

PPMI Biochemical Biomarker Update

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The Michael J Fox Foundation

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Update on Alpha-synuclein ELISA

December 2008: Transfer of assay

- MJFF evaluated several CROs for ability to transfer Schlossmacher assay

February 2009-December 2009: Optimization of assay

- Improved incubation and blocking buffer conditions
- Switched detection antibody
- improved sensitivity 20-fold

January 2010-July 2010: Generation of additional supply

- Additional polyclonal rabbit/goat antibody
- Rabbit monoclonal antibody generation

Current status: Optimized Assay with Rabbit Monoclonal

- Participant in Alpha-synuclein assay comparison study
- Developing commercialization plan for kit



Qualification Study

- Study Objectives
 - Determine diurnal fluctuations of DJ-1 and alpha-synuclein in healthy volunteer CSF
 - Determine within-subject variability and between subject variability
 - Establish a bank of biosamples (CSF and blood) to utilize for assay development and future biomarker validation
- Study Design
 - 13 healthy volunteers (ages 30-55): 9 male/4 female
 - CSF and serum/plasma collected at 11 time points over 26 hour time frame; protocol repeated two weeks later
 - CSF analyzed for DJ-1, alpha-synuclein, and hemoglobin levels

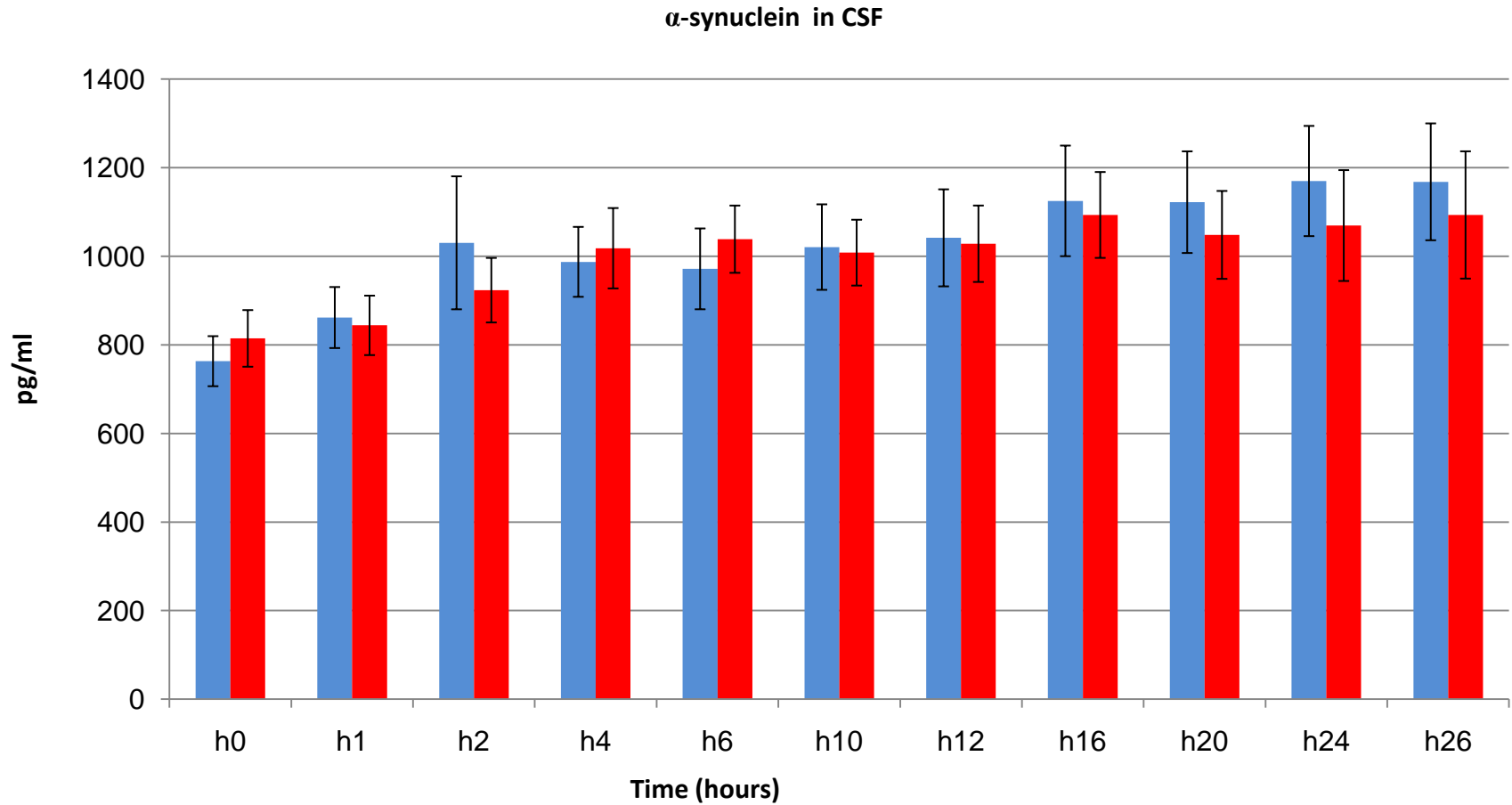


Assay Details

- Alpha-synuclein ELISA
 - Dynamic range: 4-2000pg/mL
 - Human specific alpha-synuclein
 - Polyclonal antibody capture, biotinylated-monoclonal antibody detection-appears to pick up all alpha-synuclein species present;
- DJ-1 ELISA
 - MBL commercial kit
 - Polyclonal capture, HRP-conjugated detection
- Hemoglobin Detection
 - Bethyl laboratories commercial ELISA kit



Alpha-synuclein levels over 26 hours



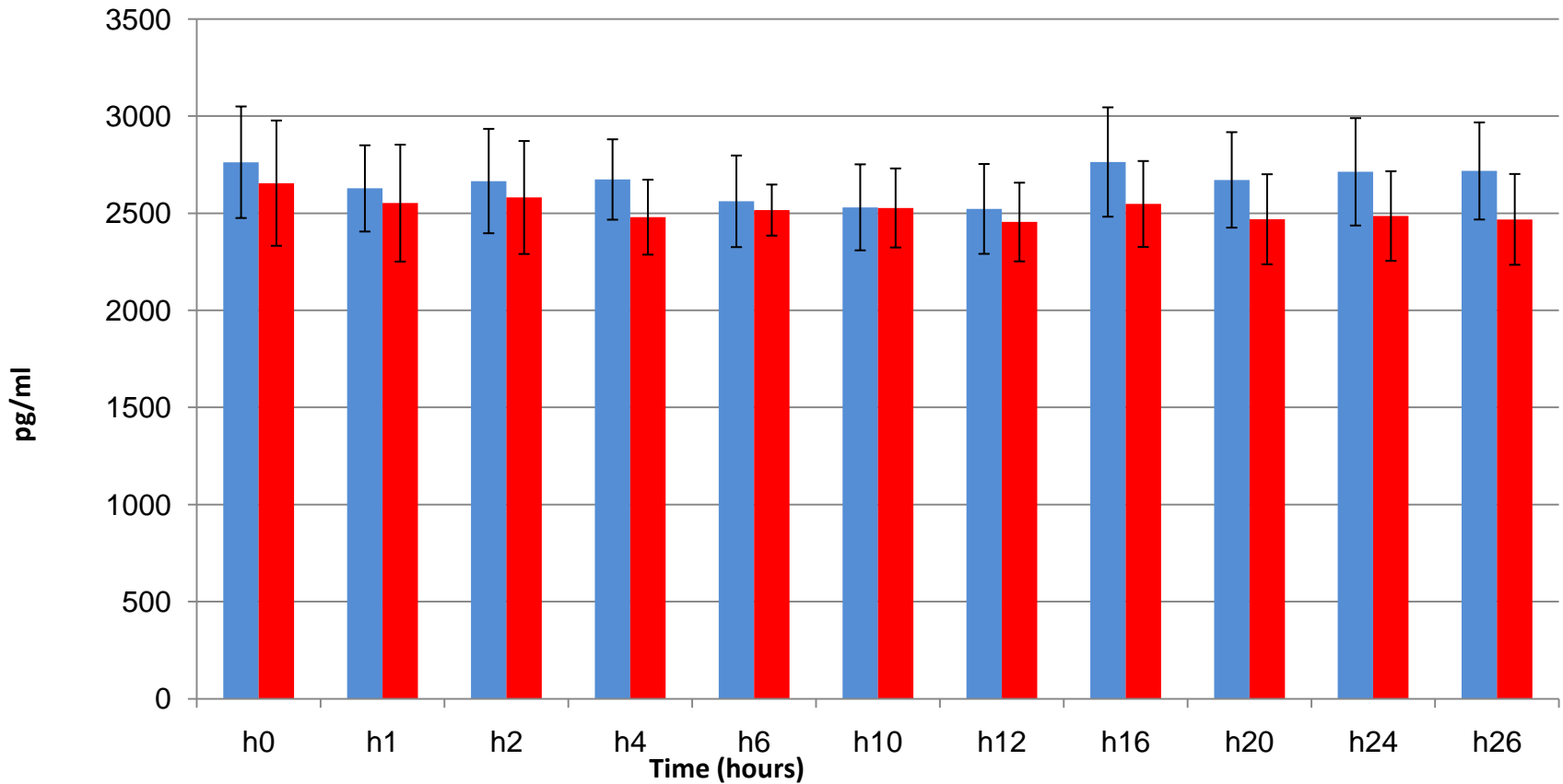
Alpha-synuclein levels over 26 hours

- Mean observed concentrations: 800 (baseline)-1100 pg/mL (26 hours)
- Alpha-synuclein significantly changed over the 26 hour period ($p < 0.0001$) but not within the two week time point
- Within subject variability: approximately 20%
- Between subject variability: 30%



DJ-1 levels over 26 hours

DJ-1 in CSF



DJ-1 levels over 26 hours

- Mean observed concentrations: between 2400-2530 pg/mL
- No change over 26 hours
- No period effect on DJ-1 levels
- Within subject variability: approximately ~20%
- Between subject variability: ~26%



Acknowledgements

- California Clinical Trials/Parexel
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- Chris Coffey/Chelsea Caspell
(University of Iowa)



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