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Original research

Impact of interrupting antiretroviral therapy started during primary HIV-1 infection on plasma neurofilament light chain protein, a marker of neuronal injury: The SPARTAC trial

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ABSTRACT

Objective: Antiretroviral therapy (ART)-conferred suppression of HIV replication limits neuronal injury and inflammation. ART interruption tests efficacy in HIV cure trials and viral rebound after ART interruption may induce neuronal injury. We investigated the impact of protocol-defined ART interruption, commenced during primary HIV-1 infection (PHI) on a biomarker of neuro-axonal injury (neurofilament light protein (NfL)), and its associations with inflammation (D-dimer and interleukin-6 (IL-6)) and HIV-1 reservoir size (total HIV-1 DNA).
Design: Retrospective study measuring plasma NfL in 83 participants enrolled in SPARTAC randomised to receive 48-weeks ART initiated during PHI, followed by ART interruption.
Methods: NfL (Simoa immunoassay, Quanterix™) was measured before ART, after 48 weeks on ART, and 12 weeks after stopping ART. Plasma D-dimer and IL-6, and total HIV-1 DNA in peripheral $CD4^+$ T-cells results were available in a subset of participants. Longitudinal NfL changes were assessed using mixed models, and associations with clinical and laboratory parameters using linear regression.
Results: NfL decreased following 48-weeks ART (geometric mean 6.9 to 5.8 pg/mL, $p = 0.006$) with no further significant change up to 12-weeks post-stopping ART despite viral rebound in the majority of participants

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(median 1.7 to 3.9 plasma HIV-1 RNA log_{10} copies/mL). Higher baseline NfL was independently associated with higher plasma HIV-1 RNA ($p = 0.020$) and older age ($p = 0.002$). While NfL was positively associated with D-dimer ($n = 48$; $p = 0.002$), there was no significant association with IL-6 ($n = 48$) or total HIV-1 DNA ($n = 51$). *Conclusions:* Using plasma NfL as a surrogate marker, a decrease in neuro-axonal injury was observed in a cohort of participants following ART initiation during PHI, with no evidence of neuro-axonal injury rebound following ART interruption for up to 12 weeks, despite viral rebound in the majority of participants.

1. Introduction

Antiretroviral treatment (ART) has improved survival for people with HIV,[1] however, upon ART cessation, plasma viral load rebound occurs in most people.[2] The source of rebounding virus originates from latently infected cells, the 'reservoir'.[3] Whilst SMART[4] identified that interrupting ART with a $CD4^+$ count re-initiation criteria was unsafe, recent HIV-1 eradication research suggests that with monitoring, analytical treatment interruption (ATI) may be safe, whilst being the best way of testing efficacy and identifying remission.[5]

The main HIV reservoir resides in resting memory $CD4^+$ T-cells, but evidence suggests that the central nervous system (CNS)[6–12] is another important site. A concern with HIV-1 eradication strategies and ATI is that viral rebound in the CNS could precipitate immuno-activation and neuro-inflammation, leading to neuronal injury.[8] Evidence demonstrates that within 4 weeks of stopping ART initiated during primary HIV-1 infection (PHI), plasma biomarkers of inflammation (IL-6 and D-dimer) which decreased during ART, return to pre-ART levels[13] and that IL-6 and D-dimer mediate neuroinflammation and may cause neuro-axonal injury.[14–16] Early ART initiation is associated with lower HIV-1 DNA (a measure of HIV-1 reservoir),[17–19] and when stopping ART, is associated with a delayed time to virus rebound.[20,21]

Reported CNS adverse events during HIV-1 eradication studies including ATI are rare.[22–26] However, in many modern studies the period off ART is carefully monitored, with ART reinitiated at early viral rebound.[27] Nonetheless, it remains imperative to ensure CNS safety. Cerebrospinal fluid (CSF) neurofilament light protein (NfL) is a validated, sensitive and dynamic biomarker of CNS neuro-axonal injury[28–30] with elevated concentrations reported in neurological disorders, including across the spectrum of HIV infection.[31–33] The neurofilament complex form a major structural component of myelinated axons and sustain the structural and functional integrity of axons.[29] Neurofilaments make up around 85% of the cytoskeleton proteins and contain four main subunits with different molecular weights: NfL (68 kDa), neurofilament medium (150 kDa), neurofilament heavy (190–210 kDa) and α-internexin (66 kDa), of which NfL is the most abundant and most soluble.[28] In conditions involving cortical neuronal injury, neurofilament proteins can be used as a biomarker of neuro-axonal injury. Following an injury, neurofilament proteins from the damaged neuro-axonal units are released proportional to the severity of injury into interstitial fluid and enters the CSF, where they can then be measured.[33] However, restricted by accessibility, frequent CSF NfL measurement is difficult. A novel Simoa assay which can reliably measure blood NfL (usually 50–100 times lower than CSF NfL) has been developed,[34] thus removing the barriers faced by CSF sampling. Preliminary data demonstrates that blood NfL correlates moderately to strongly with CSF NfL[34–38] across a variety of neurological disorders, including HIV disease[34,39–42] and a recent meta-analysis demonstrated moderate correlations between CSF and blood NfL, especially when blood NfL was measured using Simoa or electrochemiluminescence assays, further strengthening the evidence for blood NfL as a reliable surrogate marker for CSF NfL.[43]

The primary aim of our study was to determine whether stopping ART was associated with increased neuro-axonal injury. Secondary aims were to investigate associations between neuro-axonal injury, inflammation and HIV-1 reservoir size.

2. Materials and methods

2.1. Participants

Short Pulse Anti-Retroviral Therapy at Seroconversion (SPARTAC)[44] was a multicentre, randomised controlled trial comparing 12 weeks ART or 48 weeks ART, with deferred ART (standard of care at the time), amongst participants diagnosed within six months of HIV-1 seroconversion. HIV-1 viral load and $CD4^+$ count measurement was 12-weekly until $CD4^+$ count <350 cells/mm^3, reflecting international treatment guidelines at the time.[45] Stored plasma samples from participants allocated to the 48-week ART arm at baseline (before ART), week 48 (after 48-weeks ART) and week 60 (12-weeks after stopping ART) were assessed. Plasma samples from participants were aliquoted and stored at −80 °C in the Kings College London Infectious Diseases Biobank, before shipment to the UK Dementia Research Institute, University College London for analysis. All participants gave written informed consent for future use of their stored samples; the trial was approved by research ethics committees in each country.[44] Further detail on the SPARTAC study including participant characteristics has previously been described.[44]

2.2. Laboratory analyses

Samples from weeks 0, 48 and 60 were analysed at the UK Dementia Research Institute, University College London, UK using the NF-light assay on the HD-X Simoa instrument (Quanterix™, USA).[46]

Plasma D-dimer and IL-6 were measured previously in a subset of participants enrolled in Brazil, Australia, Italy and the United Kingdom at baseline, week 48 and week 60.[13] Total HIV-1 DNA from $CD4^+$ T-cells enriched from peripheral blood mononuclear cells was measured previously in participants with clade B virus at baseline and week 48.[20]

2.3. Data analysis

Statistical analyses were performed using Stata 17.0. P-values <0.05 were considered statistically significant. NfL was considered high if $>$ 10 pg/mL in participants aged <51 years, and if >15 pg/mL in participants 51–61 years.[34,47] The lower limit of quantification for HIV-1 RNA was <50 copies/mL, except in Africa, where it was <400 copies/mL which was the lower limit of detection using routine assays in Africa at the time of study. Longitudinal changes in NfL, D-dimer, IL-6 and total HIV-1 DNA were analysed using mixed models. Comparisons of high NfL between time-points was done using the exact McNemar test. Associations between baseline NfL with age, sex (sex assigned at birth), $CD4^+$ T-cell counts, $CD4^+/CD8^+$ ratio, plasma HIV-1 RNA, duration between seroconversion and randomisation, weight, creatinine clearance (Cockcroft-Gault formula), and in subgroups with D-dimer and IL-6, and total HIV-1 DNA data were analysed using linear regression. Correlations between NfL and laboratory parameters were assessed using Pearson's correlation. Missing values for baseline NfL ($n = 4$) and weight ($n = 5$) were imputed using multiple imputations by chained equations under the missing at random assumption, including all factors listed above in the imputation model and creating 20 imputed datasets. Predictors of change in NfL from baseline were analysed, adjusted for baseline NfL. A two-sample T-test of equal variance was performed to investigate whether NfL differed significantly in those with detectable

versus undetectable plasma HIV-1 RNA at all timepoints.

3. Results

Of 123 participants randomised to receive 48 weeks ART, 83 had stored plasma available from at least two timepoints and were included. Participant demographics are described in Table 1. Baseline characteristics of the participants included in this analysis were similar to the cohort of 123 participants allocated to 48 weeks ART.[44]

3.1. Longitudinal NfL

NfL geometric mean decreased from 6.9 (baseline) to 5.8 pg/mL (after 48 weeks ART), p = 0.006; (Table 2). There were no changes in NfL between weeks 48 and 60 despite plasma viral rebound in most during this time period (p = 0.70; Table 2). The proportion with high NfL was 17.7 % (14/79) at baseline, and 11.6 % (8/69), and 9.9 % (8/81) at weeks 48 and 60 (p = 0.31), respectively. 5/8 participants with high plasma NfL at week 48 also had so at week 60 (p = 1.0).

3.2. Factors associated with baseline NfL

In multivariable regression analysis, higher baseline NfL was independently associated with older age (0.13 [95% confidence interval (CI) 0.05, 0.21], $\log_{10}$ NfL per 10 years increase in age higher, p = 0.002) and baseline HIV-1 RNA (0.08 [95% CI 0.01, 0.14] $\log_{10}$ NfL per 1 $\log_{10}$ rise in plasma HIV-1 RNA higher, p = 0.020). No significant associations were seen between baseline NfL and sex (p = 0.56), baseline $CD4^+$ T-cell count (p = 0.28), creatinine clearance (p = 0.46) or weight (p = 0.88). Lower $CD4^+/CD8^+$ ratio and shorter time between seroconversion and randomisation were associated with higher NfL at baseline in univariable models, however, this was not significant when including HIV-1 RNA in the model (p = 0.23 and p = 0.10, respectively). None of the above factors were associated with change in NfL from baseline to week 48.

Table 1
Baseline characteristics of participants in the 48-week ART arm with plasma NfL measured at any time point (n = 83).

	All participants n = 83	Male n = 50	Female n = 33
Age, years	34 (27, 41)	36 (31, 46)	27 (22, 37)
Time from seroconversion to randomisation, weeks	13 (9, 15)	11 (7, 13)	14 (12, 17)
Weight, kg	73 (65, 82)	75 (68, 83)	65 (54, 79)
Creatinine clearance, mL/min	107 (97, 130)	113 (98, 128)	103 (81, 139)
Virus subtype			
- B	44 (53.0)	43 (86.0)	1 (3.0)
- C	26 (31.3)	1 (2.0)	25 (75.8)
- Other	13 (15.7)	6 (12.0)	7 (21.2)
Region			
- Europe[a]	42 (50.6)	39 (78.0)	3 (9.1)
- Africa[b]	30 (36.1)	0 (0)	30 (90.9)
- Australia & Brazil	11 (13.2)	11 (22.0)	0 (0)
Clinical manifestations of symptomatic HIV seroconversion illness	51 (61.5)	42 (84.0)	9 (27.3)
ART regimen initiated			
– 2 NRTI and bPI	75 (90.4)	42 (84.0)	33 (100)
– 2 NRTI and EFV	7 (8.4)	7 (14)	0 (0)
– 1 NRTI and bPI and T20	1 (1.2)	1 (2)	0 (0)

Values are median (IQR) or total (%).
ART = antiretroviral treatment, NfL = neurofilament light chain protein, NRTI = nucleoside reverse-transcriptase inhibitors, bPI = ritonavir-boosted protease inhibitor, EFV = efavirenz, T20 = enfuvirtide.
[a] Italy, Spain and United Kingdom.
[b] South Africa and Uganda.

Table 2
Clinical parameter trends over the study period.

	Week 0: Before starting ART	Week 48: After 48 weeks of ART	Week 60: 12 weeks after stopping ART
Plasma NfL, pg/mL[a]	N = 79 6.92 (5.97–8.01)	N = 69 5.77 (4.94–6.74)	N = 81 5.75 (5.08–6.52)
Plasma HIV-1 RNA, $\log_{10}$ copies/mL	N = 79 4.59 (4.03–5.18)	N = 68 1.70 (1.70–2.60)	N = 81 3.78 (2.82–4.51)
$CD4^+$ T-cell count, cells/μL	N = 79 608 (465–760)	N = 68 794 (597–995)	N = 80 714 (479–867)
CD4: CD8 T-cell ratio	N = 79 0.53 (0.38–0.82)	N = 68 0.98 (0.73–1.32)	N = 80 0.70 (0.47–1.00)
Subgroup analysis			
D-dimer, mg/L[a]	N = 44 0.36 (0.29–0.44)	N = 32 0.28 (0.24–0.33)	N = 45 0.31 (0.25–0.38)
IL-6, pg/mL[a]	N = 44 1.38 (1.08–1.78)	N = 34 1.42 (1.08–1.88)	N = 45 1.48 (1.22–1.78)
Total HIV DNA, $\log_{10}$ copies/10^6 $CD4^+$ T-cells	N = 45 3.79 (3.47–3.97)	N = 51 3.26 (3.09–3.44)	n/a

Values are median (IQR) unless stated otherwise.
n/a: not assessed at this timepoint.
[a] Values are geometric mean (95 % confidence interval).

HIV-1 RNA was <400 copies/mL in 7.6 % (6/79), 83.8 % (57/68), 24.7 % (20/81) of all participants at baseline, week 48, and week 60, respectively. Whereas participants with HIV-1 RNA ≥400 copies/mL had significantly higher NfL at baseline than participants with HIV-1 RNA <400 copies/mL (geometric mean 7.2 versus 4.0 pg/mL; p = 0.028), there was no significant difference at week 48 (5.2 versus 5.9 pg/mL; p = 0.57), or week 60 (6.0 versus 5.1 pg/mL; p = 0.29), respectively.

3.3. D-dimer, IL-6 and total HIV-1 DNA per $CD4^+$ T-cell analysis

D-dimer and IL-6 results were available in 48/83 (Table 2). D-dimer decreased significantly from baseline to week 48 (from geometric mean 0.36 to 0.28 mg/L, p = 0.017), with no further change to week 60 (p = 0.46). D-dimer significantly correlated with NfL at baseline (r = 0.66, p < 0.001), week 48 (r = 0.45, p = 0.010) and week 60 (r = 0.53, p < 0.001). Baseline D-dimer was also associated with baseline NfL (0.53 [95% CI 0.20, 0.85] $\log_{10}$ NfL per 1 $\log_{10}$ rise in D-dimer, p = 0.002) when included into a multivariable model with age and baseline HIV-1 RNA as independent factors, whereas there was no association between NfL and HIV-1 RNA (p = 0.91). In contrast, there was no change in IL-6 between baseline, week 48 and week 60 (Table 2), and there was no significant association between NfL and IL-6 at any timepoint. Total HIV-1 DNA results were available in 51/83 participants (Table 2); total HIV-1 DNA decreased significantly between baseline and week 48 (from geometric mean 5689 to 1730 copies/10^6 $CD4^+$ T-cells; p < 0.001, Table 2). However, there was no significant association between NfL and total HIV-1 DNA at baseline (r = 0.13, p = 0.40) or week 48 (r = 0.24, p = 0.13), or when analysing the two variables as change from baseline (r = 0.33, p = 0.064).

4. Discussion and conclusion

From this large, international cohort of individuals randomly allocated to interrupt ART initiated during PHI, we observed that despite evidence of neuro-axonal injury (using plasma NfL) during untreated PHI, we saw no evidence of recurrence in neuro-axonal injury up to 12 weeks post-ART interruption. Our results are in keeping with the study

in individuals with treated chronic HIV-1 disease, where following ATI, no evidence of increased neuronal injury was demonstrated at the first point of plasma HIV-1 RNA >1000 copies/mL.[48]

Data suggests that during very early HIV-1 infection, neuronal injury is often delayed compared to viral and inflammatory changes[49]; no detectable rise in CSF NfL was seen in participants with hyperacute HIV-1 infection[50] whereas CSF NfL was elevated in half of individuals several months after acquiring HIV-1.[51] A study of eight individuals on suppressive ART initiated during chronic infection who interrupted ART, demonstrated that while none developed neurological symptoms, three experienced significant rises in CSF NfL.[52] Taken together, evidence suggests that when closely monitored, short periods of ATIs are safe from a neurological perspective.

The upper limit of normal for plasma NfL is age-dependant[34,47]; in our study the proportion of participants with plasma NfL above the threshold considered normal remained similar and low across all timepoints. This may reflect that amongst these individuals, there is little neuronal injury due to the short duration of infection.

Our results are in keeping with published data demonstrating a positive association between NfL with age and plasma HIV-1 RNA.[34] While we saw a positive association between NfL and D-dimer (biomarker of pro-coagulation), we saw no evidence of an association with IL-6 (pro-inflammatory cytokine). Data from SPARTAC demonstrated that within 4 weeks of interrupting ART initiated during PHI, 78% had detectable plasma HIV-1 RNA ≥400 copies/mL,[53] plasma biomarkers of inflammation (IL-6 and D-dimer) which decreased during ART had returned to pre-ART levels[13] and plasma HIV-1 RNA strongly correlated with plasma D-dimer.[54] These findings suggest a biological explanation why viral transcription might lead to a pro-coagulation and pro-inflammatory milieu, resulting in neuro-axonal injury.

Strengths of our study include the protocol-indicated ART interruption, enabling us to assess the impact of ATI without risk of bias through confounding by indication. SPARTAC was an international study with 40 % female participants, thus our results are uniquely generalisable. Limitations include the relatively short follow-up period after stopping ART and not all participants had D-dimer, IL-6, and total HIV-1 DNA results available. Stronger correlations between CSF and blood NfL have been reported in conditions with higher CSF and blood NfL concentrations.[34–37] However, plasma NfL concentrations were generally low across all timepoints in this sub-study. Evidence suggests that the correlation between blood and CSF NfL is lower at lower NfL concentrations, thus the current assays may still be insufficiently sensitive to detect changes at these low concentrations, due to low signal-to-noise ratio. Furthermore, the lack of concurrent CSF NfL limits our knowledge about parallel trends in CSF NfL during this time period. Data on underlying comorbidities in the participants throughout the study period were not available to us, thus potential confounding factors which may have independently affected NfL concentrations including central and peripheral neurological conditions, could not be controlled or accounted for. Of note, the participants enrolled into the SPARTAC study were a relatively young cohort (see Table 1), and the prevalence of comorbidities in this population is expected to be generally low.

Our overall results are reassuring, but it is unclear whether they can be extrapolated to other populations, such as those undergoing HIV eradication strategies followed by ATI, with low nadir $CD4^+$ counts, chronic HIV infection or receiving more contemporary antiretroviral regimens.

When using plasma NfL as a surrogate marker, we observed a decrease in neuro-axonal injury in a cohort of participants following ART initiation during PHI, with no evidence of neuro-axonal injury rebound following ART interruption for up to 12 weeks. The ability to identify individuals undergoing ATI experiencing neuro-axonal injury with a blood test may be invaluable for ATI monitoring.

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Data statement

Data and materials may be made available upon reasonable request in writing to the corresponding author.

CRediT authorship contribution statement

Jasmini Alagaratnam: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wolfgang Stöhr:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Elizabeth Hamlyn:** Writing – review & editing, Formal analysis. **Kholoud Porter:** Writing – review & editing, Methodology, Formal analysis. **Jamie Toombs:** Writing – review & editing, Investigation. **Amanda Heslegrave:** Writing – review & editing, Investigation. **Henrik Zetterberg:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Magnus Gisslén:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **Jonathan Underwood:** Writing – review & editing. **Mauro Schechter:** Writing – review & editing, Investigation. **Pontiano Kaleebu:** Writing – review & editing, Investigation. **Giuseppe Tambussi:** Writing – review & editing, Investigation. **Sabine Kinloch:** Writing – review & editing, Investigation. **Jose M. Miro:** Writing – review & editing, Investigation. **Anthony D. Kelleher:** Writing – review & editing, Investigation. **Abdel Babiker:** Writing – review & editing. **John Frater:** Writing – review & editing, Investigation. **Alan Winston:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Sarah Fidler:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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