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Microbial translocation does not drive immune activation in Ugandan children with HIV

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Background

Immune activation (IA), potentially driven by microbial translocation (MT), is linked to increased morbidity despite ART in HIV. We investigated MT as a driver of IA in HIV-infected African children.

Methods

ART-naïve and ART-experienced children were recruited to a Ugandan site of the CHAPAS-3 Trial (ISRCTN69078957), with HIV-uninfected age-matched controls from the same communities. 19 markers (cellular and soluble) of IA, cellular proliferation inflammation, vascular injury and disordered thrombogenesis were measured at weeks 0, 48 and 96, alongside viral load at week 96 and CD4 cell count/percentage. Intestinal fatty acid binding protein (I-FABP) was used to quantify gut damage and MT was assessed using a panel of specific bacterial polymerase chain reactions (PCRs), broad-range 16S rDNA PCR and next generation sequencing (NGS)(Illumina method), with bespoke method adaptations to enable sequencing of very low bacterial DNA levels. MT and I-FABP were assessed at weeks 0, 12 and 72. Cluster analysis of IA and MT markers was performed in R.

Table 1 Biomarkers of immune activation, cellular proliferation, inflammation and vascular injury

Soluble biomarkers of inflammation and vascular injury		
Interleukin-1 receptor antagonist (IL-1 RA)	Monocyte chemoattractant protein 1 (MCP1)	Intercellular adhesion molecule 3 (ICAM-3)
C-reactive protein (CRP)	Serum amyloid A (SAA)	Tissue factor (TF)
Tumour necrosis factor-α (TNF-α)	Vascular cellular adhesion molecule (VCAM)	Soluble intercellular adhesion molecule 1 (sICAM-1)
Interleukin-10 (IL-10)	Angiopoeitin 1	Angiopoeitin 2
Interleukin-6 (IL-6)	E-selectin	Thrombomodulin
Interleukin-8 (IL-8)	P-selectin	D-Dimer
Cellular markers of immune activation and proliferation		
CD3+CD8-HLA-DR+CD38-	CD3+CD8+HLA-DR+CD38-	CD4+CD45RA-CD31+Ki67+
CD3+CD8-HLA-DR+CD38+	CD3+CD8+HLA-DR+CD38+	CD4+CD45RA+CD31+Ki67+
CD4+CD45RA-CD31-Ki67+	CD4+CD45RA+CD31-Ki67+	

Results

- 249 children were included: 120 ART naïve and 22 ART experienced (median (IQR)) age 2.8 (1.7-4.0) & 6.5 (5.9-9.2) years; median baseline CD4% 20 (14-24) & 34 (31-39), respectively) and 107 age-matched HIV-uninfected controls.
- Immune recovery was good (ART-naïve: median (IQR) CD4% change 17 (12-22)) and viral load suppression <100 copies/ml at 96 weeks was 76% (ART-naïve) and 91% (ART-experienced).
- IA decreased over time on ART. By 96 weeks in ART-naïve children, median CD4+HLA-DR+CD38+ decreased from 7% to 2%; median (IQR) CRP and TNF-a decreased from 6.4 mg/L (1.9-27.3) to 2.5 (0.8-12.0) and 9.3 pg/mL (6.5-11.9) to 4.4 (2.9-5.7) respectively (p<0.001 for all).
- Specific and broad-range PCRs for bacterial DNA were negative/very low in all groups and over time (Figures 1 & 2).
- At baseline using NGS, very low levels of microbial DNA were found in both HIV infected groups, including *Staphylococcus aureus, Enterobacteriaceae, Veillonellae* & *Clostridiales*.

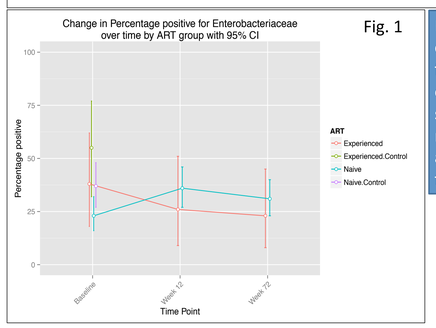

Figure 1: Widely overlapping 95% confidence intervals in percentage positive for *Enterobacteriaceae* qPCR in ART naïve, experienced and HIV-negative controls. Non-significant change over 72 weeks in ART-naïve and ART experienced groups (p=0.47 and 0.5 respectively by Wilcoxon rank sum test

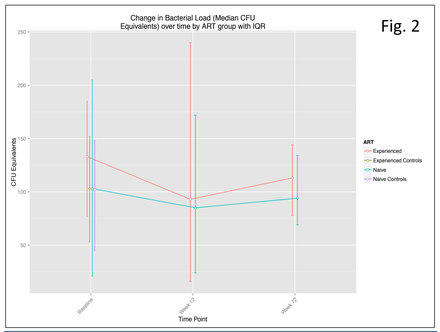

Figure 2: No significant difference in bacterial load as measured by broad range 16S rDNA PCR (median colony forming unit equivalents) between HIV-negative control groups and ART-naïve/experienced HIV-infected groups. Non-significant change in proportions positive over 72 weeks in HIV-infected groups regardless of ART-naïve/ experienced status (p=0.26 and 0.44 respectively by Fisher's exact test).

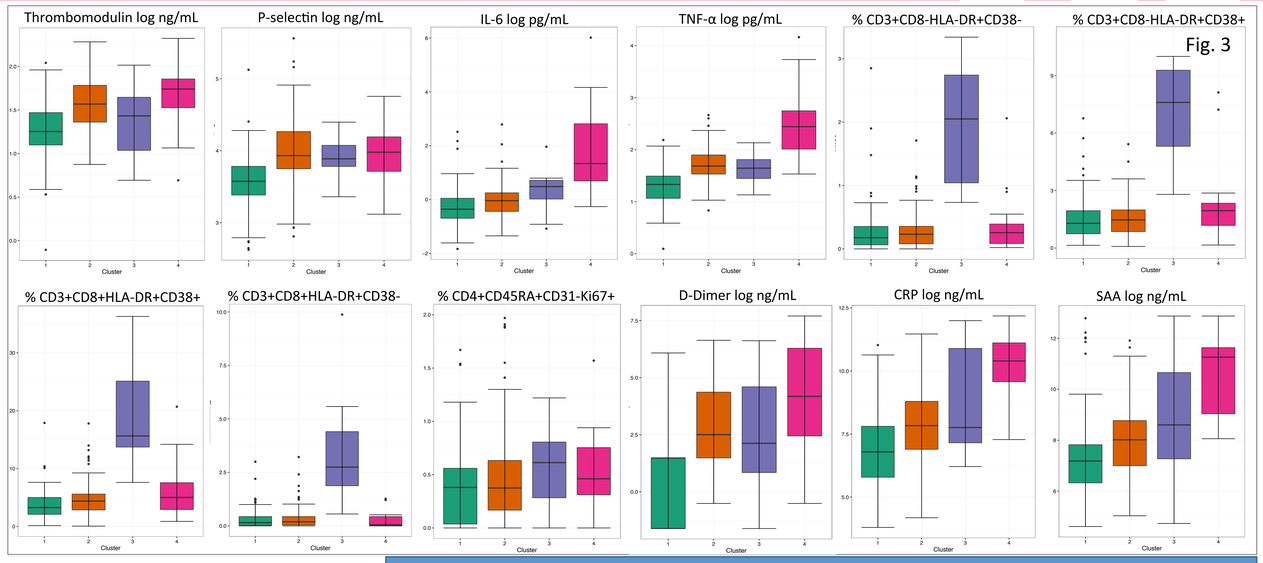

Figure 3 : Boxplots of IA markers at week 96 by clusters, showing differential expression of soluble and cellular markers between clusters. Title refers to y-axis including unit. For cellular markers, % refers to percentage of total cells. Cluster 1: green, cluster 2: orange, cluster 3: purple, cluster 4: pink.

Results continued

- Cluster analysis at Week 96 identified 4 clusters (Table 2, Figures 3, 4 & 5).
- **Cluster 1:** (n=103) lowest levels of IL-6, TNF-α thrombomodulin, P-selectin, .
- **Cluster 2:** (n=115) high CD4 %, P-selectin and thrombomodulin, but lower IL-6 and TNF-α
- **Cluster 3:** (n=10) high levels of DR+ on both CD8+ and CD4+ cells; and high CD4+CD45RA+CD31-Ki67+
- **Cluster 4:** (n=21) high IL-6 and TNF-α.
- Levels of microbial DNA at week 72 did not differ between clusters except *Enterobacteriaceae* (higher in clusters 1 & 3 compared to cluster 2 & 4(p<0.01)) (Table 2).

Table 2: Characteristics and microbial translocation markers by cluster group compared by Fisher's exact test and Student's t-test.

Characteristics		Cluster 1	Cluster 2	Cluster 3	Cluster 4	p-value
N (row %)		103 (41)	115 (46)	10 (4)	21 (8)	
Receipt of ART at baseline N (column %)	ART naive	57 (55)	49 (43)	5 (50)	9 (43)	0.02
	ART experienced	14 (14)	5 (4)	0 (0)	3 (14)	
	HIV negative	32 (31)	61 (53)	5 (50)	9 (43)	
Viral suppression at week 96 (<100 copies/ mL) N (column %)	Yes	64 (62)	33 (29)	3 (30)	3 (14)	<0.001
	No	7 (7)	12 (11)	2 (20)	8 (38)	
	HIV negative	32 (31)	61 (53)	5 (50)	9 (43)	
	VL unavailable	0 (0)	9 (8)	0 (0)	1 (5)	
I-FABP pg/mL[1]		148 (79-227)	150 (82-206)	147 (112-183)	157 (100-297)	0.99
N positive by PCR at week 72 (column %)	Assay Sensitivity					
16S rDNA	5-50 CFUs/PCR reaction[2]	98 (95)	107 (93)	9 (90)	20 (95)	0.86
Bifidobacterium spp.	0.1-10 CFUs/PCR reaction[2]	0 (0)	0 (0)	0 (0)	0 (0)	-
Staphylococcus aureus		3 (4)	5 (10)	0 (0)	0 (0)	0.53
Streptococcus pyogenes		0 (0)	0 (0)	1 (10)	0 (0)	0.04
Fusobacterium spp.		1 (1)	1 (1)	1 (10)	0 (0)	0.18
Enterobacteriaceae		44 (43)	32 (29)	5 (50)	1 (5)	0.002
Staphyloccocus spp.		0 (0)	0 (0)	0 (0)	0 (0)	-
Lactobacillus spp.		1 (1)	0 (0)	0 (0)	0 (0)	0.55

[1]I-FABP: Intestinal fatty acid binding protein; [2]CFU: colony forming units

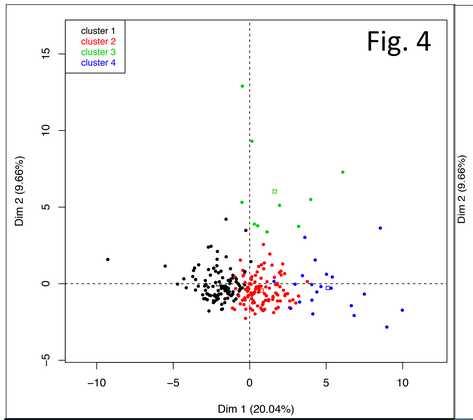

Figure 4: Principal components analysis by IA markers showing distribution of four clusters on the first and second dimensions

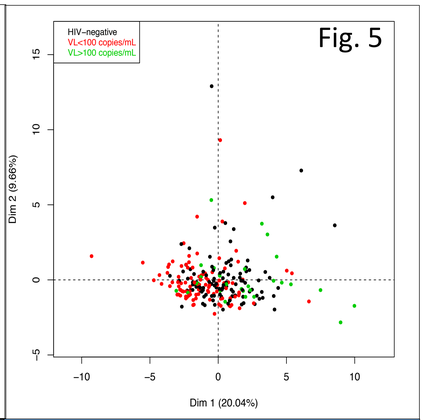

Figure 5: Overlapping distribution of those with viral suppression and similar HIV status on the first and second dimensions. VL: viral load

Conclusions

1. Immune recovery was good with concomitant decrease in IA over time on ART
2. Although there was some evidence of clustering by immune activation markers at week 96, there was no clear clustering by HIV status or VL suppression
3. Levels of bacterial DNA were low regardless of HIV/ART/IA status or time on ART
4. MT markers did not appear to be linked to HIV infection, immune status or IA.
   - MT may not be a significant driver of IA in this setting.