FEASIBILITY OF USING DRIED BLOOD SPOTS FOR BIOMARKERS OF NEUROPATHOLOGY

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BACKGROUND

- Blood based biomarkers of neuropathology are increasingly being used in several research studies
  - Blood based biomarkers have been shown to be associated with dementia
- Most studies use blood that has been collected and processed soon after collection for biomarker measurement
  - These methods cannot be practically implemented in resource poor settings
- Alternate methods that do not rely on complex processing or cold storage can improve application of these biomarkers in a more broad research context.

BIOMARKERS OF NEUROPATHOLOGY

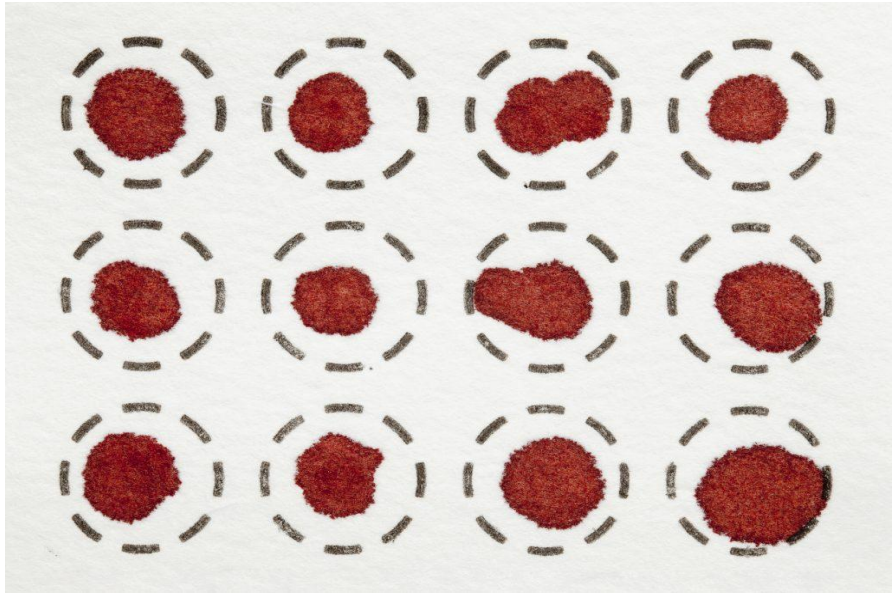
- Two previous papers have demonstrated the feasibility of using dried blood spots for measurement of neurofilament light (NfL)
  - NfL is a part of the axonal cytoskeletion and is a non-specific marker of neurodegeneration
  - Both studies showed that NfL levels are lower in DBS as compared to plasma but reasonably high levels of correlation between DBS and plasma levels were observed in both studies (r=0.76 – 0.80).

- Elution volumes and elution conditions are not standardized across studies

Alzheimer's Dement. *(2021)*;13:e12145.
Scientific Reports (2020); 10: 97

ALTERNATE METHODS: DRIED BLOOD SPOTS (DBS)

- Dried Blood Spots (DBS) is the most commonly used microsampling method
  - newborn screening programs,
  - pharmacokinetics
  - toxicology
  - infectious disease
- LIMITATIONS
  - Hematocrit effect
  - Sample heterogeneity
  - Environmental conditions
  - Labor intensive processing in the laboratory



https://www.aacc.org/cln/articles/2022/september/dried-blood-spots-and-beyond
https://autogen.com/wp-content/uploads/2019/04/Dried-Blood-Spots-1024x747.jpeg

DBS: PROCESSING METHODS

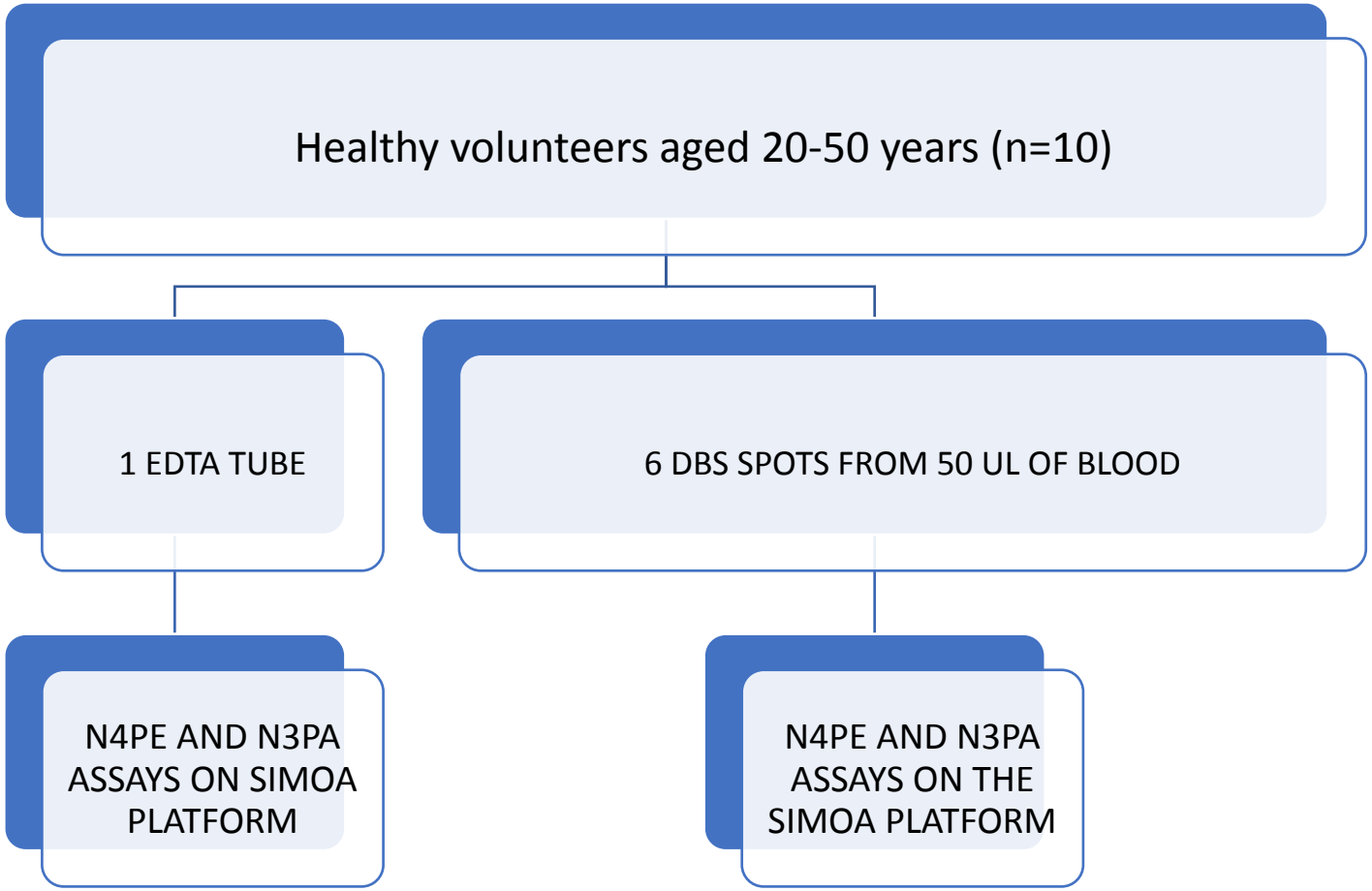
- **Method A:** Manufacturer's recommendation: DBS placed in a shaker (500 rpm) for 4 hours with 200 ul of sample diluent (sample diluent provided by Quanterix Inc.)
- **Method B:** Manufacturer's recommendation + 30 minutes of sonication
- **Method C:** Manufacturer's recommendation + 30 minutes of sonication + centrifugation for 10 minutes (9900 rcf)
- Samples were analyzed using the N4PA assay kit on the SR-X platform (Quanterix Inc.)
  - Biomarkers included NfL, GFAP, total Tau and UCHL-1
- Samples were analyzed in triplicate

RESULTS: PROCESSING CONDITIONS

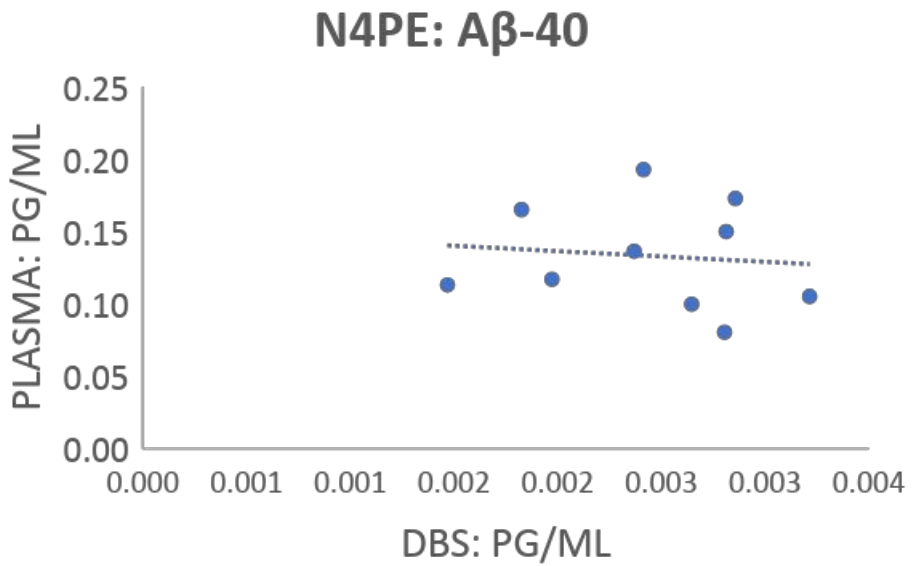
ANALYTE	METHOD A	METHOD B	METHOD C
Total Tau	8%	6%	4%
UCH L1	6%	5%	6%
Neurofilament light	17%	19%	20%
Gilial Fibrillary Acidic Protein (GFAP)	20%	13%	11%

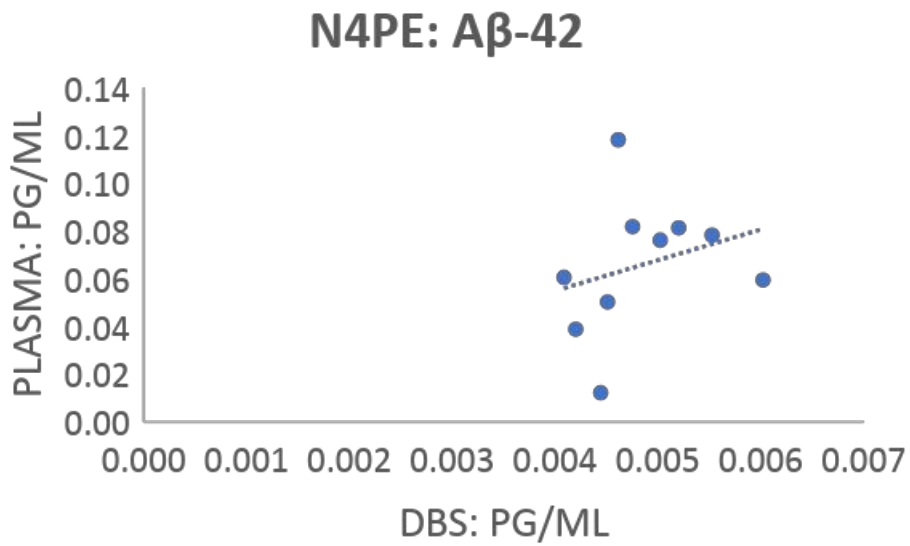
- Decided to use Method C for all future assay development using DBS

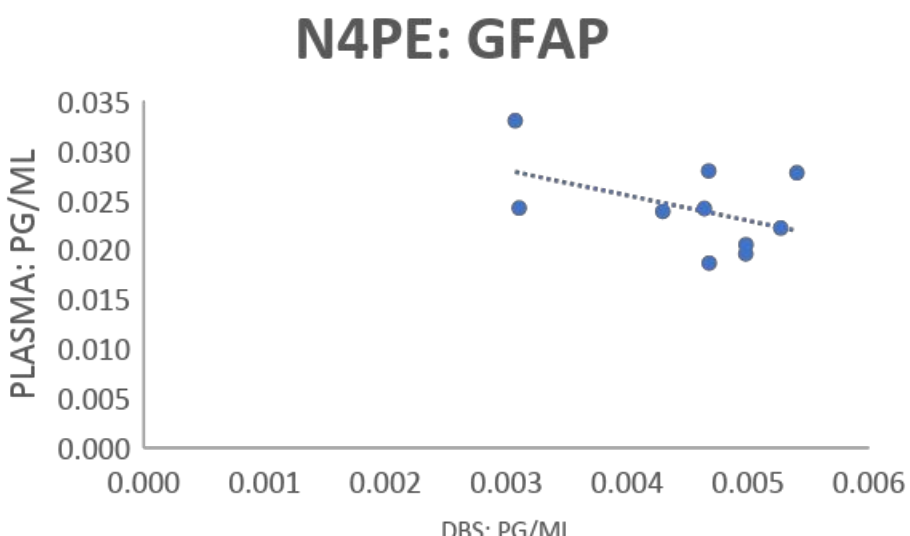
STUDY DESIGN: COMPARISON OF DBS AND PLASMA

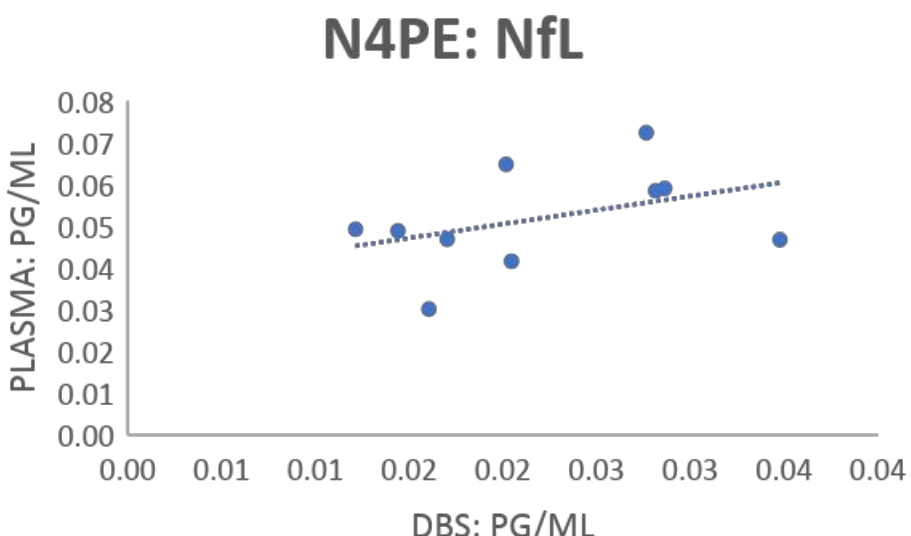


RESULTS: DBS VS. PLASMA

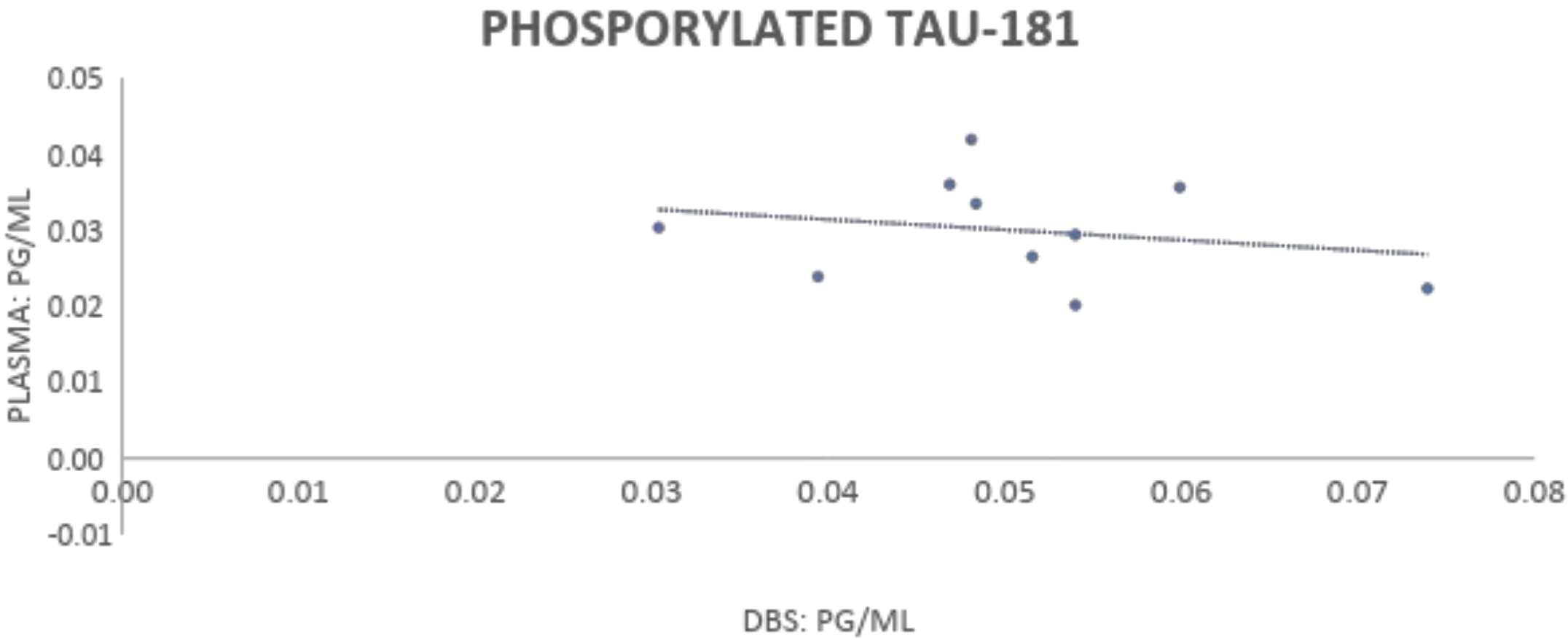




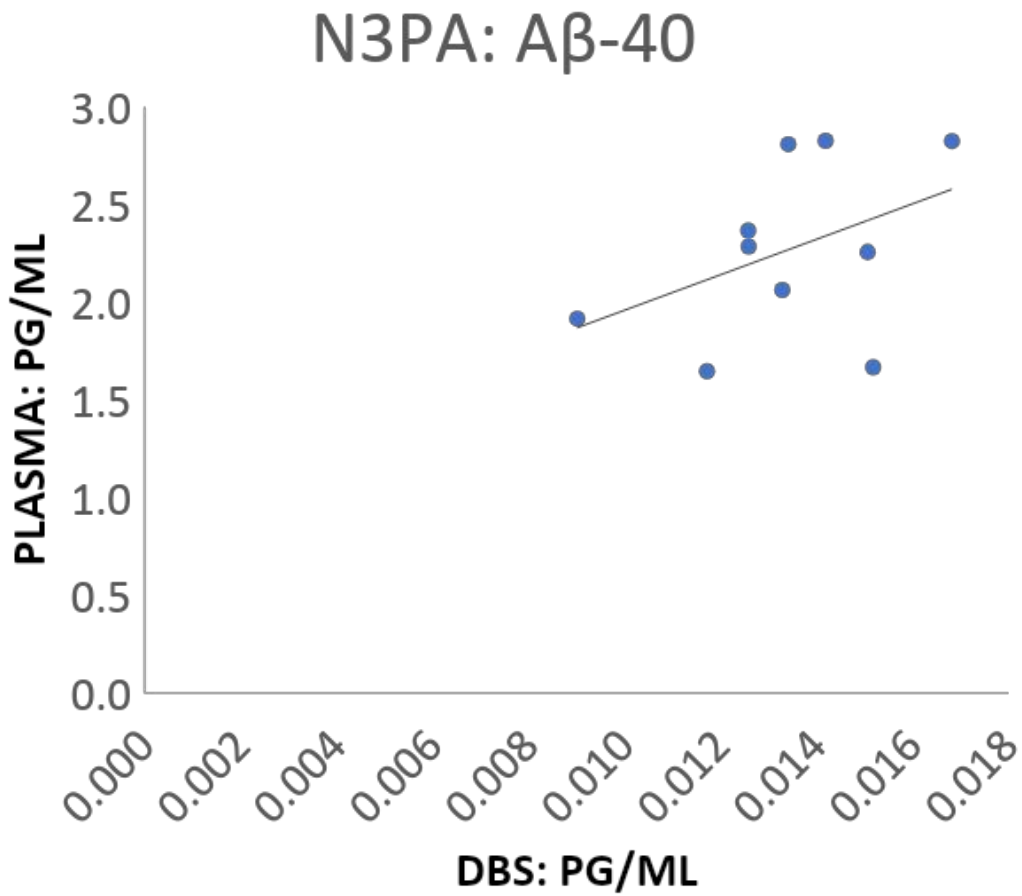


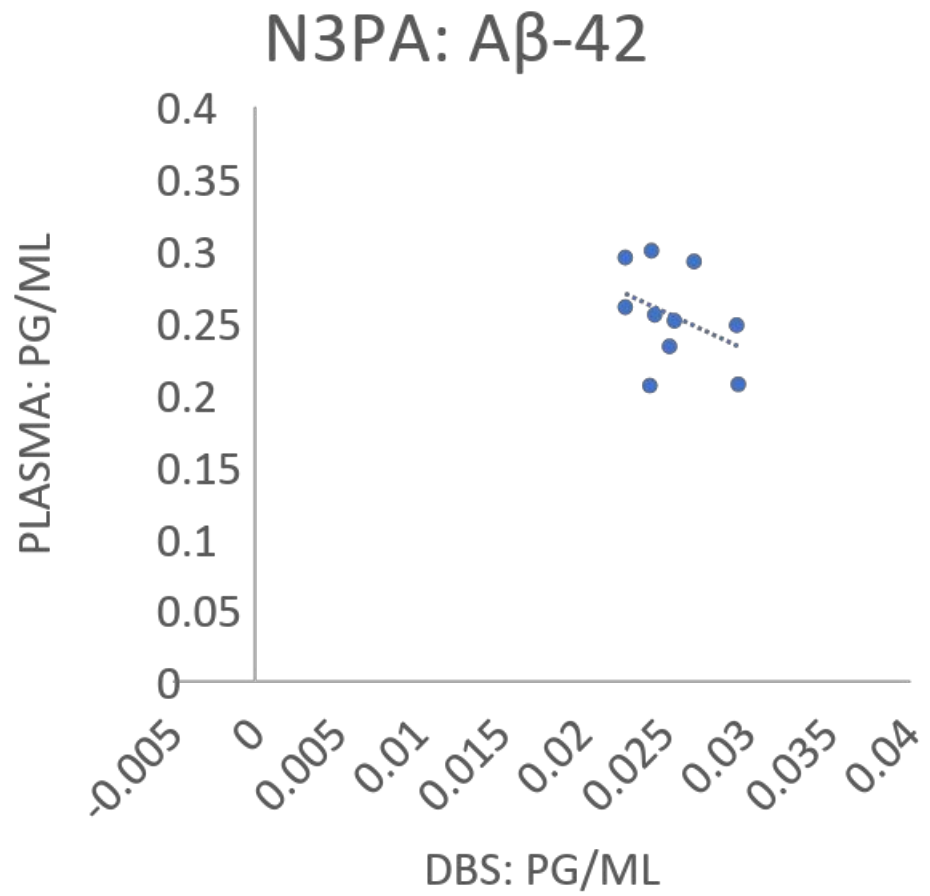


RESULTS: DBS VS. PLASMA (CONTD).....



.....AND MORE RESULTS





CONCLUSIONS AND FUTURE DIRECTIONS

- DBS values for all biomarkers are markedly lower than the corresponding plasma values
  - All values are well within the analytical range of the highly sensitive Simoa assays

- Correlation between DBS and plasma values depends on both the analyte and the antibodies used to estimate the analyte
  - Vastly different results for DBS and plasma with different assays for Aβ-40 and Aβ-42.

- Case-control studies within HRS to evaluate DBS can be used to replicate the association between neuropathological biomarkers and dementia observed with plasma samples
  - Larger range of biomarker values may change the observed correlation between DBS and plasma values

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