therapy[2]. Some of the biomarkers involved in chronic infection include intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF), endoglin, angiopoietins, and tyrosine kinase with immunoglobulin-like and EGF-like domains 1(TIE-1) [2, 13-19].

While numerous studies have been published on these markers and their correlation to HIV disease activity in adults, data on children are limited. In addition to the classical inflammatory cytokines TNF-α, IL-6, and IL-1β, other biomarkers of inflammation and those involved in endothelial activation may provide important clues about possible ongoing immune and endothelial activation despite adequate virologic control in HIV-infected infants and children.

Objective

The objective of the proposed study is to examine host proteins ("biomarkers") that may be associated with HIV-1 disease activity among children with vertically acquired HIV-1 infection. In particular, biomarkers of host response pathways (inflammation and endothelial activation) will be explored. The working hypothesis is that the biomarkers will provide evidence of ongoing inflammation and endothelial activation in HIV-1 infected children, despite excellent virologic control with combination antiretroviral therapy (cART).

Method

The proposed study made use of plasma samples from HIV-infected children that have been collected as part of the CIHR-funded Early Pediatric Initiation-Canadian Child Cure Cohort Study (EPIC, principal investigator Hugo Soudeyns). The study followed 63 patients with a viral load (VL) < 40 copies/mL while on combination antiretroviral therapy and were analyzed for the study.

Selected PIC (IL-12, TNF, IL-10, IL-6, IL1b, IL-8) were measured using commercially available cytometric bead array assay (Beckton-Dickinson). Endothelial activation was measured by quantifying plasma levels of Ang-1, Ang-2, soluble Tie-2, sICAM-1, and soluble endoglin using ELISA DuoSets (R&D Systems). ELISAs were conducted according to the manufacturer's instructions and plates were read at 450 nm with a wavelength correction of 540 nm and concentrations interpolated using a 4-parameter logistic slope curve (GraphPad Prism v5.0). All biomarkers correlations were performed using Spearman's correlation. Statistics and principal component analysis (PCA) were computed using SPSS statistical program.

Results

Patients

Peripheral blood samples from 63 children with vertically acquired HIV-1 infection were were gathered from the CIHR-funded Early Pediatric Initiation-Canadian Children Cure Cohort Study (EPIC4). Among the 63 patients, 35 were females. The median age was 13 years old with a range from 4 to 19 years old. Thirty-four subjects had initiated cART in the first year of life with subsequent SVS. The remaining 29 subjects had either initiated cART after one year of age (n=6), or had at least one regimen failure before achieving SVS (n=23). Analysis of samples show plasma HIV-viral load for all patients was below levels of detection <40 copies/mL.

Detection of proinflammatory cytokines

To investigate whether patients were experiencing persistent inflammation, we analyzed the plasma samples acquired to measure the levels of IL-12, TNF, IL-10, IL-6, IL-1b and IL-8 using cytometric bead array assay (Figure 1). The proinflammatory cytokines (PICs) of 23 patients have currently been processed. PICs were low (< 20 pg/mL) in all but 2 patients.
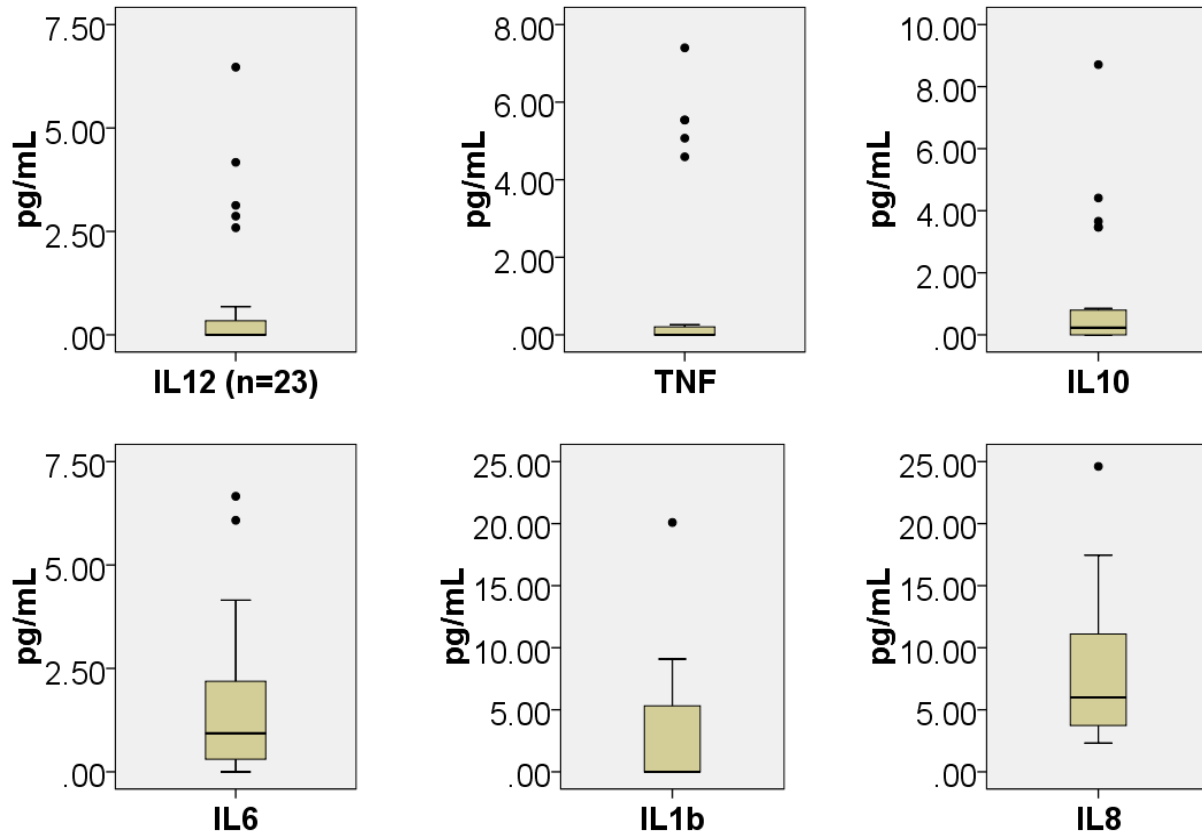

Figure 1: Low concentration of proinflammatory biomarkers detected in HIV-1 patients with sustained viral suppression. Concentrations of the biomarkers were measured using cytoplasmic bead array assay and plotted as a boxplot (n=23).

Endothelial markers detected in various levels in HIV-1 infected pediatric patients

With the induction of PICs, we also wanted to determine whether endothelial activation was also present. Levels of Ang-1, Ang-2, sICAM1, sVEGFR-1 and sEndoglin were measured using ELISA (Figure 2). The median (1st and 3rd quartile) concentration detected in the plasma samples of the patients were 18 ng/mL (13-37), 0.93 ng/mL (0.60-4.1), 77 ng/mL (68-106), 1.7 ng/mL (0.44-5.4), and 33 ng/mL (20-58) respectively.
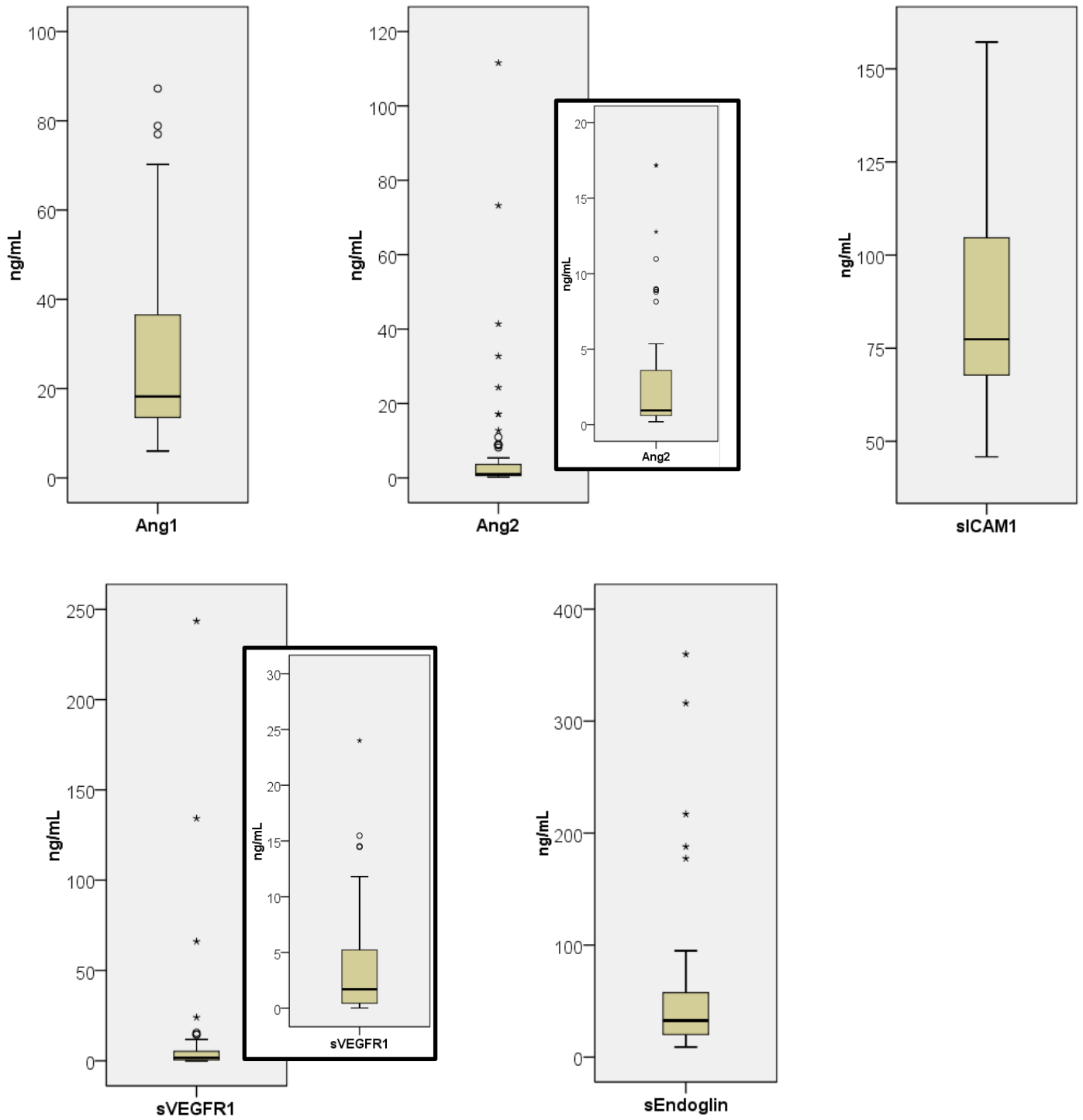
Figure 2: Concentration of various endothelial biomarkers in blood samples acquired from HIV-1 patients with sustained viral suppression. Concentrations of the biomarkers were measured using ELISA and plotted as a boxplot (n = 63).

Endothelial activation persists despite achieving viral suppression of HIV-1

      To assess whether there was evidence of overall endothelial activation, we correlated all the selected biomarkers (Figure 2). Using a Spearman rank correlation coefficient, we found a significant positive correlation between Ang-2 and sVEGFR-1 ($\rho = 0.725$, $p < 0.001$), Ang-2 and sEndoglin ($\rho = 0.509$, $p < 0.001$), and sVEGFR-1 and sEndoglin ($\rho = 0.619$, $p < 0.001$).
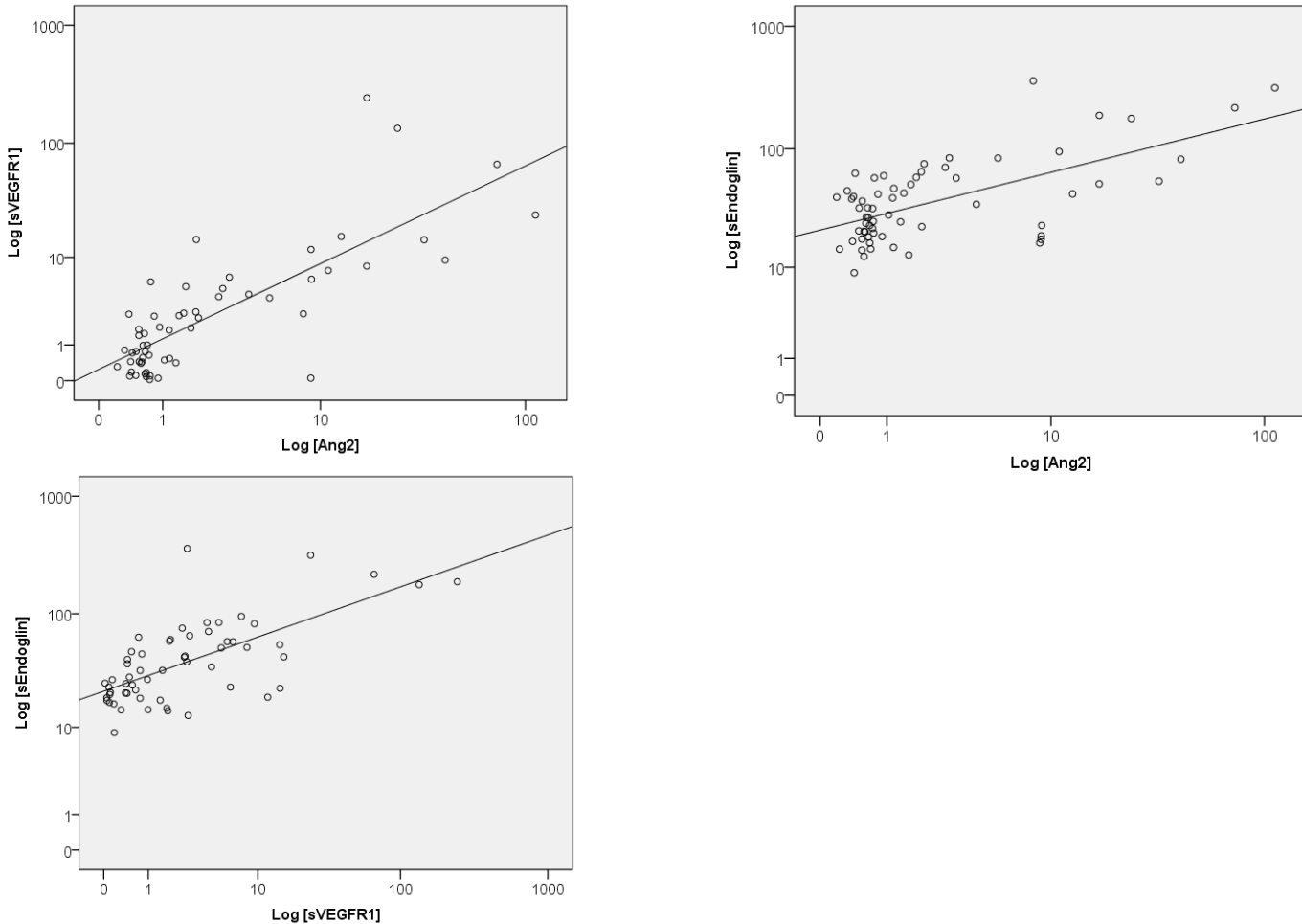

Figure 3: Ang-2, sVEGFR-1 and sEndoglin positively correlate with one another. Concentrations of the biomarkers were measured using ELISA and plotted logarithmically to demonstrate positive correlation. Spearman's correlation was conducted using SPSS statistics program.

      We also performed a PCA on the data set to model how many components would be able to explain the total variance (Table 1). It was determined that 58% of the variance could already be explained by a single component, or endothelial activation.

Table 1: Principle Component Analysis of the endothelial biomarkers. PCA was conducted using SPSS statistical program.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.908	58.156	58.156	2.908	58.156	58.156
2	.752	15.043	73.198			
3	.640	12.800	85.998			
4	.433	8.650	94.649			
5	.268	5.351	100.000			

Extraction Method: Principal Component Analysis.

Component Matrix[a]

	Component
	1
Ang1	.783
Ang2	.777
sICAM1	.707
sVEGFR1	.700
sEndoglin	.838

Extraction Method: Principal Component Analysis.

a. 1 components extracted.

Using the component matrix for component 1, we assessed to see whether there was a correlation between endothelial activation and the proinflammatory markers. The correlation was close to significant, but not statistically significant ($p > 0.05$).

Discussion

Systematic inflammation, immune activation and endothelial activation are all thought to play a significant role in the progression of HIV-infection. Although long term antiretrovirals, attenuates the response seen in infected individuals, persistence of these mechanisms are still often seen.

Analysis of the proinflammatory biomarkers using cytoplasmic bead array assay showed that many patients still experience low levels of inflammation (Figure 1). In another study, similar conclusions were observed in HIV-1 infected patients who have been on antiretroviral therapy for 12 years by using ELISA to quantify their inflammatory markers [2]. More patients may have shown persistent low-grade inflammation however due to the sensitivity of cytoplasmic bead array assay, many PICs were found to be below the level of detection. This may be one of the reasons in which the proinflammatory markers did not statistically correlate with endothelial activation. Likewise, the small amount of sample size also contributes to this problem. Additional samples will need to be processed and quantification of the PICs will be reanalyzed again using a more sensitive assay such as ELISA to deal with this issue.

Endothelial quiescence and activation is primarily regulated by the angiopoetin molecules, Ang-1 and Ang-2. Ang-1 is expressed in endothelial cells and is responsible in maintaining the vascular stability.

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Meanwhile, Ang-2 antagonizes Ang-1 and destabilizes the blood vessels. By measuring levels of Ang-1 and Ang-2, as well as other endothelial activation biomarkers such as VEGFR-1, sEndoglin and sICAM-1, we observed that many children have persistent endothelial activation (Figure 2). Levels of Ang-2, sEndoglin and VEGFR-1, all indicators of endothelial activation, positively correlate with one another based on Spearman's correlation. The principal component analysis further supports that 58.1% of the variance between biomarkers can be explained by a single component, endothelial activation.

Our study suggests that many pediatric patients who have achieved sustained viral suppression show signs of ongoing inflammation and endothelial activation, both of which have been suggested to contribute to the increased incidence of cardiovascular and thromboembolic events seen in treated HIV-1 patients. Due to the low sensitivity of the cytoplasmic bead array assays, we will switch to ELISAs to acquire more accurate measurements of proinflammatory cytokines. In addition, we will be acquiring additional samples to compare the levels of inflammatory and endothelial activation biomarkers of patients before they have initiated of cART and patients who have been HIV-exposed but uninfected. We are also keen to assess plasma samples of patients prior and after interrupting their cART and experienced a loss of virologic control.

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