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Immunochemical Detection of α-Synuclein Autoantibodies in Parkinson's Disease: Correlation between Plasma and Cerebrospinal Fluid Levels

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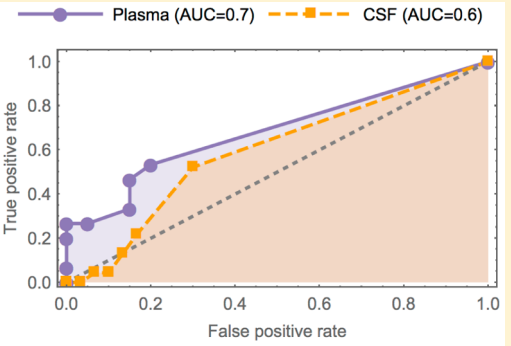
Supporting Information

ABSTRACT: Autoantibodies to Parkinson's disease (PD) amyloidogenic protein, α-synuclein, were recognized as a prospective biomarker for early disease diagnostics, yet there is inconsistency in previous reports, potentially related to PD status. Therefore, plasma and cerebrospinal fluid (CSF) of the cross-sectional cohort of 60 individuals, including recently diagnosed PD patients with mild and moderate PD and age-matched controls, were examined by enzyme-linked immunosorbent assay (ELISA). Nonparametric statistics was used for data analysis. We found significantly elevated levels of α-synuclein autoantibodies in both plasma and CSF in mild PD compared to controls, followed by some decrease in moderate PD. Receiver operating characteristic and effect size analyses confirmed the diagnostic power of α-synuclein antibodies in both plasma and CSF. For the first time, we showed the correlation between plasma and CSF α-synuclein antibody levels for mild, moderate, and combined PD groups. This indicates the potentiality of α-synuclein antibodies as PD biomarker and the increased diagnostic power of their simultaneous analysis in plasma and CSF.



KEYWORDS: *Biomarkers, blood plasma, cerebrospinal fluid, enzyme-linked immunosorbent assay (ELISA), Parkinson's disease, α-synuclein autoantibodies*

INTRODUCTION

Parkinson's disease (PD) is the second most common chronic neurodegenerative disease characterized by motor and cognitive dysfunctions. PD affects more than 1% of individuals over 60 years old and ca. 4% of those over 80 years old.[1] The increasing number of PD patients, due to growing elderly population, leads to rapidly escalating cost for the healthcare system. The major pathology of PD arises from abnormal amyloid accumulations of α-synuclein, mostly in the form of Lewy bodies and Lewy neurites, and loss of dopaminergic neurons in the *substantia nigra*, though a detailed map of disease progression remains unclear.[2] A definitive diagnosis of PD is difficult to achieve, especially in its early stage, when the accuracy does not exceed 26% for untreated or not clearly responsive subjects and 53% for subjects responsive to medication.[3] Modern neuroimaging techniques, such as dopamine transporter scans, incur a high cost, not readily accessible and therefore not optimal for a first-line PD screening. There is also a long latency between the onset of pathological processes and appearance of clinical symptoms. The latter are often not evident until some 60−80% of dopaminergic neurons have been lost, leaving very limited options for therapeutic intervention.[4] Therefore, the development of biomarker-based diagnostics capable of detecting PD in the earliest stage, when neurons at risk can be protected and general treatments can be more effective, is of major importance. The criteria proposed for these biomarkers are that they should be linked to fundamental features of PD.[5]

Serological assays have demonstrated that amyloid self-assembly of α-synuclein is allied with humoral immunity.[6−8] Autoantibodies involved in clearance pathways and tissue homeostasis are suggested to play an active role throughout PD progression and may have a protective power reducing α-synuclein level.[6]

Autoimmune responses to α-synuclein in blood plasma or sera as a marker of PD were studied previously;[6−18] however the results are mixed. Contributory problems have been the lab-to-lab variability associated with assays and sample collecting procedures as well as the diverse nature of patient cohorts.[16] As PD is an inherently heterogeneous disease, the reported variation may be related to clinical disease duration, severity, age of onset, familial or sporadic origin, and disease subtype (typical or atypical). Therefore, more studies on different PD groups are needed. The increased content of α-synuclein

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autoantibodies was reported in early versus late PD stages in different cohorts by using enzyme-linked immunosorbent assay (ELISA), immunoblotting, surface plasmon resonance, and electrochemistry.[6−8,14,15,18] Combined PD groups without differentiated disease stages[12,16] and late PD cohorts with mean clinical disease durations of ca. 10 years[10,13] have shown no difference or even a decline[13] in α-synuclein antibody levels compared to controls. Interestingly, it has been reported that the serum α-synuclein antibody concentrations in both typical PD and atypical Parkinsonism were highest in the patients with <4 year duration.[17] Comparison of familial and sporadic PD indicated that α-synuclein autoantibodies were detected in 65% of PD patients and their presence (90%) strongly correlated with an inherited PD;[18] however both cohorts were biased toward prolonged clinical disease duration with mean of ca. 9 years. It is interesting to note also that α-synuclein antibody levels in blood sera were increased during Lewy body associated dementia, which is also characterized by α-synuclein aggregate accumulation, and to lesser extent in Alzheimer's disease.[9,11]

Autoantibodies to α-synuclein in cerebrospinal fluid (CSF) have been less studied,[11,16] as CSF samples are not as easily obtainable as the blood specimens. Among available data, no differences were determined in CSF α-synuclein autoantibody levels in Lewy body dementia[11] and combined idiopathic Parkinson's syndrome group[16] compared to controls. However, the measurements of biomarkers in CSF associated with disease pathological causes proved to be very efficient for the early diagnostics of other neurodegenerative diseases such as Alzheimer's disease, starting from the early stage of mild cognitive impairment.[19,20] Since CSF is a sensitive indicator of hazardous pathological processes in the brain tissues, reflecting the delicate shifts in protein homeostasis during disease, here we compared α-synuclein antibody levels in CSF and blood of the same individuals. It is important to note that all PD cases in our study were newly diagnosed, with a time from a first diagnosis of PD to sampling of CSF and plasma of less than 3 months, and symptomatically divided into two subgroups with mild (1.5−2) and moderate (2.5−3) PD according to Hoehn and Yahr (H−Y) scaling. Therefore, these were the disease clinical stages when the disease-modifying therapeutic interventions may still be applicable compared to more advanced PD with irreversible neuronal loss.

RESULTS AND DISCUSSION

The levels of autoantibodies to α-synuclein in the blood plasma and CSF were examined by using indirect ELISA. The serial dilutions were performed and corresponding titers were used, since such approach is arbitrary to the nature of body fluid and more reliable in assessing the total autoantibody levels than the measurements at a single dilution. The titer values were determined by fitting the dilution curves with a logistic function. This approach enabled us to measure their intercepts with the background levels more accurately, significantly minimizing errors at high dilutions (Figure 1), see Supporting Information.

In order to differentiate whether parametric or non-parametric methods should be applied for statistical data analysis, we have assessed the normality of α-synuclein antibody titer distributions for each of the studied groups, including combined group of all PD patients, subgroups with mild and moderate PD, and controls. For this purpose, the quantiles of α-synuclein antibody titer distributions both in plasma and in CSF were plotted versus quantiles of the

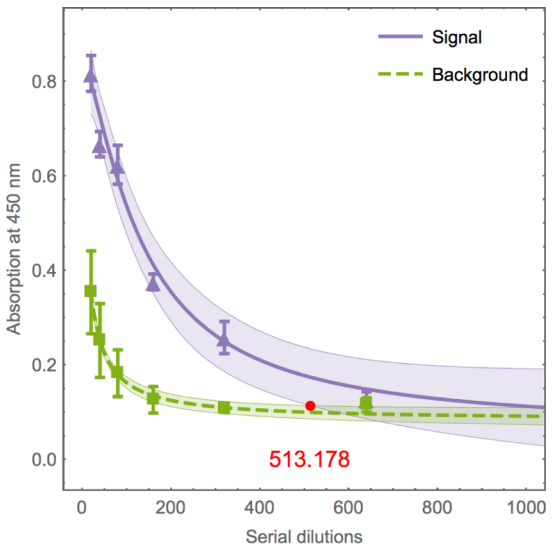

Figure 1. α-Synuclein antibody titer determination by ELISA serial dilutions and fitting with a logistic function. Serial dilution of a representative PD patient sample is shown by purple triangles. Nonspecific antibody binding in wells without immobilized α-synuclein was used as a background and shown by green squares. Fitting curves with their 95% confidence bands are shown in corresponding color coding for both serial dilutions. The titer for PD patient sample is shown by red dot at the intersection of the sample low confidence band with the background high confidence band.

corresponding normal distributions (Figure 2). The quantile−quantile plots have demonstrated that for each group the data points were deviated from the straight lines corresponding to the normal distributions, indicating that all studied data sets were not normally distributed. Consequently, the nonparametric statistical methods were applied for data analysis, the data sets for each patient group were presented by box-plots, and the median values with 95% confidence intervals (95% CIs) were used to compare them with each other (Figures 3, 4).

In the first instance, the objective was to compare the α-synuclein antibody levels in plasma and CSF samples of combined PD patient group with those of controls as shown in Figure 3A,B. In the plasma samples of the combined PD group, the median value of α-synuclein antibody titers was higher compared to controls, that is, 6.5 (95% CI 5.5−7.8) versus 5.0 (95% CI 4.3−5.3), respectively. Similarly, the CSF samples of combined PD group were also characterized by higher median value of α-synuclein antibody titers of 5.0 (95% CI 4.5−5.1) versus 4.0 (95% CI 3.4−4.7) for controls. Thus, the median α-synuclein antibody titers for both plasma and CSF samples were clearly higher in PD patients than in controls.

Since the diagnostics is particularly important for the initial disease stages, we divided PD patients in two subgroups of mild and moderate PD according to their H−Y scores, as described in Table 1 and Supporting Information. While the levels of plasma and CSF antibody titers were generally elevated in both PD subgroups compared to controls, these subgroups differed from each other (Figure 4A,B). In the blood plasma samples of the mild PD subgroup, the median value of α-synuclein antibody titers was significantly higher, 6.8 (95% CI 4.9−8.0), than the corresponding value in the control group, 4.9 (95% CI 4.3−5.3). The median value of titers decreased in the moderate PD subgroup to 6.3 (95% CI 4.1−7.2) but still remained higher

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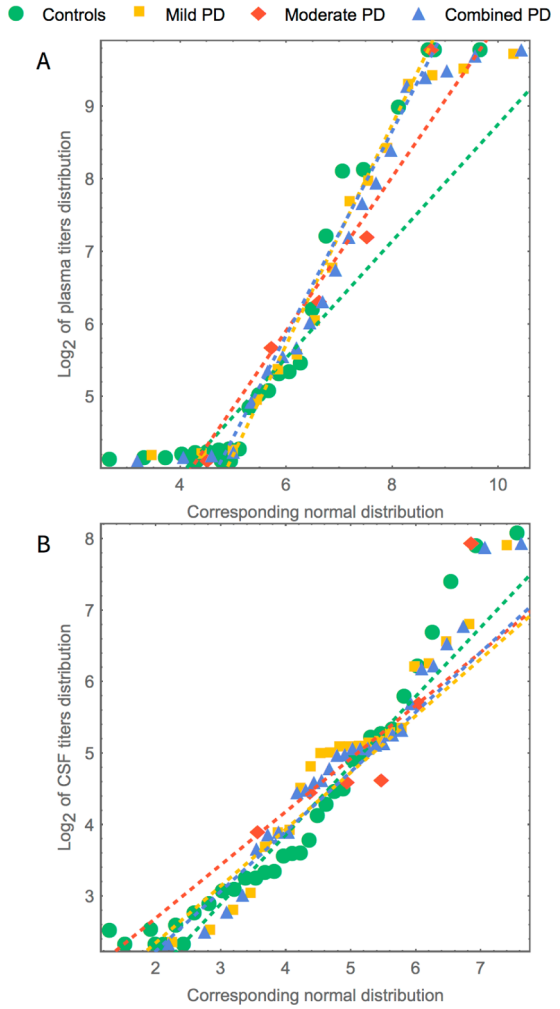

Figure 2. Quantile−quantile plots for α-synuclein antibody titer distributions for PD patients and controls measured in (A) blood plasma and (B) CSF. The data points corresponding to controls are shown by green circles, to mild PD subgroup by yellow squares, to moderate PD subgroup by red diamonds, and to combined PD group by blue triangles. The corresponding normal distributions are shown by dashed lines in the same color coding.

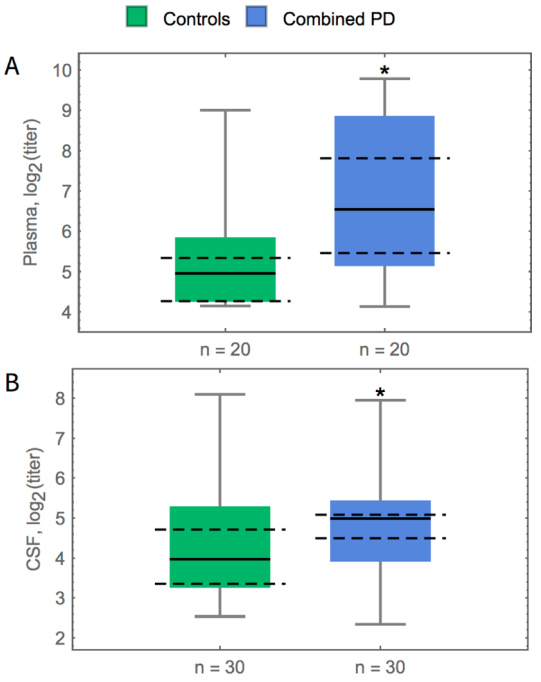

Figure 3. Box plots of α-synuclein antibody titer distributions (A) in plasma, combined PD group (n = 20) and controls (n = 20), and (B) in CSF, combined PD group (n = 30) and controls (n = 30). The data for control group are shown in green and those for combined PD group in blue boxes. Boxes include 25−75% of the data, and whiskers denote the minimum and maximum measured values. Median values are shown by solid black line and median 95% CIs by black dashed lines. *p < 0.05 calculated by Mann−Whitney U-test.

Table 1

characteristics of subjects	control	mild PD	moderate PD
number	30	24	6
male/female ratio	16/14	12/12	3/3
mean age (range), years	70.4 (57−80)	65.5 (38−79)	67.2 (56−77)
mean sampling time after diagnosis (range), months		2.8 (1−8)	3.1 (2−3.5)
H−Y score		1.5−2.0	2.5−3.0

than for controls. Importantly, the same trend was observed in CSF samples of the corresponding subgroups. The median titer of α-synuclein autoantibodies in the mild PD subgroup was 5.1 (95% CI 3.9−5.1), in the moderate PD subgroup the median decreased to 4.6 (95% CI 4.2−5.7); however both of these values were higher compared to the median titer for controls, 4.0 (95% CI 3.4−4.7).

In order to draw conclusions on the biological importance of the differences between α-synuclein antibody titers for all patient groups compared to controls, we estimated the magnitude of the observed effect[21] in addition to its statistical significance. Since all distributions of the antibody titers were non-normal, we evaluated the effect size by using Cliff's delta (δ) and its 95% CIs (Figure 4C). Cliff's delta is defined to range from −1 to 1, reflecting the extent to which one distribution tends to generally lie above or below another.[22] We used here the following scale for the effect size: 0.1 < δ < 0.3 corresponded to small effect, 0.3 < δ < 0.5 to medium, and 0.5 < δ < 1.0 to large effect.[23] Thus, the calculated effect size for the titers of α-synuclein antibodies in plasma was in the medium range and equal to 0.43 for the mild PD subgroup and 0.34 for the moderate PD subgroup compared to controls, while the effect size for antibody titers in CSF was in the medium range and equal to 0.37 for the moderate PD subgroup and in the small range of 0.20 for the mild PD subgroup compared to controls, respectively. The 95% CIs were wider for the measurements in plasma compared to CSF due to individual variability among subjects in both PD subgroups and controls. Individual variability of measurements in biofluids is a common phenomenon observed in other studies[9,16,24] and is related to potential contribution from concomitant diseases, aging, and inherent heterogeneity of PD pathologies, which should not be viewed as a single morbid entity.[25]

The fact that we have observed significant increase of the median values of α-synuclein antibody titers with the moderate effect sizes both in plasma and in CSF of the mild PD subgroup compared to controls is particularly important, since this

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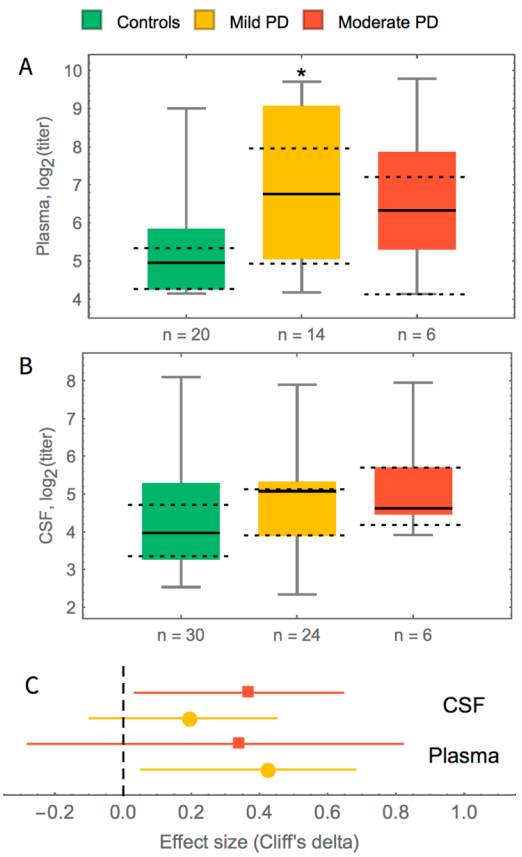

Figure 4. Distributions of α-synuclein antibody titers for mild PD, moderate PD, and controls with corresponding effect sizes (Cliff's delta). Box plots of the titer distributions (A) in plasma, controls (n = 20), mild PD (n = 14), and moderate PD (n = 6), and (B) in CSF, controls (n = 30), mild PD (n = 24), and moderate PD (n = 6). (C) The effect size (Cliff's delta) estimates and their 95% CIs for mild and moderate PD subgroups. The box plots and Cliff's delta for controls are denoted by green, for mild PD subgroup by yellow, and for moderate PD subgroup by red colors. Boxes include 25−75% of the data, and whiskers denote the minimum and maximum measured values. Median values are shown by black solid lines and median 95% CIs by black dashed lines. *p < 0.05 calculated by Mann−Whitney U-test.

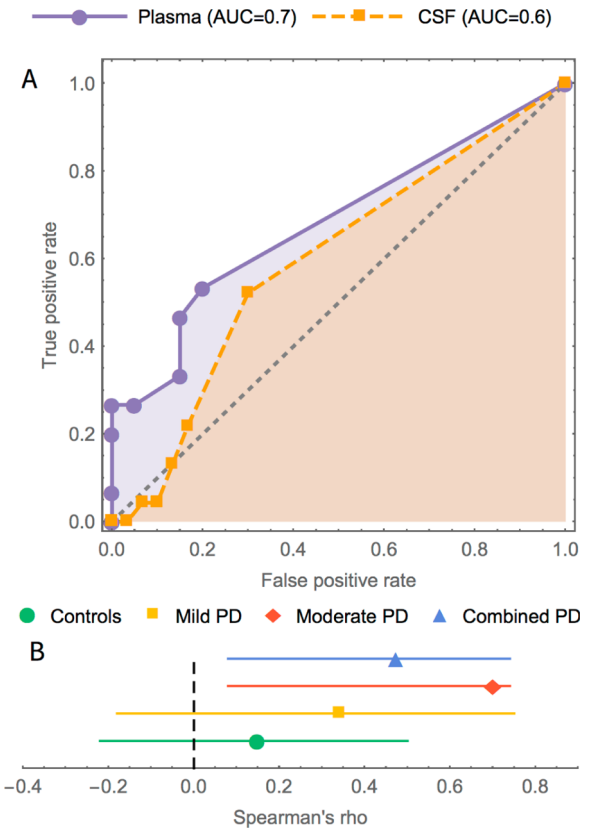

Figure 5. Assessment of diagnostic value and correlation between the levels of α-synuclein antibodies in plasma and CSF for PD patients and controls. (A) ROC curves to evaluate the diagnostic value of α-synuclein antibody measurements in blood plasma (purple circles and purple solid line) and CSF (orange squares and orange dashed line) for PD patients compared to controls. The area under the ROC curve for the CSF measurements is shown in orange and incremental area under the ROC curve for the blood plasma measurements is shown in purple. Diagonal gray dotted line indicates the results when the parameter in question has no diagnostic power and the area under the curve equals to 0.5. (B) Spearman's rho correlations, and their 95% CIs between the titers of α-synuclein antibodies in plasma and CSF of the same individuals for controls (green), mild PD (yellow), moderate PD (red), and combined PD (blue), respectively.

subgroup of PD patients with <3 months of clinical duration and initial stages of disease is most difficult to diagnose correctly.[3,25] Despite the application of stringent diagnostic criteria, misdiagnoses do occur in more than half of cases, with some patients thought clinically to have idiopathic PD turning out to have other illnesses.[3] Conversely, in other patients with atypical clinical pictures the diagnosis of PD was established but only after their death.[3,25] These highlight the need for some additional biomarkers, which can shed further light on disease origin and validate the clinical diagnostics. The mild PD patients with short clinical history are also the most susceptible to disease-modifying treatment versus advanced PD, which further underscores the importance of the application of α-synuclein antibodies as a biomarker in PD validation.

Furthermore, we have estimated the diagnostic power of α-synuclein antibody measurements in PD patients compared to controls by the receiver operating characteristic (ROC) analysis with subsequent calculations of the areas under ROC curves (Figure 5A). ROC analysis has demonstrated that indeed the assessments of α-synuclein antibody titers both in plasma and in CSF of PD patients have diagnostic powers with relatively higher strength for plasma measurements, that is, the areas under ROC curves were 0.7 vs 0.6, respectively. These observations are further signified if considering the upper boundaries (95% CI) for the median values of titers in both blood and CSF of the PD group (Figure 3), which are clearly higher for PD group than for controls.

To evaluate the strength of association between the α-synuclein antibody titers in plasma and CSF of the same individuals, Spearman's rho correlations were calculated for PD and controls (Figure 5B). We have found moderate Spearman's rho correlation for the mild PD subgroup, 0.34 (95% CI −0.18 to 0.75) and combined PD group, 0.47 (95% CI 0.08−0.74) in contrast to weak correlation for controls, 0.15 (95% CI −0.22 to 0.50), indicating that the increases of antibody levels in both body fluids are linked to PD pathology and likely to reflect similar underlying processes. It is worth noting that strong correlation (0.70) found for the moderate PD subgroup can be potentially related to its small size; for this subgroup the same 95% CI were used as for the combined PD group (Figure 5B).

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Moderate Spearman's rho correlations between plasma and CSF measurements for the same individuals in the mild and combined PD groups indicate that the simultaneous analysis of α-synuclein antibodies in both body fluids will increase its diagnostic power. It is important to note that such correlations were assessed for the first time as there is a general lack of data on the simultaneous measurements of α-synuclein antibodies in both blood and CSF for the same PD patients. Similar measurements were performed for Lewy body dementia cohort;[9] however in this study the Spearman's rho correlation was not evaluated.

We have not found correlations of the α-synuclein antibody titers either in plasma or CSF of PD patients with their age and sex. We have found a weak negative correlation (−0.14) between the CSF α-synuclein antibody titers and H−Y scales in the combined PD cohort, while no correlation was found for the plasma titers and H−Y scores in all PD groups.

In general, the nonparametric statistical analysis used in this study demonstrated that such approach can be a useful tool in statistical assessment of the patient cohorts characterized by a limited size and non-normal distribution of their data sets.

It is interesting to note that the median titer of α-synuclein antibodies is 2 times higher in plasma than in CSF, although the total concentration of IgG in plasma is ∼100 times higher than that in CSF,[26] suggesting that CSF is relatively enriched with α-synuclein antibodies. The permeability of blood−brain barrier progressing during PD[27] may contribute to the exchange between these body fluids, but the connection between them could be very complex and far from clear. CSF is viewed as a sink for brain extracellular solutes,[28] linking it with α-synuclein accumulation and humoral immune reactions in the brain. Conversely, according to the Braak hypothesis[29,30] misfolded α-synuclein first appears in the gastrointestinal tract, thus the immune system can become exposed to the accumulated misfolded protein already at an early PD stage, potentially resulting in elevated autoantibody levels in the blood. These suggest that different pathological pathways could be involved in PD progression, which is reflected in variability of α-synuclein antibody responses both in blood and in CSF and in the moderate but not strong correlation between the levels of antibodies in both body fluids.

Importantly, the similar trend of the reduction of α-synuclein antibody levels with disease progression was observed also in Lewy body dementia,[9] which may reflect the common underlying pathologies in synucleinopathies. If in the beginning of PD, the rise of antibodies to α-synuclein may be linked to inflammatory reactions[31] and an extensive α-synuclein aggregation,[2] the reduction of their levels at the later stages may be due to absorption of antibodies by tissue-deposited α-synuclein. Interestingly in AD patients, whose α-synuclein burden may rise slowly, the reversed trend was found with higher levels of α-synuclein antibodies after prolonged disease course.[9] Though multiple and yet unknown mechanisms can contribute to the reduction of α-synuclein antibody amounts in prolonged PD, among them could be also decreased numbers of T and B lymphocytes, which were reported for 5 and 10 year PD durations.[6]

Significance of the rise of α-synuclein antibody level in early PD may reflect their protective function with respect to α-synuclein aggregation and also their prospective applications as a passive immunization agent.[32] It has been shown that passive immunization with α-synuclein antibodies reduced its neuronal and glial accumulation, prevented cell-to-cell transfer, and ameliorated neurodegeneration and behavioral deficits associated with its overexpression in mouse model.[33,34] Translating immunotherapy to humans needs to consider the issue of potential autoimmunity[32] and specifically take into account the status of humoral immunity, since naturally produced autoantibodies to α-synuclein may effectively complement passive immunotherapy.

Thus, the present assessment of α-synuclein antibodies in plasma and CSF demonstrates the potentiality of α-synuclein antibodies as PD diagnostic tool aimed to validate clinical examinations and to increase diagnostic accuracy. The indirect ELISA method applied here is highly reliable and can be easily implemented at the point of care. The blood tests are easily obtainable and relatively noninvasive, and if an elevated level of α-synuclein antibodies is found in plasma, it may prompt further examination of CSF, leading to increased diagnostic power. Diagnostic biomarkers based on autoantibodies, reflecting inherent protective mechanisms in the body, may also strengthen therapeutic interventions, such as passive immunization, if PD is timely diagnosed and can be more effectively treated.

METHODS

Materials. Horseradish peroxidase labeled anti-human IgG (A8792) and bovine serum albumin was from Sigma-Aldrich. Phosphate buffered saline solution was prepared from tablets (09-8912-100, Medicago). The peroxidase substrate for ELISA (10-9405) was also obtained from Medicago. Recombinant human α-synuclein was expressed in *Escherichia coli* and purified as described previously.[35] All other chemicals were of analytical grade.

ELISA. The content of α-synuclein autoantibodies in the CSF and blood plasma samples of PD and controls were measured by indirect ELISA. The details of the protocol can be found in Supporting Information.

Human Subjects. The characteristics of PD patients are presented in Table 1. Detailed description of the cohorts can be found in Supporting Information.

Data Analysis. Statistical data analysis was performed by nonparametric methods using a *Wolfram Mathematica* 11 package as described in Supporting Information.

Ethical Approval. The study was conducted according to the Declaration of Helsinki principles and approved by the Ethical Review Board of the Faculty of Medicine, Umeå University, Sweden. All subjects gave their written informed consent prior to the investigation. Participants were identified by numbers, not by name.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.7b00063.

Detailed ELISA protocol, description of patients, sample treatment and data analysis (PDF)

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Author Contributions

I.H. and I.A.I. made equal contributions. I.H. performed the experiments. I.H., I.A.I., and L.A.M.-R. analyzed data and

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prepared the manuscript. L.F. collected and analyzed patient's samples. L.A.M.-R. and L.F. designed the project.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) de Lau, L. M., and Breteler, M. M. (2006) Epidemiology of Parkinson's disease. *Lancet Neurol. 5*, 525−535.

(2) Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., and Goedert, M. (1997) Alpha-synuclein in Lewy bodies. *Nature 388*, 839−840.

(3) Adler, C. H., Beach, T. G., Hentz, J. G., Shill, H. A., Caviness, J. N., Driver-Dunckley, E., Sabbagh, M. N., Sue, L. I., Jacobson, S. A., Belden, C. M., and Dugger, B. N. (2014) Low clinical diagnostic accuracy of early vs advanced Parkinson disease: clinicopathologic study. *Neurology 83*, 406−412.

(4) Schapira, A. H. (1999) Science, medicine, and the future: Parkinson's disease. *BMJ. 318*, 311−314.

(5) Gerlach, M., Maetzler, W., Broich, K., Hampel, H., Rems, L., Reum, T., Riederer, P., Stoffler, A., Streffer, J., and Berg, D. (2012) Biomarker candidates of neurodegeneration in Parkinson's disease for the evaluation of disease-modifying therapeutics. *J. Neural. Transm. 119*, 39−52.

(6) Gruden, M. A., Sewell, R. D. E., Yanamandra, K., Davidova, T. V., Kucheryanu, V. G., Bocharov, E. V., Bocharova, O. R., Polyschuk, V. V., Sherstnev, V. V., and Morozova-Roche, L. A. (2011) Immunoprotection against toxic biomarkers is retained during Parkinson's disease progression. *J. Neuroimmunol. 233*, 221−227.

(7) Gruden, M. A., Yanamandra, K., Kucheryanu, V. G., Bocharova, O. R., Sherstnev, V. V., Morozova-Roche, L. A., and Sewell, R. D. (2012) Correlation between protective immunity to alpha-synuclein aggregates, oxidative stress and inflammation. *NeuroImmunoModulation 19*, 334−342.

(8) Yanamandra, K., Gruden, M. A., Casaite, V., Meskys, R., Forsgren, L., and Morozova-Roche, L. A. (2011) α-synuclein reactive antibodies as diagnostic biomarkers in blood sera of Parkinson's disease patients. *PLoS One 6*, e18513.

(9) Koehler, N. K. U., Stransky, E., Shing, M., Gaertner, S., Meyer, M., Schreitmüller, B., Leyhe, T., Laske, C., Maetzler, W., Kahle, P., Celej, M. S., Jovin, T. M., Fallgatter, A. J., Batra, A., Buchkremer, G., Schott, K., and Richartz-Salzburger, E. (2013) Altered serum IgG levels to α-synuclein in dementia with Lewy bodies and Alzheimer's disease. *PLoS One 8*, e64649.

(10) Maetzler, W., Apel, A., Langkamp, M., Deuschle, C., Dilger, S. S., Stirnkorb, J. G., Schulte, C., Schleicher, E., Gasser, T., and Berg, D. (2014) Comparable autoantibody serum levels against amyloid- and inflammation-associated proteins in Parkinson's disease patients and controls. *PLoS One 9*, e88604.

(11) Maetzler, W., Berg, D., Synofzik, M., Brockmann, K., Godau, J., Melms, A., Gasser, T., Hornig, S., and Langkamp, M. (2011) Autoantibodies against amyloid and glial-derived antigens are increased in serum and cerebrospinal fluid of Lewy body-associated dementias. *J. Alzheimers Dis. 26*, 171−179.

(12) Woulfe, J. M., Duke, R., Middeldorp, J. M., Stevens, S., Vervoort, M., Hashimoto, M., Masliah, E., Chan, P., Di Monte, D. A., Langston, J. W., Petzinger, G., Hoogendoorn, H., and Munoz, D. G. (2002) Absence of elevated anti-alpha-synuclein and anti-EBV latent membrane protein antibodies in PD. *Neurology 58*, 1435−1436.

(13) Besong-Agbo, D., Wolf, E., Jessen, F., Oechsner, M., Hametner, E., Poewe, W., Reindl, M., Oertel, W. H., Noelker, C., Bacher, M., and Dodel, R. (2013) Naturally occurring α-synuclein autoantibody levels are lower in patients with Parkinson disease. *Neurology 80*, 169−175.

(14) Bryan, T., Luo, X., Forsgren, L., Morozova-Roche, L. A., and Davis, J. J. (2012) The robust electrochemical detection of a Parkinson's disease marker in whole blood sera. *Chem. Sci. 3*, 3468−3473.

(15) Xu, Q., Evetts, S., Hu, M., Talbot, K., Wade-Martins, R., and Davis, J. J. (2014) An impedimetric assay of α-synuclein autoantibodies in early stage Parkinson's disease. *RSC Adv. 4*, 58773−58777.

(16) Heinzel, S., Gold, M., Deuschle, C., Bernhard, F., Maetzler, W., Berg, D., and Dodel, R. (2014) Naturally occurring alpha-synuclein autoantibodies in Parkinson's disease: sources of (Error) variance in biomarker Assays. *PLoS One 9*, e114566.

(17) Smith, L. M., Schiess, M. C., Coffey, M. P., Klaver, A. C., and Loeffler, D. A. (2012) α-synuclein and anti-α-synuclein antibodies in Parkinson's disease, atypical Parkinson syndromes, REM sleep behavior disorder, and healthy controls. *PLoS One 7*, e52285.

(18) Papachroni, K. K., Ninkina, N., Papapanagiotou, A., Hadjigeorgiou, G. M., Xiromerisiou, G., Papadimitriou, A., Kalofoutis, A., and Buchman, V. L. (2007) Autoantibodies to alpha-synuclein in inherited Parkinson's disease. *J. Neurochem. 101*, 749−756.

(19) Horvath, I., Jia, X., Johansson, P., Wang, C., Moskalenko, R., Steinau, A., Forsgren, L., Wågberg, T., Svensson, J., Zetterberg, H., and Morozova-Roche, L. A. (2016) Pro-inflammatory S100A9 protein as a robust biomarker differentiating early stages of cognitive impairment in Alzheimer's disease. *ACS Chem. Neurosci. 7*, 34−39.

(20) Zetterberg, H. (2015) Cerebrospinal fluid biomarkers for Alzheimer's disease: current limitations and recent developments. *Curr. Opin. Psychiatry. 28*, 402−409.

(21) Nakagawa, S., and Cuthill, I. C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev. Camb. Philos. Soc. 82*, 591−605.

(22) Cliff, N. (1993) Dominance statistics: Ordinal analyses to answer ordinal questions. *Psychol. Bull. 114*, 494.

(23) Cohen, J. (1988) *Statistical power analysis for the behavioral sciences*, L. Erlbaum Associates, Hillsdale, N.J..

(24) Hall, S., Ohrfelt, A., Constantinescu, R., Andreasson, U., Surova, Y., Bostrom, F., Nilsson, C., Wildner, H., Decraemer, H., Nagga, K., Minthon, L., Londos, E., Vanmechelen, E., Holmberg, B., Zetterberg, H., Blennow, K., and Hansson, O. (2012) Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. *Arch. Neurol. 69*, 1445−1452.

(25) Hughes, A. J., Daniel, S. E., Kilford, L., and Lees, A. J. (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J. Neurol., Neurosurg. Psychiatry 55*, 181−184.

(26) Burtis, C. A., Ashwood, E. R., and Bruns, D. E. (2006) *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed., Elsevier Saunders, St. Louis, MO.

(27) Obermeier, B., Daneman, R., and Ransohoff, R. M. (2013) Development, maintenance and disruption of the blood-brain barrier. *Nat. Med. 19*, 1584−1596.

(28) Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., Benveniste, H., Vates, G. E., Deane, R., Goldman, S. A., Nagelhus, E. A., and Nedergaard, M. (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med. 4*, 147ra111.

(29) Braak, H., de Vos, R. A., Bohl, J., and Del Tredici, K. (2006) Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci. Lett. 396*, 67−72.

(30) Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z. Y., Roybon, L., Melki, R., and Li, J. Y. (2014) Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol. 128*, 805−820.

DOI: 10.1021/acschemneuro.7b00063
ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

(31) Roodveldt, C., Christodoulou, J., and Dobson, C. M. (2008) Immunological features of alpha-synuclein in Parkinson's disease. *J. Cell. Mol. Med. 12*, 1820−1829.
(32) Schneeberger, A., Mandler, M., Mattner, F., and Schmidt, W. (2012) Vaccination for Parkinson's disease. *Parkinsonism Relat. Disord. 18*, S11−S13.
(33) Bae, E. J., Lee, H. J., Rockenstein, E., Ho, D. H., Park, E. B., Yang, N. Y., Desplats, P., Masliah, E., and Lee, S. J. (2012) Antibody-aided clearance of extracellular α-synuclein prevents cell-to-cell aggregate transmission. *J. Neurosci. 32*, 13454−13469.
(34) Masliah, E., Rockenstein, E., Mante, M., Crews, L., Spencer, B., Adame, A., Patrick, C., Trejo, M., Ubhi, K., Rohn, T. T., Mueller-Steiner, S., Seubert, P., Barbour, R., McConlogue, L., Buttini, M., Games, D., and Schenk, D. (2011) Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. *PLoS One 6*, e19338.
(35) Sharma, S. K., Chorell, E., Steneberg, P., Vernersson-Lindahl, E., Edlund, H., and Wittung-Stafshede, P. (2015) Insulin-degrading enzyme prevents alpha-synuclein fibril formation in a nonproteolytical manner. *Sci. Rep. 5*, 12531.

DOI: 10.1021/acschemneuro.7b00063
ACS Chem. Neurosci. XXXX, XXX, XXX−XXX