Compartmentalized Multiplex Biomarker Correlations and Biological Pathways in HIV

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Ronald J. Ellis[1,2], Bin Tang[2], Patricia K. Riggs[3], Jennifer E. Iudicello[2], Maria Cecilia G. Marcondes[4], Violaine Delorme-Walker[4], Robert K. Heaton[2], Scott L. Letendre[2,3]

[1] Department of Neurosciences, UC San Diego, San Diego, CA, United States; [2] Department of Psychiatry, UC San Diego, San Diego, CA, United States; [3] Department of Medicine, UC San Diego, San Diego, CA, United States; [4] San Diego Brain Research Institute, San Diego, CA, United States; [5] Departments of Medicine and Psychiatry, UC San Diego, San Diego, CA, United States

BACKGROUND

- Cerebrospinal fluid (CSF) and plasma inflammatory biomarkers often used to monitor CNS and systemic disease
- Their interrelationship across the BBB remains poorly defined.
- Multiplex proteomics enables large-scale assessment of cross-compartment structure.

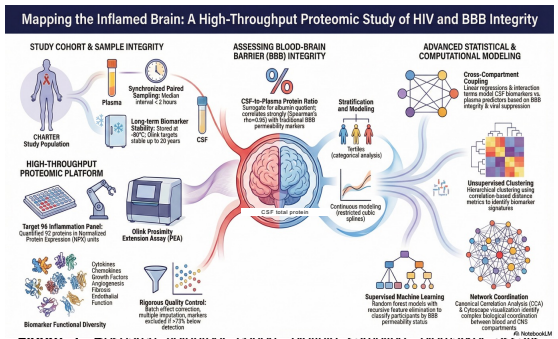


Figure 1: Research volunteer cohort, biofluid sampling, biomarker assays, quality control, correlational approach (within- and across-compartment correlation matrices) and and effect modifiers considered (BBB permeability, viral suppression, ART regimen)

Statistical and Machine Learning Approach

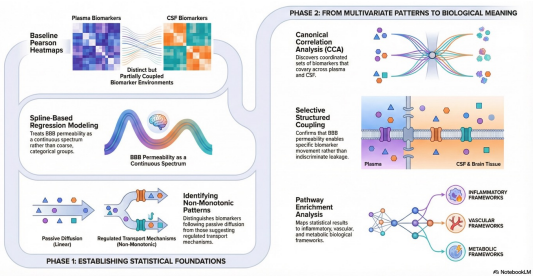


Figure 2: 3-stage analytic workflow:

1. Pearson correlation heatmaps establish that plasma and CSF function as partially coupled but distinct environments.
2. Multivariate Structure: Canonical Correlation Analysis (CCA)-unsupervised method to identify coordinated sets of biomarkers
3. Biological Interpretation: Pathway enrichment analyses (KEGG, Gene Ontology) confirm that coordinated coupling patterns map onto known inflammatory, vascular, and metabolic pathways.

- **We first demonstrated compartmentalization descriptively, then modeled effect modification of coupling, and finally identified coordinated biomarker sets and their biological pathways.**

- CSF and plasma inflammatory biomarkers were strongly co-regulated within compartments / weakly correlated across compartments
- Cross-compartment coupling was modest and only partly explained by BBB permeability and viral suppression
- Multivariate structure revealed coordinated biomarker sets mapping to inflammatory and vascular pathways

RESULTS

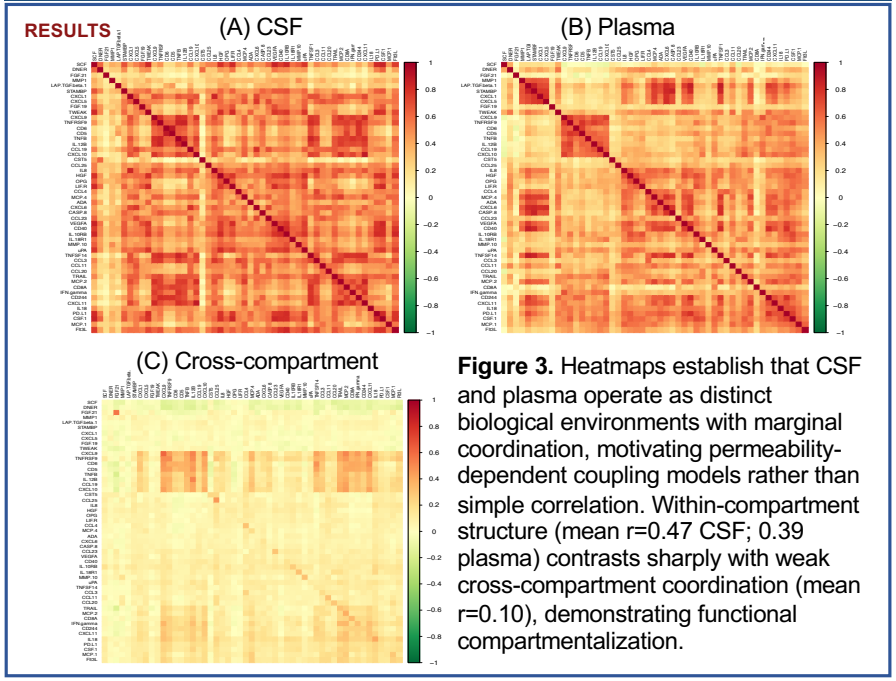


Figure 3. Heatmaps establish that CSF and plasma operate as distinct biological environments with marginal coordination, motivating permeability-dependent coupling models rather than simple correlation. Within-compartment structure (mean r=0.47 CSF; 0.39 plasma) contrasts sharply with weak cross-compartment coordination (mean r=0.10), demonstrating functional compartmentalization.

Participants: N=567. 68.0% on ART (**NOTE**: ART initiation guidelines limited to CD4+ < 350 cells/μL during recruitment 2003-2007). Median current CD4+ T-cells 418 cells/μL; nadir 183. **277 virally suppressed (see Fig 4)**; 286 unsuppressed. Mean age 43.0 years; 78.5% male; 48.1% Black.

This early ART-era cohort (2003–2007) included a substantial unsuppressed subgroup, enabling evaluation of viral suppression and BBB permeability as effect modifiers.

Fig 4. Effects of BBB permeability and viral suppression on CSF-plasma biomarker relationships showing statistically significant interaction effects in pre-specified regression models evaluating effect modification of CSF–plasma coupling. Surrogate for BBB permeability = CSF total protein (principally blood-derived albumin; the clinical gold standard, the CSF:serum albumin ratio, is is being measured in a validation subset).

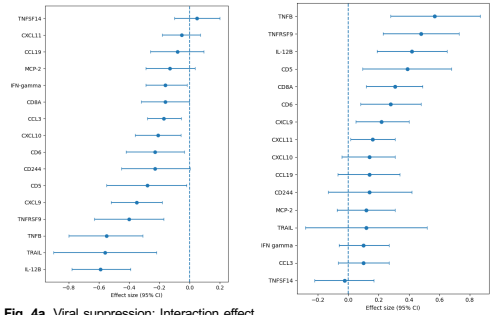


Fig. 4a. Viral suppression: Interaction effect from regression of CSF biomarkers x plasma biomarkers. Biomarkers such as IL-12B, TNFRSF9, TNFB, and CXCL9 showed stronger associations in unsuppressed participants, indicating enhanced cross-compartment interactions possibly due to blood-brain barrier compromise. Interaction coefficients were standardized as effect sizes. p-values adjusted using B-H.

Fig 4b. BBB permeability: The moderation effect of the BBB on the CSF-plasma correlation of biomarkers. The interaction was determined using multiple linear regression, controlling for covariates including age, sex, nadir CD4, viral suppression, and lifetime any substance diagnosis.

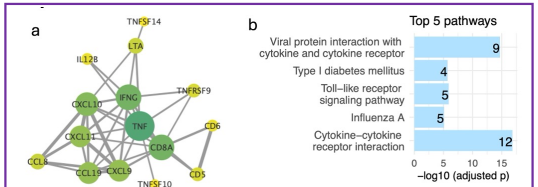


Fig. 5. Gene Ontology and KEGG pathway enrichment of 15 biomarkers most strongly contributing to cross-compartment coupling (canonical coefficient ≥ 0.4). (a) network analysis; line width = co-expression, node size and color intensity = degree of connection. Larger darker green nodes indicate greater connectivity in the network. (b) bar plot of the top five enriched pathways in KEGG, suggesting mechanisms underlying cross-compartment biomarker regulation. Pathways reflect how systemic inflammation and vascular signaling influence CNS versus systemic biomarker exchange.

PLAIN LANGUAGE SUMMARY

In people with HIV, inflammatory proteins in blood and CSF behaved as largely distinct systems. Limited cross-compartment coupling suggests regulated exchange rather than passive leakage across the BBB