The prospective relation between Hemispheric Lateralisation and CD4+ T-cells in Human Immunodeficiency Virus Type 1 (HIV-1)

- **Dr Rachel C Sumner** – School of Health Sciences & Social Care, Brunel University, Uxbridge, UK
- **Dr Alexander V Nowicky** – Centre for Rehabilitation Research, School of Health Sciences & Social Care, Brunel University, Uxbridge, UK
- **Dr Andrew Parton** – School of Social Sciences, Brunel University, Uxbridge, UK
- **Miss Carolien Wylock** – Department of Internal Medicine, Infectious Diseases Unit, Universitair Ziekenhuis Brussel, Brussels, Belgium
- **Dr. Renata Cserjesi -** Faculty of Medicine & Pharmacy, Vrije Universiteit Brussel, Belgium
- **Dr. Benjamin Fischler -** Private Practice, Brussels, Belgium
- **Dr. Patrick Lacor** – Department of Internal Medicine, Infectious Diseases Unit, Universitair Ziekenhuis Brussel, Brussels, Belgium
- **Prof Yori Gidron –** Faculty of Medicine & Pharmacy, Vrije Universiteit Brussel, Belgium

Corresponding Author:
Dr Rachel Sumner
Brunel University
Kingston Lane
Uxbridge
Middlesex
UB8 3PH
UK

Rachel.c.sumner@gmail.com
00447927407689

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All authors declare that there are no conflicts of interest.

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Objectives: Neuromodulation of the immune system has been proposed to be influenced by hemispheric lateralisation (HL). The present study tested whether HL predicted $CD4^+$ levels, statistically controlling for confounders.

Methods: Employing two assessments of HL, 68 HIV-1^+ patients were followed prospectively. Numerous exclusion criteria and confounder assessments were employed (e.g., age, medication).

Results: Left-HL significantly positively predicted $CD4^+$ levels at follow-up, and this was moderated by medication (HAART) status: Only in HAART-naïve patients, did HL predict CD4 levels Furthermore, HL significantly predicted whether patients were in a clinically significant high/low $CD4^+$ count level.

Conclusions: The present work partly corroborated the theory of HL influences on immunity, extended it to HIV immunity and identified a moderator: HAART medication, using a more rigorous methodology. Implications for future research and treatments are provided.

Introduction

Since its discovery over 30 years ago, human immunodeficiency virus (HIV) has been one of the most widely studied viruses in medical history. Whilst much is known about how prognosis varies between patients, there is still a large proportion of this variance thus far unexplained [1, 2]. One potential means of explaining some of this

variance comes from neuroimmune influences, and more specifically from Hemispheric Lateralisation (HL) and its effects on the immune system. HL refers to the stable tendency to activate or relay functions of one hemisphere more than the other (e.g. [3, 4]). A recent review has suggested an immunopotentiating role for the left hemisphere, and an immunosuppressant role for the right hemisphere [5]. However very little confounder control has been exerted in studies so far and therefore the HL-immune relationship requires substantiation with stricter methodological control, and exploration of mediating and moderating variables.

One study has examined the implications of HL on HIV prognosis by examining the longitudinal immune correlates of HL in HIV [6]. Gruzelier *et al*. found that left HL was associated with better immune outcomes (CD4$^+$, CD8$^+$ T-cells) at a 30 month follow up. Furthermore, their study also described a relatively poorer prognosis in those patients with right HL. However, their sample was small (N=26) and relatively uniform. Most importantly, Gruzelier *et al*. did not control for effects of baseline immune parameters and other confounders (e.g., mood, mode of infection, other illnesses) that may affect the central and autonomic nervous system (ANS) or the course of HIV [7]. Additionally, the study did not selectively recruit right-handed participants, a measure which is central in HL research. Left handed individuals characterise atypical interhemispheric communication and hemispheric specialisation [8, 9]. Finally, the research was conducted before the advent of highly-active antiretroviral therapy (HAART), one of the most significant developments in HIV medicine [10]. These limitations question the validity of their inferences, and the application of the findings to modern HIV patients and treatment.

The present study aimed to extend the findings of the Gruzelier group and examine the prospective relationship between HL and CD4$^+$ T-cell counts in HIV-1$^+$ patients,

with better methodological and statistical control. Methodologically, a larger sample of participants was sought from broader demographic profiles. Only right-handed participants were recruited to eliminate the heterogeneity observed in HL amongst left handed and ambidextrous people. Additionally, strict exclusion factors were implemented to reduce confounds. Statistically, the influence of relevant confounders was tested as well. The present study also aimed to identify and evaluate potential variables that may moderate or mediate the relationship between HL and HIV prognosis. Identified *a priori*, mood, HAART medication status and adherence and duration of HIV infection were selected as potential moderating or mediating variables due to their influence on immune status and trajectory in HIV disease (e.g. [11, 12]). It was hypothesised that left-HL would predict a better immune outcome in asymptomatic HIV patients.

Methods

Participants & Recruitment Criteria

The participant sample (N=68) was drawn from a clinical population of HIV^+ outpatients of a hospital located in Brussels, Belgium. Inclusion criteria for the study were: right-handedness; asymptomatic HIV-1 disease ($CD4^+$ T-cell count> 200/mm^3); good literacy and verbal fluency in Flemish, French or English; patients aged between 18 and 80 years of age; and no presence of cognitive deficits. The exclusion criteria were based on three research requirements; cognitive capability, extraneous modulation of HIV disease course and extraneous modulation of the communication between the brain and the immune system. Cognitive capability was ascertained by including patients scoring >25 on the Mini Mental State Examination (MMSE: [13]) with candidates scoring lower than 25 being excluded from further participation.

Patients who had a diagnosis of any psychiatric illness were excluded. Additionally patients who were pregnant, or had been diagnosed with a neurological disorder, an autoimmune disease, or were receiving either immune-modulating (outside of HAART regimens) or autonomic nervous system modulating drugs were excluded. Any patients that had a medical history of dependence to or abuse of alcohol, methamphetamine or cocaine were excluded. Further, any medical history of clinically meaningful brain damage was an indicator for study exclusion.

Measures & Materials

HL was assessed using two measures; the Hemispheric Preference Test (HPT: [14]) and a computerised variant of the Line Bisection Test (LBT: [15]) using prebisected lines. The HPT includes 20 items, with 10 items reflecting either right or left characteristics (e.g., visualisation, strategic thinking, respectively). A "left" index of the HPT was calculated using the formula left-HL=100x(Left-Right)/(Left+Right). In the LBT, participants estimated which side of a prebisected line was larger, when both in fact were equal. Both tests have been validated against EEG measures of HL [16, 17]. CD4$^+$ T cell count was obtained from the participants' medical files three months before study entry (T1), at study entry (T2) and three months later (T3). Mood was assessed using the Hospital Anxiety and Depression Scale (HADS: [18]) and adherence to HAART was measured using the Morisky Medication Adherence Scale [19]. Participants were surveyed for their socio-demographic background, HIV-relevant history (mode of infection, time since diagnosis etc.) and health behaviour (e.g. addictive substance use, condom use, number of sexual partners in the previous year).

Procedure

Data was collected over three time points (T1, T2, T3). A retrospective medical baseline (T1) for T-cell data was adopted, to have a longer follow-up between immune outcomes. At study entry (T2), participants were screened with the MMSE, and were given a semi-structured interview for demographic information and adherence. Participants' HL was measured by the HPT and the LBT at T2.

Follow-up data collection was conducted at approximately three months post study entry (T3).

Statistical Analysis

All data analyses were run using the calculated left HL index with a square-root transformation of the scores, and a corresponding LHL index of the LBT. Confounders in relation to $CD4^+$ at T3 were assessed statistically from the available data using bivariate Pearson's correlations for continuous data (e.g., age, time since diagnosis) and *t* test or ANOVA for categorical data (e.g. ethnicity, mode of contraction). Those variables that presented significant ($p<.05$) associations with $CD4^+$ at T3 were used as covariates in each correlation. Analyses were conducted using Spearman's *rho* correlation in order to counteract bias due to outliers within the dataset. To ensure that confounding variables were still controlled for in these tests using the Spearman's *rho*, the T3 $CD4^+$ scores (dependent variable) were residualised on medication variables and/or T1 $CD4^+$ data, which emerged as the significant confounders. The relationship between left-HL and these residualised scores were then examined by Spearman's *rho* correlations. Moderator analyses were repeated using Spearman's *rho* correlation, split by moderator subgroup (e.g. HAART/non-HAART). The statistical and clinical significance of these results was then tested

using logistic regression where the dependent variable was a clinically significant cut-off of $CD4^+$ outcome data. Participants' outcome (T3) $CD4^+$ T-cell levels were categorised into high ($CD4^+$ T-cell count> 500/mm^3) or low ($CD4^+$ T-cell count< 350/mm^3) and analysed against the T1 $CD4^+$ scores, use of HAART and left-HL scores.

Results

Sample Characteristics

The original sample size was 72, and four participants had to be excluded from final analyses. These were due to comprehension problems (*N*=3) and outlying HPT data (*N*=1). Table 1 shows the summary composition of the sample.

Hemispheric Lateralisation and Immunity in HIV

Among all confounders, only baseline (T1) $CD4^+$ T-cell count and HAART were significantly associated with T3 $CD4^+$, hence we controlled for their effects. A Spearman's *rho* correlation was conducted between the standardised prospective $CD4^+$ T-cell measurement ($CD4^+$ T3, residualised on $CD4^+$ T1 and HAART medication as covariates) and the square-root transformation of the Hemispheric Preference Test (SQRT HPT). There was a statistically significant correlation between left-HL and standardised outcome $CD4^+$ T-cell count (*rho*=.234, *p*=.047, one tailed test, *N*=61).

Moderator Analyses

<u>Ethnicity</u>

A potential moderating factor was identified in ethnicity (African or European origination), by the observation of potential relationships between this variable and several other variables (e.g. gender, mode of HIV contraction, sexual orientation). No significant difference was observed between outcome (T3) $CD4^+$ counts for these two groups ($t_{(58)}$= 1.75, p=.086). Spearman's *rho* correlations were conducted between the standardised prospective $CD4^+$ T-cell measurement ($CD4^+$ T3, residualised on $CD4^+$ T1 and HAART medication) and the square-root transformation of the Hemispheric Preference Test (SQRT HPT) as predictor, split by ethnicity group. There were no statistically significant correlations between left HPT and standardised outcome $CD4^+$ T-cell count for the European sub-sample (*rho*=.241, p=.081, one tailed test, N=35), though a trend was observed for the African sub-sample (*rho*=.402, p=.055, one tailed test, N=17).

HAART Medication

A significant difference was observed between patients with and without HAART treatment in T3 $CD4^+$ counts ($t_{(50.80)}$= 2.06, p=.045), with those taking HAART having the higher levels of outcome T-cell count. HAART was presented as a potential moderator due to its immunomodulating effects within HIV disease, and the large proportion of participants (N=48, 70.6%) reporting to be undergoing HAART treatment. Spearman's *rho* correlations were conducted between the standardised prospective $CD4^+$ T-cell measurement ($CD4^+$ T3 residualised on $CD4^+$ T1) and SQRT HPT as predictor, split by treatment group. There was no statistically significant correlation between left HPT scores and standardised outcome $CD4^+$ T-cell count for the HAART treated sub-sample (*rho*=.153, p=.170, one tailed test, N=41). In contrast, there was a statistically significant correlation between left HPT scores and

standardised outcome CD4+ in the non-HAART group (*rho*=.627, *p*=.019, one tailed test, *N*=11; see figure 1).

Assessment of HL to predict clinically significant outcome CD4+ T-cell count

In order to explore whether the clinical categorisation of T3 (outcome) CD4+ T-cell count (high= CD4+>500/mm3; low= CD4+<350/mm3) is influenced by left HL, controlling for baseline (T1) CD4+ T-cell count and HAART medication status, a binary logistic regression model was performed. The results from the logistic regression are presented in table 2 and show that both T1 CD4+ T-cell count and left-HL (SQRT HPT) scores significantly predicted the clinically-significant categorisation of CD4+ T-cell levels at T3. Additionally we tested for an interaction between HAART and left HL with either a linear regression for the continuous outcome CD4+ (T3), or a logistic regression for the categorical outcome of high versus low CD4+. Both regressions testing this interaction were non-significant (*p*>.05). Analysis of the cells of the interaction provided very small sample sizes for the non-HAART treated sub-group (low CD4+ *N*=5; high CD4+ *N*=4) against the HAART-treated sub-group (low CD4+ *N*=11; high CD4+ *N*=28).

Discussion

Hemispheric Lateralisation & Prospective CD4+ T-cell Count

The present study sought to examine the predictive ability of HL in relation to prognosis in HIV-1+ patients. The main finding showed a significant, positive relationship between left HL and prospective CD4+ T-cell count. This finding, albeit relatively modest, is in line with those found in a previous HIV+ patient sample [6] and amongst the wider literature describing a relationship between left HL and better

immunity [5]. Further, the logistic regression uncovers a clinically significant relationship between outcome CD4+ T-cell count category (high versus low) and left HL, controlling for confounders. This finding suggests that HL could serve as a clinical predictor of CD4+ T-cell loss trajectory, and therefore an indicator of potential intervention both determined by HL and targeting HL.

Moderation

The analysis for moderation by ethnicity did not attain statistical significance, however a trend was indicated. The analysis for moderation by medication group provided a significant, positive relationship between left HL and prospective immunity only in those patients who were not receiving antiretroviral treatment. This analysis indicated that approximately 39.3% of the variance observed in outcome CD4+ levels could be attributed to LHL, controlling for baseline CD4+ counts. These new findings present a new dimension to, and the effect size is clinically significant for, the current knowledge on HL in HIV disease. However, when this moderator was tested by examining interactions for both continuous and categorical CD4+ outcomes the findings were not significant. It is possible that this lack of support from these tests may be attributable to insufficient data in one or more of the cells of the interaction, but it does mean that viewing HAART as a moderator in this relationship should be viewed with caution until the findings can be replicated in a larger sample.

Why left HL may be predictive of CD4+ outcome in treatment-naïve patients, but not HAART-treated patients, is not clear. It is possible that if both left HL and HAART are working in unison toward immunopotentiation, that the effects of HL in the presence of HAART may be masked. This explanation is partially substantiated when examining other immune cells affected in the same way by HL and HIV. For

example, induction of HAART treatment has been shown to affect (amongst others) lymphocyte proliferation and neutrophil function [20, 21]. These immune measures have also been demonstrated to be asymmetrically influenced by HL [22, 23]. It is possible that lacking HAART enables HL to manifest its immunomodulatory effects. Should this finding be replicated, it has vast implications for countries where access to HAART is limited, as activating the left hemisphere can enhance immunity [24].

Limitations

The present study's sample size was restricted due to missing data, and the dataset held prevailing skewness even after data transformation. Further, the sub-sample analyses groups were not equal in type or composition. As HAART was used as a moderating variable, control based on line and type of HAART treatment may further elucidate these findings. The period of follow-up was significantly shorter (≈ 6 months) than the 30 month follow-up employed by the Gruzelier group [6]. Finally, the HL assessments themselves were a deviation from the precedent of EEG employed by Gruzelier *et al*. [6], although the measures were validated by EEG. Nevertheless, our results support the HL-immune relations reviewed recently [5] and extend them to HIV with more rigourous methodology.

Future Directions

Further assessment of this relationship within the HIV context is required, most specifically the potential moderating implication of ethnicity and of HAART. Analysis of the quality of moderation by HAART may also provide useful information (i.e. first or second line treatments, types of medication etc.). Should

more empirical evidence support the role of HL in HIV prognosis, targeted interventions can be devised at relatively low cost, with high mobility to reach those areas still under a heavy HIV burden. These include testing whether activating the left hemisphere, by focused cognitive exercises that activate that hemisphere, could possibly affect $CD4^+$ levels in patients not receiving HAART.

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Table 1. Descriptive statistics of continuous data with mean, standard deviation and number of respondents.

Characteristic		Mean	SD	*N*
Age (years)	Whole sample	43.30	8.95	68
Gender	Male			51
	Female			18
Ethnicity	African			23
	European			46
Medication	HAART-treated			48
	HAART-naive			20
Duration of HIV (years) [64.2% sexually contracted]		6.32	6.36	64
MMSE (maximum range 25-30)		29.03	1.25	68
Mean period between clinic visits (days)		109.97	33.98	65
Non-Adherence (maximum range 0-4, 4 indicating maximum non-adherence)		.55	.77	47
HADS Anxiety (maximum range 0-21)		7.22	4.37	68
HADS Depression (maximum range 0-21)		4.13	3.54	68
Line Bisection Index of Left Lateralisation (maximum range 0-10)		5.46	2.64	68
HPT Left Lateralisation Index Z Score (based on square-root transformation)		3.05	.073	61
$CD4^+$ ($cells/mm^3$)	T1: Retrospective data point (3 months)	571.31	251.92	61
	T2: Study entry	567.64	263.29	66
	T3: Prospective data point (3 months)	545.90	242.28	60

Table 2. Logistic regression analysis of outcome $CD4^+$ T-cell levels, left HL (SQRT HPT), baseline $CD4^+$ T-cell levels and HAART medication status.

		B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP (B)	
								Lower	Upper
Step	CD4 (T3)	.022	.008	7.696	1	.006	1.022	1.006	1.038
	HAART	-.715	1.464	.238	1	.625	.489	.028	8.626
	LHPT (SQRT)	.971	.428	5.139	1	.023	2.641	1.141	6.114

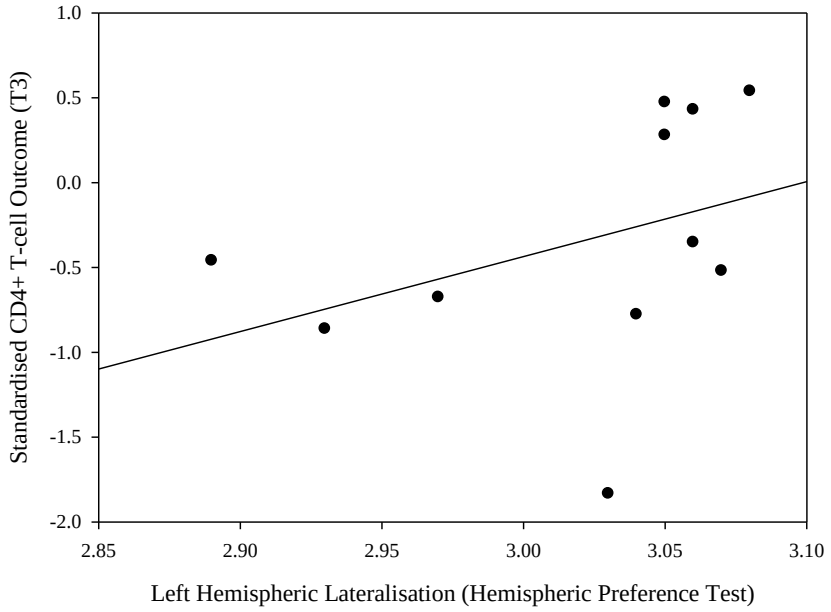

Figure 1. A scatterplot showing the correlation between left hemispheric lateralisation and standardised CD4+ T-cell count at follow-up (T3) for the non-HAART sub-sample.