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Impact of pre-analytical differences on biomarkers in the ADNI and PPMI studies: implications in the era of classifying disease based on biomarkers

Tessandra Stewart, PhD^a, Min Shi, PhD^a, Aanchal Mehrotra, PhD^a, Patrick Aro, BS^a, David Soltys, BS^a, Kathleen F. Kerr, PhD^b, Cyrus P. Zabetian, MD^{c,d}, Elaine R. Peskind, MD^{e,f}, Peggy Taylor, ScD^g, Leslie M. Shaw, PhD^h, John Q. Trojanowski, MD, PhD^{i,j}, Jing Zhang, MD, PhD^{a,*}, and Alzheimer's Disease Neuroimaging Initiative[†]

^aDepartment of Pathology, University of Washington School of Medicine, Seattle, WA, USA

^bDepartment of Biostatistics, University of Washington, Seattle, WA, United States.

^cParkinson's Disease Research and Geriatric Research, Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA

^dDepartment of Neurology, University of Washington School of Medicine, Seattle, WA, USA

^eVeterans Affairs Northwest Network, Mental Illness Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA, 98108, USA.

^fDepartment of Psychiatry and Behavioral Science, University of Washington School of Medicine, Seattle, WA, 98195, USA.

^gBioLegend, Dedham, MA, USA.

^hDepartment of Pathology & Laboratory Medicine and Center for Neurodegenerative Disease Research, Institute on Aging, University of Pennsylvania, Philadelphia, PA, USA.

ⁱCenter for Neurodegenerative Disease Research (CNDP), University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

^jDepartment of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Abstract

BACKGROUND: Neurodegenerative diseases require characterization based on underlying biology using biochemical biomarkers. Mixed pathology complicates discovery of biomarkers and characterization of cohorts, but inclusion of greater numbers of patients with different, related diseases with frequently co-occurring pathology could allow better accuracy. Combining cohorts

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*Correspondence to: Jing Zhang, Department of Pathology, University of Washington School of Medicine, Seattle, Washington, 98104-2499, USA zhangj@u.washington.edu (J. Zhang).

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collected from different studies would be a more efficient use of resources than recruiting subjects from each population of interest for each study.

OBJECTIVE: To explore the possibility of combining existing datasets by controlling pre-analytic variables in the Alzheimer's Disease (AD) Neuroimaging Initiative (ADNI) and Parkinson's (PD) Progression Markers Initiative (PPMI) studies

METHODS: Cerebrospinal fluid (CSF) was collected and processed from 30 subjects according to both the ADNI and PPMI protocols. Relationships between reported levels of AD and PD biomarkers in the same subject under each protocol were examined.

RESULTS: Protocol-related differences were observed for A β , but not t-tau or α -syn, and trended different for p-tau and pS129. Values of α -syn differed by platform. Conversion of α -syn values between ADNI and PPMI platforms did not completely eliminate differences in distribution.

DISCUSSION: Factors not captured in the pre-analytical sample handling influence reported biomarker values. Assay standardization and better harmonized characterization of cohorts should be included in future studies of CSF biomarkers.

Keywords

Alzheimer's disease; Parkinson's disease; A β ; tau; α -synuclein

Introduction

Cerebrospinal fluid (CSF) amyloid- β (A β), total tau (t-tau), and phosphorylated tau (p-tau) provide good sensitivity and specificity for diagnosis of Alzheimer's disease (AD)[1–3]. However, mixed pathology is common in clinical AD, with autopsy revealing significant contributions of α -synuclein (α -syn) aggregation (involved in Lewy body diseases such as Parkinson's disease [PD]), TDP-43, and vascular pathologies[4–9]. Different pathways also appear to interact: for example, in the Alzheimer's Disease Neuroimaging Initiative (ADNI) [3] cohort, inclusion of CSF α -syn improved the diagnostic and prognostic performance of A β 42 and t-tau in AD[10–12], while A β , t-tau, and p-tau also appear to be altered in Parkinson's disease in multiple studies, including in the Parkinson's Progression Markers Initiative (PPMI) cohort [13].

Mixed pathologies in AD have likely contributed substantially to disappointing results in clinical trials targeting a single pathway, such as A β [14–18]. Indeed, NIA has recently called for a new research framework for AD, i.e. classifying AD based on underlying biology with biomarkers of A (A β), T (p-tau), and N (t-tau), rather than clinical symptoms[19], with additional markers representing other pathologies, e.g. α -syn, TDP-43 and vascular dysfunction, to be included in the future.

Evaluating all of these markers in multiple cohorts can be costly and time-consuming, and one alternative could be combining existing cohorts, creating a “super-cohort” to increase statistical power and better identify mixed pathologies, including minor components and interacting pathways. As an initial step, the current study was designed to evaluate the effects of pre-clinical variables, including sample handling and storage[20–25], and assay platforms, on common AD and PD biomarkers. Using data collected in our own cohort,

controlling all major variables involved in ADNI vs PPMI, we explored the possibility of merging the biomarker datasets of these cohorts.

Methods.

Participants:

Ten AD, 10 PD, and 10 healthy control subjects were enrolled at the Pacific Udall Center at the Veterans Affairs Puget Sound Health Care System and the University of Washington Alzheimer's Disease Research Center. Written consent was obtained under supervision of the respective Institutional Review Boards. Inclusion criteria were previously described[26, 27]; PD and AD subjects met diagnostic criteria for UK PD Society Brain Bank[28] and NINDS-ADRDA[29] respectively. The Control group was composed of volunteers with no diagnosed neurodegenerative diseases, with similar sex and age distributions to the PD group (Table 1).

CSF collection

CSF was collected by lumbar puncture (LP) according to previously published methods[3, 13], using the syringe method. ~22 mL of CSF (~2 mL for clinical lab testing, and then 4× ~5 mL to be processed alternately by protocol, i.e., PPMI protocol for the first 5 mL, ADNI for the second, PPMI for the third, and so on) were collected from each participant. To account for any gradient effects, for half of the patients the ADNI protocol was followed for the first aliquot, and vice versa for the other half. Collected CSF was processed for transport and storage according to the protocols for ADNI[3] or PPMI[13]. A detailed description of the differences in the two protocols is provided in Table 2. The Zhang laboratory performed tasks assigned to both the biorepository and analytical sites in the ADNI/PPMI studies.

Immunoassays:

Each immunoassay plate contained standard curve points, an internal control reference sample, and subject samples. All samples originating from an individual subject were assayed on the same plate, and subjects were divided across plates to provide an even distribution of disease statuses. Data were normalized by calculating batch means for replicate internal reference CSF concentrations for each analyte with the exclusion of outliers as determined by Chauvenet's Criterion. Batches whose mean deviated $\pm 10\%$ from the mean model of all batches were normalized by application of a correction factor to all samples in a batch as determined by: *Correction factor = batch mean of internal reference CSF / mean of all batches internal reference CSF*

A β , t-tau, and p-tau:

CSF samples were assayed for t-tau, tau phosphorylated at 181 (p-tau), and A β 1–42 (A β 42) using a commercially available kit (lot: 405221; Fujirebio US, Inc., INNO-BIA AlzBio3, v21, Malvern, PA) on a Luminex analyzer (Qiagen, LiquiChip Workstation IS 200, Germantown, MD). All CSF handling steps employed low-binding micropipette tips (VWR™, 101100–416, Visalia, CA). Samples were thawed on ice <60 minutes, then briefly mixed by vortex. One hundred eighty μ L of each CSF sample was transferred to a low-binding polypropylene microcentrifuge tube (Fisher Scientific, 02–681-320, Hanover Park,

Illinois) and centrifuged at 4000x g for 10 minutes at room temperature. Seventy-five μ L of CSF supernatant/replicate was added to a 96-well, 1.2 μ m filter plate (Millipore Sigma, MSBVN1250, Burlington, Massachusetts) pre-loaded with microspheres and conjugate working solution. The standard curve was extended beyond the manufacturer's instructions with 3-fold and 9-fold dilutions below the kit's lowest standard. Two manufacturer supplied Control samples were included on each plate.

α -Syn:

Total α -syn was measured as in the ADNI study[26]. Briefly, MicroPlex® poly61 microspheres (Luminex, Austin, TX) were coupled to ASY-1 anti- α -synuclein antibody[26]. CSF was thawed on ice and treated with Protease Inhibitor Cocktail (Sigma, St. Louis, MO), then re-frozen at -80°C until the time of the immunoassay. Samples were thawed on ice and treated with RIPA buffer[26], incubated for 30 minutes on ice, and then centrifuged at 14,000 x g for 10 minutes at 4°C . The supernatant was further diluted 1:2 with 0.1% bovine serum albumin (Sigma, St. Louis, MO) in PBS prior to plating. Samples were added to a 96-well filter plate (Luminex, Austin, TX) containing microspheres and incubated for 3 hours at room temperature with shaking. CSF α -syn was detected with biotinylated anti-human α -syn antibody (R&D Systems, Minneapolis, MN) and streptavidin RPE (Prozyme, Hayward, CA). Analytes were measured as with the AlzBio3 assay.

Total α -syn levels in CSF were additionally measured by ELISA as in the PPMI study[13] (lot: B248418; Biolegend, San Diego, CA). Briefly, CSF samples were thawed overnight at 4°C and diluted 1:10 in assay diluent. Samples were measured on a microplate reader and analyte concentrations were extrapolated from a 4-parameter non-linear fit with integrated software (Promega, Glomax Multi Detection System with Instinct Software, Madison, WI).

pS129.

α -Synuclein phosphorylated at S129 (pS129) levels were measured as previously described[30]. Briefly, MicroPlex poly61 microspheres and CSF samples were prepared as for the total α -syn assay. Samples were thawed on ice and centrifuged at 14,000xg for 10 minutes at 4°C . Seventy-five μ L of CSF supernatant was added to 25 μ L of 0.1% bovine serum albumin (Sigma, St. Louis, MO) in PBS prior to plating on a 96-well filter plate containing microspheres and incubated for 3 hours at room temperature, with shaking. CSF pS129 was detected with biotinylated anti-human pS129 antibody (Biolegend, San Diego, CA) and streptavidin RPE (Prozyme, Hayward, CA), using a LiquiChip Luminex 200 Workstation.

Hemoglobin:

Hgb contamination was measured using Human Hemoglobin ELISA Quantitation Kit (Bethyl Lab Inc., Montgomery, TX, USA) according to the manufacturer's instructions. CSF samples were thawed on ice, centrifuged for 10 minutes at 14,000xg, and the supernatants diluted 1:20 in assay diluent. Assay plates were measured on a microplate/cuvette reader and Hgb concentrations were extrapolated from a 4-parameter non-linear fit standard curve (Molecular Devices, SpectraMax M2e with Softmax Pro v5.4.4, San Jose, CA).

Statistical analysis:

Analyses were performed using GraphPad Prism 6 and R v3.4.3. Group means were compared using paired t-tests. Association between values was assessed by Deming regression. Agreement of assays between cohorts was determined by Bland-Altman analysis. Some data used in the preparation of this article were obtained from the ADNI and PPMI databases, (data available at <http://adni.loni.usc.edu/data-samples/access-data/> and <https://www.ppmi-info.org/access-data-specimens/>). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For up-to-date information, see www.adni-info.org. For up-to-date information on the PPMI, visit www.ppmi-info.org. PPMI – a public-private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners.

Comparison with study database cohorts was performed by selecting control and disease subjects for which values of all selected analytes were available, from the baseline visits of subjects from both studies. ADNI A β 42, t-tau, and p-tau values were the first occurring value for the baseline data, using the raw values, from the ADNI1 set. For PPMI, where multiple measures were available, the data collected in 2013 were used. Models were based on Deming regression on the raw or log-transformed values. To estimate the error of these models, leave one out cross validation (LOOCV) was performed, and the absolute error for each iteration recorded. The mean absolute error was then divided by the mean of the real measured values to estimate the coefficient of variation (CV). A p-value less than 0.05 was considered significant.

Results:

Differences in ADNI and PPMI collection protocols.

The ADNI and PPMI protocols were compared by examination of the published materials and direct communication with study personnel, and included differences in methods and materials in CSF collection, storage materials, sample handling at the collection and storage sites, and processing immediately prior to assay performance. A comparison of sample collection and processing procedures is presented in Table 2.

Description of the test cohort.

Thirty subjects (10 each of PD, AD, and healthy control) were recruited and their CSF collected. The age and sex distributions of each group are presented in Table 1.

Measurement of blood contamination of CSF samples:

The level of Hgb detected in CSF collected according to the ADNI protocol ("ADNI CSF") was substantially higher than that collected according to the PPMI protocol ("PPMI CSF"), although this difference did not reach statistical significance (468.0 ± 304.80 vs 11.02 ± 3.26 , paired t-test $p=0.14$; Fig. 1A), probably due to the large variability and small number of

cases included. More of the ADNI than PPMI CSF samples exceeded the 200 ng/ml exclusion criterion used in previous studies of α -syn[10, 26] (17% vs 0; Fisher's exact, $p=0.05$). Deming regression did not detect an association between Hgb concentrations in ADNI vs PPMI CSF (Fig. 1B).

Analysis of classical Alzheimer's Disease markers:

T-tau, p-tau and A β 42 were compared using the Inno-BIA AlzBio3 kit, as in the initial phases of both the ADNI and PPMI studies. No differences were observed between protocols for t-tau ($p=0.54$), while p-tau trended higher in the PPMI CSF samples ($p=0.20$), and higher p-tau values were reported in the PPMI protocol for most subjects (Fig 1C, E). Scatterplots and Deming regression revealed a strong linear association between the values for each analyte ($p<0.0001$; Fig 1D, F). For A β , the values of PPMI CSF were on average 85.0 ± 69.8 pg/ml lower than for ADNI CSF ($p<0.0001$; Fig 1G, H). Tau/A β was an average of 0.075 ± 0.105 ($P=0.0006$) higher in PPMI than ADNI samples, with significant association between protocols ($p<0.0001$; Fig 1 I-J). Slope and intercept of Deming regressions, and bias and limits of agreement for Bland-Altman analysis of each analyte are presented in Table 3.

Analysis of α -syn markers:

Total α -syn was measured using both the Luminex assay used by ADNI[11], and the Biologend ELISA assay used by PPMI. No significant differences were found between protocols in either assay (ELISA $p=0.72$, Luminex $p=0.26$; Fig 2 A, D). While the association of values between protocols was significant for both assays (ELISA $p<0.0001$, Luminex $p<0.0001$; Fig 2 B, E), the correlation was better for the ELISA assay. Exclusion of samples with Hgb>200 ng/ml improved the association between protocols (Fig 2 C, F).

We compared the assay platforms to each other (Fig 2 J-O), using either all 60 samples (2 protocols for 30 subjects) measured by ELISA vs Luminex, or ADNI CSF measured by Luminex vs PPMI CSF measured by ELISA (to mimic the condition of comparing the ADNI to PPMI studies). The ELISA reported much higher values than Luminex (Fig 2 J, M; Table 3), and the correlation, though significant, was not as strong as for the classical AD markers (Fig. 2 K,N). Exclusion of the values with high Hgb improved the correlation (Fig. 2 L,O).

α -Syn phosphorylated at serine 129 (pS129) was also measured (Fig 2 G-I). The value was on average 11.8 ± 49.3 pg/ml higher in PPMI CSF ($p=0.045$), although the difference in values between paired samples were less consistently in the same direction than in other analytes. However, the samples were significantly associated ($p<0.0001$). Exclusion of high Hgb samples had little effect (Fig 2I).

Slope and intercept of Deming regressions, and bias and limits of agreement for Bland-Altman analysis of each analyte are presented in Table 3.

Comparison of markers by diagnostic group.

The distributions of markers, compared by diagnostic group, are presented in Fig 3. As expected, AD patients had higher t-tau, p-tau, and tau/A β , and lower A β , than controls or

PD patients; however, there was little difference between control and PD subjects for these markers. Little difference in total or pS129 α -syn was observed, regardless of whether samples with high Hgb were excluded.

Application of test cohort results to the ADNI and PPMI cohort datasets.

Next, we sought to determine whether the relationships between protocols observed in our study could be used to transform the values reported in the ADNI and PPMI studies. Data were available from 103 PPMI control subjects and 113 ADNI1 control subjects, as well as 92 AD and 130 PD patients. The ADNI subjects were older than PPMI subjects, and the PPMI cohort consisted of more male subjects in both the control and PD groups (Table 1).

Because both studies used healthy older adults as controls, they should, in theory, be relatively similar, despite differences in the age and sex distributions of the cohort. We reasoned that if the primary differences between studies are imparted by the collection/processing protocols, then the relationship between the distributions in the control groups for ADNI and PPMI should reflect the same relationship as comparing ADNI CSF vs PPMI CSF in our test cohort. Therefore, we first compared the relationships for the control subjects in ADNI and PPMI, and in our test cohort. For A β and p-tau, the direction of the difference was opposite to that observed in the test cohort, while for t-tau, a difference was observed between the database samples, but not between protocols in the test cohort (Fig 4 A-C). We concluded that no conversion derived from the test cohort could suitably transform the database data to make the study cohorts similar, likely due to intrinsic cohort differences. However, the α -syn values for the PPMI database samples were significantly higher than the ADNI database samples, similar to the ADNI CSF Luminex values compared to the PPMI CSF ELISA values in the test cohort (Fig. 4D). Because correlation between appropriate groups, e.g., ADNI Luminex vs PPMI ELISA (see Fig. 2E-F), was improved by exclusion of high Hgb samples, only this subset of the data was used for further analysis.

We then generated conversion models by performing Deming regression on either the raw or log10 transformed values. To estimate the error of the transformations, we performed LOOCV. By this method, transformation of the raw ADNI values to approximate the PPMI methodology resulted in a mean absolute error of 132.2 pg/ml and an estimated CV of 19.0%, and transformation of PPMI values to approximate the ADNI method a mean error of 225.7 pg/ml and CV of 14.6%. The same procedure on the log-transformed values resulted in a mean error and CV of 122.3 pg/ml and 17.6% for ADNI samples, and 230.2 pg/ml and 14.9% for PPMI, respectively. These transformations were then applied to the control and disease subjects from the ADNI and PPMI databases. The resulting distributions remained notably different following transformation, and are presented in Figure 5A-B. To account for the possibility that the relationship between protocols was not the same between diagnostic states, we also transformed the data using models generated by Deming regression of just the test cases with the same diagnosis (i.e., the database control values were transformed using a model derived from only the control test cases, while the database AD values were transformed using a model derived from only the AD test cases). The resulting transformations were largely similar to those derived from the test cohort as a whole, and are presented in Supplemental Figure 1.

Discussion

The A/T/N framework of neurodegenerative disorders classifies AD according to its underlying, heterogeneous pathology. Its application would be facilitated by pooling the resources of multiple large studies, especially those with longitudinal data, such as ADNI and PPMI, to generate “super-studies”. However, there are major differences in pre-analytic steps involved in the collection, processing and storage of CSF archived in ADNI vs. PPMI, similar to those reported to influence some reported analyte values in other studies[20–25, 31]. In this investigation, among classical AD markers, t-tau and p-tau did not significantly differ between groups, though p-tau trended higher in PPMI. In contrast, A β 42 was significantly lower in PPMI CSF for most individuals; the opposing directions may be explained by the differing hydrophobicity of the two species altering their interaction with tube surfaces[24]. Use of the tau/A β ratio, a reliable marker for AD[32, 33], did not mitigate A β 42 disparities between protocols. Although the A β 42 differences may be largely reduced with a simple linear correction, the results emphasized the importance of standardization of procedures, including the type of tubes for the storage of CSF samples. An additional caveat is that the AlzBio3 kit reports different values between lots, with the ADNIGO/2 results being closer to those collected in PPMI. We used the original ADNI 1 values for these markers, because this is the same data set for which α -syn values were measured. However, the conclusions were the same whether ADNI 1 or ADNIGO/2 data sets were used.

Evidence of cross-talk between CSF biomarkers suggests that addition of PD markers (α -syn) might improve classifying and/or monitoring of AD, beyond what is possible using A β , t-tau, and p-tau[10–12, 34], consistent with the modern A/T/N framework view of neurodegenerative disease, which requires consideration of the underlying pathology.

CSF α -syn itself did not differ by collection protocol, but, as in previous studies[35], the ELISA platform reported higher values. This can likely be attributed to differences in antibody-analyte interactions, and absolute quantification requires confirmation by alternative methods, such as quantitative mass spectrometry. As with the AD markers, correction for analytical differences may be unnecessary for studies using the same platform, and possible if differing platforms are used. Additionally, CSF α -syn requires careful quality control, as contamination with highly abundant[27], blood-derived α -syn can easily mask differences in CSF α -syn between groups, necessitating exclusion of contaminated samples[26, 36]. One major protocol-related effect observed in this study was on CSF Hgb levels, an index of blood contamination in CSF. Consistent with previous studies noting relatively high Hgb in the ADNI cohort, all five samples with >200 ng/ml Hgb were obtained using the ADNI protocol. Interestingly, because samples were collected from a single LP, this means that the likely greater blood contamination of the ADNI CSF arises from the post-collection CSF processing. A possible explanation is that the ADNI protocol, unlike the PPMI protocol, does not involve centrifugation immediately after collection, meaning that any red blood cells present in the CSF may be lysed during the first freezing cycle. The values of pS129 varied between protocols less consistently, suggesting simple linear correction may be less suitable than with other markers. Unlike total α -syn, pS129 is less influenced by blood contamination[30], meaning that the greater variability may be unrelated to this variable, but rather arise from a more complicated combination of factors.

Moreover, it was not possible to compare pS129 between study cohorts, as no values are currently available for the PPMI cohort.

We next explored the possibility of combining the ADNI and PPMI study data based on our findings in the test cohort, with careful consideration of various variables. However, t-tau, a marker that appeared more resistant to the pre-analytic variations in the test cohort, did differ between ADNI and PPMI. A β 42, which showed significant protocol effects, differed in the opposite direction from the database cohorts. Only α -syn differed between protocol/platforms similarly to the ADNI vs PPMI study control subjects. Application of the derived α -syn models to the ADNI and PPMI studies diminished, but did not eliminate, differences between the distributions of the database cohorts. For all analytes, whether or not correction was necessary, differences remained in the database cohorts, suggesting variability introduced by factors other than the pre-analytical handling, such as age, site effects, or other unknown variables [26, 37–39]. Notably, the differing age distributions should result in a predictable difference in some markers, such as α -syn and tau, which both have been shown to increase in CSF with age [26, 40], and in fact the trend of higher α -syn and tau with age could be observed within each group. While this suggests that correction by age might be necessary in future attempts at conversion between studies, in this study, correction is unlikely to eliminate the differences: the ADNI database subjects, who were older (and should be expected to have higher α -syn based on age) had lower mean α -syn values, even after correction using the conversion models, so further correction by age would increase the differences. In contrast, tau did not differ between protocols in the test group. Therefore, the differences observed in the database cohorts further emphasize that, while correction based on collection protocol is not necessary, careful matching of comparison cohorts in terms of demographic characteristics will help to optimize accuracy. Moreover, in our cohort, to minimize effects of variability of the assays themselves, we ran the samples together (same plates, same day, and same assay operator). Thus, differences that might occur due to, e.g., lot-to-lot differences in the assays, or differences in collection/processing by site, could not be replicated in our design. Additionally, unknown differences not captured in the basic demographic data, likely contributed to the distributions. Therefore, for future attempts to combine the datasets for analysis, intrinsic cohort differences should be considered. Such combinations might be more suitable for population-based studies; for case-control studies, more careful selection of subject subsets (including age- and sex- matching) might be needed.

Together, our results demonstrated that the effects of the two sample collection and storage protocols on common AD and PD biomarker candidates were minimal or may be largely corrected, but any attempts to combine the datasets for analysis should proceed with caution. Additionally, these findings suggest that, after confirmation in in bigger test cohorts, any strategy for generation of merged “super studies” must take into consideration not only pre-analytic factors, but also characteristics of cohorts and site-effects. They further suggest potential strategies for mitigating these variables: large consortia should collaborate to share optimized, standardized protocols (e.g., for minimizing blood contamination and avoiding circadian variation in samples), and use uniform platforms. Commercial kits, tested between lots to achieve minimal variability, should be adopted. Careful and shared attention to such factors, and recruitment from broader pools of subjects, would be useful in generating study

populations that can be included as modular “units” to future, collaborative studies of neurodegenerative diseases. Additionally, rich clinical data must be captured and shared, to allow for controlling of these factors. Despite the challenges, these measures are worth undertaking, for the broader ability to measure heterogeneous neurodegenerative diseases based on underlying biological factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. ADNI vs PPMI levels of blood contamination reflected by elevated Hgb levels and AD analytes.

Levels of analyte and Deming regression for samples collected according to ADNI and PPMI protocols for: A-B) Hgb. C-D) total tau. E-F) p-tau 181. G-H) A β 42. I-J) Tau/A β ratio. Red bars represent mean values. Inset in B) shows overlapping low Hgb points.

Figure 2. Values and Deming regression for samples collected by ADNI and PPMI protocols for α -syn analytes.

A-B) Total α -syn measured by Biolegend ELISA assay. C) Measured by ELISA with samples with Hgb>200 excluded. D-E) Total α -syn measured by Luminex assay. F) Luminex with Hgb>200 excluded. G-H) pS129 α -syn. I) pS129 with Hgb>200 excluded. J-O) Relationship between total α -syn measured by ELISA and Luminex either comparing J-K) all 60 samples including or L) excluding Hgb>200. M-O) Comparison of ADNI protocol samples measured by Luminex vs the PPMI protocol samples measured by ELISA, with (M,N) or without (O) inclusion of samples with Hgb>200.

Figure 3.

Comparison of markers by diagnostic group. Distributions of measured values for ADNI and PPMI test samples for A) tau; B) A β ; C) p-tau; D) tau/A β ; E) pS129; F) ELISA α -syn; G) Luminex α -syn; H) Luminex α -syn excluding samples with Hgb>200; I) ELISA α -syn excluding samples with Hgb>200

Figure 4. Comparison data distributions for ADNI vs PPML.

Differences in analyte distribution were compared between the test cohort, and control subjects in the database cohorts, for A) Aβ, B) t-tau, C) p-tau, and D) α-syn.

Figure 5. Correction of database α -syn data based on models derived in the test cohort.

A) Effect of correction using Deming regression of ADNI protocol Luminex measured α -syn and PPMI protocol ELISA-measured α -syn in the test cohort to the ADNI database control and AD subjects. “PPMI Converted” refers to PPMI database values converted to approximate ADNI methods, and “ADNI Converted” refers to ADNI database values converted to approximate PPMI methods. Circles: control subjects. Triangles: disease subjects (AD and PD for ADNI and PPMI, respectively). Dark red indicates ADNI samples, while light red indicates PPMI values converted to approximate ADNI values. Similarly, dark blue indicates PPMI samples, while light blue indicates ADNI values converted to approximate PPMI values. B) Effect of correction using Deming regression based on log-transformed α -syn values in the test cohort.

Table 1.

Demographic description of cohorts

	ADNI		PPMI		ADNI vs PPMI Protocol Test Cohort		
	Con	AD	Con	PD	Con	AD	PD
N	110	92	103	130	10	10	10
Age ± SD	75.8 ± 5.2	74.5 ± 8.5	60.2 ± 12.1	62.0 ± 9.3	67.0±4.8	62.1±5.2	64.3±8.4
% male	51%	57%	61%	75%	60%	40%	70%

Table 2.

Comparison of CSF collection/processing protocols from ADNI and PPMI studies.

	ADNI	PPMI
Initial collection	In 5-cc syringes (1481729, Fisher Scientific) if suction LP, or 7-cc polypropylene collection tubes (14-956- 1C, Fisher Scientific) if drip LP ^I	In 3-ml plastic syringe (part of LP tray, A3772-24, Smiths medical), 1–2 mL transferred to cryovial for local laboratory testing, then in 6 ml polypropylene syringes (A3772-24, Smiths Medical)
Transfer	Directly from 5-cc syringes or with sterile transfer pipets (1371120, Fisher Scientific) from 7-cc collection tubes to 13 ml polypropylene transfer tubes (60.541.004, Sarstedt), the 1 st 2 ml transferred to 2 cryovials (1050026, Fisher Scientific) for local laboratory testing	To 15 ml conical polypropylene tubes (430701, Corning)
Processing at collection site	None.	Invert tubes 3–4 times, centrifuge (2,000 × g, 10 min, 4°C) within 15 minutes of collection, supernatant transferred to a new 15 ml conical polypropylene tube (430791, Corning), inverted 3–4 times, 1.5 ml aliquots in 2 ml pre-cooled polypropylene tubes (72.694.006, Sarstedt)
Freezing, shipping and storage	13 ml transfer tubes frozen directly on dry ice as soon as possible and shipped	Aliquots frozen directly on dry ice within 60 minutes of collection, stored at –80°C or shipped on dry ice
Further processing at biodepository site	Samples in 13-mL tubes thawed at room temperature with gentle rotation, transferred to 30 ml polypropylene tubes (62.543.001, Sarstedt), mixed by gentle rotation for 5 minutes, 0.5 ml aliquoted in 0.5 ml polypropylene copolymer tubes (3428000005 & 3428210116, Fisher Scientific), aliquots transferred directly to –80°C	Pre-chill labeled 0.5 ml siliconized cryovials at –80°C. Thaw 1.5 ml CSF aliquots at 4°C. Transfer 0.5 ml tubes to dry ice. Mix 1.5 ml CSF aliquots by inverting the tube 6–8 times (not vortexing or mixing by pipette). Sub- aliquot 250 µl CSF into each pre-cooled tube. Transfer to –80°C freezer.
Long term storage	–80°C	–80°C
Analytical site processing	For Aβ and tau: thaw 0.5 ml sub-aliquots on ice before assay; For α-syn and pS129: thaw 0.5 ml sub-aliquots on ice, add 10% of protease inhibitor cocktail (10×), further sub-aliquot into 0.11-mL aliquots and frozen at –80°C. Thaw aliquots on ice before assay.	Thaw 0.25 ml sub-aliquots on ice before assay

^IThe syringe method was used for all subjects in this study.

Table 3.

Statistics of values measured by assay.

Analyte	Mean±SD		T-test p-value ¹	Regression equation X=ADNI, Y=PPMI (p-value) ²	Bland-Alman Bias ± SD (95% LOA)
Hgb	468.0±1642	11.02±17.58	0.13	Y=0.0024X+9.9 (0.23)	−457.0±1666 (−3722, 2808)
t-tau	98.39±67.98	97.64±69.12	0.52	Y=1.02X-2.40 (<0.0001)	−0.75±6.69 (−13.66, 12.15)
p-tau	48.15±27.84	59.95±33.78	0.20	Y=1.22X+1.0 (<0.0001)	11.80±10.96 (−9.68, 33.29)
Aβ	506.5±189.3	421.5±177.2	<0.0001	Y=0.93X-50.38 (<0.0001)	−85.03±69.83 (−221.9, 51.84)
Tau/Aβ	0.26±0.27	0.34±0.37	0.0004	Y=1.37X-0.02 (<0.0001)	−0.08±0.11 (−0.28, 0.13)
ELISA α-syn	1506±522.2	1521±532.1	0.72	Y=1.02X-16.77 (<0.0001)	14.78±226.4 (−429.0, 458.5)
High Hgb Excluded	1506±520.9	1521±541.2	0.19	Y=1.10±−111.0 (<0.0001)	−42.25±157.9 (−351.7, 267.3)
Luminex α-syn	629.8±244.2	643.1±220.3	0.50	Y=0.84X+91.72 (<0.0001)	−14.3±107.5 (−224.8, 196.6)
High Hgb Excluded	615.4±229.8	643.1±224.3	0.66	Y=0.95+37.02 (<0.0001)	−7.05±73.76 (−151.6, 137.5)
pS129	80.42±16.49	85.35±13.30	0.04	Y=0.72X+27.04 (<0.0001)	4.35±10.09 (−15.43, 24.13)
	Luminex	ELISA			
ELISA vs Luminex α-syn	636.6±232.5	1513±527.2	<0.0001	Y=0.45X-21.44 (<0.0001)	1056±467.1 (140.5, 1972)
High Hgb Excluded	630.6±225.0	1514±527.2	<0.0001	Y=0.44−14.17 (<0.0001)	851±296.4 (269.9, 1432)
ADNI Luminex vs PPMI ELISA	629.8±244.2	1521±532.1	<0.0001	Y=2.15X+88.45 (<0.0001)	−812.5±303.3 (−1407, −218.1)
High Hgb Excluded	615.4±229.8	1548±571.8	<0.0001	Y=2.33+27.23 (<0.0001)	−842.5±307.7 (−1446, −239.5)

¹ Paired t-tests.² Deming regression. LOA: limits of agreement