Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

Methods for bead-based xMAP technology

A monoclonal antibody (MAb), 9B6, IgG1, recognising a human specific α-syn C-terminal epitope in exon 5, was used as capture antibody. The antibody was covalently coupled to carboxylated beads (region 126) according to the protocol supplied by the manufacturer (Luminex, Austin, Texas). MAb, 4D8, IgG1, an antibody recognising an N-terminal epitope in exon 3, was used as detector in its biotinylated format. The synuclein assay was standardized using recombinant α-synuclein from rPeptide. The bead assay was combined with sandwich immuno-assays, measuring Aβ_{1-42} (21F12/82E1bio, bead 110, standardized with full-size Aβ_{1-42}), T-tau (BT2-42F8, bead 106, standardized with a synthetic peptide containing the epitopes), P-tau (AT270- 42F8bio, bead 169, standardized with a synthetic phosphorylated peptide) and a bead controlling heterophilic antibody interference (Aspecific MAb, bead 150). Heterophilic antibodies are a common problem in immunoassays and has been useful to exclude samples in plasma studies. While the problem of heterophilic antibodies has also been acknowledged in CSF studies, in none of the 453 CSF samples analyzed in this study we observed heterophilic antibodies with an arbitrary cut-off of MFI of 150. The format of the multi-assay, including selected antibodies and standards, is shown in the table below. After sonication and vortexing, a mixture (100 µL/well) of the beads was added to 96 well filter plates. After draining the wells using a vacuum manifold, standards, blanks or CSF samples (diluted 1:3 with a buffer containing detergent) were added (75 µL/well) together with the biotinylated detector_MAb mixture (25µL/well) and incubated overnight at +2–8 °C in the dark on a plate shaker (600 rpm). After washing of the wells, phycoerythrine-labeled streptavidine was added (100 µL/well) and incubated on a plate shaker (600 rpm) for 1 h at room temperature. After a second wash step, 100 µL of phosphate-buffered saline was added. The assays were analysed on a Luminex 100IS.
instrument. For each set of microspheres, 100 beads were analysed, and the median fluorescence intensities (MFI) were used for quantification. MFI from the individual sample result was converted to concentration using the 5 PL curve fitting combined with power of law variance weighting from the Bioplex software (Software version 4.1) with $b$ fixed at 1.8 and an auto-calculated per run.

Luminex data for $\text{A}_1\beta_{1-42}$ and $\text{P-tau}$ were normalized to ELISA concentrations running approximately 200 samples using both methods. The equations used to convert the levels were $y=0.11x+69.4$ for $\text{A}_1\beta_{1-42}$ and $y=0.31x+34.3$ for $\text{P-tau}$. ELISA measurements of $\text{A}_1\beta_{1-42}$ and $\text{P-tau}$ were performed as described previously $^5, 6$. The levels of many CSF biomarkers are known to differ between different clinical sites due to differences in pre-analytical handling of samples $^7$ and consequently one often need to adjust levels between centers $^8$. In the present study, the levels for $\alpha$-synuclein, $\text{A}_1\beta_{1-42}$, $\text{T-tau}$ significantly differed between Skåne University Hospital and Sahlgrenska University Hospital. Therefore, the levels for these analytes in the material from Sahlgrenska University Hospital were adjusted to levels at Skåne University Hospital by establishing conversion factors. To compensate for these differences we selected the group with largest overlap in individuals between clinical sites, i.e. the patient group with PD. In the PD group, the levels for $\alpha$-syn, $\text{A}_1\beta_{1-42}$, $\text{T-tau}$ significantly differed between Lund/Malmö and Gothenburg. Therefore the levels for these analytes in the Gothenburg material were adjusted to Lund/Malmö levels by calculation of a conversion factor, i.e. analyte value $X$ [mean of the analyte within the PD group (Lund/Malmö)/mean of the analyte within the PD group (Gothenburg)]. The conversion factors for $\alpha$-synuclein, $\text{A}_1\beta_{1-42}$, $\text{T-tau}$ were determined to 0.79, 0.77, and 1.22, respectively. The reason for the slight but significant differences between clinical sites might be due to somewhat different handling procedures of the samples. The CSF from Gothenburg was stored in the original tubes at -80
degrees until analyses. However, the samples in Lund were thawed and aliquoted into new tubes and refrozen before shipment to Gothenburg for analyses.
eReferences


eTable. Human multi-analyte assay format.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Capture mAb</th>
<th>Biotinylated Detection mAb</th>
<th>Calibrator</th>
<th>Region</th>
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<td></td>
<td>169</td>
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<td>AspecificMAb</td>
<td>9H10C6</td>
<td>*</td>
<td>HeterophilicAb interference</td>
<td>150</td>
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</table>
**eFigure.** Diagnostic accuracy of CSF biomarkers in patients with dementia or parkinsonism when using CSF biomarker levels unadjusted for differences between clinical centres. Multivariate discriminant analysis (DA) was performed using the orthogonal projections to latent structures (OPLS) algorithm. Receiver operating characteristic curves were calculated for both OPLS-DA and for each analyte, including α-synuclein (α-syn), β-amyloid1-42 (Aβ1-42), total tau (T-tau) phosphorylated tau (P-tau), and neurofilament light chain (NF-L). The areas under the curves (AUC) are given. Panel A, patients with AD (N=48) were compared to patients with DLB and PDD (N=103). Panel C, patients with PD (N=90) were compared to patients with atypical parkinsonism, i.e. PSP, CBD and MSA (N=105). Corresponding variable importance in projection (VIP) plots (B, D) illustrate the relative contributions of the analytes to the separation between the patient groups. Error bars represent the 95% confidence interval.