Inflammatory Cytokines in ART-Naive Kenyan Children Diagnosed With HIV at Hospital Admission Predicts Increased Risk of Mortality in Kenyan Children with Untreated HIV Infection

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Background

- HIV infection leads to persistent inflammation and immune activation which contributes to both immediate and long-term morbidity and mortality.
- A cascade of inflammatory responses begins after HIV infection, prompting cytokine production and leading to a persistent inflammatory state which is associated with an increased risk of adverse outcomes and death.
- While many studies have found higher cytokine levels in PLHIV, and associations between high inflammatory cytokines and death, to date there are sparse data on children living with HIV (CLHIV) in the critical care setting.

- **Hypothesis**:
  - We hypothesized that elevated levels of pro-inflammatory cytokines -- IL-1β, IL-2, IL-6, and TNF-α -- predict poorer clinical outcomes including death within 6-months of admission, combined endpoint of death or continued hospitalization at 15-days, and mean number of days spent in hospital.

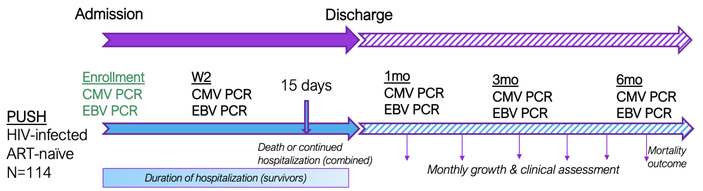
- **Objective**:
  - We aimed to assess baseline serum proinflammatory cytokine levels and their association with hospitalization outcomes in a cohort of severely-ill ART-naïve Kenyan children living with HIV (CLHIV).

KEY TAKEAWAY

- ✓ IL-6 was detected in 103 (99.04%) children, IL-1β in 7(6.31%), IL-2 in 26(25%), and TNF-α in 47(45.19%).
- ✓ **IL-1β detection** was independently associated with higher $\log_{10}$ cytomegalovirus (CMV) DNA-level (p<0.01) and pneumonia diagnosis (p<0.01). Receipt of steroids was associated with lower prevalence of IL-1β detection (p<0.01).
- ✓ Total neutrophil count (p<0.01), hemoglobin level (g/dL) (p<0.01), and CD4% (p<0.01) were independently associated with decreased **IL-2 detection**.
- ✓ Higher C-reactive protein (p<0.01) and higher CD4% (p<0.01) were associated with higher **IL-6 levels**.
- ✓ Each doubling of IL-6 levels was associated with a 27% increased hazard of death (aHR=1.27 (95%CI=1.07, 1.50), p<0.01).
- ✓ No correlates of **TNF-α** were identified.
- ✓ No association between any cytokines and hospitalization duration or the combined outcome were found.

Methods and Population Characteristics

- The analysis utilized admission serum samples of 104 of hospitalized ART naïve Kenyan children aged 0-12 years newly-diagnosed with HIV in the Pediatric urgent start of HAART (PUSH) study (1).
- All children initiated ART and were followed up for 6 months (with visits at 2 weeks, 1, 3, and 6 months) during which plasma, serum, and PBMC samples were collected (Fig. 1).
- We measured baseline levels of IL-1β, IL-2, IL-6, and TNF-α for the 104 serum samples.



- **Outcomes**:
  - Death
  - Continued hospitalization at 15 days
  - 6-month mortality
  - Duration of hospitalization

Fig. 1. PUSH study flow chart

Table 1. POPULATION CHARACTERISTICS	Total (N)*	Median (IQR) / n(%)
Age (at enrollment in years)	104	2.05 (0.99, 6.02)
Female	104	48 (46.15%)
WAZ <-2SD	99	62 (62.63%)
Severe acute malnutrition (SAM)	73	21 (28.77%)
Baseline WHO stage III/IV	103	74 (71.84%)
Total neutrophil count	99	3.17 (1.73, 5.11)
Severe immunosuppression (age and CD4%)	103	82 (79.61%)
Log_{10} HIV RNA copies/ml	95	5.76 (5.15, 6.27)
Died before 6 months	104	25 (15.3%)
Died or continued hospitalization at 15 days	104	44 (42.31%)
Continued hospitalization among survivors (day 15)	80	20 (25%)
Median duration of hospitalization (days)[a]	95	11 (6, 17)

*A total of 104 (57.5%) of 181 children enrolled in the PUSH study had serum available for baseline cytokine measurement.

Results

Table 2: Concurrent EBV and CMV viremia at admission (Correlates and Survival Outcomes)

		Cytokine exposure variable			
Outcomes	**Measure of association**	**IL-1β detection**	**IL-2 detection**	**log_2 IL-6 level**	**TNF-α detection**
		7 (6.31%) children detected	26 (25%) children detected	103 (99.04%) children detected	47 (45.19%) children detected
Combined endpoint	IRR (95% CI)	1.01 (0.41, 2.47), p=0.98	1.4 (0.88, 2.20), p=0.15	1.05 (0.94, 1.19), p=0.33	1.45 (0.92, 2.28), p=0.10
	aIRR (95% CI)	0.94 (0.38, 2.30), p=0.90	1.27 (0.78, 2.08), p=0.32	1.05 (0.94, 1.16), p=0.34	1.44 (0.92, 2.25), p=0.10
6-month mortality	HR (95% CI)	1.17 (0.27, 5.01), p=0.82	1.50 (0.64, 3.52), p=0.34	**1.32 (1.10, 1.59), p=0.002**	1.86 (0.82, 4.19), p=0.13
	aHR (95% CI)	1.04 (0.24, 4.44), p=0.96	0.94 (0.38, 2.32), p=0.90	**1.27 (1.07, 1.50), p=0.006***	1.78 (0.79, 4.01), p=0.16
Mean days hospitalized	Coeff (95%CI)	1.67 (-9.28, 12.63), p=0.76	3.43 (-2.75, 9.61), p=0.27	-0.73 (-2.14, 0.67), p=0.30	1.87 (-3.47, 7.23), p=0.48
	aCoeff (95%CI)	1.50 (-9.59, 12.59), p=0.78	3.70 (-2.87, 10.28), p=0.26	-0.73 (-2.18, 0.71), p=0.31	1.89 (-3.51, 7.30), p=0.48

Notes. All analyses adjusted for age and sex at enrollment. *Additional adjustment for HIV RNA level did not change findings for any cytokines except IL-6. Each doubling of IL-6 levels was associated with a 39% increased hazard of death (aHR = 1.39 (1.12, 1.72), p=0.003).

Conclusion and Future Work

- In this cohort of Kenyan children diagnosed with HIV at hospitalization, each doubling of IL-6 levels was associated with a 39% increased hazard of death (aHR = 1.39 (1.12, 1.72), p=0.003).
- These findings provide novel insights into drivers of inflammation in severely-ill ART-naïve CLHIV and suggest IL-6 is a prognostic marker for increased risk of death in this population.

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References:

[1]Njuguna Lancet HIV (2018)