Sensitivity and specificity of a seed amplification assay for diagnosis of multiple system atrophy: a multicentre cohort study

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Summary

Background The pathological hallmarks of multiple system atrophy and Parkinson's disease are, respectively, misfolded-α-synuclein-laden glial cytoplasmic inclusions and Lewy bodies. CSF-soluble misfolded α-synuclein aggregates (seeds) are readily detected in people with Parkinson's disease by α-synuclein seed amplification assay (synSAA), but identification of seeds associated with multiple system atrophy for diagnostic purposes has proven elusive. We aimed to assess whether a novel synSAA could reliably distinguish seeds from Lewy bodies and glial cytoplasmic inclusions.

Methods In this multicentre cohort study, a novel synSAA that multiplies and detects seeds by fluorescence was used to analyse masked CSF and brain samples from participants with either clinically diagnosed or pathology-confirmed multiple system atrophy, Parkinson's disease, dementia with Lewy bodies, isolated rapid eye movement sleep behaviour disorder (IRBD), disorders that were not synucleinopathies, or healthy controls. Participants were from eight available cohorts from seven medical centres in four countries: New York Brain Bank, New York, USA (NYBB); University of Pennsylvania, Philadelphia, PA, USA (UPENN); Paracelsus-Elena-Klinik, Kassel, Germany (DeNoPa and KAMSA); Hospital Clinic Barcelona, Spain (BARMSA); Universität Tübingen, Tübingen, Germany (EKUT); Göteborgs Universitet, Göteborgs, Sweden (UGOT); and Karolinska Institutet, Stockholm, Sweden (KIMSA). Clinical cohorts were classified for expected diagnostic accuracy as either research (longitudinal follow-up visits) or real-life (single visit). Sensitivity and specificity were estimated according to pathological (gold standard) and clinical (reference standard) diagnoses.

Findings In 23 brain samples (from the NYBB cohort), those containing Lewy bodies were synSAA-positive and produced high fluorescence amplification patterns (defined as type 1); those containing glial cytoplasmic inclusions were synSAA-positive and produced intermediate fluorescence (defined as type 2); and those without α-synuclein pathology produced below-threshold fluorescence and were synSAA-negative. In 21 pathology-confirmed CSF samples (from the UPENN cohort), those with Lewy bodies were synSAA-positive type 1; those with glial cytoplasmic inclusions were synSAA-positive type 2; and those with four-repeat tauopathy were synSAA-negative. In the DeNoPa research cohort (which had no samples from people with multiple system atrophy), the novel synSAA had sensitivities of 95% (95% CI 88–99) for 80 participants with Parkinson's disease and 95% (76–100) for 21 participants with IRBD, and a specificity of 95% (86–99) for 60 healthy controls. Overall (combining BARMSA, EKUT, KAMSA, UGOT, and KIMSA cohorts that were enriched for cases of multiple system atrophy), the novel synSAA had 87% sensitivity for multiple system atrophy (95% CI 80–93) and specificity for type 2 seeds was 77% (67–85). For participants with multiple system atrophy just in research cohorts (BARMSA and EKUT), the novel synSAA had a sensitivity of 84% (95% CI 71–92) and a specificity for type 2 seeds of 87% (74–95), whereas cases from real-life cohorts (KAMSA, KIMSA, and UGOT) had a sensitivity of 91% (95% CI 80–97) but a decreased specificity for type 2 seeds of 68% (53–81).

Interpretation The novel synSAA produced amplification patterns that enabled the identification of underlying α-synuclein pathology, showing two levels of fluorescence that corresponded with different pathological hallmarks of synucleinopathy. The synSAA might be useful for early diagnosis of synucleinopathies in clinical trials, and potentially for clinical use, but additional formal validation work is needed.

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Research in context

Evidence before this study

We did a search in PubMed using the following terms: "Lewy body disease (LBD)", "Parkinson's disease (PD)", "multiple system atrophy (MSA)", "glial cytoplasmic inclusions (GCI)", "MSA mimic", "synucleinopathies", "real-time quaking-induced conversion (RT-QuIC)", "protein misfolding cyclic amplification (PMCA)", AND "seed amplification assay (SAA)" for articles published in English on or before Nov 2023, in any field. Studies have shown that misdiagnosis rates for different synucleinopathies during early disease stage are very high given the overlap between their symptoms. Although the α-synuclein seed amplification assay (synSAA) that uses CSF has been established as a robust biomarker test for Lewy body pathology, its sensitivity for glial cytoplasmic inclusion pathology is inconsistent, probably because of variable synSAA conditions. Thus, it is not fully understood whether misfolded α-synuclein (seeds) in CSF are a reliable, or even viable, biomarker for multiple system atrophy that could be used for diagnostic purposes.

Added value of this study

We developed a novel synSAA that uses standard SAA equipment and was calibrated with a fluorescent dye to measure fluorescence values that could be compared across instruments. The synSAA consistently generated different fluorescence values when analysing CSF and brain samples from people with multiple system atrophy or Parkinson's disease and dementia with Lewy bodies. We tested the synSAA in a multicentre study, including eight independent cohorts from seven different institutions, which to our knowledge is the largest synSAA study focused on the detection of glial cytoplasmic inclusion-related seeds in CSF from people with multiple system atrophy. In addition to masked analysis of CSF samples from clinically diagnosed cases with different levels of diagnostic accuracy, this study includes masked analysis of brain and pathology-confirmed CSF samples. Another important feature of our study is that control participants included multiple system atrophy mimics, such as progressive supranuclear palsy and adult-onset progressive cerebellar ataxia, which represent excellent examples of clinically challenging cases that could benefit from a biomarker test such as synSAA.

Implications of all the available evidence

Our results show that a novel synSAA can detect α-synuclein seeds in CSF samples from people with multiple system atrophy, with high sensitivity and specificity, and can differentiate them from seeds in Parkinson's disease and dementia with Lewy bodies cases. Discriminating synucleinopathies on the basis of their underlying pathology by means of a CSF biomarker test has many implications, the most obvious being significantly shortening the time to final diagnosis and reducing the misdiagnosis rate. An additional implication is the identification of very early cases that might better respond to experimental drugs in clinical trials.

Introduction

Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy are neurodegenerative diseases pathologically characterised by accumulation of misfolded α-synuclein aggregates—collectively referred to as synucleinopathies. Lewy bodies are the pathological hallmark of the Parkinson's disease and dementia with Lewy bodies clinicopathological spectrum,[1] whereas differently misfolded α-synuclein deposits known as glial cytoplasmic inclusions are found in oligodendrocytes of people with multiple system atrophy.[2] Unlike Parkinson's disease, multiple system atrophy is a fast progressing fatal neurodegenerative disease; differentiation of these two disorders is important for initiating appropriate treatment and for prognosis in terms of disability and survival.[3] Accurate diagnosis is also important for the prognosis of individuals with prodromal synucleinopathy syndromes, such as pure autonomic failure and rapid eye movement sleep behaviour disorder (RBD). Because the early clinical presentation overlaps for synucleinopathies and other diseases, diagnosis is challenging and misdiagnosis happens frequently.[4–7] For example, Parkinson's disease can be misdiagnosed as progressive supranuclear palsy or multiple system atrophy, and progressive supranuclear palsy is often misdiagnosed as multiple system atrophy owing to the presence of cerebellar ataxia.[5] Biomarkers are inaccurate for early distinction between Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and progressive supranuclear palsy.[8–10]

In the past few years, α-synuclein seeds have been detected in CSF by α-synuclein seed amplification assay (synSAA) and have been shown to be a robust biomarker for Parkinson's disease and dementia with Lewy bodies.[11–15] The synSAA works by mechanically fragmenting seeds and elongating them into fibrils at the expense of monomeric recombinant α-synuclein protein (substrate). These fibrils are detected by a fluorescent dye, thioflavin T. If seeds are not present, there is no amplification and fluorescence is below threshold. The synSAA results for multiple system atrophy have been inconsistent and often irreproducible, with sensitivities ranging from 6% to 80%.[11,16] The large sensitivity variability in multiple system atrophy could be a reflection of the many synSAA variations reported to date and scant understanding of experimental conditions that amplify glial cytoplasmic inclusion-related seeds. synSAA sensitivity inconsistency is not the only issue; fluorescence patterns that discriminate multiple system atrophy from Parkinson's disease at the individual level have been reported, but not independently replicated, and some

Panel: Cohorts included in the study

Pathology-confirmed cohorts

New York Brain Bank at Columbia University, New York, NY, USA (NYBB)

- The cohort consisted of 23 participants, of whom ten (43%) were male and 13 (57%) were female; the median age of all participants was 74·0 years (IQR 70·0–82·5)
- Brain samples (cingulate cortex) were collected between 2002 and 2021 (post-mortem intervals of 1 h 17 min to 29 h 57 min)
- Pathological findings included cases with Lewy bodies, glial cytoplasmic inclusions (multiple system atrophy), and no α-synuclein pathology
- Samples were assessed by standard pathological evaluation including Luxol fast blue-haematoxylin and eosin staining, Bielschowsky staining, immunostaining against amyloid β, ubiquitinated proteins, α-synuclein, phosphorylated-tau, and TDP-43

The University of Pennsylvania, Philadelphia, PA, USA (UPENN)

- The cohort consisted of 21 participants, of whom 15 (71%) were male and six (29%) were female; the median age of all participants was 66·9 years (IQR 62·3–71·8)
- CSF samples were collected between November, 2007 and September, 2021
- Pathological findings included cases of progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, Lewy bodies, and glial cytoplasmic inclusions (multiple system atrophy)
- Samples were assessed by standard procedures, including haematoxylin and eosin and thioflavin S staining, as well as immunostaining against amyloid β, α-synuclein, phosphorylated-tau, and TDP-43

Clinically defined research cohorts

De novo Parkinson's disease at Paracelsus-Elena-Klinik, Germany (DeNoPa)

- The cohort consisted of 175 participants, of whom 114 (65%) were male and 61 (35%) were 175 female; the median age of all participants was 66·0 years (IQR 59·5–70·5)
- Baseline CSF samples were collected between July, 2009 and August, 2019[13,18]
- Participants were followed up either annually or biannually for up to 10 years
- Diagnoses included Parkinson's disease, isolated rapid eye movement sleep behaviour disorder, and non-synucleinopathies; healthy controls were also included
- Diagnostic workup included dopamine-transporter-SPECT, clinical evaluations, levodopa challenge, and assessments of cognitive function
- Isolated rapid eye movement sleep behaviour disorder was diagnosed through video polysomnography by experienced raters on two consecutive nights, according to established criteria[19] and all participants were examined by movement disorder specialists

Barcelona MSA at Hospital Clinic Barcelona, Spain (BARMSA)

- The cohort consisted of 47 participants, of whom 23 (49%) were male and 24 (51%) were female; the median age of all participants was 67·0 years (IQR 62·6–72·3)
- Participants were recruited into either the Catalan Multiple System Atrophy Registry (n=15) or the QUICK plus PARK cohort (n=32), both with identical clinical enrolment criteria
- Baseline CSF samples were collected between October, 2015 and August, 2022[20,21]
- Diagnoses included multiple system atrophy and Parkinson's disease; healthy controls were also included
- Participants with multiple system atrophy were followed up every 6 months for at least 2 years and had an average disease duration of 5·24 years (IQR 3·24–6·90)

Eberhard Karls Universität Tübingen, Germany (EKUT)

- The cohort consisted of 61 participants, of whom 31 (51%) were male and 30 (49%) were female; the median age of all participants was 63·9 years (IQR 58·0–74·5)
- Baseline CSF samples were collected between June, 2006 and March, 2022
- 1-year follow-up was available for 93% of participants; 2-year follow-up was available for 66% of participants
- Diagnoses included multiple system atrophy, Parkinson's disease, dementia with Lewy bodies, and non-synucleinopathies
- Multiple system atrophy participants had an average disease duration of 3 years (IQR 1·3–13·9)
- Diagnostic workup included MRI and levodopa challenge for parkinsonism-predominant multiple system atrophy, when clinically appropriate
- Cases with Parkinson's disease and dementia with Lewy bodies were prescreened with alternative synSAA conditions and enriched in samples without α-synuclein seeding activity (appendix pp 3–4)[11]

Clinically defined real-life cohorts

Kassel MSA, Paracelsus-Elena-Klinik, Germany (KAMSA)

- The cohort consisted of 63 participants, of whom 31 (49%) were male and 32 (51%) were female; the median age of all participants was 67·5 years (IQR 57·9–76·2)
- CSF samples were collected between September, 2009 and August, 2023
- Diagnoses were based on a single visit and included multiple system atrophy, Parkinson's disease, dementia with Lewy bodies, and non-synucleinopathies
- Multiple system atrophy was diagnosed according to the 2022 Movement Disorder Society criteria (MSA-MDS22); non-synucleinopathy cases were mimics of multiple system atrophy (eg, adult-onset progressive cerebellar ataxia or

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See Online for appendix

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progressive supranuclear palsy) that fit an exclusion criterion (appendix p 3)[22]
- Detailed review of medical records was done if participants were initially diagnosed before MSA-MDS22 release

Göteborgs Universitet, Sweden (UGOT)
- The cohort consisted of 15 participants
- CSF samples were collected
- Diagnoses were based on a single visit and all were multiple system atrophy

Karolinska Institutet MSA, Sweden (KIMSA)
- The cohort consisted of 36 participants, of whom 21 (58%) were male and 15 (42%) were female; the median age of all participants was 63·0 years (IQR 59·0–71·0)
- CSF samples were collected between 2006 and 2021
- Diagnoses were based on a single visit and included multiple system atrophy, Parkinson's disease, and non-synucleinopathies; healthy controls were also included
- Average disease duration at CSF collection for people with multiple system atrophy was 2·5 years

reports showed such differences only at the group level.[16,17]

We hypothesised that glial cytoplasmic inclusion-related seeds in CSF are a biomarker for multiple system atrophy that can be amplified, detected, and reliably differentiated from Lewy body-related seeds. Here, we describe a novel synSAA and evaluate its use in distinguishing underlying α-synuclein pathology and synSAA fluorescence patterns in pathology-confirmed CSF and brain samples. We also report the sensitivity and specificity of this novel synSAA using CSF samples from clinical cohorts with different diagnostic accuracies.

Methods

Study design and participants

This multicentre cohort study included participants recruited into eight convenience cohorts defined clinically or pathologically, which were established by seven independent medical centres in four countries (panel). Clinical cohorts were classified on the basis of their expected diagnostic accuracy into either research cohorts (cross-sectional CSF samples from a consecutive series of cases with longitudinal clinical follow-ups) and real-life cohorts (cross-sectional CSF samples from a convenience series of cases with single-visit clinical evaluations). All clinical and pathological diagnoses were established at each of the medical centres. Multiple system atrophy was clinically diagnosed following the 2008 Second Consensus Statement (MSA-SCS08),[23] Parkinson's disease was diagnosed following the 2015 Movement Disorder Society criteria,[24] and dementia with Lewy bodies was diagnosed following the fourth consensus report of the dementia with Lewy bodies consortium,[25] unless otherwise specified. Multiple system atrophy classification into parkinsonism-predominant (MSA-P) or cerebellar-predominant (MSA-C) was based on cerebellar signs (gait ataxia with cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction). Recruitment and sample collection was approved by the institutional review board at each of the seven centres. All participants provided written informed consent for this study to their respective centres.

Procedures

The novel synSAA was developed by modifying previously reported synSAA conditions,[13,14,26] using larger Si_3N_4 beads, a higher temperature, more frequent fragmentation cycles, sarkosyl, and reducing the total reaction volume (appendix p 2). We tested the novel synSAA with unmasked CSF samples from healthy controls and cases with a synucleinopathy (either commercially available or kindly provided by BM and HZ). Samples were analysed in triplicate and maximum fluorescence (F_{max}) was determined for each replicate. CSF samples from people with Parkinson's disease, dementia with Lewy bodies, and isolated rapid eye movement sleep behaviour disorder (IRBD) produced three replicates with high F_{max} (≥45 000 relative fluorescence units [RFU], defined as type 1); multiple system atrophy samples presented two or three replicates with intermediate F_{max} (3000–45 000 RFU, defined as type 2); and control samples presented two or three replicates with F_{max} below threshold (<3000 RFU, defined as negative; appendix pp 5–6). Determination criteria with dual output for specimens were, thus, defined: the first output established the detection or non-detection of α-synuclein seeds; the second output established the type of seeds present in synSAA-positive samples (appendix pp 2–3). Briefly, samples with three type 1 replicates were deemed synSAA-positive type 1. Samples with two or three type 2 replicates were deemed synSAA-positive type 2. Samples with two type 1 replicates and one type 2 were deemed synSAA-positive undetermined. Samples with two or three negative replicates were deemed synSAA-negative. Other samples were deemed inconclusive and undetermined and were retested if sample volume allowed. CSF subaliquots were analysed with five different production batches of substrate to evaluate reproducibility of the novel synSAA. The novel synSAA yielded consistent results across batches of substrate, with synSAA-positive type 2 results obtained for multiple system atrophy samples, synSAA-positive type 1 results obtained for Parkinson's disease samples, and synSAA-negative results obtained for healthy control samples (appendix p 6). Thus, the modifications made to develop the novel synSAA resulted in higher

reproducibility, improved detection of multiple system atrophy, and a reduction in runtime, compared with the previous synSAA (appendix pp 5–6). After confirming reproducibility and defining output criteria, we evaluated the amplification pattern of α-synuclein seeds from brain samples containing different types of pathology (ie, Lewy bodies, glial cytoplasmic inclusions, or no α-synuclein pathology). To this end, a masked cohort of pathology-confirmed brain samples (obtained from the NYBB cohort) were homogenised (appendix p 3) and analysed with the novel synSAA. We defined specificity for type 1 seeds as the agreement between synSAA-positive type 1 results and Lewy bodies (or clinically defined Parkinson's disease, dementia with Lewy bodies, and IRBD), and specificity for type 2 seeds as the agreement between synSAA-positive type 2 results and glial cytoplasmic inclusions (or clinically defined multiple system atrophy). We next evaluated sensitivity, specificity, and specificity for type 1 or type 2 seeds using CSF samples from pathology-confirmed cases (comprising all the UPENN cohort and some cases from DeNoPa and KIMSA). Lastly, the novel synSAA was evaluated by use of clinically defined CSF samples from six cohorts (DeNoPa, BARMSA, EKUT, KAMSA, UGOT, and KIMSA): specificity, sensitivity, and specificity for type 1 seeds were established by analysing CSF samples from the DeNoPa research cohort; and specificity, sensitivity and specificity for type 2 seeds were established by analysing CSF samples from the BARMSA and EKUT research cohorts and from the UGOT, KIMSA, and KAMSA real-life cohorts. The EKUT cohort included CSF samples from people with Parkinson's disease and dementia with Lewy bodies that were independently prescreened to lack α-synuclein seeding activity, using an alternative synSAA (appendix pp 3–4).[11] The KAMSA cohort used the 2022 Movement Disorder Society multiple system atrophy diagnostic guideline (MSA-MDS2022)[25] instead of MSA-SCS08, hence, some results from this cohort are presented separately from the other real-life cohorts.

CSF samples were collected according to internal protocols from each participating centre, pseudonymised, divided into 150–500 µL aliquots in polypropylene tubes and stored at –80°C until shipping in dry ice. All synSAA analyses were done at Amprion (San Diego, CA, USA), and synSAA operators were masked to all samples. Results were returned to each centre before unmasking, except for DeNoPa since diagnoses were available to Amprion but not to synSAA operators. Clinical and pathological determinations were masked to synSAA results. For analytical comparison purposes, some samples were also analysed with previously reported synSAA conditions.[13,14,26] Neurofilament light chain has been proposed to be elevated in multiple system atrophy compared with Parkinson's disease[20] and was available for analysis in some cases (appendix pp 4–5).

Statistical analysis

Sample size was determined by sample availability. Sensitivity was calculated as the number of synSAA-positive cases divided by the number of people diagnosed with a given synucleinopathy (ie, Parkinson's disease, multiple system atrophy, IRBD, or dementia with Lewy bodies). Specificity was calculated as the number of synSAA-negative cases divided by the number of people without a synucleinopathy diagnosis (ie, healthy controls or non-synucleinopathy cases). Specificity for type 1 (or type 2) seeds was calculated as the number of people with synSAA-positive type 1 (or type 2) divided by the number of people with synSAA-positive Parkinson's disease, dementia with Lewy bodies, or IRBD (or multiple system atrophy). Seed specificity was compared with diagnostic accuracy by comparing research cohorts (highest diagnostic accuracy) with the real-life cohort that used the updated MSA-MDS2022 (KAMSA), and to the real-life cohorts that used MSA-SCS08 (lowest diagnostic accuracy). 95% CIs for sensitivity, specificity, and seed specificity were calculated by use of the Clopper–Pearson exact method. Association between categorical variables (MSA-P *vs* MSA-C sensitivity, research *vs* real-life cohort seed specificity, research cohorts *vs* KAMSA seed specificity, and novel *vs* previous synSAA sensitivity) was evaluated by means of two-sided Fisher's exact test. ANOVA with multiple comparisons was used to evaluate group differences in neurofilament light chain levels and F_{max} violin-plots (Kruskal–Wallis). All graphs and statistical analyses (including F_{max} outlier identification [ROUT, Q=1]) were done with GraphPad version 10.2.2.

Role of the funding source

Employees of the funder (YM, CMF, HN, and LC-M) had a role in study design, data collection, data analysis, data interpretation, writing of the report, and the decision to submit the paper for publication.

Results

Figure 1 shows the eight cohorts included in the study and the numbers of cases and diagnoses or pathologies in each cohort. The cohorts are described in the panel. Two cohorts from the USA were pathology confirmed (NYBB and UPENN). A convenience series of 23 brain samples (cingulate cortex) were included from NYBB. Samples were from individuals with pathological findings of Lewy bodies (n=5) and glial cytoplasmic inclusions (n=12) and from people with non-synucleinopathies (n=6). A convenience series of 21 CSF samples from UPENN included cases with *intravitam* diagnoses of multiple system atrophy mimics (eg, dementia with Lewy bodies, Parkinson's disease, Parkinson's disease dementia, primary progressive aphasia, corticobasal syndrome, progressive supranuclear palsy, frontotemporal dementia, and frontotemporal lobar degeneration). Primary pathologies consisted of 4R-tauopathies (nine progressive supranuclear palsy, six corticobasal

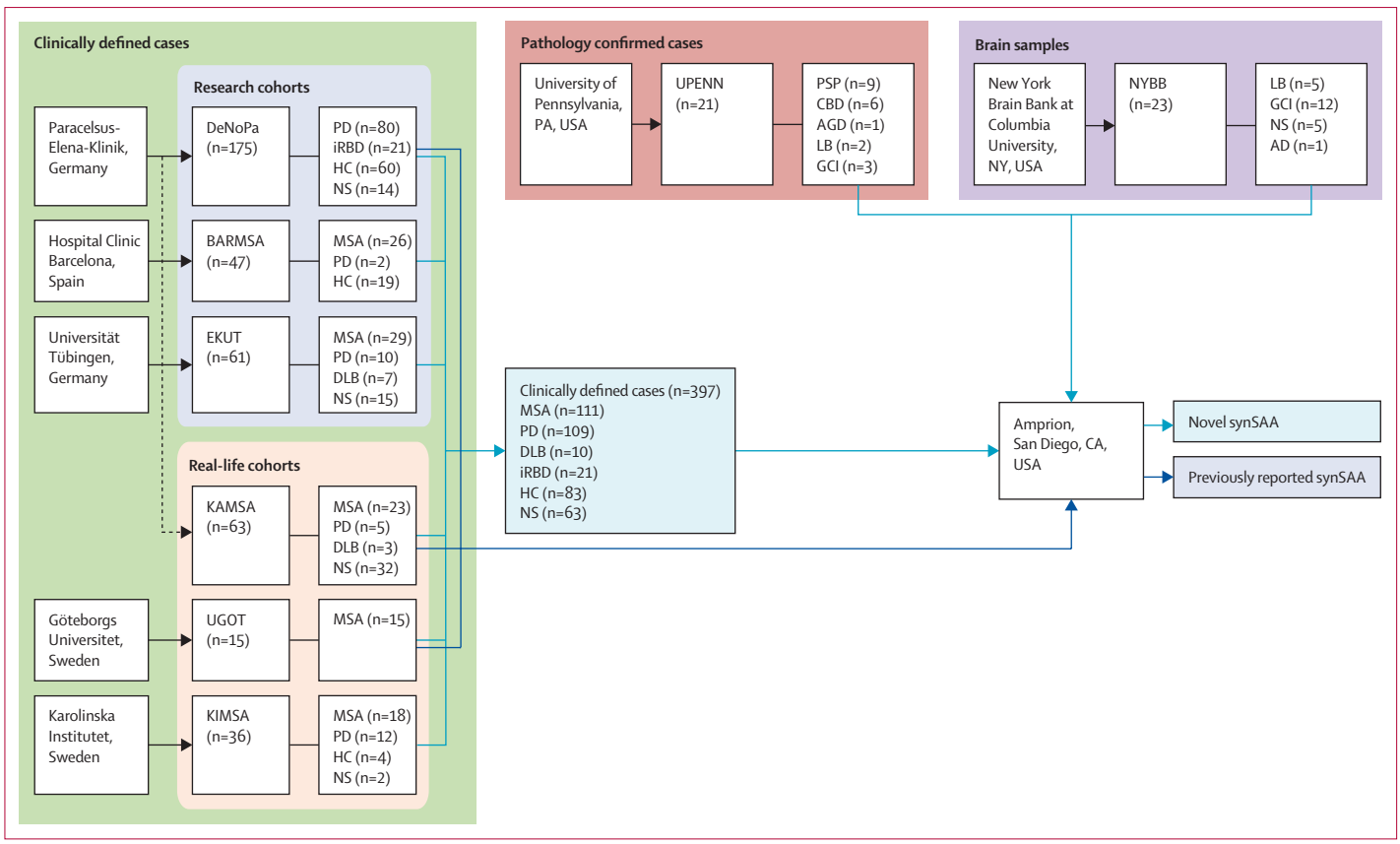


Figure 1: **Flowchart showing cohorts, diagnoses and pathologies, and sample analysis**
Samples included in this study were kindly provided by the institutions described in this diagram, which included clinically defined CSF samples (green shading), pathology-confirmed CSF samples (red shading), or brain samples (purple shading). Among the clinical samples, three cohorts were classified as research (grey shading) and three as real-life (orange shading) given their different levels of characterisation and expected diagnostic accuracy. Samples were all shipped to Amprion (San Diego, CA, USA) for masked synSAA testing. All the samples were analysed with the novel synSAA (light blue line), and some with sufficient sample volume were also tested with a previously reported synSAA (dark blue line).The novel synSAA is the index test, whereas the previously reported synSAA was used for comparison purposes only; both synSAAs used the clinical diagnosis or pathological findings (if available) as the reference standard. DeNoPa=De Novo Parkinson's disease at Paracelsus-Elena-Klinik, Germany (de novo Parkinson's disease cohort). BARMSA=Barcelona MSA at Hospital Clinic Barcelona, Spain. EKUT=Eberhard Karls Universität Tübingen, Germany. KAMSA=Kassel MSA, Paracelsus-Elena-Klinik, Germany. UGOT=Göteborgs Universitet, Sweden. KIMSA=Karolinska Institutet MSA, Sweden. UPENN=University of Pennsylvania, PA, USA. NYBB=New York Brain Bank, Columbia University, NY, USA. PD=Parkinson's disease. iRDB=isolated rapid eye movement sleep behaviour disorder. HC=healthy controls. NS=non-synucleinopathy. MSA=multiple system atrophy. DLB=dementia with LB. GCI=glial cytoplasmic inclusions. LB=Lewy bodies. AD=Alzheimer's disease. PSP=progressive supranuclear palsy. CBD=corticobasal degeneration. AGD=argyrophilic grain disease.

degeneration, and one argyrophilic grain disease), two with Lewy bodies, and three with glial cytoplasmic inclusions (appendix p 8). Three clinically defined research cohorts were from Germany and Spain (DeNoPa, BARMSA, and EKUT). The DeNoPa research cohort comprised 175 CSF samples and included 80 people with Parkinson's disease (four pathology confirmed), 21 with IRBD, 14 with non-synucleinopathies (four had progressive supranuclear palsy, five essential tremor with dystonia, two restless leg syndrome, and three vascular Parkinson's disease), and 60 healthy controls. The BARMSA research cohort consisted of 47 CSF samples, including 26 cases of multiple system atrophy, two Parkinson's disease, and 19 healthy controls. The EKUT research cohort comprised 61 baseline CSF samples, including 29 with multiple system atrophy, ten with Parkinson's disease, seven dementia with Lewy bodies, and 15 non-synucleinopathies (six peripheral neuropathy, seven pseudotumor cerebri, one spinal stenosis, and one multifactorial gait disorder). Finally, three clinically defined real-life cohorts were from Germany and Sweden (KAMSA, UGOT, and KIMSA). The KAMSA real-life cohort consisted of 63 CSF samples from 23 people with multiple system atrophy, five with Parkinson's disease, three dementia with Lewy bodies, and 32 non-synucleinopathies (23 had adult-onset progressive cerebellar ataxia, six progressive supranuclear palsy, one normal pressure hydrocephalus, one normal pressure hydrocephalus with RBD, and one dystonia with parkinsonism). The UGOT real-life cohort included CSF samples from 15 people with multiple system atrophy. The KIMSA real-life cohort included 36 CSF samples from 18 people with multiple system atrophy (two were pathology confirmed), 12 Parkinson's disease, two non-synucleinopathies (one temporary Bell paresis, one paraesthesia of the legs), and four healthy controls.

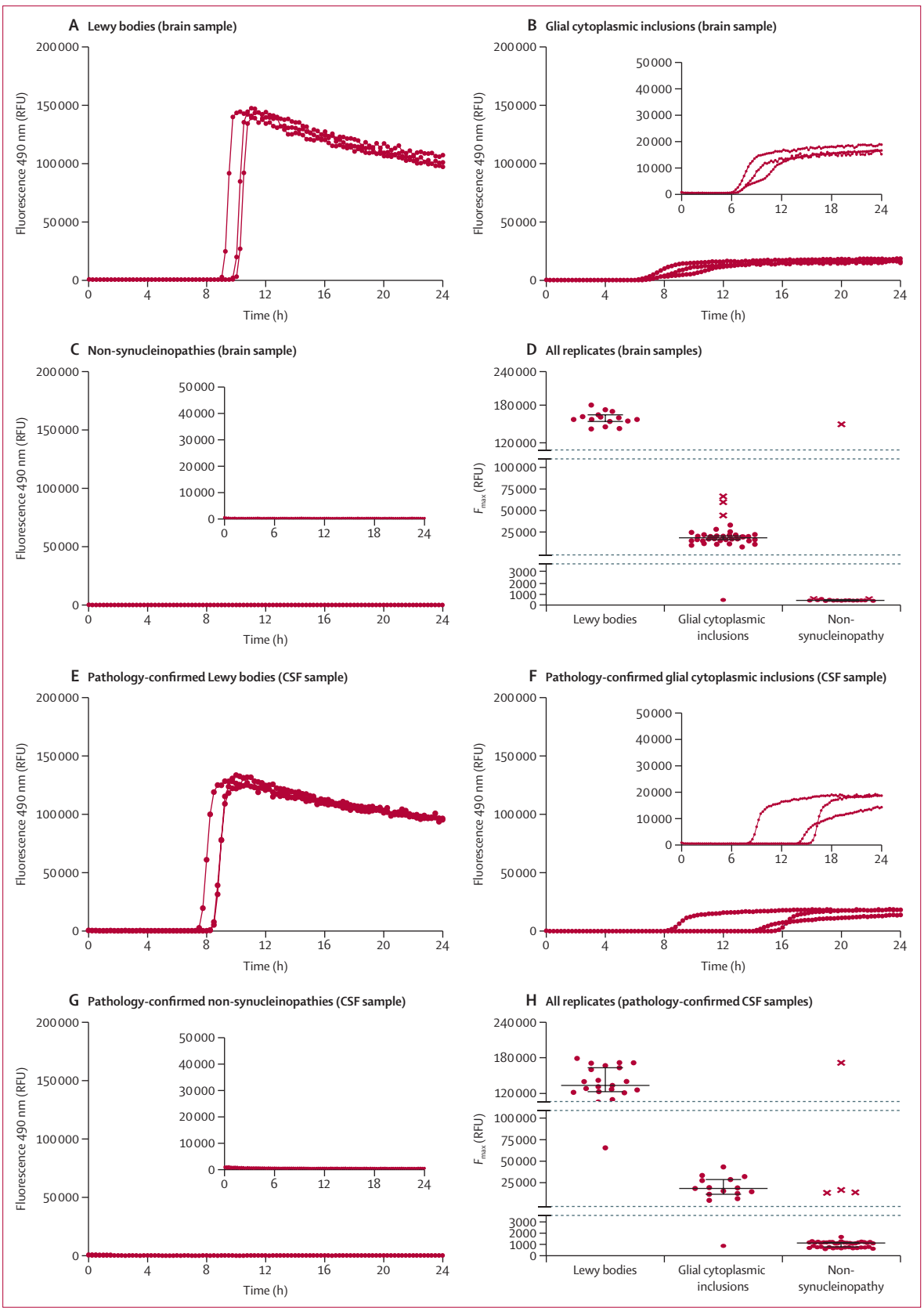


Figure 2: **Amplification patterns of brain samples and pathology-confirmed CSF samples**
Representative fluorescence traces produced by brain samples with Lewy bodies (A), glial cytoplasmic inclusions (B), non-synucleinopathy (C), and F_{max} of all replicates (D) from 23 pathology-confirmed brain samples (five Lewy bodies, 12 glial cytoplasmic inclusions, and six non-synucleinopathies). Outliers are represented with an x. Representative fluorescence traces produced by pathology-confirmed CSF samples with Lewy bodies (E), cytoplasmic inclusions (F), non-synucleinopathy (G), and F_{max} of all replicates (H) from 27 pathology-confirmed CSF samples (seven Lewy bodies, five glial cytoplasmic inclusions, and 15 non-synucleinopathies). Outliers are represented with an x.

Demographic and biomarker data for all cohorts with CSF samples are presented in the appendix (pp 9–12).

The novel synSAA analysis of 23 brain samples (from the NYBB cohort) showed synSAA-positive type 1 results for the five cases with Lewy bodies (figure 2A), synSAA-positive type 2 for the 12 cases with glial cytoplasmic inclusions (figure 2B), and synSAA-negative results for the six non-synucleinopathy cases (figure 2C), in 100% agreement with pathology (figure 2D). Evaluation of the 27 pathology-confirmed CSF samples from three cohorts (UPENN, DeNoPa, and KIMSA) showed that samples from six cases with Lewy bodies and one case of corticobasal degeneration with Lewy bodies were deemed synSAA-positive type 1 (figure 2E), samples from five cases with glial cytoplasmic inclusions were deemed synSAA-positive type 2 (figure 2F), and synSAA-negative results were obtained for nine progressive supranuclear palsy cases, five corticobasal degeneration cases, and one argyrophilic grain disease without evidence of α-synuclein pathology (figure 2G), also yielding 100% specificity, sensitivity, and seed specificity compared with pathology findings (figure 2H).

The novel synSAA was next tested in 175 CSF samples from the clinically defined DeNoPa research cohort. The novel synSAA reached 95% sensitivity for both Parkinson's disease (95% CI 88–99; 76 of 80) and for IRBD (76–100; 20 of 21). Specificity for type 1 seeds was 100% for both Parkinson's disease (95% CI 95–100; 76 of 76) and for IRBD (83–100; 20 of 20; table). Specificity was 86% for non-synucleinopathy cases (95% CI 57–98; 12 of 14) and 95% for healthy controls (86–99; 55 of 58 [two cases were inconclusive]). These results for the novel synSAA were compared with the previously reported synSAA and both assays reached the same result in 172 (98%) of 175 samples (appendix p 13).

The novel synSAA was further evaluated for sensitivity and specificity for type 2 seeds in the five clinically defined cohorts that were enriched for multiple system atrophy cases (BARMSA, EKUT, KAMSA, UGOT, and KIMSA). In total, there were 111 samples from multiple system atrophy cases, 29 from people with Parkinson's disease, ten cases of dementia with Lewy bodies, 49 with non-synucleinopathies, and 23 healthy controls (table). Overall, the novel synSAA had 87% sensitivity for multiple system atrophy (95% CI 80–93; 96 of 111 samples were synSAA-positive) and specificity for type 2 seeds was 77% (67–85; 74 of 96).

Specificity for type 2 seeds varied according to the expected diagnostic accuracy of each cohort. In the BARMSA longitudinal research cohort, participants were evaluated every 6 months for at least 2 years, and results of the novel synSAA showed 85% sensitivity (95% CI 65–96; 22 of 26) and 91% specificity for type 2 seeds (71–99; 20 of 22). In the EKUT longitudinal research cohort, participants had one or two annual evaluations, and the novel synSAA showed 83% sensitivity (95% CI 64–94; 24 of 29) and 83% specificity for type 2 seeds (63–95; 20 of 24). The KAMSA real-life cross-sectional cohort was the only cohort to use MSA-MDS22 diagnostic criteria and reached 87% sensitivity (95% CI 66–97; 20 of 23) and 80% specificity for type 2 seeds (56–94; 16 of 20). The KIMSA and UGOT real-life cohorts (single visit diagnosis using MSA-SCS08 diagnostic criteria) had respective sensitivities of 100% (95% CI 81–100; 17 of 17 [one case was inconclusive]) and 87% (60–98; 13 of 15), but specificity for type 2 seeds was 53% (28–77; nine of 17) and 69% (38–91; nine of 13).

The combined specificity of the novel synSAA for type 2 seeds in research cohorts (ie, with the highest diagnostic accuracy; BARMSA and EKUT) was 87% (95% CI 74–95; 40 of 46) and sensitivity was 84% (71–92). The combined specificity for type 2 seeds in real-life cohorts (KAMSA, KIMSA, and UGOT) was 68% (95% CI 53–81) and sensitivity was 91% (80–97). In the two real-life cohorts that used the MSA-SCS08 criteria and had the lowest expected diagnostic accuracy (KIMSA and UGOT), combined specificity for type 2 seeds was 60%; 95% CI 41–77; 18 of 30). Specificity for type 2 seeds was associated with expected diagnostic accuracy (research cohorts [BARMSA and EKUT] *vs* real-life [KIMSA and UGOT], p=0·0019). However, no difference in specificity for type 2 seeds was noted between research cohorts (BARMSA and EKUT) and the KAMSA real-life cohort that used the MSA-MDS22 guideline (p=0·19). MSA-P and MSA-C classification was not associated with the sensitivity of the novel synSAA in any of the clinically defined cohorts (p≥1·0 for BARMSA, EKUT, and KIMSA, p=0·56 for KAMSA).

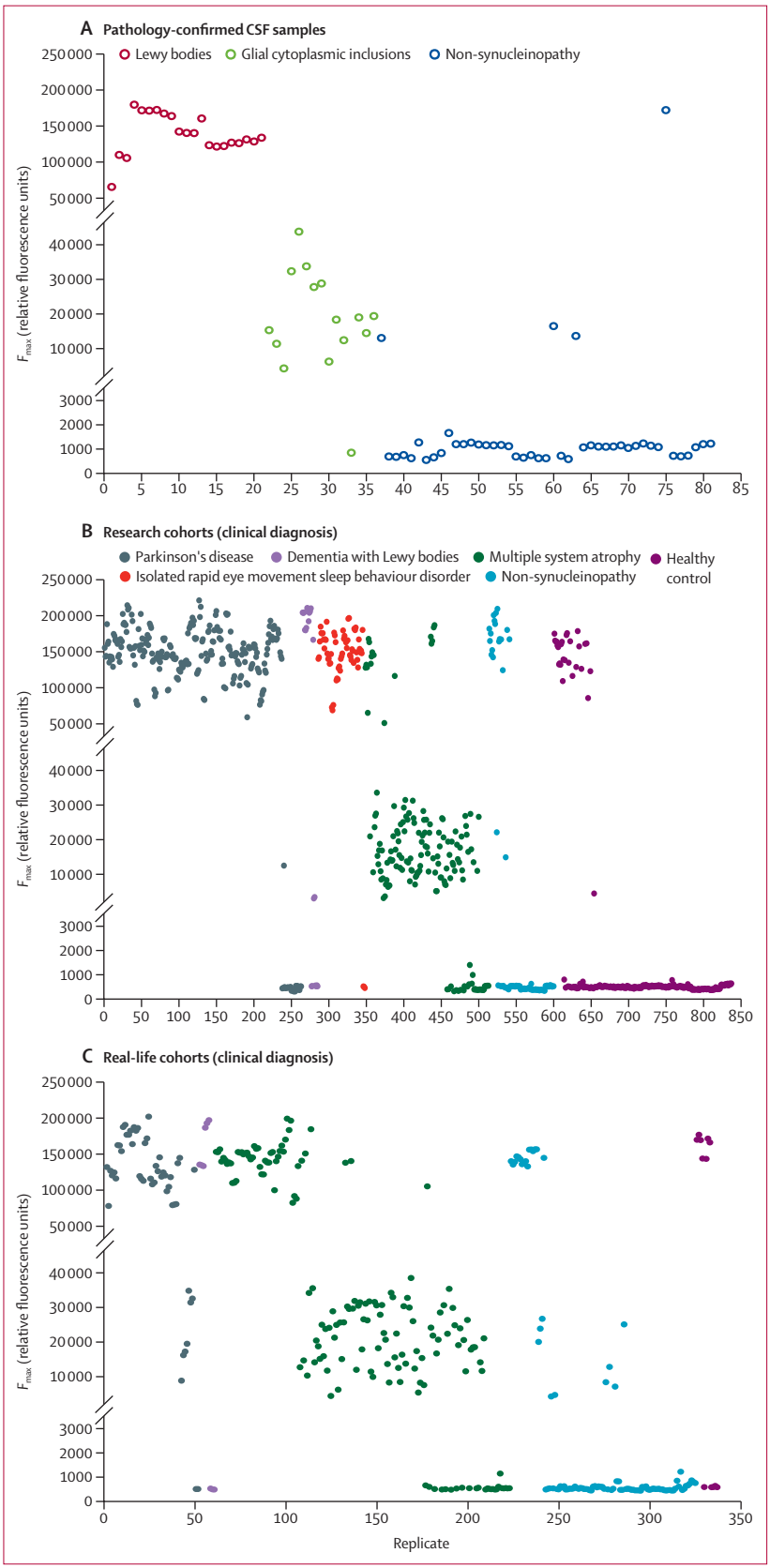
31 CSF samples from people with multiple system atrophy were of sufficient volume for additional testing (21 from KAMSA and ten from UGOT) and, thus, were analysed with the previously reported synSAA. The sensitivity of the previous synSAA was 33% (95% CI 13–59; six of 18 [three cases were inconclusive]) in KAMSA and 30% (7–65; three of ten) in UGOT, confirming the higher sensitivity of the novel synSAA for multiple system atrophy (appendix p 14). Post-hoc analysis of 14 MSA-P cases from KIMSA that presented synSAA-positive type 1 results showed that seven had an initial diagnosis of Parkinson's disease that changed to multiple system atrophy once they presented dysautonomia.

The five cohorts that were enriched for multiple system atrophy cases (BARMSA, EKUT, KAMSA, UGOT, and KIMSA) also included some samples from people with Parkinson's disease and dementia with Lewy bodies (table; appendix pp 3–4). Non-synucleinopathy participants were also included in these cohorts, and the novel synSAA produced a synSAA-negative result for 12 of 14 samples in EKUT (specificity 86%, 95% CI 75–98 [one sample was inconclusive]), 26 of 32 in KAMSA (81%, 64–93), and two of two in KIMSA (100%, 16–100). The novel synSAA was negative for 20 of 23 cases of adult-onset progressive cerebellar

	DeNoPa	BARMSA	EKUT	KAMSA	KIMSA	UGOT	Total
Multiple system atrophy							
n	0	26	29	23	18	15	111
Sensitivity (95% CI)	NA	85% (65–96)	83% (64–94)	87% (66–97)	100%* (81–100)	87% (60–98)	87%* (80–93)
Type 1 (95% CI)	NA	5% (0–23)	8% (1–27)	20% (6–44)	41% (18–76)	31% (9–61)	19% (12–28)
Type 2 (95% CI)	NA	91% (71–99)	83% (63–95)	80% (56–94)	53% (28–77)	69% (39–91)	77% (67–85)
Undetermined (95% CI)	NA	5% (0–23)	8% (1–27)	0 (0–17)	6% (0–29)	0 (0–25)	4% (1–10)
Multiple system atrophy—parkinsonian							
n	0	11	13	13	14	0	51
Sensitivity (95% CI)	NA	91% (59–100)	85% (55–98)	92% (64–100)	100%* (75–100)	NA	92%* (81–98)
Type 1 (95% CI)	NA	0 (0–31)	18% (2–52)	25% (5–57)	54% (25–81)	NA	26% (14–41)
Type 2 (95% CI)	NA	90% (56–100)	82% (48–98)	75% (43 -95)	46% (19–75)	NA	72% (57–84)
Undetermined (95% CI)	NA	10% (0–45)	0 (0–28)	0 (0–26)	0 (0–25)	NA	2% (0–12)
Multiple system atrophy—cerebellar							
n	0	7	16	10	4	0	37
Sensitivity (95% CI)	NA	86% (42–100)	81% (54–96)	80% (44–98)	100% (40–100)	NA	84% (68–94)
Type 1 (95% CI)	NA	17% (0–64)	0 (0–25)	13% (0–53)	0 (0–60)	NA	6% (1–21)
Type 2 (95% CI)	NA	83% (36–100)	85% (55–98)	88% (47–100)	75% (19–99)	NA	84% (66–95)
Undetermined (95% CI)	NA	0 (0–46)	15% (2–45)	0 (0–37)	25% (1–81)	NA	10% (2–26)
Parkinson's disease							
n	80	2	10†	5	12	0	109
Sensitivity (95% CI)	95% (88–99)	100% (16–100)	50% (19–81)	100% (48–100)	92% (62–100)	NA	91% (84–96)
Type 1 (95% CI)	100% (95–100)	100% (16–100)	100% (48–100)	80% (28–99)	82% (48–98)	NA	97% (91–99)
Type 2 (95% CI)	0 (0–5)	0 (0–84)	0 (0–52)	20% (1–72)	9% (0–41)	NA	2% (0–7)
Undetermined (95% CI)	0 (0–5)	0 (0–84)	0 (0–52)	0 (0–52)	9% (0–41)	NA	1% (0–6)
Dementia with Lewy bodies							
n	0	0	7†	3	0	0	10
Sensitivity (95% CI)	NA	NA	71% (29–96)	67% (9–99)	NA	NA	70% (35–93)
Type 1 (95% CI)	NA	NA	80% (28–99)	100% (16–100)	NA	NA	86% (42–100)
Type 2 (95% CI)	NA	NA	20% (1–72)	0 (0–84)	NA	NA	14% (0–58)
Undetermined (95% CI)	NA	NA	0 (0–52)	0 (0–84)	NA	NA	0 (0–41)
Isolated rapid eye movement sleep behaviour disorder							
n	21	0	0	0	0	0	21
Sensitivity (95% CI)	95% (76–100)	NA	NA	NA	NA	NA	95% (76–100)
Type 1 (95% CI)	100% (83–100)	NA	NA	NA	NA	NA	100% (83–100)
Type 2 (95% CI)	0 (0–17)	NA	NA	NA	NA	NA	0 (0–17)
Undetermined, % (95% CI)	0 (0–17)	NA	NA	NA	NA	NA	0 (0–17)
Non-synucleinopathy							
n	14	0	15	32	2	0	63
Specificity (95%CI)	86% (57–98)	NA	86%* (75–98)	81% (64–93)	100% (16–100)	NA	84%* (72–92)
Type 1 (95% CI)	100% (16–100)	NA	50% (1–99)	83% (36–100)	0 (NA)	NA	80% (44–97)
Type 2 (95% CI)	0 (0–84)	NA	0 (0–84)	17% (0–64)	0 (NA)	NA	10% (0–45)
Undetermined (95% CI)	0 (0–84)	NA	50% (1–99)	0 (0–46)	0 (NA)	NA	10% (0–45)
Healthy controls							
n	60	19	0	0	4	0	83
Specificity (95% CI)	95%‡ (86–99)	95% (74–100)	NA	NA	50%‡ (1–99)	NA	94%§ (86–98)
Type 1 (95% CI)	100% (29–100)	100% (3–100)	NA	NA	100% (3–100)	NA	100% (48–100)
Type 2 (95% CI)	0 (0–71)	0 (0–98)	NA	NA	0 (0–98)	NA	0 (0–52)
Undetermined (95% CI)	0 (0–71)	0 (0–98)	NA	NA	0 (0–98)	NA	0 (0–52)

DeNoPa=De Novo Parkinson's disease at Paracelsus-Elena-Klinik, Germany. BARMSA=Barcelona MSA at Hospital Clinic Barcelona, Spain. EKUT=Eberhard Karls Universität Tübingen, Germany. KAMSA=Kassel MSA, Paracelsus-Elena-Klinik, Germany. KIMSA=Karolinska Institutet MSA, Sweden. UGOT=Göteborgs Universitet, Sweden. *One inconclusive sample. Not retested owing to lack of sample volume, excluded from sensitivity–specificity calculation. †Group enriched in synSAA negative samples evaluated with alternative assay conditions. ‡Two inconclusive samples. Not retested due to lack of sample volume, excluded from specificity calculation. §Four inconclusive samples. Not retested due to lack of sample volume, excluded from specificity calculation.

Table: **Sensitivity and specificity estimations for the novel synSAA with clinically diagnosed samples**



ataxia (87%, 66–97) and four of six cases of progressive supranuclear palsy (67%, 22–96) in KAMSA (appendix p 15). 19 CSF samples in BARMSA and four in KIMSA were from healthy controls, and the novel synSAA had respective specificities of 95% (95% CI 74–100; 18 of 19) and 50% (1–99; one of two [two cases were inconclusive]).

F_{max} measurements for all CSF replicates were classified according to the expected diagnostic accuracy of each cohort into pathology-confirmed, research cohorts, and real-life cohorts (figure 3). Despite the difference in diagnostic accuracy of each group, F_{max} values from all individual replicates were not normally distributed but fell into the three categories that defined type 1, type 2, and negative replicates (appendix p 7). Overall, 76 (94%) of 81 (95% CI 86–98) of the pathology-confirmed replicates, 703 (84%) of 837 (81–86) of the research replicates, and 215 (64%) of 336 (59–69) of the real-life replicates presented patterns that agreed with the suspected underlying pathology. In pathology-confirmed samples (from UPENN, DeNoPa, and KIMSA; figure 3A), 21 (100%) of 21 (95% CI 84–100) of the Lewy body replicates (primary or secondary pathology) were type 1, 14 (93%) of 15 (68–100) of the glial cytoplasmic inclusion replicates were type 2 (one [7%] of 15 were negative), and 41 (91%) of 45 (79–98) of the control replicates (no primary or secondary α-synuclein pathology) were negative (three [7%] of 45 were type 2, one [2%] of 45 were type 1). In the research group (comprising DeNoPa [four pathology-confirmed Parkinson's disease samples excluded], BARMSA, and EKUT cohorts; figure 3B), 237 (90%) of 264 (95% CI 86–93) of the Parkinson's disease replicates were type 1 (26 [10%] of 264 were negative and <1% [one of 264] were type 2). Among the dementia with Lewy bodies replicates, 13 (62%) of 21 (38–82) were type 1 (six [29%] of 21 were negative and two [10%] of 21 were type 2). 60 (95%) of 63 (87–99) of the IRBD replicates were type 1 (three [5%] of 63 were negative). Of the multiple system atrophy replicates, 116 (70%) of 165 (63–77) were type 2 (32 [19%] of 165 were negative, 17 [10%] of 165 were type 1). 68 (78%) of 87 (68–86) of the non-synucleinopathy replicates were negative (17 [20%] of 87 were type 1 and two (2%) of 87 were type 2). Finally, 209 (88%) of 237 (83–92;) of the healthy control replicates were negative 27 (11%) of 237 were type 1 and one (<1%) of 237 were type 2). In the real-life group (comprising KAMSA, UGOT, and KIMSA

Figure 3: **Visualisation of F_{max} values collected from all evaluated CSF samples**
F_{max} values for all 1254 replicates analysed in this study. (A) F_{max} from individual replicates (n=81) generated by synSAA analysis of 27 pathology-confirmed CSF samples. (B) F_{max} from individual replicates (n=837) generated by synSAA analysis of 279 clinically defined CSF samples from research cohorts. (C) F_{max} from individual replicates (n=336) generated by synSAA analysis of 112 clinically defined CSF samples from real-life cohorts. The *y*-axis is broken at the 3000 RFU and 45 000 RFU thresholds and each part of the axis is scaled to show dispersion of the measurements within each of the three fluorescence ranges. RFU=relative fluorescence unit.

[two pathology-confirmed multiple system atrophy samples excluded] cohorts; figure 3C), 42 (82%) of 51 (95% CI 69–92) of the Parkinson's disease replicates were type 1 (seven [14%] of 51 were type 2 and two [4%] of 51 were negative). Among the dementia with Lewy bodies replicates, six (67%) of nine (30–93) were type 1 (three [33%] of nine were negative). Of the multiple system atrophy replicates, 85 (53%) of 162 (45–60) were type 2 (52 [32%] of 162 were type 1 and 25 [15%] of 162 were negative). 77 (76%) of 102 (66–83) of the non-synucleinopathy replicates were negative (16 [16%] of 102 were type 1 and nine [9%] of 102 were type 2). Finally, five (42%) of 12 (15–72) of the healthy control replicates were negative (seven [58%] of 12 were type 1).

Discussion

The goal of this multicentre cohort study was to investigate whether CSF α-synuclein seeds related to glial cytoplasmic inclusions (the pathological hallmark of multiple system atrophy) could be reliably amplified, detected, and differentiated from seeds related to Lewy bodies (found in Parkinson's disease and dementia with Lewy bodies). Using pathology-confirmed CSF and brain specimens, we showed that a novel synSAA can detect two types of α-synuclein seeds that corresponded 100% with underlying Lewy bodies (defined as type 1 seeds) or glial cytoplasmic inclusions (type 2 seeds). The differentiation of these seeds was reproducible when the novel synSAA was tested with different batches of substrate, indicating that α-synuclein seed typing is robust and compatible with diagnostic applications. Moreover, F_{max} values for all studied CSF samples were distributed into three types of replicates (type 1, type 2, and negative), showing that these patterns emerge from the different α-synuclein seeding activities.

The novel synSAA was evaluated by use of CSF samples from individuals with clinically defined synucleinopathies from well characterised longitudinal research cohorts and real-life cross-sectional cohorts. Agreement between the novel synSAA and the clinical diagnosis varied depending on the expected diagnostic accuracy of each cohort. For example, participants in the DeNoPa cohort had many years of follow-up plus DAT-SPECT for Parkinson's disease and polysomnography for IRBD. Thus, the diagnosis of these participants is probably very accurate, and the novel synSAA reached 95% sensitivity for Parkinson's disease and IRBD, and 95% specificity for healthy controls, confirming the clear detection of the disease-associated biomarker. Type 1 α-synuclein seeds were detected in all samples from people with Parkinson's disease and IRBD who were synSAA-positive, consistent with their expected underlying Lewy body pathology. Specificity for type 2 seeds in individuals with multiple system atrophy was higher in research than in real-life cohorts, probably owing to greater agreement between clinical diagnosis and neuropathology when participants are followed up.[27] In particular, diagnosis of multiple system atrophy according to MSA-SCS08 is 40% sensitive in early disease stages, and it increases to 76% after 3 years.[27] Therefore, a proportion of synSAA-negative and synSAA-positive type 1 cases was expected in clinically defined multiple system atrophy cohorts if the synSAA were to correspond to underlying pathology. Additional studies have shown that only 62% of clinically diagnosed multiple system atrophy cases match pathology findings, and the most common pathological findings among individuals with misdiagnoses were Lewy bodies and progressive supranuclear palsy.[5] In our study, pathology-confirmed participants with Lewy bodies who had been misdiagnosed with corticobasal syndrome were synSAA-positive, and cases with pure 4R-tauopathy who had been misdiagnosed with either dementia with Lewy bodies or Parkinson's disease and mild cognitive impairment were synSAA-negative. Moreover, the KAMSA cohort contained people with adult-onset progressive cerebellar ataxia, which is another clinically relevant control group because this disorder is a mimic of multiple system atrophy, and most cases were synSAA-negative. Therefore, our results provide evidence that the novel synSAA conditions reported here can detect and differentiate type 2 α-synuclein seeds that are associated with glial cytoplasmic inclusion pathology and are consistent with a multiple system atrophy diagnosis.

Our findings are important because they highlight the importance of biological disease, rather than clinical presentation. Biological definitions of diseases associated with α-synuclein (ie, on the Parkinson's disease and dementia with Lewy bodies continuum) have been proposed,[28,29] with α-synuclein seeds being the earliest reported disease-specific biomarker. In the research criteria that define neuronal α-synuclein disease,[27] misfolded α-synuclein accumulates in neurons and is identified in CSF by use of a synSAA, given the established detection of α-synuclein seeds related to Lewy bodies.[13,14] In the SynNeurGe research criteria,[29] the use of neurofilament light chain and MRI is recommended to further identify cases that might have multiple system atrophy, but these criteria acknowledge the lack of specificity for neurofilament light chain and sensitivity for MRI. The novel synSAA conditions reported here will greatly increase the specificity of these research diagnostic criteria, because type 2 α-synuclein seeds could be used as an exclusion criterion, thereby enriching study populations with cases with type 1 α-synuclein seeds (or vice versa). Although independent reproduction of our results in additional cohorts is necessary, our work provides a technological foundation to further advance the biological definitions of synucleinopathies, with the goal of enabling very early detection and accelerating drug development.

In our study, we identified novel synSAA conditions to consistently amplify type 2 α-synuclein seeds, but we did not elucidate the mechanism causing the type 2 pattern. We have shown reproducible detection of type 1 α-synuclein seeds with a previously reported synSAA,[13,14]

but robust amplification of type 2 α-synuclein seeds required the more stringent novel synSAA conditions that are reported here. The difference in seeding activity between these types of α-synuclein seeds could be related to the two protofilaments in multiple system atrophy and the single protofilament in Parkinson's disease and dementia with Lewy bodies, described in studies by use of cryogenic electron microscopy.[30,31] Lateral interactions between multiple system atrophy protofilaments might make type 2 α-synuclein seeds more stable and difficult to fragment than type 1 α-synuclein seeds. Similarly, these lateral interactions could block thioflavin T binding pockets, generating lower F_{max} values. Alternatively, the different protofilament conformation might generate thioflavin T binding pockets slightly more exposed to the aqueous solution, which could reduce thioflavin T fluorescence by quenching.

The limitations of our study include the small number of brain specimens and pathology-confirmed CSF samples, which should be expanded in future studies. Additionally, the cohort that included CSF samples from people with adult-onset progressive cerebellar ataxia was not followed up longitudinally, and numbers in the tauopathy group were low. Thus, additional samples—preferably pathology-confirmed or longitudinal cases to increase diagnostic accuracy—should be analysed to fully characterise the value of the novel synSAA in differential diagnosis. Lastly, it is unknown whether type 1 and type 2 α-synuclein seeds detected by the novel synSAA correspond to cryogenic electron microscopy-resolved structures, or whether type 1 and type 2 α-synuclein seeds can be further stratified.

In summary, our study describes a novel synSAA with high sensitivity for synucleinopathies that can differentiate glial cytoplasmic inclusions and Lewy bodies during life. The novel synSAA could facilitate differential diagnosis of synucleinopathies and potentially augment clinical trials of disease-modifying therapies.

Contributors

PK, MSy, YC, BM, and LC-M contributed to the conceptualisation. YM, CMF, and LC-M contributed to the formal analysis. YM, CMF, SW, HN, TCM, DM, PP, MSy, YC, PS, and LC-M contributed to the investigation. YM, CMF, HN, and LC-M contributed to the methodology. SW, SS, AP-S, DMG, MF, MSo, AC, CP, EM, FV, MJM, JCl, PK, TCM, RNA, BFG, KBl, HZ, JCo, DM, VK, KBr, TFT, AS, MSy, UJK, YC, PS, and BM contributed to resources. SW, VK, MSy, UJK, YC, PS, and LC-M contributed to the validation. YM, CMF, SW, and LC-M contributed to the writing—original draft. CMF and LC-M contributed to the visualisation. LC-M contributed to the funding acquisition, project administration, and supervision. YM, CMF, SW, SS, HN, AP-S, DMG, MF, MSo, AC, CP, EM, FV, MJM, JCl, PK, TCM, RNA, BFG, KBl, HZ, JCo, DM, VK, PP, KBr, TFT, AS, MSy, UJK, YC, PS, BM, and LC-M contributed to the writing—review and editing. YM, CMF, SW, and LC-M had access to, reviewed, and verified all the data. LC-M, BM, MSy, YC, and PS were responsible for the decision to submit. All authors had full access to the data in the study and agreed to submit for publication.

Declaration of interests

YM, CMF, HN, and LC-M are Amprion employees and declare employee stock option ownership and invention of patents related to SAA assigned to Amprion. YM declares patent or patent application numbers US11970520B2, US20230084155A1. CMF declares patent or patent application numbers US11970520B2, US11959927B2, and US20230084155A1. HN declares patent or patent application numbers US20230084155A1. LC-M declares patent or patent application numbers US11970520B2, US11959927B2, US20190353669A1, US20230084155A1, and US20210223268A1. UJK is on the scientific advisory board of Amprion, on the data monitoring committee for UCB, and a consultant for NurrOn. MSy has received consultancy honoraria from Ionis, UCB, Prevail, Orphazyme, Servier, Reata, GenOrph, AviadoBio, Biohaven, Zevra, Solaxa, and Lilly, all unrelated to the present manuscript. HZ has served at scientific advisory boards or as a consultant, or both, for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche; is chair of the Alzheimer's Association Global Biomarker Standardization Consortium; and is a co-founder of Brain Biomarker Solutions in Gothenburg, which is a part of the GU Ventures Incubator Program. RNA received consultation fees from Biogen, Biohaven, Capsida, Gain Therapeutics, Genzyme/Sanofi, Servier, Takeda, and Vanqua Bio. TFT serves as a clinical trial advisory board member for Bial. KBr is on the advisory boards of F Hoffman La Roche, VanquaBio, and the Michael J Fox Foundation. PS is a DSMB member in Amulet study in MSA for Lundbeck. BM has received honoraria for consultancy or educational presentations, or both, from GE, Bial, Roche, Biogen, and AbbVie, and is member of the executive steering committee of the Parkinson Progression Marker Initiative of the Michael J Fox Foundation for Parkinson's Research and has received research funding from Aligning Science Across Parkinson's disease Collaborative Research Network. CP has received honoraria financed by Bial. AS declares consultancy for Acadia, Boehringer-Ingelheim, Mitzubishi, GE Healthcare, and Capsida. AS declares participation on a data safety monitoring board or advisory board for Wave Life Sciences, Inhibikase, Prevail, Alterity, Healy ALS Consortium (Massachusetts General Hospital), and Huntington Study Group. KBl has served as a consultant or on an advisory board for Abbvie, AriBio, ALZpath, BioArctic, AC Immune, Biogen, Eisai, Lilly, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers, and produced or participated in educational programmes for Biogen, Eisai, and Roche Diagnostics.

Data sharing

All relevant data generated during this study are included in this Article, and additional information could be made available on reasonable request to the corresponding author.

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