Cerebrospinal fluid cytokines and neurodegeneration-associated proteins in Parkinson's Disease

Ruwani S. Wijeyekoon MRCP, PhD[1], Sarah F. Moore MB BChir[1,6], Krista Farrell MRCP, MPhil[1], David P. Breen MRCP, PhD[2,3,4], Roger A. Barker MRCP, PhD[1,5], Caroline H. Williams-Gray MRCP, PhD[1]

1 John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, UK.

2 Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, Scotland, UK

3 Anne Rowling Regenerative Neurology Clinic, University of Edinburgh, Edinburgh, Scotland, UK

4 Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, Scotland, UK

5 Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

6 University of Exeter Medical School, University of Exeter, Exeter, UK

*Corresponding author - Ruwani S. Wijeyekoon

Address - John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, E.D. Adrian Building, Forvie Site, Robinson Way, Cambridge CB2 0PY, UK.

Tel. – 01223 331160

Email - rsw27@cam.ac.uk

Word count – 1590

Running Title - Cerebrospinal fluid in Parkinson's Disease

Key words – Parkinson's disease, cerebrospinal fluid, cytokine, tau, alpha-synuclein

Financial Disclosure/Conflicts of Interest - The authors report no conflicts of interests concerning the research related to the manuscript.

Funding - Funding for this work was provided by the Rosetrees Trust (M369-F1), Addenbrooke's Charitable Trust (PF15/CWG) and the NIHR Cambridge Biomedical Research Centre Dementia and Neurodegeneration Theme (146281). RSW was supported by a Fellowship from Addenbrooke's Charitable Trust (RG77199). SFM was supported by the Transeuro EU FP7 grant (242003) and is now an NIHR Academic Clinical Fellow (ACF-2015-23-501). DPB is supported by a Wellcome Trust Clinical Research Career Development Fellowship (214571/Z/18/Z). RAB is an NIHR Senior Investigator (NF-SI-0616-10011) and is supported by the Wellcome Trust-MRC Cambridge Stem Cell

Institute (203151/Z/16/Z). CHWG holds a RCUK/UKRI Research Innovation Fellowship awarded by the Medical Research Council (MR/R007446/1) and receives support from the Cambridge Centre for Parkinson-Plus.

ABSTRACT

Introduction- Immune markers are altered in Parkinson's disease (PD), but relationships between cerebrospinal fluid (CSF) and plasma cytokines, and associations with neurodegeneration-associated proteins remain unclear.

Methods- CSF and plasma samples and demographic/clinical measures were obtained from 35 PD patients. CSF samples were analysed for cytokines (together with plasma), and for alpha-synuclein, amyloid beta(1-42) peptide, total tau and phospho(Thr231)-tau.

Results- There were no CSF-plasma cytokine correlations. IL-8 was higher and IFN-γ, IL-10 and TNF-α were lower in CSF versus plasma. In CSF, total tau correlated positively with IL-8 and IL-1β, while alpha-synuclein correlated positively with amyloid beta(1-42) and negatively with semantic fluency (known marker of PD dementia risk).

Discussion- CSF and peripheral cytokine profiles in PD are not closely related. Associations between CSF IL-8 and IL-1β, and tau suggest CSF inflammatory changes may relate to tau pathology within PD. CSF alpha-synuclein/amyloid beta may reflect the risk of developing PD dementia.

BACKGROUND

Parkinson's disease (PD) is associated with central and peripheral immune changes[1]. However, the relationships between these changes and central neurodegeneration are unclear. The cerebrospinal fluid (CSF) is in close contact with the central nervous system (CNS) and studying CSF immune markers and neurodegeneration-associated proteins, alongside paired plasma immune markers may provide additional insights into these relationships in PD.

The key neurodegeneration-associated protein involved in PD is alpha-synuclein, with multiple factors leading to abnormal aggregation and pathology. CSF total alpha-synuclein concentration is generally decreased in PD compared to controls[2], possibly reflecting intracellular accumulation/aggregation. PD patients with cognitive impairment and dementia additionally have decreased CSF amyloid beta and increased total tau levels[3].

Increased levels of inflammatory cytokines (e.g. Interleukin(IL)-1β, IL-6, IL-18 and Tumour Necrosis Factor(TNF)-α) have been detected in PD CSF compared to controls[4][5][6] and many studies have reported elevated levels of inflammatory cytokines in the serum/plasma of PD patients compared to controls[7]. A more 'pro-inflammatory' serum cytokine profile has further been associated with more rapid disease progression in early PD[8] and while peripheral cytokine transport across the blood-brain barrier, with mediation of microglial activation and neuronal damage, could impact on disease course, a consistent relationship between peripheral and central cytokine levels has not been demonstrated in PD.

The relationships between CSF cytokine changes and neurodegeneration-associated protein levels are largely unknown. Currently, limited studies have investigated this in PD[9] and no studies have investigated CSF alpha-synuclein, amyloid beta, total tau and phospho-tau alongside CSF cytokines in a single PD cohort.

This study aimed to examine the relationships between central and peripheral cytokine levels as well as CSF cytokine and neurodegeneration-associated protein associations in a well characterised moderate-stage PD cohort, in order to provide further insight into the drivers of central immune activation in PD.

METHODS

Patient recruitment

Ethical approval was obtained from the Cambridgeshire-2 Research Ethics Committee (08/H0308/331). Patients were recruited from the PD Research Clinic at the John van Geest Centre for Brain Repair in Cambridge. Following screening for contraindications to lumbar puncture, written informed consent was obtained. Clinical data gathered included demographic, medical/drug history, Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Addenbrooke's Cognitive Examination-Revised (ACE-R), semantic fluency (predictive of dementia in PD)[10] and Beck Depression Inventory (BDI) scores .

Sample collection and processing

Lumbar punctures were performed in the left lateral position at the L3/4 or 4/5 space using aseptic technique, 1% lignocaine as local anaesthetic and a 22G spinal needle. ~2-5 ml of CSF was collected. A subset of patients (n=22) had concurrent EDTA venous blood sampling.
CSF samples were centrifuged at 3000g for 15 minutes. Supernatant was stored in ~500ul aliquots at -80ºC. Plasma was extracted from blood by centrifugation at 2000rpm for 15 minutes and stored at -80ºC.

Cytokine and protein analysis

Samples were analysed using the Mesoscale Discovery (MSD) electrochemiluminescence platform. Assays were performed in duplicate, at 1:2 dilution, according to the manufacturer's instructions (https://www.mesoscale.com/)-V-PLEX Pro-inflammatory panel-1 cytokines (Interferon(IFN)-γ, Interleukin(IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, Tumour Necrosis Factor(TNF-α)) in CSF and plasma, and alpha-synuclein, phospho(Thr231)-tau, total tau and amyloid beta(1-42) in CSF. Plates were read using the MSD SECTOR Imager. Data was processed using MSD Discovery Workbench software.

Statistical analysis

Data was analysed using IBM SPSS version 25 and Graph Pad Prism 7. Cytokine and protein variables within the CSF and plasma with assay-detected values in >75% of participants were included in the analysis. Log_{10} transformation was performed due to the non-parametric distribution of variables.
Plasma and CSF cytokine profiles were compared using a repeated measures ANOVA and paired t-tests, with Bonferroni correction for multiple testing as appropriate. Bivariate correlations were assessed between CSF cytokines and neurodegeneration-associated proteins, and between CSF markers and clinical measures. Variables with uncorrected significant correlations ($p<0.05$) were included in linear regression analyses with adjustment for relevant confounders.

RESULTS

Participant demographics

Demographic and clinical measures in the PD cohort (n=35) were expressed as Mean(Standard Deviation): age 65.4(7.6) years; gender 48.6% male; years of education 18.7(3.9); disease duration 5.4(5.6) years; MDS-UPDRS part III (on treatment) 31.0(12.1); ACE-R score 90.3(9.4); BDI score 9.1(7.7) and semantic fluency score 23.9(8.4).
CSF samples from all patients and paired plasma samples from 22 patients were available for analysis.

CSF and plasma cytokines

The cytokines IFN-γ,IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70 and TNF-α in CSF, and IFN-γ, IL-6, IL-8, IL-10 and TNF-α in plasma, had measurable values in >75% of the analysed samples and were used for further analysis.

Bivariate analysis of the detected cytokines in both CSF and plasma (IFN-γ, IL-6, IL-8, IL-10 and TNF-α) indicated no correlations between CSF and plasma levels in this cohort. Repeated-measures ANOVA indicated a significant overall difference between CSF and plasma profiles ($F=32.75, p<0.001$). Paired comparisons between CSF and plasma values indicated that IL-8 was significantly higher, while IFN-γ, IL-10 and TNF-α were significantly lower, in CSF versus plasma following Bonferroni correction for multiple testing ($p<0.005$)(Figure 1A).

CSF cytokines and neurodegeneration-associated proteins

Alpha synuclein and amyloid beta(1-42) were the most abundant measured neurodegeneration-associated proteins in CSF, whereas total tau and phospho-tau were present at low concentrations (Figure 1B). Bivariate correlation analyses between all measured CSF neurodegeneration-associated proteins and CSF cytokines revealed significant relationships between alpha-synuclein and amyloid beta(1-42) (Pearson's r=0.499,p=0.004), total tau and IL-1β (Pearson's r=0.554,p=0.002), total tau and IL-8 (Pearson's r=0.591,p<0.001), and phospho-tau and IL-2 (Pearson's r=-0.667,p=0.001)(Table S1)(Figure 1D-G).

Multivariate linear regression analyses with each neurodegeneration-associated protein as the dependent variable confirmed significant positive relationships between tau and IL-8 (B=0.779,p=0.001), tau and IL-1β (B=0.338,p=0.041), and alpha-synuclein and amyloid beta(1-42) (B=0.605,p=0.010), with age included as covariate (Table 1).

CSF markers and clinical variables

Bivariate correlation analyses revealed a significant relationship between semantic fluency and alpha synuclein which withstood correction for multiple testing (r=-0.53,p=0.003) as well as nominally significant associations (p<0.05) between semantic fluency and IL-6; ACE-R and IL-6; and age and both IFN-γ and IL-1β (Table S2). There were no associations with measures of motor severity.

Multivariate linear regression analyses with semantic fluency and ACE-R score as dependent variables, including age as covariate, confirmed the significant relationship between semantic fluency and alpha-synuclein (B=-8.82,p=0.023), but not the associations between ACE-R and CSF variables (Figure 1C,Table S3).

DISCUSSION

This study has demonstrated that the cytokine profile in PD CSF does not relate closely to that seen in the periphery, suggesting that factors within the CNS may play a role in influencing local CNS inflammation. In keeping with this, our data also revealed positive correlations between CSF proinflammatory cytokines (IL-8, IL-1β) and CSF total tau in PD. In addition, we have confirmed a positive correlation between CSF alpha-synuclein and amyloid beta(1-42) and found a negative correlation between CSF alpha-synuclein and semantic fluency- a key clinical predictor of dementia in PD[10].

Previous studies investigating relationships between CSF and serum/plasma cytokines in PD have reported elevated TNF-α in CSF compared to serum and correlations between CSF and serum levels of IL-6, IL-1β and IL-10 in PD and controls[11]. The different findings compared to our study may reflect variations in cohort demographics, disease stage and methodology. The lack of CSF-peripheral cytokine correlations in our results suggests that central and peripheral cytokine levels may behave independently of each other and that CSF changes may not simply reflect passive diffusion of circulating cytokines to the CNS. Contributory factors to CSF cytokine levels may include cytokine production from CNS cells (e.g.microglia, astrocytes and neurons), peripheral-derived immune cells trafficking into the CNS, and active transport across the blood brain barrier[12]. Thus, CSF cytokines may better reflect CNS pathology, compared to peripheral cytokines, which may be driven by factors including peripheral alpha-synuclein aggregation/pathology, systemic infections/inflammation and microbial changes (e.g. gut microbiome)[13], with separate relevance to PD and disease progression[8].

Within PD CSF, the 'pro-inflammatory' cytokines IL-1β and IL-8 correlated positively with total tau, while the 'anti-inflammatory' IL-2 correlated negatively with phospho-tau. Potential causal links between tau species and immune changes are unclear, but inflammation has been shown to influence tau production/pathology[14]. CSF tau/phospho-tau levels have also been linked to cognitive progression and dementia within PD[3], while post-mortem and genetic studies have connected increased tau pathology and expression with higher risk of PD cognitive dysfunction/dementia[15][16]. The current findings linking CSF tau to inflammatory cytokines in PD may, therefore, be of some relevance in terms of the biological basis of cognitive heterogeneity within PD, regardless of findings in healthy controls. However, control studies will be essential in order to gain a more complete understanding of the tau-cytokine relationships within the CSF.

The positive correlation between CSF alpha-synuclein and amyloid beta is consistent with previous studies[17]. Higher CSF alpha-synuclein levels are associated with worse (lower) semantic fluency, which is predictive of the development of PD-associated dementia[10]. Although the relationship between CSF alpha-synuclein and cognitive function in PD is complex, this result is consistent with previous studies in similar-stage PD, linking posterior cortical impairment to increased CSF alpha-synuclein[18]. These observations further support the importance of alpha-synuclein in the development of PD dementia, as has been demonstrated in post-mortem studies [15].

Limitations of this study include the small sample size, lack of plasma samples in all subjects, and absence of matched healthy control samples. Furthermore, not all cytokines could be adequately measured in the CSF and plasma using this assay, and higher sensitivity assays may be needed for improved resolution of low-level cytokines. However, the assessment of multiple neurodegeneration-associated proteins and cytokines in simultaneously obtained CSF and plasma samples from a clinically well-characterised cohort has uniquely allowed interrelationships between these factors to be explored further in PD. Longitudinal assessments of these relationships in larger PD cohorts and matched healthy controls would now be of interest.

ACKNOWLEDGEMENTS

We gratefully acknowledge the participation of all our patient volunteers. We acknowledge the support and assistance of the Core Biochemical Assay Laboratory at Cambridge University Hospitals.

COMPETING INTERESTS

The authors report no competing interests.

FUNDING

Funding for this work was provided by the Rosetrees Trust (M369-F1), Addenbrooke's Charitable Trust (PF15/CWG) and the NIHR Cambridge Biomedical Research Centre Dementia and Neurodegeneration Theme (146281). RSW was supported by a Fellowship from Addenbrooke's Charitable Trust (RG77199). SFM was supported by the Transeuro EU FP7 grant (242003) and is now an NIHR Academic Clinical Fellow (ACF-2015-23-501). DPB is supported by a Wellcome Trust Clinical Research Career Development Fellowship (214571/Z/18/Z). RAB is an NIHR Senior Investigator (NF-SI-0616-10011) and is supported by the Wellcome Trust-MRC Cambridge Stem Cell Institute (203151/Z/16/Z). CHWG holds a RCUK/UKRI Research Innovation Fellowship awarded by the Medical Research Council (MR/R007446/1) and receives support from the Cambridge Centre for Parkinson-Plus.

AUTHOR'S ROLES

1) Research project: A. Conception, B. Organization, C. Execution;

2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique;

3) Manuscript: A. Writing of the first draft, B. Review and Critique.

RSW – 1)A,B,C; 2)A,B,C; 3)A,B

SFM – 1)A,B,C; 2)C; 3)B

KF – 1)B,C; 2)C; 3)B

DPB – 1)B,C; 2)C; 3)B

RAB – 1)A,B; 2)C; 3)B

CHWG – 1)A,B;C; 2)A,C; 3)B

PATIENT CONSENT FOR PUBLICATION

Not required

DATA SHARING STATEMENT

Data are available upon reasonable request.

REFERENCES

1 Su X, Federoff HJ. Immune Responses in Parkinson's Disease: Interplay between Central and Peripheral Immune Systems. *Biomed Res Int* 2014;**2014**:1–9. doi:10.1155/2014/275178

2 Eusebi P, Giannandrea D, Biscetti L, *et al.* Diagnostic utility of cerebrospinal fluid α-synuclein in Parkinson's disease: A systematic review and meta-analysis. *Mov Disord* 2017;**32**:1389–400. doi:10.1002/mds.27110

3 Hu X, Yang Y, Gong D. Changes of cerebrospinal fluid Aβ42, t-tau, and p-tau in Parkinson's disease patients with cognitive impairment relative to those with normal cognition: a meta-analysis. *Neurol Sci* 2017;**38**:1953–61. doi:10.1007/s10072-017-3088-1

4 Mogi M, Harada M, Narabayashi H, *et al.* Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in

juvenile parkinsonism and Parkinson's disease. *Neurosci Lett* 1996;**211**:13–6. doi:10.1016/0304-3940(96)12706-3

5 Chen X, Hu Y, Cao Z, *et al.* Cerebrospinal fluid inflammatory cytokine aberrations in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis: A systematic review and meta-analysis. Front. Immunol. 2018;**9**. doi:10.3389/fimmu.2018.02122

6 Zhang P, Shao X-Y, Qi G-J, *et al.* Cdk5-Dependent Activation of Neuronal Inflammasomes in Parkinson's Disease. *Mov Disord* 2016;**31**:366–76. doi:10.1002/mds.26488

7 Qin X-Y, Zhang S-P, Cao C, *et al.* Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease. *JAMA Neurol* 2016;**73**:1316. doi:10.1001/jamaneurol.2016.2742

8 Williams-Gray CH, Wijeyekoon R, Yarnall AJ, *et al.* Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov Disord* 2016;**31**:995–1003. doi:10.1002/mds.26563

9 Eidson LN, Kannarkat GT, Barnum CJ, *et al.* Candidate inflammatory biomarkers display unique relationships with alpha-synuclein and correlate with measures of disease severity in subjects with Parkinson's disease. *J Neuroinflammation* 2017;**14**:164. doi:10.1186/s12974-017-0935-1

10 Williams-Gray CH, Foltynie T, Brayne CEG, *et al.* Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. *Brain* 2007;**130**:1787–98. doi:10.1093/brain/awm111

11 Karpenko MN, Vasilishina AA, Gromova EA, *et al.* Interleukin-1β, interleukin-1 receptor antagonist, interleukin-6, interleukin-10, and tumor necrosis factor-α levels in CSF and serum in relation to the clinical diversity of Parkinson's disease. *Cell Immunol* 2018;**327**:77–82. doi:10.1016/j.cellimm.2018.02.011

12 Becher B, Spath S, Goverman J. Cytokine networks in neuroinflammation. Nat. Rev. Immunol. 2017;**17**:49–59. doi:10.1038/nri.2016.123

13 Main BS, Minter MR. Microbial Immuno-Communication in Neurodegenerative Diseases. *Front Neurosci* 2017;**11**:151. doi:10.3389/fnins.2017.00151

14 Barron M, Gartlon J, Dawson LA, *et al.* A state of delirium: Deciphering the effect of inflammation on tau pathology in Alzheimer's disease. *Exp Gerontol* 2017;**94**:103–7. doi:10.1016/j.exger.2016.12.006

15 Irwin DJ, Grossman M, Weintraub D, *et al.* Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol* 2017;**16**:55–65. doi:10.1016/S1474-4422(16)30291-5

16 Williams-Gray CH, Evans JR, Goris A, *et al.* The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. *Brain* 2009;**132**:2958–69. doi:10.1093/brain/awp245

17 Buddhala C, Campbell MC, Perlmutter JS, *et al.* Correlation between decreased CSF α-synuclein and Aβ1–42 in Parkinson disease. *Neurobiol Aging* 2015;**36**:476–84.

doi:10.1016/j.neurobiolaging.2014.07.043

18 Compta Y, Valente T, Saura J, *et al.* Correlates of cerebrospinal fluid levels of oligomeric- and total-α-synuclein in premotor, motor and dementia stages of Parkinson's disease. *J Neurol* 2015;**262**:294–306. doi:10.1007/s00415-014-7560-z

FIGURE LEGENDS

Figure 1 –

(A) Paired comparisons of analysed CSF and plasma cytokines in patients with PD (n=22).

(B) Mean levels of CSF neurodegenerative proteins in patients with PD (n=35).

(C) Scatter plot of Semantic Fluency score versus log_{10}CSF alpha-synuclein.

(D,E,F,G) Graphs demonstrating relationships between CSF markers.

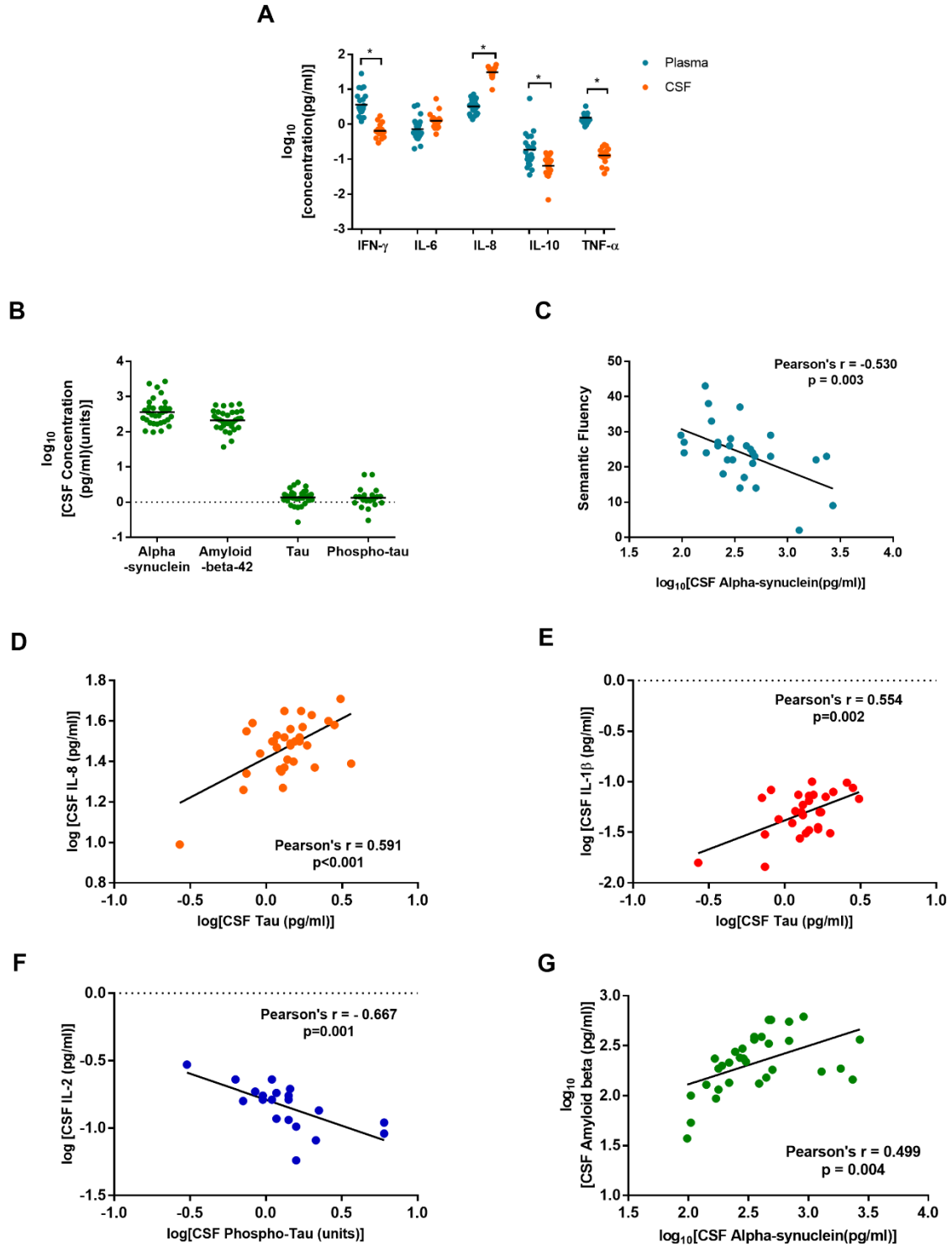

Figure 1 –

(A) Paired comparisons of analysed CSF and plasma cytokines in patients with PD (n=22).

(B) Mean levels of CSF neurodegenerative proteins in patients with PD (n=35).

(C) Scatter plot of Semantic Fluency score versus log_{10}CSF alpha-synuclein.

(D,E,F,G) Graphs demonstrating relationships between CSF markers.

Variable	Beta Coefficient (B)	Significance	95% Confidence Interval for B	
			Lower	Upper
Dependent variable - log_{10} CSF total tau				
Age	0.001	0.869	-0.008	0.009
log_{10} CSF IL-1β	**0.338**	**0.041***	**0.014**	**0.661**
log_{10} CSF IL-8	**0.779**	**0.001***	**0.332**	**1.225**
Dependent variable - log_{10} CSF phospho-tau				
Age	0.002	0.874	-0.020	0.024
log_{10} CSF IL-1β	-0.499	0.122	-1.150	0.152
log_{10} CSF IL-2	-0.692	0.104	-1.548	0.164
log_{10} CSF TNF-α	-0.197	0.606	-1.005	0.610
Dependent variable - log_{10} CSF alpha-synuclein				
Age	0.007	0.455	-0.011	0.024
log_{10} CSF amyloid beta(1-42)	**0.595**	**0.011***	**0.144**	**1.046**

Table 1 - *Linear regression analysis, with log_{10} CSF total tau, log_{10} CSF phospho-tau and log_{10} CSF alpha-synuclein as the dependent variables. *p<0.05.*

SUPPLEMENTARY DATA

Factors (log_{10})	**CSF cytokine or neurodegeneration-associated protein**	**Correlation (Pearson's r)**	**Significance (p)**
CSF alpha-synuclein	log_{10} CSF amyloid beta	0.499	0.004*
CSF amyloid beta(1-42)	log_{10} CSF IL-4	-0.731	0.025
CSF tau	log_{10}CSF IL-1β	0.554	0.002*
	log_{10}CSF IL-8	0.591	<0.001*
CSF phospho-tau	log_{10}CSF IL-1β	-0.537	0.022
	log_{10}CSF IL-2	-0.667	0.001*
	log_{10}CSF TNF-α	-0.456	0.043

Table S1 - *Summary of significant correlations between neurodegeneration-associated proteins and cytokines. *=Remained significant post Bonferroni correction.*

Clinical Variable	CSF marker	Correlation (Pearson's r)	Significance (p)
Semantic Fluency	log_{10}CSF IL-6	0.418	0.021
	log_{10} CSF alpha-synuclein	-0.530	0.003*
ACE-R score	log_{10}CSF IL-6	0.366	0.043
Age	log_{10}CSF IFN-γ	-0.410	0.027
	log_{10}CSF IL-1β	-0.369	0.049

***Table S2** – Significant correlations between clinical variables and CSF markers on bivariate analysis. * = Remained significant post Bonferroni correction.*

Variable	Beta Coefficient (B)	Significance	95% Confidence Interval for B	
			Lower	Upper
Dependent variable – ACE-R				
Age	-0.408	0.058	-0.830	0.014
log_{10}CSF IL-6	14.006	0.080	-1.784	29.797
Dependent variable – Semantic fluency				
Age	-0.273	0.215	-0.714	0.169
log_{10}CSF IL-6	10.184	0.129	-3.178	23.546
log_{10}CSF alpha-synuclein	**-8.825**	**0.023***	**-16.346**	**-1.304**

***Table S3 -** Linear Regression with ACE-R score and semantic fluency as the dependent variables. *p<0.05.*