Minimally invasive autopsy confirms HIV-1 persistence in multiple compartments despite prolonged cART

Adriaan Basson[1], Nadia Sabet[1], Tanvier Omar[2], Melanie Moodie[1], Monique Nijhuis[1,3], Anne Marie Wensing[1,3], Caroline Tiemessen[1], Francois Venter[1], Ebrahim Variava[1], Neil Martinson[1], Maria Papathanasopoulos[1]

[1]University of the Witwatersrand, Johannesburg, South Africa; [2]National Health Laboratory Services, Johannesburg, South Africa; [3]University Medical Center Utrecht, Utrecht, The Netherlands

BACKGROUND

HIV cure strategies require sophisticated knowledge of the formation and maintenance of latent and active reservoirs of HIV-infected cells. The overall paucity of individuals undergoing autopsy have hampered investigation of the anatomical distribution of infected cells during prolonged combination antiretroviral therapy (cART).

To date, only three studies have reported on autopsy findings: the Last Gift study (n=6; Chaillon *et al*., 2020, JCI), CanCure (n=2; Dufour *et al*., 2023, Cell Reports) and a Zambia Cohort (n=8; Liu *et al.,* 2022, JCI Insights). In 2018, we conducted a pilot study (FIND 1.0) and in 2019, we started the FIND 2.0 study - the largest postmortem study using minimally invasive autopsy on deceased virologically suppressed and unsuppressed adult persons with HIV (PWH) to characterise viral reservoirs.

METHODS

Recruitment site: Admitted adult PWH who died at Klerksdorp Tshepong Hospital, North West Province, South Africa, between September 2019 to April 2022.

Recruitment procedures: Relatives of eligible deceased PWH provided informed consent, ultrasound-guided autopsy was performed ≤16hrs hours of death. Documented demographic, clinical data, causes of death.

Ultrasound-guided core needle biopsies were used to sample a broad range of body fluids and organs postmortem. Trocars were used for minimal contamination. (Figure 1).

Statistical Analysis: using Stata v16.0.

HIV proviral DNA was detected in ALL liquid and deep tissue samples tested, in virologically suppressed (n=37) and viremic (n=22) deceased PWH (Figure 2).

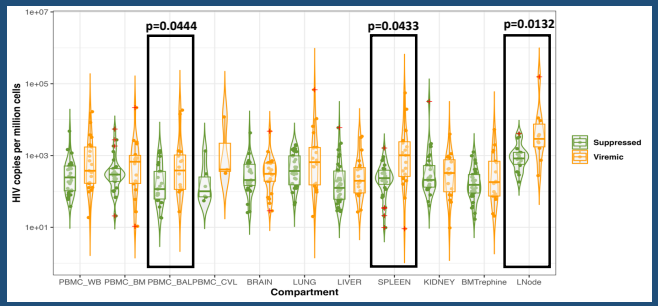


Figure 2: HIV proviral quantification for each autopsy sample type, per viremic status of the 60 FIND 2.0 deceased PWH

Of all compartments tested, only the **spleen, lymph nodes and PBMC_BAL** of viremic patients had *significantly higher* proviral DNA concentrations.

RESULTS

We conducted minimally invasive autopsies on 60 deceased individuals (Tables 1 and 2), collected 875 unique specimens from 18 distinct anatomical compartments and analysed 519 unique specimens from 11 anatomical compartments for the presence of HIV DNA (Table 3). Total HIV reservoir size/burden (within body) and % contribution of each compartment is shown in Tables 4 and 5.

Table 1: Demographic and Clinical Data of 60 deceased PWH enrolled on the FIND 2.0 study.

		Virologically Suppressed	Viremic	p values
Total # of deceased individuals		38	22	
Gender (Female)		50% (n=19)	31.8% (n=7)	0.171
Age in years (mean ±SD)		**53.0 ± 11.46**	**42.5 ± 14.4**	**0.004**
Ethnicity- Black African		92.1% (n=35)	100% (n=22)	0.29
Months since HIV diagnosis		**91.5 (IQR 63.8-121.9)**	**29.6 (IQR 0.6-124.8)**	**0.041**
ART treatment status		**100% on cART & Compliant**	**41% (n=9) on cART; 33.3% (n=3) compliant 59% (n=13) cART naive**	**<0.001**
Last VL (RNA copies/ml)		**0.2 (IQR 0.2-0.2)**	**98078 (IQR 1830-269150)**	**<0.0001**
Last CD4 counts (cells/mm³)		**236 (IQR 108-438)**	**46 (IQR 17-178)**	**0.003**
Clinical @ admission	Current OI's	36.8% (n=14)	63.6% (n=14)	0.055*
	Current TB	23.1% (n=9)	22.7% (n=5)	0.93
	Neurological Disease	18.4% (n=7)	18.2% (n=4)	0.63
	Respiratory Symptoms	44.7% (n=17)	59.0% (n=13)	0.28
	Abnormal Chest X ray	79.9% (n=30)	95% (n=21)	0.39
Time to autopsy (hours)		10.5 (IQR 5.7-14.8)	8.4 (IQR 6.6-12.2)	0.197

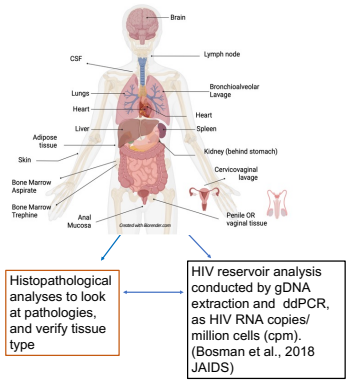


Figure 1: Liquid and Tissue samples collected from the FIND 2.0 deceased PWH.

Table 2: ARV regimens of FIND 2.0 deceased PWH.

Treatment Regimen	Virologically Suppressed	Viremic
TDF/FTC/EFV	22	2
TDF/FTC/LPV/r	2	0
TDF/3TC/LPV/r	1	1
AZT/3TC/EFV	1	0
TDF/3TC/DTG	8	0
ABC/FTC/EFV	1	0
AZT/3TC/DTG	3	0
AZT/3TC/LPV/r	0	2
ABC/3TC/DTG	0	1
Unknown	0	3
Total on cART	**38**	**9**

CONCLUSIONS

Core needle biopsies are an acceptable, and effective method for autopsy of deceased PWH. Results confirm that HIV reservoirs persist (and are detectable) in all the anatomical compartments tested, in virologically suppressed and viremic deceased PWH.

The data implies that successful, prolonged cART largely impacts the size of the viral reservoir in PBMC BAL and secondary lymphoid tissues (LN and spleen), since there was no significant differences detected in proviral DNA concentrations in the other anatomical compartments. This should be verified in a larger sample size.

Multiple anatomical sites can act as hubs for HIV dissemination within the body. This poses the question of whether PBMC_BAL and the major secondary lymphoid tissues (LN and spleen) harbor a greater replication competent reservoir, responsible for viral rebound, and repopulating tissues throughout the body. Ongoing work is evaluating viral sequences and the presence of replication competent HIV in the different compartments.

Findings provide new insights on the anatomical location of HIV-infected cells that persist despite prolonged cART to be targeted for effective cure interventions.

Acknowledgements

We thank the next-of-kin for providing informed consent to enrol their deceased relatives onto this study. This research was funded by the South African Medical Research Council through its Grants Innovations and Product Development business unit.

Table 4: Comparison of *within body* total HIV reservoir size, median (IQR)

Virologically Suppressed	Viremic	p value
n=37 2906.8 (1523.0 - 6385.9)	n=22 6220.4 (1984.4- 15643.7)	**0.044**

Table 5: % contribution of each compartment to *within body* total HIV reservoir*, median (IQR)

	Virologically suppressed	Viremic	p value
Spleen	n=32 7.3 (3.6 -11.5)	n=19 16.7 (2.9-16.7)	**0.0076**
CVL	n=6 6.2 (2.5-7.0)	n=3 21.9 (16.6-41.5)	**0.039**
Kidney	n=27 8.1 (3.1-17.4)	n=19 4.2 (2.9-9.2)	**0.03**

*The % contribution of all other anatomical sites to the total HIV reservoir were not significantly different

Table 3: Biorepository of different liquid and tissue samples collected (multiple vials stored), for the 60 FIND 2.0 deceased PWH

Compartment	Whole Blood	BM Aspirate	CSF	BAL	CVL	BM Trephine	Brain	Liver	Lung	Kidney	Spleen	Heart	Lymph Node	Skin	Adipose Tissue	Anal Mucosa	Penis	Vagina	TOTAL
# Collected	59	55	58	54	14	59	60	60	60	60	58	58	55	57	56	9	16	9	**857**
# Processed	56	51	ND	48	12	52	59	59	55	48	51	ND	28	ND	ND	ND	ND	ND	**519**

Author Contact information

Adriaan.Basson@wits.ac.za; Maria.Papathanasopoulos@wits.ac.za