CRISPR-detected cell-free DNA for pediatric TB diagnosis and treatment response

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BACKGROUND

Children with HIV (CHIV) are at high risk for tuberculosis (TB)-related morbidity and mortality but are often missed by assays testing respiratory samples.

We developed a CRISPR-based TB assay (CRISPR-TB) that detects an *M. tuberculosis* (Mtb)-specific multicopy insertion element (IS6110) in cell-free DNA (cfDNA) isolated from 200µl serum samples.

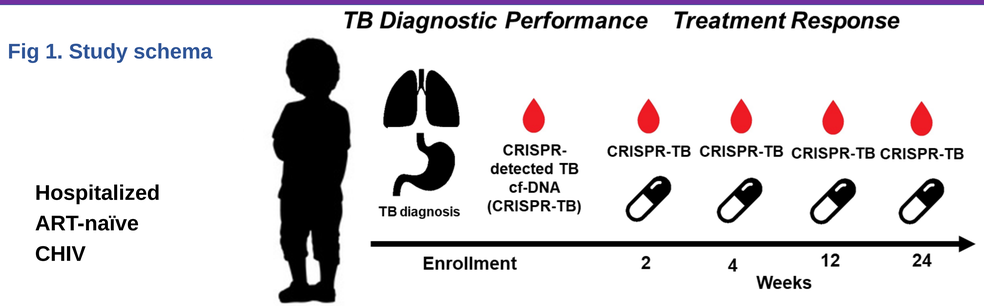
METHODS

- Cryopreserved sera were collected from ART-naïve hospitalized CHIV ≤12 years of age in Kenya at baseline and follow-up
- Children underwent baseline TB evaluation: CXR, TST, sputum or gastric aspirates Xpert/culture, stool Xpert
- Children were post-hoc classified by NIH pediatric TB case definitions: Confirmed, Unconfirmed, Unlikely TB

Outcome: Diagnostic performance of CRISPR-detected cfDNA for TB diagnosis and treatment response in CHIV

CRISPR-TB ***identified all children with confirmed TB*** and has potential for ***treatment response monitoring*** in children with HIV, including those at greatest risk of death.

Fig 1. Study schema



RESULTS

Table 1. Characteristics of hospitalized CHIV

N=153	n or median (% or IQR)
Age, years	2.0 (0.8-5.1)
Female	73 (47.7)
Severe immunosuppression	104 (68·4)
Signs/symptoms of TB	115 (75.2)
TST+ or TB exposure	29 (19)
CXR suggestive of TB	86 (58.5)
Resp cx or Xpert+	12 (7.8)
Stool Xpert+	8 (5.9)
TB treatment initiated	58 (37.9)
TB treatment response	47 (81.0)
Died	28 (18.3)

Fig 2. Study flow

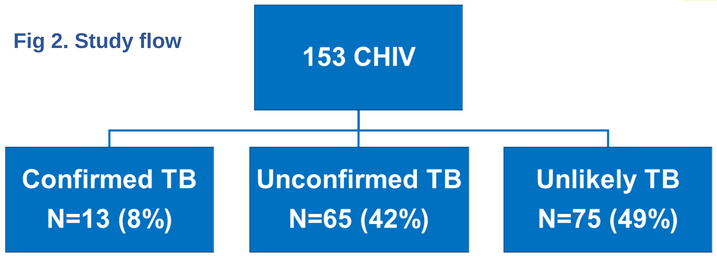


Table 2. *Mtb*-cfDNA detection for TB diagnostic performance

	Confirmed TB	Unconfirmed TB (N=65)		Unlikely TB (N=75)	
	Microbiologic confirmation N=13	Clinical TB diagnosis N=46	No clinical TB diagnosis N=19	Any NIH TB Criteria N=61	No NIH TB Criteria N=14
***Mtb* cfDNA+ children #**	13	39	13	34	3
Sensitivity - % (95% CI)	100 (75.3-100)	84.8 (71.1-93.7)	68.4 (43.4-87.4)	--	--
Specificity - % (95% CI)	--	--	--	44.3 (31.5-57.6)	78.6 (49.2-95.3)
Deaths - TB untreated	100%; 2/2	--	42.1%; 8/19	16.7%; 10/60	0%; 0/14
Deaths - TB treated	36.4%; 4/11	8.7%; 4/46	--	0%; 0/1	--
***Mtb* cfDNA (×10⁶ a.u.) [a] - Median (IQR)**	5.8 (3.2, 9.0)	4.3 (2.0, 7.3)	3.0 (1.1, 6.5)	2.0 (1.1, 5.4)	1.1 (0.99, 1.2)
p-value [b]	<0.001	0.002	0.05	0.06	Reference

[a] The *Mtb*-cfDNA level as detected by CRISPR-TB, positive cutoff threshold is 1.4 ×10⁶ a.u.
[b] Wilcoxon rank sum test compared to Unlikely TB with No NIH Criteria

CRISPR-TB detected all children with confirmed TB, and most with unconfirmed TB
Mtb cfDNA higher among confirmed and unconfirmed TB vs. unlikely TB (with no NIH TB criteria)

Table 3. Median *Mtb*-cfDNA levels before and after TBTx initiation

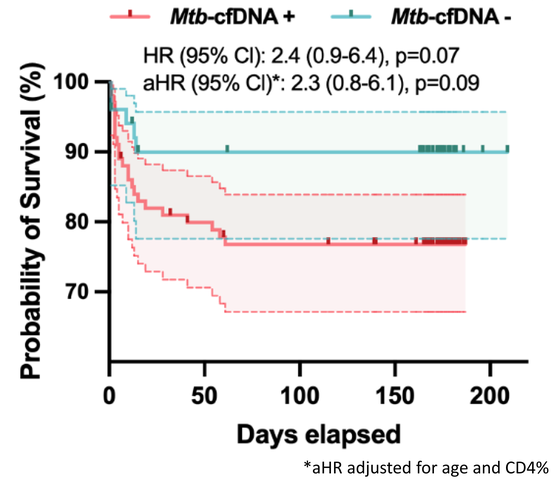
	Pre TBTx *Mtb* cfDNA (×10⁶ a.u.) – Median (IQR)	Post TBTx initiation [a] *Mtb* cfDNA (×10⁶ a.u.) – Median (IQR)	p-value [b]
All (N=51)	4.7 (2.6-8.4)	1.7 (1.2-4.1)	<0.001
Confirmed TB (N=9)	7.0 (5.8-9.6)	2.8 (1.4-5.1)	0.008
Unconfirmed TB (N=41)	4.3 (2.5-7.9)	1.5 (1.1-3.0)	<0.001
Unlikely TB (N=1)	9.2	3.8	n/a

[a] Median of 5.6 months (IQR 3.2-5.8) post-TB treatment initiation
[b] Wilcoxon matched-pairs signed-rank test

CRISPR-TB detected Mtb cfDNA decreased with TB treatment

RESULTS (cont.)

Fig 3. 6-month mortality by baseline Mtb cfDNA status



*aHR adjusted for age and CD4%

Trend for increased 6-month mortality among CHIV with Mtb cfDNA+ at enrollment

CONCLUSIONS

- In CHIV at high risk for TB, CRISPR-TB detected all children with confirmed TB, and identified an additional 85% of children with unconfirmed TB missed by microbiologic methods
- Mtb cfDNA levels decreased following TB treatment initiation, with near or complete Mtb-cfDNA clearance by 6-months post-TB treatment initiation
- CHIV with positive CRISPR-TB results at enrollment had a 2.3-fold higher risk of mortality by 6-months

COLLABORATORS

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University of Washington Department of Global Health
Tulane University School of Medicine
Emory University
Kenya Medical Research Institute KEMRI
Global WACh Woman · Adolescent · Child · health
University of Nairobi

Funding

DOD W8IXWH1910926; NICHD R01HD090927, R01HD103611 R01HD023412; NIAID K23AI120793, R21AI143341, R01AI162152; CFAR P30AI027757

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