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Preanalytical stability of plasma/serum brain-derived tau

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Abstract

INTRODUCTION: We investigated effects of matrix type and reagent batch changes on diagnostic performances and longitudinal trajectories of brain-derived tau (BD-tau).

METHODS: We evaluated (i) cohort-1: paired plasma and serum from Alzheimer biomarker-positive versus controls (*n*=26); and (ii) cohort-2: n=79 acute ischemic stroke patients with 265 longitudinal samples across four timepoints.

RESULTS: In cohort-1, plasma and serum BD-tau were strongly correlated (rho=0.96,P<0.0001) with similar diagnostic performances (AUCs >99%) and correlations with CSF total-tau (rho=0.94-0.95, P<0.0001). However, absolute concentrations were ~40% higher in plasma versus serum. In cohort-2, first and repeated BD-tau measurements showed a near-perfect correlation (rho=0.96,P<0.0001), with no significant between-batch concentration differences. In longitudinal analyses, substituting ~10% of the first-run concentrations for the re-measured values showed overlapping estimated trajectories without significant differences at any timepoint.

DISCUSSION: BD-tau has equivalent diagnostic accuracies, but uninterchangeable absolute concentrations, in plasma versus serum. Furthermore, the analytical robustness is unaffected by batch-to-batch reagent variations.

1. BACKGROUND

Existing plasma total-tau (t-tau) assays have poor diagnostic performances[1–4], and either do not correlate or do so weakly with paired CSF t-tau measures which rather reliably reflects neural injury and degeneration[5–7]. The high expression of tau in multiple peripheral sources is thought to underlie this observation, with an estimated 80% of plasma t-tau signal originating from non-central nervous system (CNS) sources[1]. In effect, the remaining 20% brain-derived signal is masked by the highly abundant peripheral tau. We recently developed a novel blood-based assay that is selective for tau of CNS origin[8]. This assay – referred to as brain-derived tau (BD-tau) – uses an antibody that binds a defined peptide at the contiguous exon 4-5 junction of the *MAPT* gene, avoiding peripherally-expressed tau which has the exon 4a insert between exons 4 and 5[9,10]. We described technical validation of the assay and its clinical performance to identify autopsy-verified Alzheimer' disease (AD) pathophysiology[8]. Importantly, plasma/serum BD-tau correlated strongly with CSF t-tau while plasma/serum t-tau did not, which together with biochemical and mass spectrometric data further support its selectively and specificity to CNS tau. Besides AD, acute neurological disorders such as acute ischemic stroke (AIS) are associated with high increases in CSF and plasma t-tau at admission[11], and thus it is anticipated that BD-tau levels will have prognostic utility in this condition.

Despite its high diagnostic and prognostic performances, the potential effects of preanalytical handling procedures on BD-tau levels and repeatability are unknown. Here, we examined matrix effects by comparing BD-tau levels and diagnostic performances in paired plasma and serum samples. We additionally evaluated the effects of reagent batch changes on cross-sectional and longitudinal measurements.

2. METHODS

2.1 Study participants and sample collection

The Neurochemistry cohort (cohort-1; n=26), Sahlgrenska University Hospital, Gothenburg, Sweden, included paired ethylenediaminetetracetic acid (EDTA) plasma and serum from neurochemically-defined biomarker-positive AD participants (n=15) and biomarker-negative controls (n=11) selected based on their CSF biomarker profile[6,12].

The stroke cohort (cohort-2) included n=79 AIS patients from the Biostroke study, Centro Hospitalar Universitário do Porto (CHUPorto), Porto, Portugal [11]. These patients were prospectively evaluated with longitudinal blood collection for biomarker assessments. EDTA plasma samples were collected at four timepoints: on admission (n=77), upon treatment (n=75), at 24 hours (n=73) and 72 hours (n=40) post-treatment.

The studies were approved by the local ethical review boards at Gothenburg (EPN140811) and Porto (CHUPorto 123-DEFI/122-CES).

Whole blood was obtained by venipuncture into Vacuette® tubes (Greiner Bio-One) and processed concurrently into EDTA plasma or serum (plasma only for cohort-2) following published protocols [13,14] In brief, the tubes were stored in cold conditions and centrifuged within 2 h of collection at $2000{\times}g$ for 10 min and stored at $-80°C$ until use.

2.2 Biomarker measurements

Blood was collected and processed following recommended procedures [13–15]. For BD-tau, the sheep monoclonal antibody TauJ.5H3 (Bioventix Plc, Surrey, United Kingdom) was used for capture and an N-terminal-tau mouse monoclonal antibody for detection. Recombinant full-length tau-441 (#TO8-50FN, SignalChem) was used as calibrator. Blood samples and calibrators were diluted with Homebrew buffer (#101556, Quanterix, MA, USA). Plasma/serum samples were diluted 4 times with the Homebrew buffer. Assay development and validation procedures, including dilution linearity, were described recently[8]. Plasma p-tau181 was measured with a validated in-house method on Simoa HD-X[12] while CSF Aβ42, p-tau181 and t-tau were analyzed with commercial INNOTEST immunoassays[16], all at the University of Gothenburg, Mölndal, Sweden. Intra- and inter-run repeatability results were <10%.

2.3 Statistical analyses

GraphPad Prism version 9.3.1, IBM SPSS Statistics 28.0.0, and Rstudio 4.2.1 with the packages ggstatsplot [17] 0.9.5, ggplot2 3.4.0, beeswarm 0.4.0, and mcr 1.3.1 were used for plot generation and statistical analyses. Non-parametric tests were used for non-normally distributed data. Spearman correlation and the Chi square test were used for continuous and categorical variables respectively. Diagnostic performances were evaluated with Receiver Operating Curves (ROC) and the respective Area Under the Curve (AUC) assessments. Fold changes were examined by comparing biomarker values with the mean of the control group. Group differences were examined using two-tailed Mann-Whitney or Kruskal-Wallis tests with Dunn's multiple comparison (for two versus more groups, respectively). The

batch effects on cross-sectional data were studied using Passing Bablok regression and Bland-Altman plots. We studied the trajectories over time of the natural logarithm of plasma BD-tau concentration by fitting a generalized Linear Mixed effects Model using restricted maximum likelihood estimation. Significance was set at $P<0.05$.

3. RESULTS

The cohort demographic and biomarker details are presented in Tables S1 and S2.

3.1 Cross-sectional performance in plasma versus serum

In the Neurochemistry cohort, where all participants had paired EDTA plasma and serum, BD-tau was increased in Aβ-positive AD dementia versus Aβ-negative controls when measured in either matrix processed from the same blood draw ($P<0.0001$; Fig. 1A-B).

The mean fold differences in the AD versus control groups were 7.36 for plasma and 6.20 for serum, compared with 2.39 for plasma p-tau181. Similar to CSF t-tau, the BD-tau fold differences reduced to ~2.40 when using median values (Table S1), given the non-normal distribution. When comparing BD-tau levels in sample pairs across the entire cohort, the absolute mean values were 10.28 pg/ml for plasma and 5.99 pg/ml for serum, suggesting that for every plasma BD-tau value the corresponding level in serum was approximately 40% lower on average (Fig. 1C). Similar results were recorded when using medians instead of means; 4.19 pg/ml for plasma and 2.32 pg/ml for serum. Nonetheless, BD-tau levels in both plasma and serum were above the assay's lower limit of quantification of 0.03 pg/ml (lowest recorded values were 0.48 pg/ml for serum and 0.63 pg/ml for plasma for the same individual).

Plasma vs. serum BD-tau were strongly correlated (Spearman rho=0.96, P<0.0001; Fig. 1D). In agreement, plasma and serum BD-tau had similar diagnostic accuracies of 100% and 99.4% (95% confidence interval [CI]=97.5%-100%) respectively. Furthermore, plasma and serum BD-tau showed similarly strong correlations with CSF t-tau (rho=0.94 vs. 0.93;P<0.0001; Fig. 1E); excluding the high BD-tau value >50 pg/ml did not affect the strength of the correlations which became rho =0.94 and 0.92 respectively. Plasma and serum BD-tau additionally correlated equivalently with CSF Aβ42 (rho= −0.70 vs. −0.69;P 0.0001), p-tau181 (rho=0.90 vs. 0.89;P<0.0001) and, as well as plasma p-tau181 (rho=0.75 vs. 0.74;P<0.0001).

3.2 Batch effects on cross-sectional and longitudinal data

In the stroke cohort, we first measured plasma BD-tau in 265 longitudinal samples from n=79 AIS patients. Later, 28 samples selected to reflect low, medium, and high concentrations were evaluated with a different reagent batch (different lots of bead-coated capture and biotin-conjugated detection antibodies). There was a strong correlation (rho=0.96,P<0.0001) between the 28 samples independently analyzed twice (Fig. 2A-B). In cross-sectional analysis, we observed no difference between batches when comparing the first-run concentrations for the whole 265 measures with another set where the values for the 28 repeated samples had been substituted for those in the rerun experiment (Fig. 2A-B). In longitudinal analyses, the first-run versus re-measured value comparisons showed

overlapping estimated trajectories and no significant difference at any timepoint (Fig. 2C-E). The Passing Bablok regression equation was: *Second method=0.93 (95%CI 0.77 to 0.99) x First method - 0.55 (95%CI –0.98 to 0.72*; Fig. 2F). Bland-Altman analysis showed high agreement between the BD-tau measures especially at low to average concentrations. The mean bias was 9.14 (95% CI:1.94 to 16.33). There was also a proportional bias between the two measures, which is explained by 24/28 re-analyzed samples having slightly lower but statistically indifferent values in the second analysis (Fig. 2G). This bias was within the expected range of unavoidable analytical error that is observed for any biofluid biomarker and technical platform [18].

4. DISCUSSION

An important criterion in evaluating the utility of a blood biomarker is to establish potential effects of preanalytical factors including the coagulation method and batch-to-batch reagent variation[18]. We have characterized these preanalytical factors for BD-tau, leading to the findings that the biomarker is reliably measurable in either plasma or serum to reflect the same CNS-derived tau changes to support diagnostic decision-making.

Importantly, the high diagnostic performance to differentiate Aβ-positive AD dementia individuals from Aβ-negative controls was independent of the use of plasma or serum. Moreover, paired plasma and serum BD-tau correlated strongly and equivalently with CSF t-tau, demonstrating that the superior performance of BD-tau over current blood-based t-tau assays regarding association with CSF t-tau[8] is independent of matrix type. Additionally, comparable plasma vs. serum BD-tau correlations with CSF Aβ42 and p-tau181 show that the new biomarker detects AD pathophysiology in peripheral blood irrespective of matrix source. However, concentrations in serum were ~40% lower than those in paired plasma. This means that absolute concentrations measured in plasma and serum are not identical and thus uninterchangeable. To this end, BD-tau evaluation in cohort studies should be restricted to either plasma or serum samples collected with an identical protocol, without switching between matrix types. These results agree with previous reports for blood-based p-tau181, p-tau231 and t-tau[12,14,15] but not p-tau217 which does not work well in serum in an AD context. Furthermore, these results suggest similar diagnostic capabilities between plasma and serum, which has not been reported for any of the other currently available t-tau assays.

Longitudinal biofluid biomarker analyses can be complicated by the need to re-measure baseline samples with the same reagent batch as the follow-up ones to avoid batch effects [19]. Here, we evaluated a workaround of measuring a small, representative selection of baseline samples along with the follow-up ones (~10% of the total samples), to improve efficiency and save time and resources. Plasma BD-tau concentrations in the first and repeated runs were near-identical, with minor analytical errors that were within range of expectation. These variations are unavoidable in clinical chemistry, however, the extent to which they affect assay performance is a reflection of how robust that biomarker is [18]. In this study, these analytical errors were insignificant to the overall biomarker performance, with the longitudinal trajectories of the whole cohort in the first run equivalent to when the second measures were substituted in.

While BD-tau appears to show specificity to AD pathology vs. other neurodegenerative diseases [8], its increases in AIS suggest release into the bloodstream following acute neurological insults. This indicates that, similar to CSF t-tau, plasma BD-tau would be additionally important for predicting clinical outcome after AIS [20].

Strengths of the study include (pre)analytical comparison of BD-tau performances in paired plasma vs. serum, and evaluation of the impact of reagent batch changes. Limitations include the small sample sizes especially in cohort-1, given the known challenges in obtaining both plasma and serum from participants with CSF biomarker characterization.

Altogether, the results indicate that BD-tau is an analytically robust biomarker, supporting its further evaluation as a novel indicator of CNS-tau in peripheral blood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding sources

Conflicts

Abbreviations:

Aβ	amyloid beta
AD	Alzheimer' disease
AIS	acute ischemic stroke
AUC	area under the curve
BD-tau	brain-derived tau
CI	confidence interval
CNS	central nervous system
CSF	cerebrospinal fluid
EDTA	ethylenediaminetetraaceticacid
p-tau	phosphorylated tau
t-tau	total tau

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RESEARCH IN CONTEXT

Systematic review:

We found one publication on plasma/serum brain-derived tau (BD-tau) on PubMed i.e., Gonzalez-Ortiz *et. al.,* (2023) Brain, which reported analytical validation, including linearity of sample signal proportional to dilution fold, recoverability of BD-tau signal from exogenously added samples, and day-to-day stability of the assay. In this study, we investigate impacts of matrix type and reagent batch switches on BD-tau.

Interpretation:

Equivalence in BD-tau diagnostic performances when measured in paired EDTA plasma versus serum samples indicates that both matrix sources can be clinically used to measure this marker, the first for any blood-based non-phosphorylated tau assay. However, higher concentrations in plasma highlights lack of interchangeability with serum. Moreover, nearly identical plasma BD-tau concentrations from two measurement rounds that also had overlapping longitudinal trajectories shows that signals are reproducible across reagent batches.

Future directions:

BD-tau can be measured in cohorts with plasma or serum samples, and using different reagent batches.
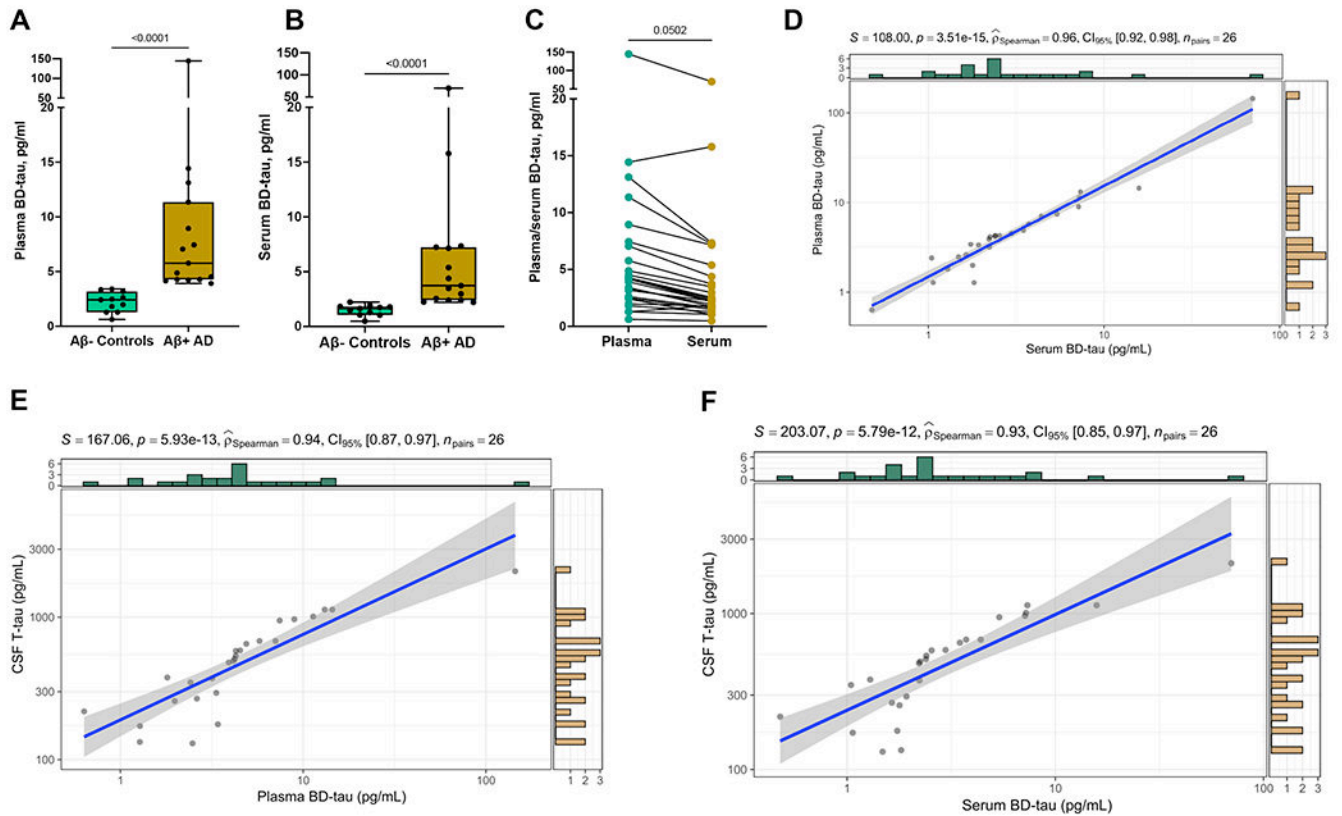
Fig. 1. Comparison of BD-tau concentrations measured in paired EDTA plasma versus serum collected from the same individuals at identical clinical visits (cohort-1).

(A) BD-tau levels measured in plasma. (B) BD-tau levels measured in serum. (C) Pairs of BD-tau measures in plasma versus serum samples from the same individuals. (D) Scatter plot showing correlation between the paired measures of BD-tau in the plasma versus serum samples. (E) and (F) Scatter plots and Spearman correlation of CSF t-tau versus each of plasma BD-tau and serum BD-tau The scales of the scatter plots in (D), (E) and (F) are log10. However, the biomarker concentrations are provided in absolute pg/ml values. The histograms in green and yellow show the marginal distributions of the two variables being compared. The information provided at the top of each scatter plot are as follows: The S value is the sum of the square differences between each variable's rank. P-value is the p-value associated with the hypothesis test carried out to see if the correlation coefficient is significant. rho (shown as $\hat{\rho}$) is the Spearman correlation coefficient (ρ/rho). n_{pairs} = number of observations.
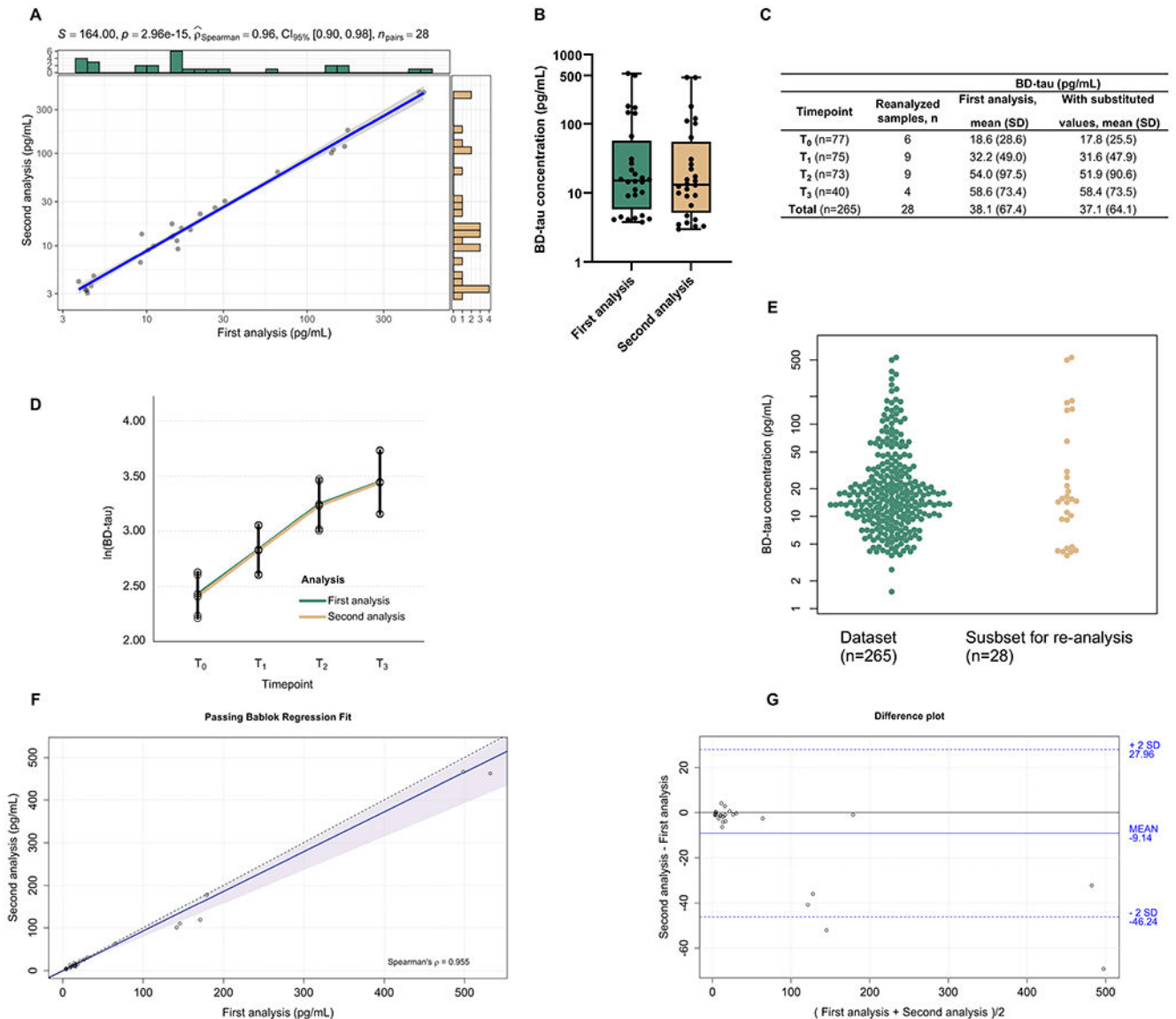
Fig. 2. Re-measurement of ~10% plasma samples from acute ischemic stroke patients using new reagent batches and the effect on the full cohort (cohort-2).

(**A**) Correlation plot of the 28 samples that were measured twice. (**B**) Boxplot representing the distribution of the samples first and second measurements. (**C**) Descriptive table of the first-run concentrations for the whole 265 samples divided by timepoints with the second set where the values for the 28 repeated samples were respectively substituted. (**D**) Longitudinal profile of BD-tau with estimated means and 95% confidence levels based on the linear mixed effects model. (**E**) Swarm plot depicting the selected samples (n=28) from the total dataset (n=265) for the second analysis. Agreement analysis of both measurements are shown in (**F**) Passing bablok regression analysis and (**G**) Bland-Altman plot considering the absolute values of both analysis. For the Passing Bablok regression, the solid and the shadow indicate the regression line and confidence interval for the regression line, respectively. The 0.95-confidence bounds of regression fit line were calculated with the bootstrap (quantile)

method. In the Bland-Altman plot, the solid line indicates the mean difference between the methods and the dashed lines the confidence interval calculated by multiplying the standard deviation by 2. The scales of the scatter plots in (A) is log10. However, the biomarker concentrations are provided in absolute pg/ml values. The histograms in green and yellow show the marginal distributions of the two variables being compared. The information provided at the top of each scatter plot are as follows: The *S* value is the sum of the square differences between each variable's rank. P-value is the p-value associated with the hypothesis test carried out to see if the correlation coefficient is significant. rho (shown as $\hat{p}$) is the Spearman correlation coefficient (ρ/rho). n_{pairs} = number of observations.