Movement Disorders
CLINICAL PRACTICE

REVIEW

α-Synuclein Seed Amplification Assays in the Diagnosis of Synucleinopathies Using Cerebrospinal Fluid—A Systematic Review and Meta-Analysis

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ABSTRACT: Background: Real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA) have been developed to detect minute amounts of amyloidogenic proteins via amplification techniques and have been used to detect misfolded α-synuclein (αSyn) aggregates in the cerebrospinal fluid (CSF) and other source materials of patients with Parkinson's Disease and other synucleinopathies.

Objectives: The aim of this systematic review and meta-analysis was to evaluate the diagnostic accuracy of αSyn seed amplification assays (αSyn-SAAs), including RT-QuIC and PMCA, using CSF as source material to differentiate synucleinopathies from controls.

Methods: The electronic MEDLINE database PubMed was searched for relevant articles published until June 30, 2022. Study quality assessment was performed using the QUADAS-2 toolbox. A random effects bivariate model was exploited for data synthesis.

Results: Our systematic review identified 27 eligible studies according to the predefined inclusion criteria, of which 22 were included in the final analysis. Overall, 1855 patients with synucleinopathies and 1378 non-synucleinopathies as control subjects were included in the meta-analysis. The pooled sensitivity and specificity to differentiate synucleinopathies from controls with αSyn-SAA were 0.88 (95% CI, 0.82–0.93) and 0.95 (95% CI, 0.92–0.97), respectively. Evaluating the diagnostic performance of RT-QuIC in a subgroup analysis for the detection of patients with multiple system atrophy the pooled sensitivity decreased to 0.30 (95% CI, 0.11–0.59).

Conclusions: While our study clearly demonstrated a high diagnostic performance of RT-QuIC and PMCA for differentiating synucleinopathies with Lewy bodies from controls, results for the diagnosis of multiple system atrophy were less robust.

Synucleinopathies is used as an umbrella term for a class of neurodegenerative diseases characterized by the misfolding and aberrant accumulation of α-synuclein leading to the formation of Lewy bodies in Parkinson's disease (PD) and dementia with Lewy bodies (DLB) or to the appearance of oligodendroglial cytoplasmic inclusion in multiple system atrophy (MSA).[1] Pure autonomic failure (PAF) or idiopathic rapid-eye movement sleep behavior disorder (iRBD) may precede synucleinopathies and are increasingly recognized as the prodromal stage of these neurodegenerative diseases.[2,3] The differential diagnosis of PD from atypical Parkinsonian disorders (APDs), including MSA and progressive supranuclear palsy (PSP), which represents a tauopathy, remains a clinical challenge. Particularly in the early stages patients are frequently misdiagnosed, even by movement

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Keywords: protein misfolding cyclic amplification (PMCA), real-time quaking-induced conversion (RT-QuIC), alpha-synuclein, synucleinopathy, biomarker.

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disorder experts.[4] Therefore, validated tools and biomarkers are urgently needed to correctly classify these patients early in the disease enabling adequate care, counseling and whenever available initiate disease-modifying therapies.

In recent years, a prion-like propagation of α-synuclein, spreading from neuron to neuron in synucleinopathies, has been demonstrated experimentally in vitro and in vivo.[5,6] Importantly, there is no evidence of transmissibility in humans which is a prerequisite for a prion disease.[7] Nevertheless, the similarities opened an avenue for the development of novel diagnostic and therapeutic approaches in the field of neurodegenerative diseases. With regards to diagnostic biomarkers, the real-time quaking-induced conversion assay (RT-QuIC), a well-established tool in the diagnosis of Creutzfeld-Jakob disease (CJD),[8] appeared to be of particular interest. Green and his group[9] were the first to use their expertise in the RT-QuIC assay to adopt it for the detection of α-synuclein aggregates in the cerebrospinal fluid (CSF) of PD patients and patients with RBD. Shortly afterwards Groveman et al.[10] reported about optimized reaction conditions for completing the α-synuclein RT-QuIC assay in a significantly shorter amount of time. Both of these two initial studies[9,10] yielded an excellent sensitivity and specificity for discriminating synucleinopathies from controls.

Already small amounts of misfolded α-synuclein can be detected by RT-QuIC via incubation of a pathogenic seed derived from biological fluids or tissues with a reaction buffer containing recombinant α-synuclein, which acts as a substrate.[11,12] Protein misfolding cyclic amplification (PMCA) is another concept of seeding assays working in a similar way to RT-QuIC with some methodical differences and was first introduced for the detection of α-synuclein aggregates in CSF by Soto et al.[13]

So far, α-synuclein seeding activity has mainly been tested with CSF as a seed for discriminating patients with synucleinopathies from controls via RT-QuIC or PMCA. Therefore, the aim of this systematic review and meta-analysis was to evaluate the diagnostic accuracy of αSyn-SAAs using CSF as source material for the diagnosis of synucleinopathies.

Methods

Literature Search Strategy

This meta-analysis followed the PRISMA statement. Two reviewers (AG, GH) systematically searched the electronic MEDLINE database PubMed by the following search strategy:

((“real-time”[All Fields] AND “quaking-induced”[All Fields] AND (“conversion”[All Fields] OR “conversions”[All Fields])) OR “RT-QuIC”[All Fields]) AND (“lewy body disease”[MeSH Terms] OR (“Lewy”[All Fields] AND “body”[All Fields] AND “disease”[All Fields]) OR “lewy body disease”[All Fields] OR (“Lewy”[All Fields] AND “body-associated”[All Fields] AND (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR “synucleinopathy”[All Fields])) OR (“parkinson disease”[MeSH Terms] OR (“parkinson”[All Fields] AND “disease”[All Fields]) OR “parkinson disease”[All Fields]) OR (“pharmacology”[MeSH Subheading] OR “pharmacology”[All Fields] OR “pd”[All Fields]) OR ((“dementia”[MeSH Terms] OR “dementia”[All Fields] OR “dementias”[All Fields] OR “dementia s”[All Fields]) AND (“lewy bodies”[MeSH Terms] OR (“Lewy”[All Fields] AND “bodies”[All Fields]) OR “lewy bodies”[All Fields] OR (“Lewy”[All Fields] AND “body”[All Fields]) OR “lewy body”[All Fields])) OR “DLB”[All Fields] OR (“lewy bodies”[MeSH Terms] OR (“Lewy”[All Fields] AND “bodies”[All Fields]) OR “lewy bodies”[All Fields] OR (“Lewy”[All Fields] AND “body”[All Fields]) OR “lewy body”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR “synucleinopathy”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR “synucleinopathy”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR “a synucleinopathies”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR “a synucleinopathy”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR (“alpha”[All Fields] AND “synuclein”[All Fields] AND “pathology”[All Fields]) OR “alpha synuclein pathology”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR (“alpha”[All Fields] AND “synuclein”[All Fields] AND “pathologies”[All Fields]) OR “alpha synuclein pathologies”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR (“alpha”[All Fields] AND “synucleinopathies”[All Fields]) OR “alpha synucleinopathies”[All Fields]) OR ((“alpha”[All Fields] OR “alpha s”[All Fields] OR “alphas”[All Fields]) AND “Synucleinopathie”[All Fields]) OR (“synucleinopathies”[MeSH Terms] OR “synucleinopathies”[All Fields] OR (“alpha”[All Fields] AND “synucleinopathy”[All Fields]) OR “alpha synucleinopathy”[All Fields])).

The final search was conducted on June 30, 2022 and resulted in a total of 134 articles.

Inclusion and Exclusion Criteria

For this systematic review, studies had to fulfill the following predefined criteria: (1) manuscripts were required to be published in English language; (2) patients with synucleinopathies were included; (3) the source material for the aggregation assays was CSF; (4) the detection method for α-synuclein aggregates was RT-QuIC or PMCA; (5) studies had to report either true positive (TP), true negative (TN), false positive (FP), and false negative (FN) rates, or overall sample size and sensitivity and specificity values. The criteria for exclusion were as follows: (1) Non-diagnostic test studies; (2) When subjects of two studies overlapped, the study with the smaller sample size was excluded from further analysis. Sample sizes informing the decisions on study inclusion were determined individually for each subgroup analysis resulting in a difference in the composition of studies in different subgroup analyses.

Data Extraction

Two investigators (AG, GH) independently extracted data on the (1) first author; (2) year of publication; (3) overall sample size; (4) number of participants in each group; (5) diagnostic criteria for the clinical diagnosis of PD, DLB, MSA, RBD, PAF, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and Alzheimer's disease (AD); (6) αSyn-SAA used (RT-QuIC or PMCA); (7) cut-off values to be considered as positive samples; (8) TP, TN, FP, and FN rates, or alternatively, sensitivity and specificity.

Quality Assessment

The Quality Assessment Tool for Diagnostic Accuracy Studies 2 (QUADAS-2),[14] which was rated and documented using Review Manager 5.3 (Nordic Cochrane Centre, Copenhagen, Denmark), was used to assess each study's methodological quality concerning risk of bias and applicability.

Data Analysis

Statistical analyses were performed for the following subgroups: (1) synucleinopathies versus non-synucleinopathies; (2) synucleinopathies with Lewy bodies (LBs) versus non-synucleinopathies; (3) synucleinopathies with LBs versus healthy controls (HC); (4) synucleinopathies with LBs versus PSP/CBD; (5) established PD/DLB versus non-synucleinopathies; (6) MSA versus non-synucleinopathies; (7) MSA versus PSP/CBD; (8) prodromal stages of synucleinopathies versus non-synucleinopathies; (9) RBD versus non-synucleinopathies.

Synucleinopathies comprised patients with PD, DLB, MSA, prodromal disease stages of synucleinopathies, and patients with neuropathologically confirmed existence of Lewy bodies mixed with other pathologies. Prodromal stages of synucleinopathies included patients with RBD, PAF or mild cognitive impairment with Lewy bodies (MCI-LB) as well as non-manifesting carriers (NMC) of genetic mutations known to cause PD.

Synucleinopathies with LBs included patients with PD, genetic forms of PD, DLB, and neuropathologically confirmed existence of LBs mixed with other pathologies. Prodromal disease stages were not included in the patient group for these analyses as they could also proceed to MSA.

Non-synucleinopathies included patients with other neurodegenerative and neurological disorders as well as HC.

For data synthesis, MetaDTA, an online toolbox applying a random effects bivariate model, was used.[15,16] Forest plots were created in Stata 16.1. Hierarchical summary receiver operating characteristics curves (HSROC) with 95% confidence intervals (95% CI) were mapped to summarize sensitivities and specificities of each study.[17] Furthermore, to assess heterogeneity of sensitivities and specificities, Chi-square tests were applied, the null hypothesis being in both cases that all studies are equal.

Results

Study Selection

A total of 134 articles were identified in the final search. In total, 27 articles were eligible according to our predefined inclusion criteria and 22 articles, comprising 1855 patients with synucleinopathies and 1378 non-synucleinopathies as controls (566 HC), were included in the main analysis. A detailed overview about the study selection process is given in Figure 1.

Two studies[18,19] were excluded from all analyses. In one of these studies[18] sensitivity and specificity values were only given according to ROC analysis and no TP/FN/FP/TN values were reported. In the other study[19] there was no control group for non-synucleinopathies.

For the main analysis of the diagnostic value of αSyn-SAAs in the differential diagnosis of synucleinopathies versus non-synucleinopathies two studies[20,21] were excluded as the subjects overlapped with other studies[22,23] and the sample size was smaller. An additional study[9] was excluded as CSF samples were obtained from the same longitudinal cohort used in another study[24] and an overlap of subjects could not be excluded.

Study Characteristics

The criteria used for the clinical diagnosis of PD, DLB, PSP, and AD were not consistent between the studies: For PD diagnosis, the UK Brain Bank diagnostic criteria[25] were used in ten studies,[9,13,18,20,22,24,26–29] the MDS clinical diagnostic criteria[30] were used in eight studies,[10,19,21,23,31–34] the diagnostic criteria of the National Institute of Neurological Disorders and Stroke (NINDS)[35] were used in one study[36] and three studies[37–39] did not explicitly state which criteria for PD diagnosis were used. One study[26] used the CSF from PD patients enrolled in the NINDS Parkinson's Disease Biomarker Program, in which diagnostic criteria were only explicitly mentioned for one site.

For DLB diagnosis, two studies[10,36] used the third report of the DLB consortium[40] and ten studies[19,21,23,28,32,33,38,39,41,42] used the fourth report of the DLB consortium.[43] Two studies[13,31] did not explicitly state which diagnostic criteria for DLB were used.

For the clinical diagnosis of PSP, five studies[21,23,31,34,42] used the MDS criteria,[44] two studies[36,39] used the criteria proposed by the NINDS and Society for PSP (SPSP) international workshop,[45] and two studies[10,26] did not explicitly state diagnostic criteria for PSP.

For the clinical diagnosis of AD, three studies[10,41,42] used the National Institute on Aging and Alzheimer's Association (NIA-AA) 2011 criteria,[46] one study[23] used the International Working Group (IWG)-2 criteria,[47] one study[39] used the CSF "Alzheimer profile" proposed by Duits et al.[48] and two studies[13,32] did not explicitly state the diagnostic criteria used.

For the clinical diagnosis of MSA, all studies[13,19,21,23,24,29,34,36,38,39,41] except one[31] used the second consensus statement.[49] The latter[31] did not explicitly report which diagnostic criteria were used.

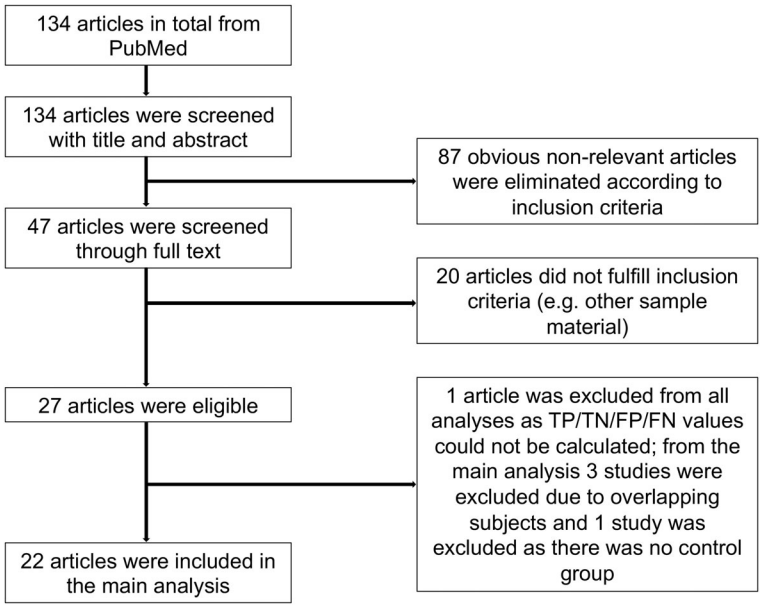

Figure 1. Flowchart for the identification of included studies.

For the clinical diagnosis of CBD, all studies[21,23,31,34,42] used the Armstrong et al. criteria.[50]

One study[51] included patients with MCI in their cohort and classified them into three groups: MCI-LB, MCI-AD, and unspecified MCI. MCI-LB was diagnosed when the current criteria for probable MCI-LB[52] were fulfilled and MCI-AD was diagnosed when in vivo evidence of AD pathology was present.[47,48,53]

The clinical diagnosis of RBD was confirmed by polysomnography in two studies[9,32] and explicitly established according to the International classification of sleep disorders-third edition (ICSD3)[54] in three studies.[21,23,24]

Seven studies[9,10,23,33,41,55,56] included a cohort with neuropathologically confirmed diagnoses as well, in which pathologies with incidental LBs could also be detected.

RT-QuIC was used as Syn-SAA in 22 studies[9,10,20–24,26–28,31,33,34,36,37,39,41,42,51,55,57] and PMCA was used in five studies.[13,18,19,29,38] One study[56] used a quantitative RT-QuIC approach.

Details about TP/TN/FP/FN values, sensitivity, specificity and cut-off values to be considered as positive samples in each included study are given in Table 1. Table S1 gives a detailed overview about patient and control groups in each of the eligible studies.

Quality Assessment

Quality assessment results based on the QUADAS-2 tool[14] are represented in Figure 2. The overall risk of bias concerning the reference standard and index test was low, whereas for flow and timing the risk of bias was rated as high or unclear in most of the eligible studies. Overall, concerns regarding applicability were low in the majority of eligible studies.

Meta-Analysis

The analysis of this systematic review showed a pooled sensitivity and specificity of αSyn-SAAs, including RT-QuIC and PMCA, for differentiating synucleinopathies from non-synucleinopathies of 0.88 (95% CI, 0.82–0.93) and 0.95 (95% CI, 0.92–0.97), respectively (Fig. 3). Figure 4 represents the HSROC for this analysis.

Furthermore, we performed several subgroup-analyses to assess the diagnostic value of αSyn-SAAs for synucleinopathies with LB pathology. The pooled sensitivity and specificity were 0.91 (95% CI, 0.87–0.95) and 0.96 (95% CI, 0.93–0.98), respectively, for differentiating synucleinopathies with LBs from non-synucleinopathies. Considering only patients with established PD or DLB according to diagnostic criteria, the pooled sensitivity showed a slight increase to 0.92 (95% CI, 0.88–0.95). The pooled specificity for differentiating synucleinopathies with LBs from HC and from PSP/CBD was 0.97 (95% CI, 0.92–0.99) and 0.94 (95% CI, 0.79–0.98), respectively.

Additionally, the pooled sensitivity and specificity of αSyn-SAAs was evaluated for the differential diagnosis of MSA versus non-synucleinopathies. The pooled sensitivity and specificity were 0.57 (95% CI, 0.26–0.83) and 0.96 (95% CI, 0.91–0.99), respectively. The pooled sensitivity decreased to 0.30 (95% CI, 0.11–0.59) when only studies using RT-QuIC as seeding method to differentiate MSA from non-synucleinopathies were included. For the differentiation of MSA from PSP/CBD

TABLE 1 *Diagnostic results and methodological aspects for all included studies in the analysis of synucleinopathies versus non-synucleinopathies*

Author	TP	FN	FP	TN	Sens	Spec	Assay	Cut-off value
Groveman et al. 2018	27	2	0	31	0.93	1.00	RT-QuIC	Mean of all samples +3SD
Manne et al. 2019	15	0	2	14	1.00	0.88	RT-QuIC	Mean of all samples +10SD
Garrido et al. 2019	18	23	2	8	0.44	0.80	RT-QuIC	Mean of negative controls +2SD
Van Rumund et al. 2019	62	21	5	72	0.75	0.94	RT-QuIC	Mean of negative controls +2SD
Bongianni et al. 2019	43	11	2	57	0.80	0.97	RT-QuIC	Mean of all samples +3SD
Rossi et al. 2020	166	39	10	224	0.81	0.96	RT-QuIC	Mean of neuropathological controls +30SD
Orru et al. 2020	105	3	11	74	0.97	0.87	RT-QuIC	10% of maximum value
Donadio et al. 2021	6	2	0	26	0.75	1.00	RT-QuIC	Mean of negative controls +3SD
Iranzo et al. 2021	47	5	4	47	0.90	0.92	RT-QuIC	Mean of negative controls +2SD
Bargar et al. 2021	143	3	0	68	0.98	1.00	RT-QuIC	Mean background fluorescence +5SD
Rossi et al. 2021	77	4	20	188	0.95	0.90	RT-QuIC	Mean of negative controls +30SD
Russo et al. 2021	24	4	1	29	0.86	0.97	RT-QuIC	10% of maximum value
Brockmann et al. 2021	244	54	2	24	0.82	0.92	RT-QuIC	Mean of negative controls +30SD
Poggiolini et al. 2021	113	30	2	53	0.79	0.96	RT-QuIC	Mean of initial fluorescence at 120 h + 5SD
Mammana et al. 2021	22	1	0	57	0.96	1.00	RT-QuIC	15% of maximum value
Perra et al. 2021	16	0	3	29	1.00	0.91	RT-QuIC	Mean fluorescence during initial 10 h + 10SD
Sokratian et al. 2021	27	4	0	14	0.87	1.00	qRT-QuIC	>200 FFUs/ml
Compta et al. 2022	19	38	4	51	0.33	0.93	RT-QuIC	Mean of negative controls +2SD
Hall et al. 2022	68	13	13	49	0.84	0.79	RT-QuIC	10% of maximum value
Shahnawaz et al. 2017	85	11	12	85	0.89	0.88	PMCA	≥50 FU
Singer et al. 2020	87	4	0	29	0.96	1.00	PMCA	≥150 AU
Shahnawaz et al. 2020	153	16	0	56	0.91	1.00	PMCA	≥50 FU

Abbreviations: SD, standard deviation; WT, wild type; FFUs, fibril forming units; FU, fluorescence units; AU, arbitrary units.

αSyn-SAAs yielded a pooled sensitivity and specificity of 0.18 (95% CI, 0.08–0.37) and 0.90 (95% CI, 0.70–0.97), respectively.

For the differentiation of patients with prodromal signs of synucleinopathies from non-synucleinopathies αSyn-SAAs yielded a pooled sensitivity and specificity of 0.74 (95% CI, 0.36–0.93) and 0.93 (95% CI, 0.89–0.96), respectively. The diagnostic performance of αSyn-SAAs for patients with RBD showed sensitivity rates of 0.64 (95% CI, 0.50–0.77),[24] 0.80 (95% CI, 0.58–0.92)[32] and 1.00 (95% CI, 0.82–1.00).[23] Pooled sensitivity and specificity rates could not be computed for this analysis due to the small number of included studies.

Forest plots for all analyses can be found in the supplemental material (Data S1).

Discussion

In this systematic review and meta-analysis, our aim was to systematically evaluate the diagnostic accuracy of αSyn-SAAs, including RT-QuIC and PMCA, for the differential diagnosis of synucleinopathies versus non-synucleinopathies. Furthermore, we performed several subgroup analyses to address pertinent research questions.

Overall, our meta-analysis showed a pooled sensitivity and specificity of 0.88 (95% CI, 0.82–0.93) and 0.95 (95% CI, 0.92–0.97), respectively, to distinguish synucleinopathies from non-synucleinopathies. There is one previous meta-analysis[58] assessing the performance of RT-QuIC in the diagnosis of LBDs reporting a pooled sensitivity and specificity of 0.91 and 0.95, respectively, which is similar to the results of our analysis. Extending the previous meta-analysis,[58] we have also included studies using PMCA methodology and our study also differs in further methodological aspects: (1) MSA patients were included in the patient group in our main analysis, as α-synuclein aggregates in the form of oligodendroglial cytoplasmic inclusions represent the histopathological hallmark of the disease[1]; (2) Non-manifesting carriers of genetic mutations associated with synuclein pathology known to cause PD and patients with MCI-LB were also included as they represent prodromal disease stages, in which diagnostic utility of αSyn-SAAs may be of particular relevance for future clinical trials and clinical practice; (3) For the main

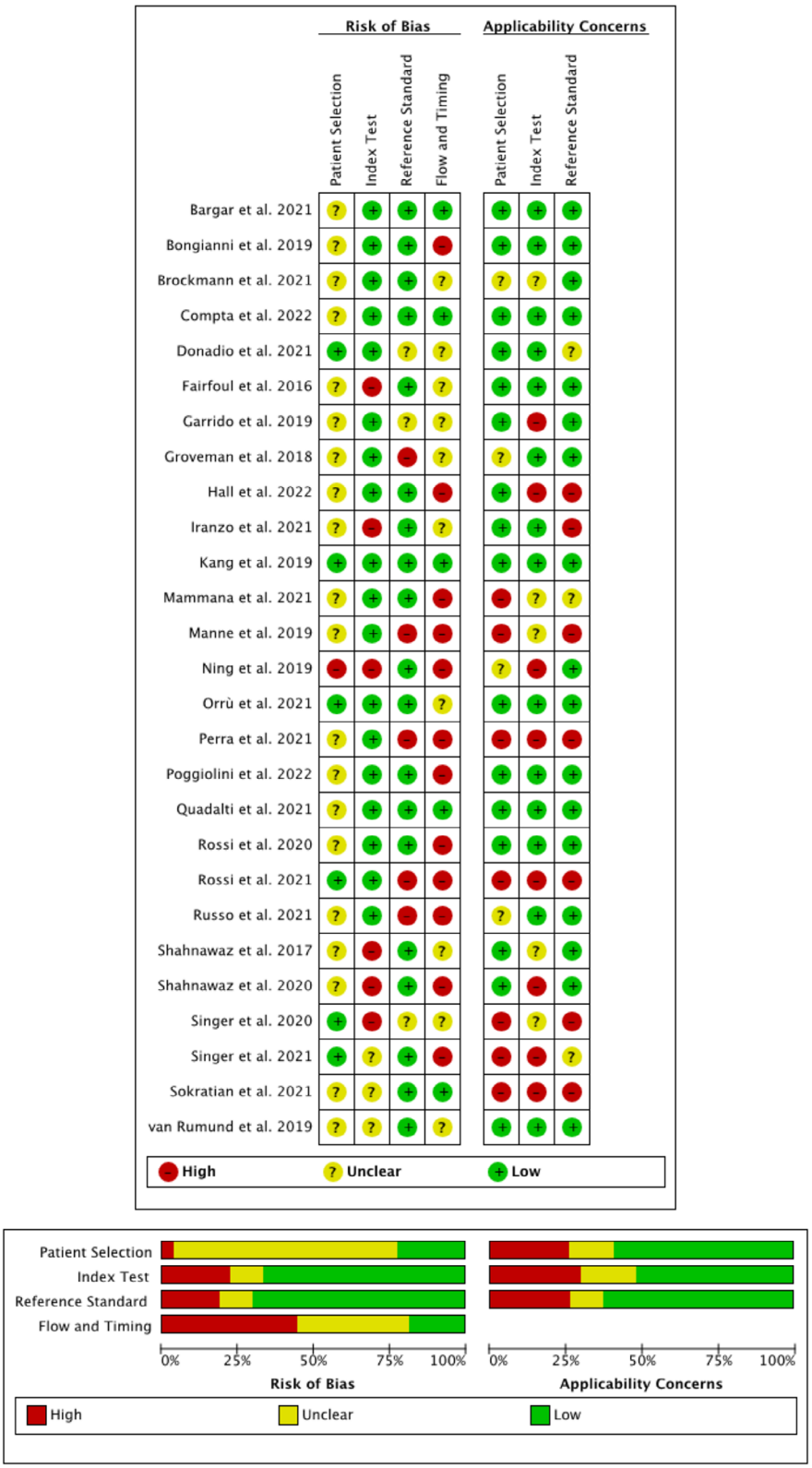

Figure 2. Quality assessment of all eligible studies based on QUADAS-2.

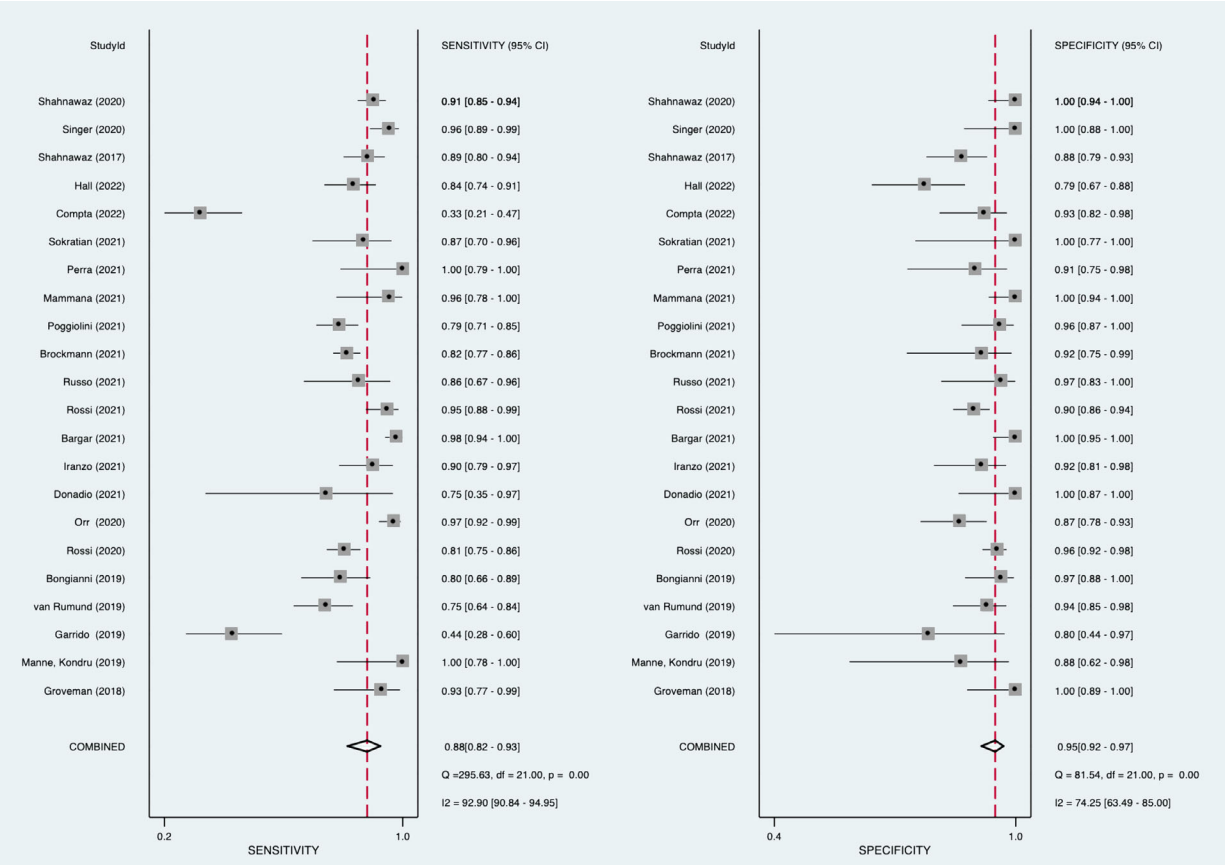

Figure 3. Forest plot of sensitivity and specificity of αSyn-SAAs for the diagnosis of synucleinopathies versus non-synucleinopathies.

analysis of the diagnostic accuracy of αSyn-SAAs in the differential diagnosis of synucleinopathies versus non-synucleinopathies, we included four more studies[34,36,51,56] using RT-QuIC, and three more studies[13,29,38] using PMCA, while we excluded one study[9] of the former meta-analysis because subjects were from the same longitudinal cohort reported in another study[24]; (4) We performed several subgroup-analyses focusing on different patient cohorts to generate more profound data on the diagnostic utility of RT-QuIC and PMCA in specific clinical settings.

Regarding the quality of included studies the overall risk of bias was adequate, only for flow and timing the risk was rated as high in a substantial number of eligible studies (see Fig. 2). Concerns regarding applicability were particularly high or unclear in seven eligible studies.[19,26,33,38,42,51,56] Regarding the risk of bias in this tool the categories "reference standard" and "index test" refer to the conduction and interpretation of the tests. Knowledge of the results of one out of the index or reference test may influence interpretation of the other test, hence, a blinded rating is preferred. "Patient selection" refers to the recruitment of included patients, for example whether they were enrolled consecutively or whether a random sample was chosen. "Flow and timing" can introduce a risk of bias when for example the interval between the index test and reference standard is inappropriate or when patients are excluded. This is particularly important since improvement or deterioration of the condition (eg, disease progression or symptom alleviation through symptomatic therapies) may cause misclassification and thereby impacts the diagnostic accuracy. Concerns regarding applicability address the question, whether the patient selection, index test and reference standard match the specific research question. Moreover, diagnostic criteria used as a reference standard for the clinical diagnosis of PD, DLB, PSP, and AD were not consistent between the studies, which yields the risk of misdiagnosis in some cases. This aspect needs be taken into consideration when interpreting the obtained values for the overall sensitivity and specificity in each analysis. Particularly, post-mortem verification of the clinical diagnosis of a synucleinopathy was only obtained in a minority of patients in a few studies.[9,10,23,33,41,55,56] Cut-off values for samples to be considered as positive were also not the same across included studies, which should be consistently defined in future harmonized protocols. Indeed, standardization of diagnostic and pre-analytical procedures are vital for the establishment of uniform cut-off values.

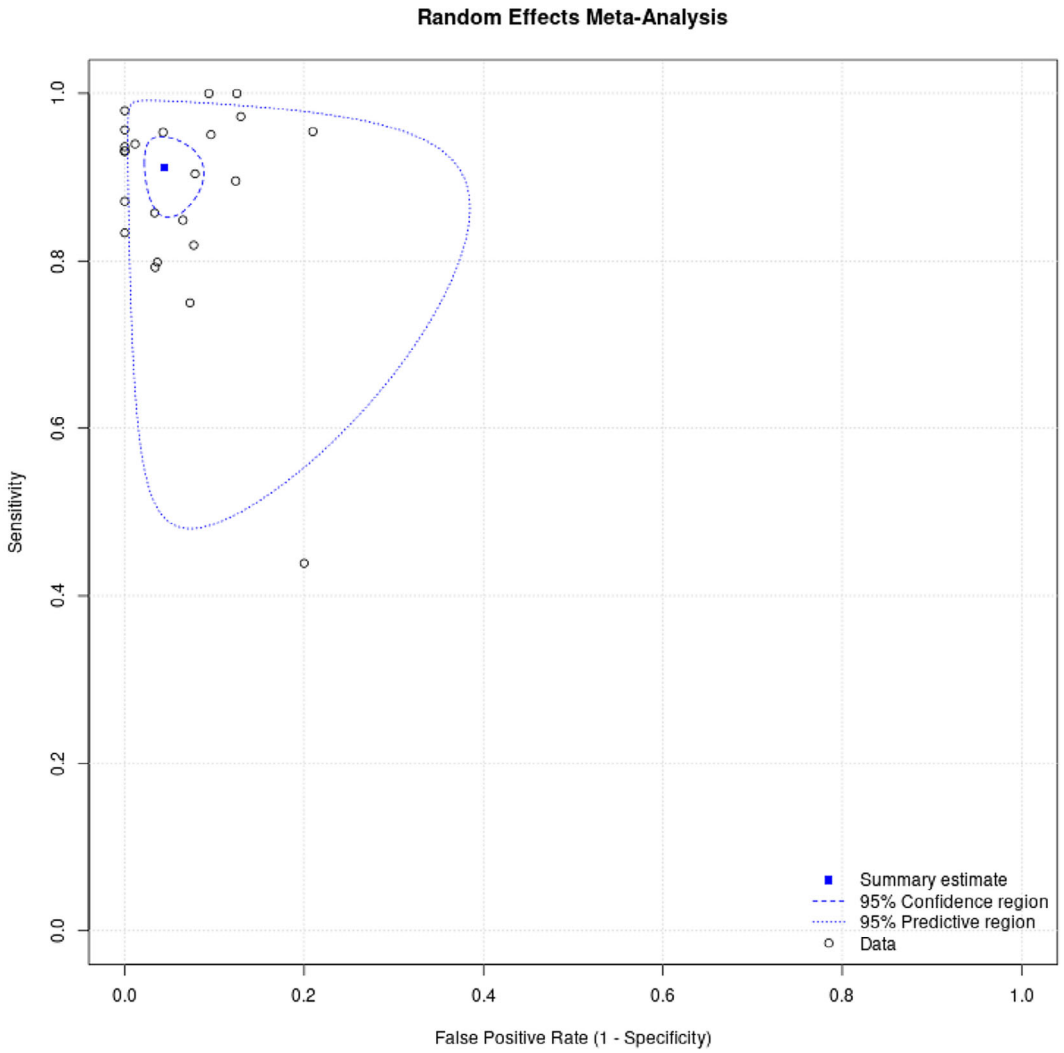

Figure 4. Summary receiver operating characteristic curve of αSyn-SAAs for the diagnosis of synucleinopathies versus non-synucleinopathies.

The results of the present and of the previous meta-analysis[58] demonstrate that αSyn-SAAs represent a highly sensitive method for the diagnosis of synucleinopathies and support their usefulness as a diagnostic biomarker in clinical routine work-up. The pooled specificity for differentiating synucleinopathies with LBs from PSP/CBD was high as well with 0.94 (95% CI, 0.79–0.98), although sample sizes for PSP/CBD were rather small in most of the included studies.

In the analysis of synucleinopathies versus non-synucleinopathies, two studies[27,34] showed markedly lower sensitivity rates with 0.44 (95% CI, 0.28–0.60) and 0.33 (95% CI 0.21–0.47), respectively. In one study,[27] non-manifesting carriers of the p.G2019S mutation in the LRRK2 gene were negative for α-synuclein aggregates by RT-QuIC and only 40% of manifest LRRK2-PD patients presented with a positive RT-QuIC result. This is consistent with the pleiotropic pathology associated with the p.G2019S mutation,[59,60] which may lack α-synuclein pathology.[61–63]

One study in MSA patients,[34] also reported a high proportion of patients presenting with negative CSF α-synuclein RT-QuIC results. Overall, CSF αSyn-SAAs have shown inconsistent results in MSA so far[21,31,34,36] with detection rates ranging from <10% to 35%, except for one study[24] demonstrating a sensitivity of 75% for MSA. Two studies[39,41] only included a very small number of MSA patients, hence results are difficult to interpret.

Studies using PMCA as seed amplification assay for α-synuclein in MSA patients showed better diagnostic performance with sensitivities of 80% to 97%.[13,19,29,38] The pooled overall sensitivity for RT-QuIC in the diagnosis of MSA was estimated to be 0.30 (95% CI, 0.11–0.59) in our analysis. When studies using PMCA were included, the pooled sensitivity

increased to 0.57 (95% CI, 0.26–0.83). A possible explanation for this observed discrepancy could be the use of different reaction buffers and structural differences in α-synuclein strains of MSA patients as described in one study.[64] Overall, MSA patients showed markedly lower maximum fluorescence values compared to PD patients in the PMCA assay.[13,19,29,38] Notably, differences in Thioflavin T (ThT) fluorescence do not seem to simply reflect different amounts of misfolded aggregates at the end of the reaction, but may rather be due to structural differences of α-synuclein aggregates ('strains') in PD and MSA patients, leading to differences in the accessibility of the mode of interaction of ThT with aggregates.[29] By investigating different thiophene-based ligands, it was shown that different ligands prefer binding to either PD or MSA α-synuclein aggregates.[29] These findings illustrate a limitation of the current clinical applicability of RT-QuIC and further research is required to better understand the discrepancies between RT-QuIC and PMCA as well as to improve the differential diagnostic yield of these tests.

Intriguingly, one study[65] using olfactory mucosa as seeding material for RT-QuIC reported a totally different behavior of MSA from the parkinsonian subtype (MSA-P) and MSA from the cerebellar subtype (MSA-C) with the latter being almost unresponsive to RT-QuIC, while 90% of MSA-P samples showed a positive seeding activity. The authors of this study[65] suggest that this again may be due to differences in α-synuclein strains possessing different tropism for peripheral tissues.

Moreover, two studies[10,55] included in our meta-analysis suggest a distinct pattern of RT-QuIC fluorescence kinetics between CSF samples from PD and DLB patients, with PD samples showing a slower seeding reactivity with a lower maximum ThT fluorescence value. Again, variants in strains of α-synuclein between PD and DLB patients were mentioned as a possible explanation for the observed differences in RT-QuIC kinetics.[10]

As MSA and PSP/CBD can be difficult to discriminate in the early disease stages, we assessed the diagnostic performance of αSyn-SAAs to differentiate between these two types of APD as well. Only studies using RT-QuIC could be included in this analysis and showed a pooled sensitivity of 0.18 (95% CI, 0.08–0.37). Therefore, our results do not support the use of this assay to differentiate MSA from PSP/CBD.

The time required for PMCA is significantly longer with 13–15 days[13,19] instead of 1–5 days for RT-QuIC, depending on the protocol used,[9,10] which is a factor to be considered in future clinical routine use.

More recently, α-synuclein RT-QuIC has also been tested in sample materials other than CSF, including skin homogenates,[33,39,66,67] olfactory mucosa,[42,65,68,69] submandibular glands,[70] and most recently also neuronally derived exososomes in peripheral blood.[71] Generally, results have been encouraging and less invasive sampling techniques for α-Syn-SAAs like skin biopsies, nasal swabs or, ultimately blood may be preferable over CSF sampling in future clinical routine.

The present results also demonstrate that αSyn-SAAs may be able to detect prodromal stages of synucleinopthies and differentiate this patient group from non-synucleinopathies with a pooled sensitivity and specificity of 0.74 (95% CI, 0.36–0.93) and 0.93 (95% CI, 0.89–0.96). respectively. In patients with RBD sensitivity rates ranged from 0.64–1.00.[23,24,32] The use of αSyn-SAAs might have significant implications in the clinical diagnosis of prodromal synucleinopathies, including prodromal PD, and in designing future clinical trials with disease-modifiying therapies, which should target patients at pre-clinical stages of the disease. However, with seven studies[23,24,27,28,32,39,51] the number included in our analysis is rather small. Therefore, more studies with larger numbers of this patient group and a longitudinal follow-up to assess conversion into a synucleinopathy are needed.

Conclusion

Our systematic review and meta-analysis clearly demonstrated a high diagnostic performance of CSF α-synuclein RT-QuIC and PMCA for differentiating synucleinopathies with LBs from non-synucleinopathies, which should be considered when framing supporting criteria for the clinical diagnosis of PD or DLB. Results in MSA have so far been inconsistent and it is unclear, whether differences exist between performance in MSA-P versus MSA-C, which clearly needs further investigation. Furthermore, our analysis provides evidence for the potential usefulness of CSF αSyn-SAAs in identifying prodromal stages of synucleinopathies. Importantly, alternative and less invasive sampling approaches for these assays have provided encouraging results that may suggest non-inferiority to CSF tests. Ultimately, α-synuclein SAAs on peripheral blood may become the future diagnostic tool of choice for the identification of synucleinopathies.

Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution. (2) Statistical analysis: A. Design, B. Execution, C. Review and Critique. (3) Manuscript preparation: A. Writing of the first draft, B. Review and Critique.

A.G.: 1A, 1B, 1C, 2A, 3A
G.H.: 1C, 3B
F.K.: 2A, 2B, 3B
M.P.: 1C, 2C, 3B
A.D.: 1A, 2C, 3B
W.P.: 1A, 2C, 3B
K.S.: 1A, 1B, 2A, 2C, 3B
B.H.: 1A, 1B, 1C, 2A, 2B, 3B

Disclosures

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Supporting Information

Supporting information may be found in the online version of this article.

Table S1. Detailed overview about patient and control groups in each of the eligible studies

Data S1. Forest plots for all subgroup analyses