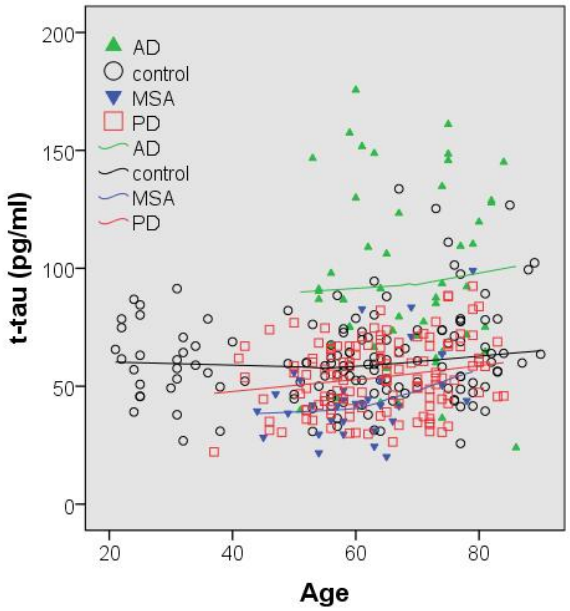
II. Supplementary Figures and Tables

A



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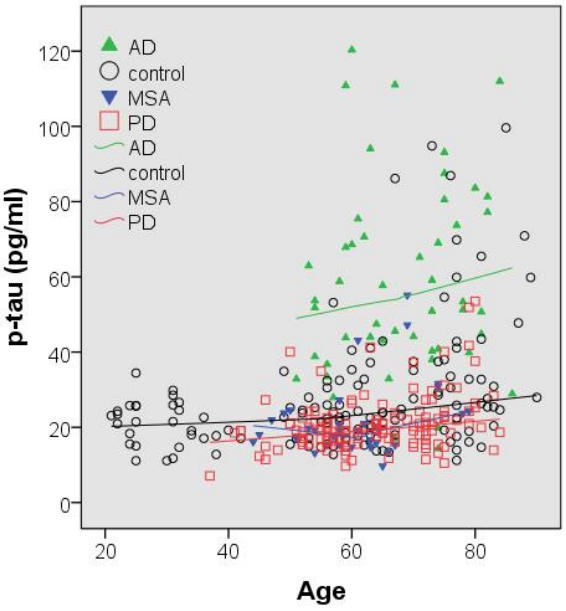

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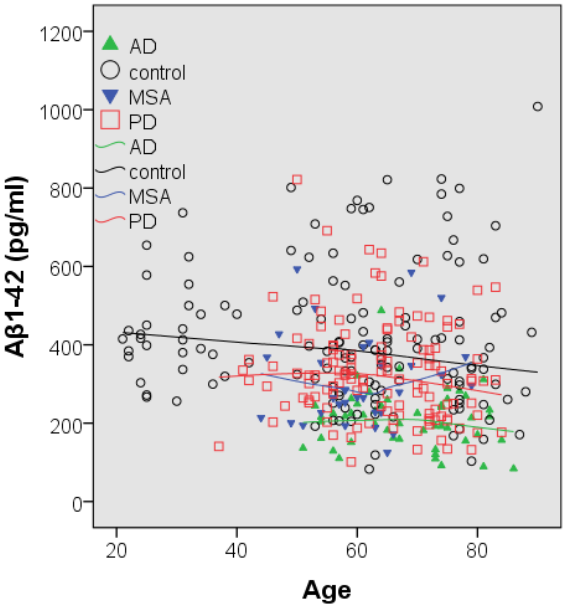

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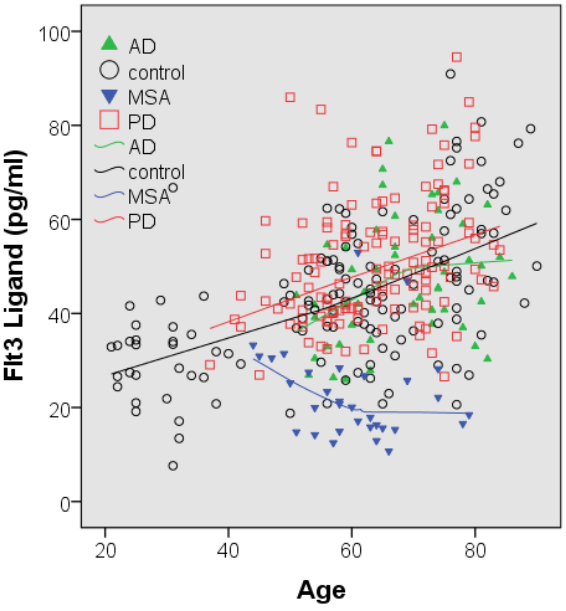

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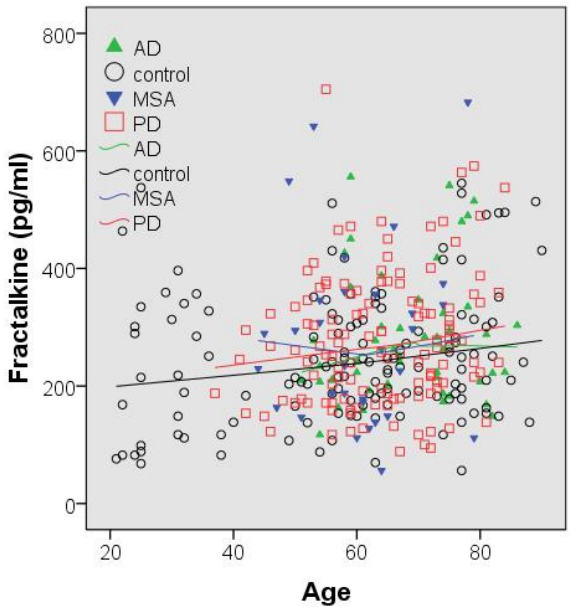
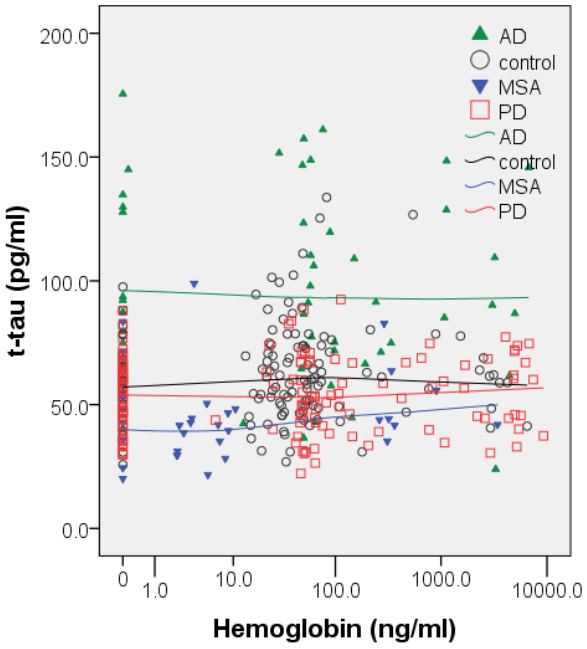


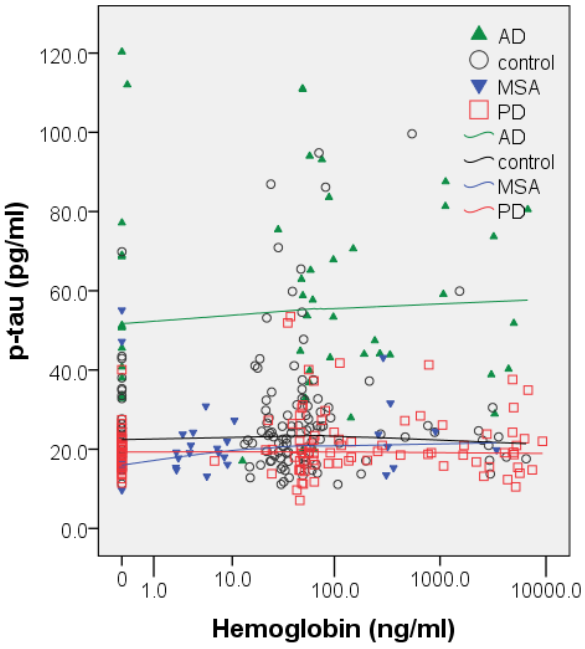
Supplementary Figure 1. Age dependence of t-tau, p-tau, $A\beta_{1-42}$, Flt3 lignad and fractalkine in CSF.

CSF total tau (t-tau) (**A**), phosphorylated tau (p-tau) (**B**), amyloid beta peptide 1-42 ($A\beta_{1-42}$) (**C**), Flt3 ligand (**D**) and fractalkine (**E**) levels were measured in individual normal control and disease (AD, Alzheimer disease; PD, Parkinson disease; and MSA, multiple system atrophy) samples by Luminex. Levels of t-tau(**A**), along with p-tau (**B**) in CSF, tended to increase with age for all groups studied, with statistical significance achieved in MSA and PD patients for t-tau (MSA: r= 0.44, $P < 0.05$; PD: r = 0.21, $P < 0.05$) and in controls and PD patients for p-tau (control: r = 0.37, $P < 0.0001$; PD: r = 0.33, $P < 0.001$). Levels of Flt3 ligand (**D**) and fractalkine (**E**) also increased with age in control, PD, and/or AD groups: Flt3 ligand (control: r = 0.57, $P < 0.0001$; PD: r = 0.34, $P < 0.0001$; AD: r =0.31, $P < 0.05$), fractalkine (CTL: r = 0.19, $P < 0.05$). Concentrations of $A\beta_{1-42}$ (**C**) were stable with respect to age.

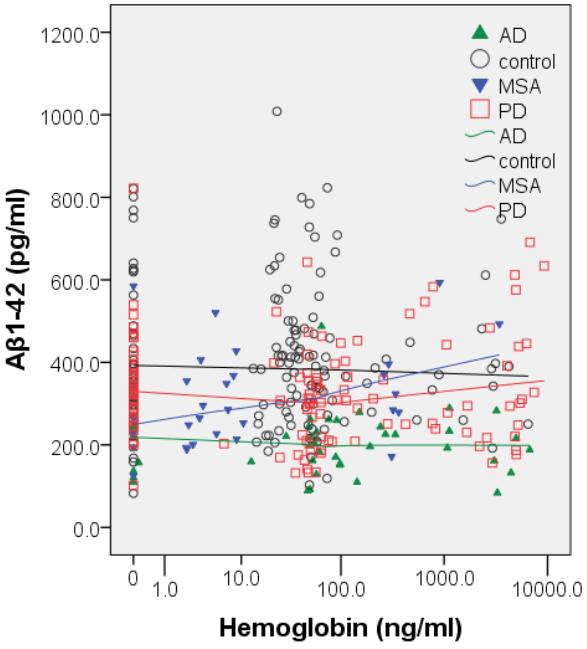
A


AD
control
MSA
PD
AD
control
MSA
PD
t-tau (pg/ml)
200.0
150.0
100.0
50.0
0.0
0
1.0
10.0
100.0
1000.0
10000.0
Hemoglobin (ng/ml)

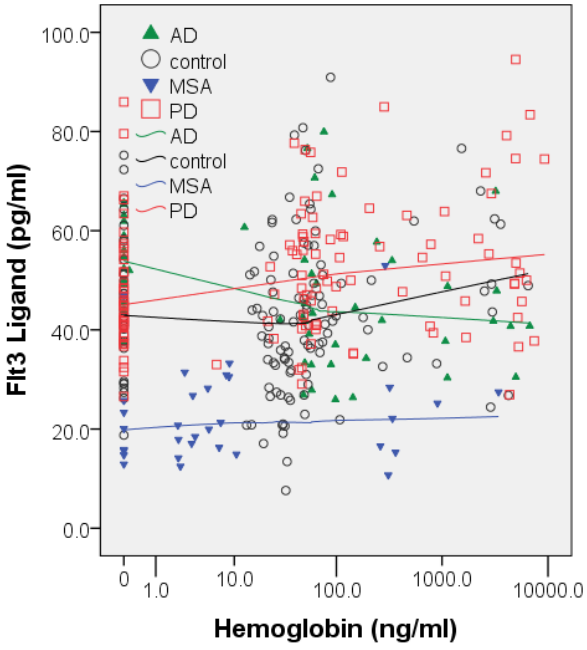
B


AD
control
MSA
PD
AD
control
MSA
PD
p-tau (pg/ml)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0
1.0
10.0
100.0
1000.0
10000.0
Hemoglobin (ng/ml)

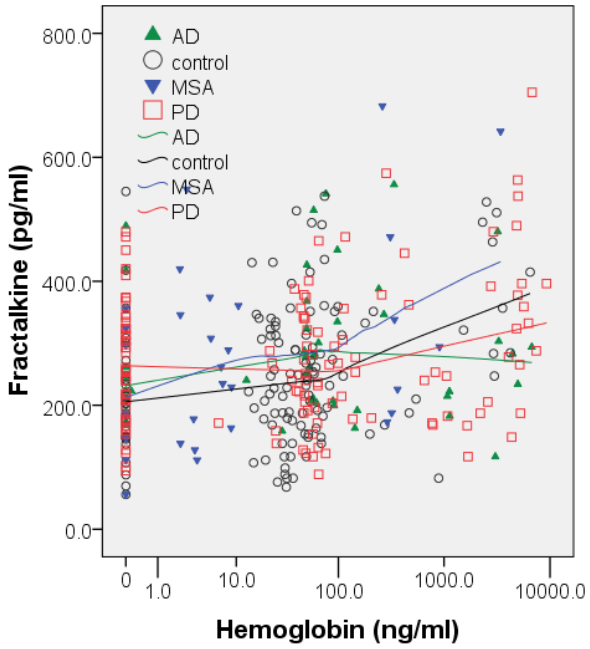
C


AD
control
MSA
PD
AD
control
MSA
PD
Aβ1-42 (pg/ml)
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
0
1.0
10.0
100.0
1000.0
10000.0
Hemoglobin (ng/ml)

D


AD
control
MSA
PD
AD
control
MSA
PD
Flt3 Ligand (pg/ml)
100.0
80.0
60.0
40.0
20.0
0.0
0
1.0
10.0
100.0
1000.0
10000.0
Hemoglobin (ng/ml)

E



Supplementary Figure 2. The effects of blood contamination on t-tau, p-tau, $A\beta_{1-42}$, Flt3 ligand and fractalkine levels in CSF.

CSF total tau (t-tau) (**A**), phosphorylated tau (p-tau) (**B**), amyloid beta peptide 1-42 ($A\beta_{1-42}$) (**C**), Flt3 ligand (**D**) and fractalkine (IL-8) (**E**) levels were measured in individual normal control and disease (AD, Alzheimer disease; PD, Parkinson disease; and MSA, multiple system atrophy) samples by Luminex, while the hemoglobin levels (as a index of red blood cell contamination in CSF) were measured using an ELISA kit. No association was observed between levels of CSF HGB and t-tau (**A**), p-tau (**B**) or Flt3 ligand (**D**); however, $A\beta_{1-42}$ (**C**) and fractalkine (**E**) levels started to increase appreciably at high HGB concentrations: $A\beta_{1-42}$ (MSA: $r = 0.38$, $P < 0.05$; PD: $r = 0.19$, $P < 0.05$), fractalkine (control: $r = 0.33$, $P < 0.001$; PD: $r = 0.33$, $P < 0.001$; MSA: $r = 0.47$, $P < 0.01$).

Supplementary Table 1. Summary of demographics and characterizations of included MSA patients

Sample ID	Age	Sex	Race	Diagnosis	Dx. Detail *	Definite? **	On Sinemet?	Septal FDA	Interpretation
		M=1	*White-1*	*Final*	*Referral*		*1=On*	*nCi-kg/cc-mCi*	
MSA92	79	1	1	MSA	MSA+OH	Yes	0	9928	Normal
MSA94	62	1	1	MSA	MSA	Yes	1	9121	Normal
MSA95	54	1	1	MSA	MSA+OH	Yes	0	12037	Normal
MSA145	69	0	1	MSA	MSA+OH	Yes	1	9068	Normal
MSA142	60	1	1	MSA	MSA+OH	Yes	0	10299	Normal
MSA96	47	1	1	MSA	MSA+OH	Yes	0	12582	Normal
MSA97	56	1	1	MSA	MSA	Yes	1	4680	Dec. septum, Nl. free wall†
MSA98	64	0	1	MSA	MSA	Yes	0	11467	Normal
MSA100	65	0	1	MSA	MSA+OH	Yes	1	9684	Normal
MSA101	69	0	1	MSA	MSA+OH	No: vs. PD	0	10929	Normal
MSA102	74	0	1	MSA	MSA	Yes	0	10508	Normal
MSA103	51	0	1	MSA	MSA+OH Cbl.	Yes: Cerebellar ataxia	0	11342	Normal
MSA104	63	1	1	MSA	MSA+OH	Yes: Ataxia, urin. retent., no parkinsonism	0	10664	Normal
MSA105	58	1	1	MSA	MSA+OH	Yes: Progressive to death	0	9817	Normal
MSA125	61	0	1	MSA	MSA+OH	Yes	1	8055	Normal
MSA110	78	1	1	MSA	MSA+OH	Yes: Sinemet-unresponsive	0	7837	Normal
MSA112	66	1	1	MSA	MSA+OH	No: vs. PD (Sinemet-responsive)	1	11241	Normal
MSA113	67	1	1	MSA	MSA+OH	No: vs. PD (Sinemet-responsive)	1	11677	Normal
MSA2	53	1	1	MSA	MSA+OH	Yes: Dysarthria, Sinemet-unresponsive, F-DOPA	0	No PET (Synth. Problem)	Normal
MSA23	58	0	B	MSA	MSA+OH	Yes: Dysarthria, ataxia, Sinemet-unresponsive	0	17356	Normal
MSA32	49	1	O	MSA	MSA	Yes: Insecticide	0	8689	Normal
MSA33	44	1	1	MSA	MSA+OH	No: vs. PD (Sinemet-responsive)	0	7690	Normal
MSA35	57	0	B	MSA	MSA+OH Cbl.	Yes: Dysarthria, Sinemet-unresponsive, F-DOPA	0	11335	Normal
MSA21	64	1	1	MSA	MSA+OH Cbl.	Yes: Ataxia, urin. retent., no parkinsonism	0	9598	Normal
MSA18	58	0	1	MSA	MSA+OH	No: vs. PD (Sinemet-responsive)	0	10740	Normal
MSA34	58	1	1	MSA	MSA+OH Cbl.	Yes: Dysarthria, no tremor, no Sinemet R, progressive, urin. retent.	0	10832	Normal
MSA10	45	1	1	MSA	MSA+OH	No: vs. PD (Sinemet-responsive)	0	10242	Normal
MSA25	63	1	1	MSA	MSA	Yes: Dysarthria, no Sinemet R, progressive, urin. retent.	0	11722	Normal
MSA13	54	1	O	MSA	MSA+OH	No: No resting tremor, no Sinemet trial	0	9855	Normal
MSA45	61	0	ME	MSA	MSA-P	No: ± Sinemet R	0	10226	Normal
MSA44	74	1	1	MSA	MSA-P	No: vs. PD (Sinemet-resp., no slur)	0	8875	Normal
MSA41	50	1	1	MSA	MSA+OH Cbl.	Yes: Dysarthria, no parkinsonism, cerebellar atrophy, progressive	0	7813	Normal

* OH: orthostatic hypotension; OH Cbl.: OH and signs of cerebellar failure.

** "no Sinemet R": no Sinemet response; "urin. Retent.": symptoms of urinary retention.

† This patient had decreased 6-^{18}F-fluorodopamine-derived radioactivity in the interventricular septum and normal radioactivity in the left ventricular free wall. The decreased septal radioactivity may have reflected decreased local blood flow.

Supplementary Table 2. Receiver operating characteristic (ROC) analysis of cerebrospinal fluid individual or combinations of biomarkers

	ALL cases					Cases with age < 65					Cases with age ≥ 65				
	AUC	p-value	cutoff value	sens (%)	spec (%)	AUC	p-value	cutoff value	sens (%)	spec (%)	AUC	p-value	cutoff value	sens (%)	spec (%)
PD vs Control															
DJ-1	0.77	< .001	40	94	50	0.72	<.001	40	98	34	0.86	< .001	40	90	73
α-syn	0.71	<.001	0.5	92	38	0.65	< .05	0.5	92	23	0.83	< .001	0.5	93	41
Flt3 ligand	0.57	0.104				0.62	<.05	37.15	90	27	0.52	0.71			
fractalkine	0.56	0.163				0.61	0.056				0.52	0.716			
t-tau	0.61	<.05	71.41	91	25	0.58	0.167	71.41	94	16	0.65	< .05	71.41	83	47
p-tau	0.65	0.001	30	90	33	0.63	< .05	30	98	18	0.70	0.001	30	80	48
$A\beta_{1-42}$	0.62	<.05	464.44	92	29	0.59	0.151	464.44	92	71	0.68	< .05	464.44	90	30
p-tau/t-tau	0.62	<.05	0.49	83	30	0.59	0.109				0.64	<.05	0.49	73	41
p-tau/$A\beta_{1-42}$	0.56	0.141				0.55	0.44				0.60	0.113			
t-tau/$A\beta_{1-42}$	0.52	0.625				0.53	0.67				0.64	0.574			
DJ-1 & Flt3 ligand [A]	0.84	<.001	26.11	94	60	0.81	<.001	26.11	94	52	0.88	<.001	26.11	93	70
p-tau, t-tau, $A\beta_{1-42}$ [B]	0.69	<.001	583.55	91	41	0.64	<.05	583.55	90	29	0.77	< .001	583.55	93	56
MSA vs Control															
DJ-1	0.85	<.001	37.6	94	55										
α-syn	0.88	<.001	0.46	94	70										
Flt3 ligand	0.95	<.001	28.26	95	90										
fractalkine	0.47	0.664													
t-tau	0.77	<.001	71.17	90	26										
p-tau	0.68	<.05	30.89	90	32										
$A\beta_{1-42}$	0.70	<.05	407.01	90	39										
p-tau/t-tau	0.70	<.05	0.34	88	31										
p-tau/$A\beta_{1-42}$	0.70	<.05	0.06	88	53										
t-tau/$A\beta_{1-42}$	0.49	0.867													
DJ-1, p-tau/t-tau [C]	0.93	<.001	11	95	70										
AD vs Control															
DJ-1	0.50	0.998				0.64	0.079				0.64	0.077			
α-syn	0.63	<.05	0.35	90	20	0.80	<.001	0.35	94	30	0.54	0.569			
Flt3 ligand	0.54	0.435				0.45	0.555				0.60	0.179			
fractalkine	0.57	0.211				0.53	0.622				0.58	0.313			
t-tau	0.77	<.001	64.34	82	65	0.79	<.001	64.34	89	18	0.72	< .05	64.34	78	71
p-tau	0.83	<.001	19.81	90	63	0.94	<.001	19.81	100	45	0.74	0.001	19.81	91	20
$A\beta_{1-42}$	0.86	<.001	312.28	92	65	0.84	<.001	312.28	89	65	0.88	<.001	312.28	96	67
p-tau/t-tau	0.77	<.001	0.4	92	41	0.89	<.001	0.4	94	49	0.67	<.05	0.4	91	
p-tau/$A\beta_{1-42}$	0.91	<.001	0.098	95	78	0.94	<.001	0.098	94	84	0.88	<.001	0.098	96	72
t-tau/$A\beta_{1-42}$	0.92	<.001	0.23	85	78	0.91	<.001	0.23	89	88	0.93	<.001	0.23	96	72
PD vs MSA															
DJ-1	0.67	<.05	17.06	92	20										
α-syn	0.72	<.05	0.26	91	25										
Flt3 ligand	0.98	<.001	29.9	99	95										
fractalkine	0.57	0.351													
t-tau	0.70	<.05	35.81	90	40										
p-tau	0.57	0.321	14.72	90	10										
$A\beta_{1-42}$	0.61	0.135	187.13	90	5										
p-tau/t-tau	0.72	<.01	0.54	90	40										
p-tau/$A\beta_{1-42}$	0.65	<.05	0.08	80	20										
t-tau/$A\beta_{1-42}$	0.60	0.172													
α-syn, p-tau/t-tau [D]	0.85	<.001	0.154	90	65										
PD vs AD															
DJ-1	0.76	< .001	39.39	94	55	0.84	< .001	39.39	98	61	0.70	< .05	39.39	88	54
α-syn	0.82	< .001	0.5	92	62	0.89	< .001	0.5	92	78	0.75	0.001	0.5	80	55
Flt3 ligand	0.52	0.723				0.65	0.057				0.41	0.222			
fractalkine	0.50	0.956				0.58	0.355				0.46	0.612			
t-tau	0.84	< .001	72.89	92	68	0.83	< .001	72.89	94	67	0.83	< .001	72.89	88	68
p-tau	0.91	< .001	32.01	93	87	0.97	< .001	32.01	98	89	0.86	< .001	32.01	85	86
$A\beta_{1-42}$	0.81	<.001	184.23	91	40	0.83	< .001	184.23	94	22	0.79	< .001	184.23	88	50
p-tau/t-tau	0.86	<.001	0.6	93	50	0.94	<.001	0.6	98	61	0.80	<.001	0.6	88	46
p-tau/$A\beta_{1-42}$	0.94	<.001	0.1351	93	90	0.95	< .001	0.1351	98	94	0.92	< .001	0.1351	85	86
t-tau/$A\beta_{1-42}$	0.94	<.001	0.297	92	84	0.93	< .001	0.297	96	89	0.92	< .001	0.297	85	82
α-syn & $A\beta_{1-42}$ [E]	0.95	<.001	169.1	93	84	0.98	< .001	169.1	98	94	0.91	< .001	169.1	85	77
α-syn & DJ-1 [F]	0.82	<.001	160.2	92	63	0.90	< .001	160.2	92	72	0.75	0.001	160.2	90	59

	ALL cases					Cases with age < 65					Cases with age ≥ 65				
	AUC	p-value	cutoff value	sens (%)	spec (%)	AUC	p-value	cutoff value	sens (%)	spec (%)	AUC	p-value	cutoff value	sens (%)	spec (%)
MSA vs AD															
DJ1	0.83	<.001	20.74	90	35										
α-syn	0.92	<.001	0.32	95	70										
Flt3 ligand	0.95	<.001	28.25	95	90										
fractalkine	0.47	0.664													
t-tau	0.89	<.001	43.77	92	60										
p-tau	0.90	<.001	19.81	92	65										
$A\beta_{1-42}$	0.75	<.05	282.3	90	40										
p-tau/t-tau	0.70	<.05	0.44	87	30										
p-tau/$A\beta_{1-42}$	0.96	<.001	0.1	95	90										
t-tau/$A\beta_{1-42}$	0.96	<.001	0.2331	95	90										
α-syn & $A\beta_{1-42}$ [G]	0.99	<.001	229.05	95	95										

AD, Alzheimer disease; MSA, multiple system atrophy; PD, Parkinson disease.

$A\beta_{1-42}$, amyloid beta peptide 1-42; α-syn, α-synuclein; p-tau, phosphorylated tau; t-tau, total tau. AUC, area under curve; Sens, sensitivity; Spec, specificity.

All the differential diagnostic testing listed was done with subjects younger than 50 and/or samples with high blood contamination (hemoglobin ≥ 200 ng/mL) excluded. For the combinations of biomarkers, values provided were derived from models based on logistic regression analysis. The models were then subjected to ROC to determine AUC, cutoff, sensitivity and specificity. Model equations are as the following:

A. 2[DJ-1] – [Flt3 ligand]
B. 17[p-tau] + [$A\beta_{1-42}$] – 5[t-tau]
C. [DJ-1] – 49[p-tau/t-tau]
D. [p-tau/t-tau] – [α-syn]
E. 895[α-syn] - [$A\beta_{1-42}$]
F. 243[α-syn] + [DJ-1]
G. 1279[α-syn] - [$A\beta_{1-42}$]