Sensitivity and Specificity of the Diagnostic Method for Detecting α-Synuclein as a Histological Marker for Parkinson's Disease in Salivary Gland Tissues: a Systematic Review and Meta-analysis

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Abstract

Immunohistochemistry of α-synuclein (α-syn), a marker for Parkinson's disease, in salivary gland (SG) biopsy specimens has been actively studied as a method of verification and early diagnosis. This systematic review and meta-analysis ***aim*** *to analyze characteristics of study designs and evaluate pooled sensitivity and specificity.*

The review included publications that were found by keyword search and met inclusion criteria. The meta-analysis of comparative studies was conducted using a univariate random-effects model to calculate pooled specificity and sensitivity.

The systematic review and meta-analysis included 16 and 13 clinical studies, respectively. Antibodies against modified α-syn, double detection, and incisional biopsy specimens of SGs were the most common approaches used in the studies. There is a need for clinical studies with quantitative data analysis. Approximately 15% of patients experienced adverse events, which were more common in case of fine-needle aspiration biopsy specimens of SGs. Pooled sensitivity and specificity (regardless of the anti-α-syn antibody type and SG size) were 76.6% and 98.0%, respectively. Sensitivity (76.3%) and specificity (99.3%) were higher when antibodies against phosphorylated α-syn and major SGs were used.

The most promising variant of the method involved double detection using antibodies against modified α-syn and markers of nerve fibers in incisional biopsy specimens of major SGs and quantitative data analysis. The meta-analysis revealed a possibility of developing this diagnostic method and implementing it into routine practice owing to its high sensitivity and specificity. Further studies employing quantitative data analysis are required to gain deeper insight into the method's role in verifying Parkinson's disease and informing the severity of neurodegeneration and disease prognosis.

Keywords: *Parkinson's disease; salivary gland biopsy; immunohistochemistry; α-synuclein; meta-analysis; systematic review*

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Анализ чувствительности и специфичности метода детекции α-синуклеина в ткани слюнных желёз в качестве диагностического гистологического маркера болезни Паркинсона: систематический обзор и метаанализ

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Аннотация

Иммуногистохимическое исследование маркера болезни Паркинсона α-синуклеина (α-syn) в биоптатах слюнных желёз (СЖ) – один из активно изучаемых методов верификации и ранней диагностики заболевания. ***Цель*** *систематического обзора и метаанализа – проанализировать особенности дизайнов клинических исследований (КИ) и оценить объединённую чувствительность и специфичность метода.*

В обзор включались публикации, найденные по заданным ключевым словам и соответствующие критериям включения. Метаанализ проводился только для сравнительных КИ с использованием универсантной модели случайных эффектов с целью вычисления объединённой специфичности и чувствительности.

В систематический обзор включены 16 КИ, в метаанализ – 13 КИ. Наиболее часто в КИ использовали антитела (АТ) к модифицированному α-syn и двойную детекцию, а также инцизионные биоптаты СЖ. Выявлена необходимость проведения КИ с количественной оценкой результатов. Доля пациентов с нежелательными явлениями составила около 15%, они чаще отмечались при использовании тонкоигольной биопсии СЖ. Объединённая чувствительность и специфичность метода (без учёта вида АТ к α-syn и размера СЖ) составили 76,6 и 98,0% соответственно. При использовании АТ только к фосфорилированному α-syn и крупных СЖ показаны бóльшие чувствительность (76,3%) и специфичность (99,3%).

Наиболее перспективным вариантом методики является двойная детекция с АТ к модифицированному α-syn и маркерам нервных волокон в инцизионном материале крупных СЖ с количественной оценкой результатов. Метаанализ продемонстрировал возможность развития и внедрения метода в клинику как диагностического из-за его высокой чувствительности и специфичности. Необходимы дальнейшие КИ с количественной оценкой для получения полного представления о значимости метода не только для верификации болезни Паркинсона, но и для получения представлений о выраженности нейродегенеративного процесса и прогнозе течения заболевания.

Ключевые слова: *болезнь Паркинсона; биопсия слюнной железы; иммуногистохимия; α-синуклеин; метаанализ; систематический обзор*

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Introduction

Postmortem histology with α-synuclein (α-syn) detection in the substantia nigra is the gold standard in diagnosing Parkinson's disease (PD) [1]. The α-synuclein protein is predominantly expressed in the nervous system and located in presynaptic terminals, where it is involved in vesicle transport, regulation of dopamine release, and intracellular calcium homeostasis [2].

Exogenous and endogenous factors that trigger α-syn modification and aggregation, as well as molecular processes directly causing accumulation of α-syn aggregates have yet to be elucidated. To date, there have only been assumptions about potential effects of polymorphisms in risk genes, chemicals, physical forces (eg, brain injuries), and radiation on the activation of oxidative stress and neuroinflammation, inducing α-syn aggregation [3]. Modified (phosphorylated or nitrosylated) α-syn disrupts intracellular transport and neurotransmission, increases oxidative stress due to mitochondrial dysfunction, and causes microglial activation. These processes underlie neuronal dysfunction, which results in clinical manifestations of PD as the α-syn aggregates continue to accumulate [4]. Thus, despite a large number of unexplored factors and processes contributing to the PD pathogenesis, a key role in PD development was attributed to modified α-syn that can be used as a histological marker for PD [4, 5].

More than 20 years ago H. Braak et al. hypothesized the gut-brain axis in PD and formation of α-syn aggregates in the peripheral nervous system long before their appearance in the substantia nigra and typical motor symptoms of PD [6]. Clinical studies showed that such aggregates are detected by histological evaluation of specimens not only of the brain but also of other organs with abundant peripheral innervation, which explains non-motor symptoms of PD that are associated with peripheral nervous system dysfunction and tend to predate motor impairments [5, 7]. Findings of these studies suggest a possibility of antemortem histological diagnosis of sporadic PD by detecting its main marker, pathological α-syn, in biopsy specimens of skin, intestine, and salivary glands (SG) [5, 8, 9].

A systematic review of English-language articles came to the conclusion that biopsy and histology of the skin and SGs could be potential diagnostic tools in PD, whereas intestinal biopsy and histology were excluded due to the low rate of aggregate detection, safety concerns, and high complexity of sampling [9]. However, other researchers highlight a number of limitations to skin biopsy as a diagnostic tool [10, 11]. A relatively high rate of detecting α-syn aggregates during skin examinations is highly dependent on sampling sites and requires examining multiple biopsy sites to obtain adequate results. Furthermore, most skin examinations use a frozen section technique, a less common and accessible laboratory procedure worldwide compared with paraffin sections [10].

Some researchers believe that main issues of using SG specimens for α-syn detection can be linked to frequent sampling of surrounding tissues (muscles, adipose tissue, lymph nodes) in addition to SG tissues [9]. It should be noted that the mentioned drawbacks are associated with fine-needle aspiration biopsy (FNAB) of minor SGs, which is a minimally invasive procedure that obtains only a small volume of tissue (7–34 mm^3 according to the studies) [12, 13]. However, utilization of major SGs and incisional biopsy (IB), which obtains an SG tissue volume of 84–390 mm^3 via a small (< 1 cm) incision, significantly mitigates the issue of insufficient study material [14–17]. Currently, there are no Russian meta-analyses of studies examining characteristics of various immunohistochemistry (IHC) techniques for SG biopsy specimens and safety data. The only similar meta-analysis was conducted by foreign researchers approximately 5 years ago and did not include Russian studies [9].

Our systematic review and meta-analysis of Russian and foreign clinical studies **aimed** to evaluate pooled sensitivity and specificity of IHC in terms of detecting modified α-syn in SG tissues. Furthermore, we assessed safety and analyzed characteristics of study designs to determine the most promising technique.

Methodology

This systematic review included all clinical studies (in English and Russian) on α-syn detection in SG tissues of patients with verified PD (both antemortem and postmortem). PubMed and Google Scholar were searched using the following keywords: "болезнь Паркинсона," "биопсия," "слюнная железа," "синуклеин," "Parkinson's disease," "biopsy," "salivary gland," and "synuclein". We did not consider articles without full-text access due to the inability to analyze study designs and findings. We did not include duplicate publications and articles concerning only histological examination of SG biopsy specimens in PD patients that did not have statistical analysis or data analysis. For the meta-analysis we pooled articles that met the above criteria and selected comparative studies involving PD patients and controls.

We took into account data on the number of participants and their diagnoses, methods of diagnosis verification, use of various tests to assess the condition of PD patients, studied specimens and sampling techniques, IHC markers, data analysis (qualitative/semiquantitative/quantitative), number and characteristics of adverse events (AE) caused by an intervention, as well as data to evaluate sensitivity and specificity. We disregarded data concerning α-syn aggregates in specimens of patients with other neurodegenerative disorders to calculate pooled sensitivity and specificity because such patients could not be assigned either to a control group (due to the high likelihood of α-syn detection in their specimens compared with healthy volunteers) or a group of PD patients.

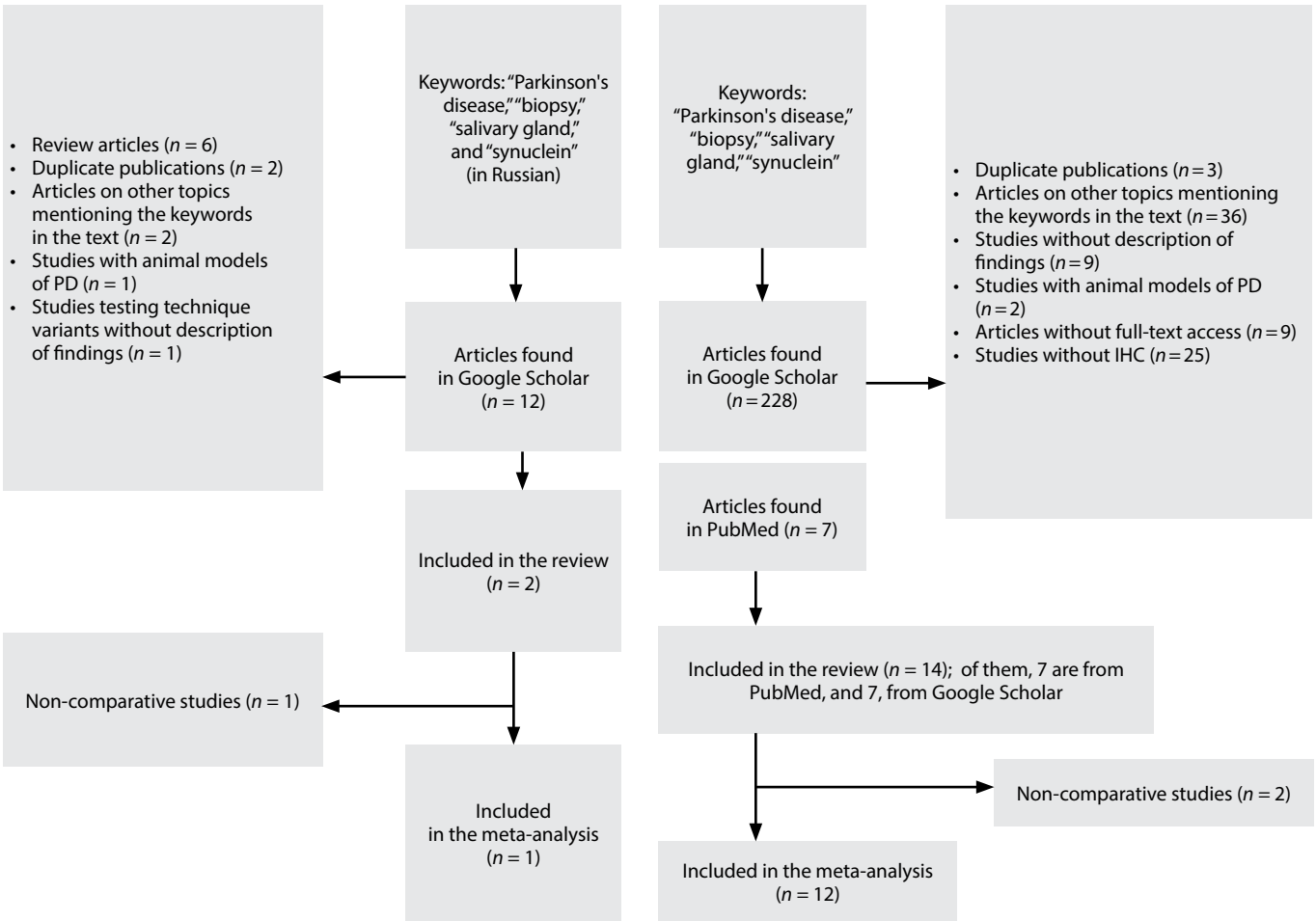

Fig. 1. Study selection for systematic review and meta-analysis.

Statistical analysis

Statistical analysis and data processing were performed using the Python programming language, SciPy module[1], and MetaDiSc 2.0, a software to perform test accuracy meta-analysis [18]. We calculated medians, interquartile ranges (IQR), and percentages. We used a univariate random-effects model, which is suitable for analyzing a small number of heterogeneous studies, to calculate pooled specificity and sensitivity, diagnostic odds ratio (DOR), false positive rate (FPR), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). Heterogeneity was measured using the I^2 test (range from 0% to 100%) that describes the percentage of variability between the studies.

General characteristics of study designs and participants

We analyzed 16 publications that met the inclusion criteria: of them, 14 articles (87.5%) were published in English between 2010 and 2023 and included in PubMed [14–17, 19–28] (Table, Fig. 1). All the studies were open and non-randomized. The comparative studies involving controls who underwent the same procedures as a study group accounted for 81.2% ($n = 13$) [13–15, 17, 19–27]. Their findings were used for 2×2 tables and meta-analysis of sensitivity and specificity. The findings of non-comparative studies were used to analyze safety and examine characteristics of different techniques.

The total number of participants in 16 clinical studies was 762. Of them, 712 underwent all examinations, including SG biopsy with subsequent IHC. Clinically verified diagnosis (PD) was made in 288 patients, of whom 260 underwent all examinations. In 15 studies, PD was sporadic (idiopathic), while in one study, it was genetic, which was confirmed by genetic testing. In 6 studies, PD diagnosis was made only by the Movement Disorder Society diagnostic criteria, whereas in 5 studies, by findings of dopamine transporter single-photon emission computed tomography. In 3 studies, patients underwent brain magnetic resonance imaging (MRI) and transcranial sonography of the

[1] Python SciPy scientific computation library. URL: https://scipy.org

Data from the articles meeting the inclusion criteria

No.	Source	Number of patients	Number of patients undergoing biopsy and immunohistochemistry	Number of PD patients undergoing biopsy and immunohistochemistry	PD diagnosis verification (beyond the Movement Disorder Society diagnostic criteria)	Hoehn and Yahr stage	PD etiology	Number of controls undergoing biopsy and IHC	Sampling site	Sampling technique	Ab	Ab for double detection	True-positive result	False-positive result	Data analysis
1	et al. (2011)	6	6	3	NA	2	Idiopathic	3	Minor SGs	IB	α-syn	Neurofilaments	2	0	Scale +/–
2	Adler C.H. et al. (2014)	15	12	12	NA	NA	Idiopathic	NA	Submandibular/ minor SGs	FNAB	pS129-α-syn	NA	9	NA	Scale +/–
3	Adler C.H. et al. (2016)	35	28	19	DAT scan	NA	Idiopathic	9	Submandibular SG	FNAB	pS129-α-syn	NA	14	2	Scale +/–
4	Beach T.G. et al. (2016)	228	228	46	NA	NA	Idiopathic	79	Sublingual SG	IB	pS129-α-syn	NA	42	0	Scale +/–
5	Folgoas E. et al. (2013)	27	27	16	NA	NA	Idiopathic	9	Minor SGs	IB	pS129-α-syn	NA	3	2	0–5 scale
6	Gao L. et al. (2015)	26	26	13	DAT scan	1,7	Idiopathic	13	Minor SGs	IB	α-syn	NA	9	0	Scale +/–
7	Vilas D. et al. (2016)	71	47	12	NA	2	Idiopathic	26	Submandibular SG	FNAB	α-syn/pS129-α-syn	Tyrosine hydroxylase	8	0	Scale +/–
8	Carletti R. et al. (2017)	14	14	7	NA	NA	Idiopathic	7	Minor SGs	IB	α-syn/pS129-α-syn	NA	5	0	Quantitative
9	Shin J. et al. (2019)	16	16	16	NA	1,8	Idiopathic	14	Submandibular SG	FNAB/IB	pS129-α-syn	S100	8	0	Scale +/–
10	Iranzo A. et al. (2018)	118	118	13	Transcranial sonography of the substantia nigra	2,4	Idiopathic	33	Minor SGs	IB	α-syn/pS129-α-syn	Tyrosine-hydroxylase/ PGP 9.5 Iodotyrosine deiodinase	7	1	0–4 scale

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No.	Source	Number of patients	Number of patients undergoing biopsy and immunohistochemistry	Number of PD patients undergoing biopsy and immunohistochemistry	PD diagnosis verification (beyond the Movement Disorder Society diagnostic criteria)	Hoehn and Yahr stage	PD etiology	Number of controls undergoing biopsy and IHC	Sampling site	Sampling technique	Ab	Ab for double detection	True-positive result	False-positive result	Data analysis
11	Fernández-Espejo E. et al. (2021)	80	75	45	DAT scan	2,1	Idiopathic	30	Parotid SG	FNAB	Nitrosylated α-syn	Iodotyrosine deiodinase	45	0	0–5 scale
12	Ma L.-Y. et al. (2019)	26	15	8	DAT scan	1,6	Idiopathic	7	Minor SGs	IB	Nitrosylated α-syn	NA	8	0	Scale +/–
13	Mangone G. et al. (2022)	61	61	27	Magnetic resonance imaging	2	Idiopathic	18	Minor SGs	FNAB	α-syn	NA	15	7	Scale +/–
14	Shin J.H. et al. (2020)	3	3	3	NA	NA	Familial	NA	Minor SGs	FNAB	pS129-α-syn	Neurofilaments	0	NA	0–4 scale
15	Khudoerkov R.M. et al. (2016)	10	10	8	NA	2,1	Idiopathic	2	Major SGs	IB	pS129-α-syn/α-syn	β-Tubulin/ tyrosine hydroxylase	6	1	0–4 scale
16	Khacheva K.K. et al. (2023)	12	12	12	Transcranial sonography of the substantia nigra	2,3	Idiopathic	NA	Sublingual gland	IB	pS129-α-syn	PGP 9,5	12	NA	Quantitative
	Всего	748	698	260	NA	2 [1,85; 2,1]		236							

substantia nigra [22, 25, 28]. Hoehn and Yahr stages of PD were described in 10 (62.5%) publications (median, 2 [IQR, 1.85–2.10]) [17, 21, 23, 24, 28]. The mean PD duration (6.51 ± 3.70 years) was indicated in 15 studies [12, 13, 15, 19–21, 23–28].

Characteristics of immunohistochemistry techniques for assessment of salivary glands specimens

Minor SGs were sampled in a half of the studies [15, 16, 20, 22, 23, 25–27] (Table). Among the studies with major SG specimens, submandibular (4 studies), sublingual (2), and parotid (1) SGs were examined. In one study, the exact sampling site of major SGs was not indicated [12–14, 17, 19, 21, 24, 28].

In 15 (93.7%) studies, antemortem biopsy specimens were used, whereas in 1, postmortem specimens. In 10 studies, SG tissues were sampled by IB; in 6 studies, by FNAB. The publications with FNAB (especially without ultrasound guidance) demonstrated a high likelihood of sampling material unrelated to SGs, thus causing to repeat a procedure or exclude a patient from the study [13, 21]. The small volume of collected tissues usually allowed only to determine the presence of α-syn inclusions, and authors could not fully assess the extent or characteristics of their spread [15, 16, 20, 23, 25, 27]. IB did not have such drawbacks and allowed for sufficient amount of SG specimens.

Two (11%) studies additionally compared findings of SG histology and neuroimaging (MRI and transcranial sonography of the substantia nigra) [26, 28]. Both studies did not find any correlation between the neuroimaging and histology findings.

In 8 studies, motor and non-motor functions of PD patients were additionally assessed by various tests and questionnaires: the Unified Parkinson's Disease Rating Scale, Non-Motor Symptoms Scale, Non-Motor Symptoms Questionnaire, Parkinson's Disease Quality of Life Questionnaire, Montreal Cognitive Assessment, Mini-Mental State Examination, Epworth Sleepiness Scale, and University of Pennsylvania Smell Identification Test [13, 16, 20, 21, 23, 25, 26, 28]. Only one study investigated a potential relationship between clinical symptoms and histology findings and demonstrated a correlation between the spread of inclusions of α-Syn phosphorylated at serine 129 (pS129-α-syn) and severity of non-motor symptoms, sleep disorders, and emotional disorders [28]. This study did not find any correlation with Hoehn and Yahr stages.

In all the studies, IHC examination for various α-syn forms in SG tissues was performed: pS129-α-syn (11 studies; 68.7% of all the studies), nitrosylated α-syn (2 studies; 12.5%), and α-syn without posttranslational modifications (7 studies; 43.7%).

Seven (43.7%) studies used double detection with antibodies (Ab) against α-syn and markers of nerve fibers: tyrosine hydroxylase (3 studies), PGP 9.5 (2 studies), neurofilaments (2 studies), and β-tubulin (1 study) [14, 15, 17, 21, 23, 27, 28]. In all the studies, paraffin-embedded tissues were used for IHC. The studies in which pS129-α-syn was detected used 3 types of Abs: anti-pS129-α-syn mouse monoclonal Abs (WAKO, clone No. pSyn#64; Abcam, clone No. 81A, ab184674) and/or anti-pS129-α-syn rabbit monoclonal Abs (Abcam, clone No. EP1536Y). The studies were characterized by high variability of histology and IHC techniques and Ab clones. The highest number of true-positive results (>70% of positive results in all PD patients) was observed in the studies using double detection and nitrosylated α-syn and pS129-α-syn.

In 5 (31.2%) studies, semiquantitative analysis was used to assess the spread of α-syn inclusions; in 9 (56.2%) studies, qualitative analysis was used, taking into account only the presence or absence of inclusions. Only 2 (12.5%) studies employed quantitative analysis [22, 28]. In the former study, ImageJ software was used to measure the spread, and the authors calculated ratios of pS129-α-syn and unmodified α-syn inclusions to nerve fibers labeled with anti-S100 Abs and to the total specimen area. Calculations revealed that the ratio of unmodified α-syn to nerve fibers was lower in PD patients compared with controls due to nerve fiber degeneration, whereas the presence of pS129-α-syn and its high ratio relative to nerve fibers was characteristic of PD patients only [22]. In the latter study, measurement was performed with a software written in Python 3.9 using the Open CV library for calculations and scikit-image for image processing. The authors calculated the absolute value of the pS129-α-syn inclusion area and the ratio of the inclusion area to the area of nerve fibers labeled with anti-PGP 9.5 Abs. pS129-α-syn was detected in all PD patients, and the ratio directly correlated with the results of the clinical condition assessment and severity of non-motor symptoms [28].

In all the studies, α-syn inclusions in various modifications were detected in SG specimens of PD patients. PD patients with true-positive results (ie, inclusions of modified α-syn) accounted for more than 70% in 8 (50%) studies, 6 of which (46.1%) were comparative [12, 13, 17, 19, 22, 24, 25, 28]. Among these 8 studies, the studies with IB and IHC of major SG specimens accounted for 62.5% and 75%, respectively.

There were 100% of positive results in 3 (18.75%) studies, 2 of which (15% of all comparative studies) were comparative [24, 25, 28]. Among these 3 studies, 2 studies were conducted using major SG specimens [24, 28].

In 5 (31.2%) studies, rare α-syn inclusions were detected in controls without PD. In half of these studies, unmodified α-syn without any phosphorus or nitrogen residues was used as the main marker [13, 17, 20, 23, 26]. Such cases were attributed to potential early (preclinical) PD or defects of IHC staining [13, 17, 22]. Such assumption is supported by the fact that Lewy bodies are found during postmortem examination in approximately 10% to 20% of people older than

60 years without any signs of parkinsonism or dementia [29]. Unmodified α-syn detected in controls (2 studies) is shown to be a relatively common and normal finding in the peripheral nervous system of healthy individuals without neurodegeneration [17, 23].

Accumulation of modified α-syn was observed in some patients with other neurodegenerative disorders. Positive results (presence of pS129-α-syn and nitrosylated α-syn) were characteristic of patients with Alzheimer's disease and dementia with Lewy bodies in 4 studies [16, 21, 23, 26].

In 3 studies, α-syn aggregates were found in 43.8%, 50%, and 89% of patients with idiopathic rapid eye movement sleep behavior disorder [21, 23, 26]. Idiopathic rapid eye movement sleep behavior disorder without other symptoms is considered a prodromal phase of neurodegeneration and eventually progresses into clinically established PD in 80% of cases [21]. Thus, positive results in this group are likely to be caused by an early stage of synucleinopathy.

Safety analysis was based on 15 studies with in vivo assessment of α-syn inclusions in SG specimens. AEs were reported in 4 (26.6%) studies, 3 of which were conducted using FNAB (50% of all studies with FNAB), and 1 of which, using IB (10% of all studies with IB). The total number of patients with AEs was 77 (14.8% of all the participants [$n = 520$] who underwent IB in 15 studies). No severe AEs were observed. The most common AEs were transient edema, minor hemorrhage, and pain at the biopsy site. Moderate throat pain and minor hemorrhage at the biopsy site (in case of FNAB) were less common. All the AEs were transient and mild and resolved without any medical or surgical intervention.

Sensitivity and specificity of immunohistochemistry techniques for α-syn detection in salivary glands

Of 16 clinical studies for the meta-analysis, we selected 13 comparative studies that compared the rates of detecting modified and unmodified α-syn using Abs in minor and major SG specimens of patients with PD and controls without neurodegenerative disorders [13–17, 19–26]. The total number of participants undergoing biopsy was 685; of them, PD was clinically diagnosed in 223 patients.

Pooled sensitivity across the 13 comparative studies with a control group, regardless of anti-α-syn Abs and specimens (i. e., all the studies using minor and major SG specimens), was 0.749 (95% CI, 0.575–0; $I^2 = 52\%$); specificity, 0.984 (95% CI, 0.855–0.999; $I^2 = 0.0$); DOR, 188.33 (95% CI, 15.42–2299.96); FPR, 0.016 (95% CI, 0.001–0.145); PLR, 48.02 (95% CI, 4.6–501.25); NLR, 0.255 (95% CI, 0.14–0.46) (Fig. 2).

Pooled sensitivity across the 8 comparative studies [13, 14, 17, 19–23] using only anti-pS129-α-syn Abs was 0.66 (95% CI, 0.476–0.800; $I^2 = 70.6\%$); specificity, 0.974 (95% CI, 0.840–0.996; $I^2 = 0.0$); DOR, 71.4 (95% CI, 8.81–578.76); FPR, 0.026 (95% CI, 0.004–0.160); PLR, 24.96 (95% CI, 3.67–169.50); NLR, 0.349 (95% CI, 0.211–0.578) (Fig. 3).

Pooled sensitivity across the 5 comparative studies [13, 14, 17, 19, 21] using anti-pS129-α-syn Abs and major SG specimens was 0.761 (95% CI, 0.608–0.993; $I^2 = 55.6\%$); specificity, 0.993 (95% CI, 0.197–1.000; $I^2 = 0.0$); DOR, 460.08 (95% CI, 0.75–281555.50); FPR, 0.007 (95% CI, 0–0.803); PLR, 110.67 (95% CI, 0.196–62405.980); NLR, 0.241 (95% CI, 0.139–0.420) (Fig. 4).

Thus, the highest sensitivity (76.3%) and specificity (99.3%) were observed with major SG specimens and IHC for pS129-α-syn. Nevertheless, even with a lack of detailed description of techniques, biopsy sensitivity and specificity in terms of PD marker detection were 75% and 98.4%, respectively.

Discussion

The systematic review that included 16 studies with 260 patients who underwent SG biopsy and IHC examination of α-syn inclusions (a marker for PD) demonstrated relatively high significance of the method in idiopathic PD diagnosis. The majority of the studies analyzed and compared findings with those of controls. In over 80% of the studies, ICH involved Abs against modified forms of α-syn, whereas 50% of the studies employed the most effective method: double detection. Most studies used only qualitative data analysis. Semiquantitative analysis was less common, whereas quantitative analysis was described only in 2 recent studies. The majority of studies employed standard and effective histological and IHC techniques, which are straightforward to replicate in anatomical pathology laboratories and have no constraints on their extensive utilization. Nevertheless, at the outset of the method's investigation, the absence of quantitative techniques for analyzing the extent of α-syn inclusion spread may impede further research and the integration of the method into clinical practice.

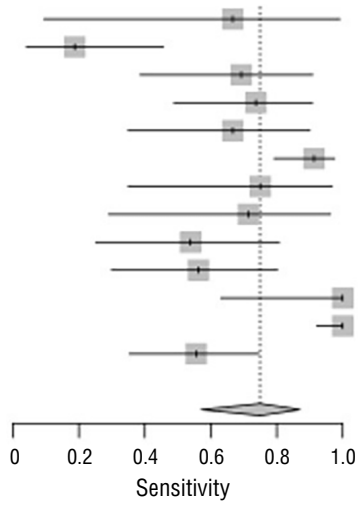
Regardless of qualitative or quantitative data analysis, the IHC effectiveness in PD verification using Abs against modified α-syn was relatively high: true-positive results exceeded 70% in half of all studies with or without controls and in 46% of the comparative studies. In one fifth of all studies, the positive results accounted for 100%.

In vivo biopsy safety was satisfactory: there were approximately 15% of patients with mild AEs across all the studies. AEs were more common in the studies using FNAB than those with IB.

The meta-analysis revealed that the pooled sensitivity and specificity of the method for detecting the PD marker in SG specimens (regardless of the anti-α-syn Abs and SG size) were 76.6% and 98%, respectively. We observed a decrease in sensitivity (66%) and specificity (97%) when analyzing the studies

A

Study	TP	Total (TP + FN)	Sensitivity	95% CI
Cersósimo M.G. et al. (2011)	3		0.67	[0.09; 0.99]
Folgoas E. et al. (2013)	16		0.19	[0.04; 0.46]
Gao L. et al. (2015)	13		0.69	[0.39; 0.91]
Adler C.H. et al. (2016)	19		0.74	[0.49; 0.91]
Vilas D. et al. (2016)	12		0.67	[0.35; 0.90]
Beach T.G. et al. (2016)	46		0.91	[0.79; 0.98]
Khudoerkov R.M. et al. (2016)	8		0.75	[0.35; 0.97]
Carletti R. et al. (2017)	7		0.71	[0.29; 0.96]
Iranzo A. et al. (2018)	13		0.54	[0.25; 0.81]
Shin J. et al. (2019)	16		0.56	[0.30; 0.80]
Ma L.-Y. et al. (2019)	8		1.00	[0.63; 1.00]
Fernández-Espejo E. et al. (2021)	45		1.00	[0.92; 1.00]
Mangone G. et al. (2022)	27		0.56	[0.35; 0.75]
Random-effects model			0.75	[0.57; 0.87]



B

Study	TN	Total (TN + FP)	Specificity	95% CI
Cersósimo M.G. et al. (2011)	3	3	1.00	[0.29; 1.00]
Folgoas E. et al. (2013)	7	9	0.78	[0.40; 0.97]
Gao L. et al. (2015)	13	13	1.00	[0.75; 1.00]
Adler C.H. et al. (2016)	7	9	0.78	[0.40; 0.97]
Vilas D. et al. (2016)	26	26	1.00	[0.87; 1.00]
Beach T.G. et al. (2016)	79	79	1.00	[0.95; 1.00]
Khudoerkov R.M. et al. (2016)	1	2	0.50	[0.01; 0.99]
Carletti R. et al. (2017)	7	7	1.00	[0.59; 1.00]
Iranzo A. et al. (2018)	32	33	0.97	[0.84; 1.00]
Shin J. et al. (2019)	14	14	1.00	[0.77; 1.00]
Ma L.-Y. et al. (2019)	7	7	1.00	[0.59; 1.00]
Fernández-Espejo E. et al. (2021)	30	30	1.00	[0.88; 1.00]
Mangone G. et al. (2022)	11	18	0.61	[0.36; 0.83]
Random-effects model			0.98	[0.87; 1.00]

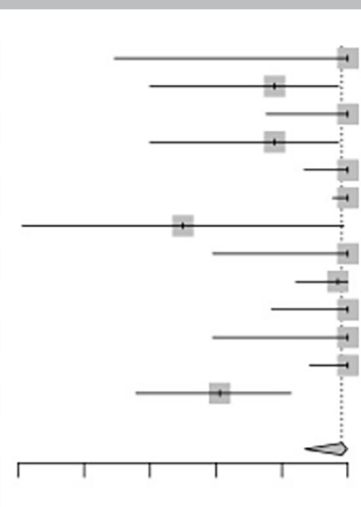
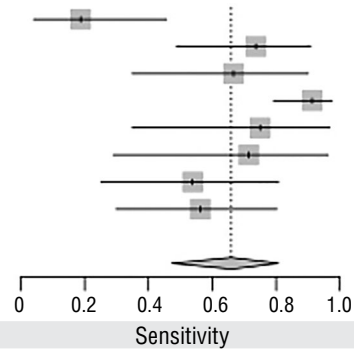


Fig. 2. Pooled sensitivity (*A*) and specificity (*B*) across all the comparative studies for methods of detecting α-syn in SGs of PD patients and controls, regardless of anti-α-synuclein Abs.
Here and in Fig. 3 and 4: TP, true-positive result; TN, true-negative result; FP, false-positive result; FN, false-negative result.

that used anti-pS129-α-syn Abs and did not specify the SG size. The studies that used anti-pS129-α-syn Abs and major SG specimens demonstrated greater sensitivity (76.3%) and specificity (99.3%) compared with the rest. This can be linked to the higher likelihood of detecting aggregates in specimens with more abundant monoaminergic innervation, as well as to significantly higher prevalence of α-syn forms with post-translational modifications among patients with neurodegenerative disorders compared with healthy individuals [31]. The latter conclusion is evidenced not only by the literature data but also by the pooled PLR of 110.6, indicating that likelihood of detecting modified α-syn using biopsy and IHC is 100-fold higher in PD patients than in healthy individuals. The fact that PLR was significantly higher than the generally accepted value of 10 allows us to assume the high diagnostic ability of the method in terms of detecting the studied PD marker. The meta-analysis revealed that the DOR of anti-pS129-α-syn Ab detection in major SG specimens was high (460.08), which suggests the potentially high effectiveness in differentiating participants by the presence or absence of PD.

A

Study	TP	Total (TP + FN)	Sensitivity	95% CI
Folgoas E. et al. (2013)	3	16	0.19	[0.04; 0.46]
Adler C.H. et al. (2016)	14	19	0.74	[0.49; 0.91]
Vilas D. et al. (2016)	8	12	0.67	[0.35; 0.90]
Beach T.G. et al. (2016)	42	46	0.91	[0.79; 0.98]
Khudoerkov R.M. et al. (2016)	6	8	0.75	[0.35; 0.97]
Carletti R. et al. (2017)	5	7	0.71	[0.29; 0.96]
Iranzo A. et al. (2018)	7	13	0.54	[0.25; 0.81]
Shin J. et al. (2019)	9	16	0.56	[0.30; 0.80]
Random-effects model			0.66	[0.47; 0.81]



B

Study	TN	Total (TN + FP)	Specificity	95% CI
Folgoas E. et al. (2013)	7	9	0.78	[0.40; 0.97]
Adler C.H. et al. (2016)	7	9	0.78	[0.40; 0.97]
Vilas D. et al. (2016)	26	26	1.00	[0.87; 1.00]
Beach T.G. et al. (2016)	79	79	1.00	[0.95; 1.00]
Khudoerkov R.M. et al. (2016)	1	2	0.50	[0.01; 0.99]
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Iranzo A. et al. (2018)	32	33	0.97	[0.84; 1.00]
Shin J. et al. (2019)	14	14	1.00	[0.77; 1.00]
Random-effects model			0.97	[0.83; 1.00]

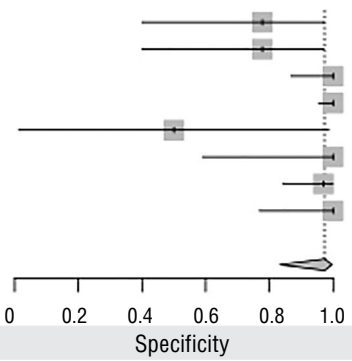


Fig. 3. Pooled sensitivity (*A*) and specificity (*B*) across the comparative studies for methods of detecting α-syn in SGs of PD patients and controls using anti-pS129-α-syn Abs.

It should be noted that there was no significant fluctuation in the pooled specificity values (from 0.974 to 0.993) owing to the sequential exclusion of the studies, depending on the Ab and specimen types.

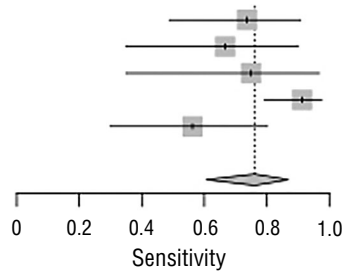
Interpreting sensitivity data with regard to clinical practice, we can conclude that 76% of patients who underwent IHC for pS129-α-syn inclusions in major SG are highly likely to get confirmed diagnosis of PD, previously established by the Movement Disorder Society diagnostic criteria. Based on the specificity data, the likelihood of false-positive results in healthy individuals is only 0.7%. However, we cannot rule out that participants with false-positive results are at risk of developing PD, and detection of modified α-syn inclusions may indicate the onset of neurodegeneration, which apparently starts in the nervous tissue decades before the typical clinical manifestations [32].

Our study is the first Russian meta-analysis, assessing the diagnostic significance of IHC in α-syn detection. The foreign meta-analysis of the English-language articles did not include Russian studies and some early publications with the findings of postmortem IHC examination of SG specimens sampled by IB [9]. Overall, the pooled sensitivity of the 5 comparative studies in our study and the 3 studies in another meta-analysis were comparable in terms of the pooled specificity: 0.99 in our meta-analysis and 0.96 in the 2019 meta-analysis [9]. The difference between the pooled sensitivity values in this study compared with those in the previous meta-analysis was 10.3% (76.3% vs 66%, respectively). The difference is probably due to the slightly larger number of studies in our analysis.

Despite the advantages of this meta-analysis and its value for critical evaluation of the diagnostic IHC, our study has limitations. First, the original studies included in the analysis had small sample sizes and some heterogeneity, which could have to some extent impacted our findings. Second, some publications did not meet the inclusion criteria due to the lack of full-text access, therefore some clinical studies and their results that could impact the sensitivity and specificity were not assessed.

A

Study	TP	Total (TP + FN)	Sensitivity	CI
Adler C.H. et al. (2016)	14		0.74	[0.49; 0.91]
Vilas D. et al. (2016)	8		0.67	[0.35; 0.90]
Khudoerkov R.M. et al. (2016)	6		0.75	[0.35; 0.97]
Beach T.G. et al. (2016)	42		0.91	[0.79; 0.98]
Shin J. et al. (2019)	9		0.56	[0.30; 0.80]
Random-effects model			0.76	[0.61; 0.87]



B

Study	TN	Total (TN + FP)	Specificity	CI
Adler C.H. et al. (2016)	7	9	0.78	[0.40; 0.97]
Vilas D. et al. (2016)	26	26	1.00	[0.87; 1.00]
Khudoerkov R.M. et al. (2016)	1	2	0.50	[0.01; 0.99]
Beach T.G. et al. (2016)	79	79	1.00	[0.95; 1.00]
Shin J. et al. (2019)	14	14	1.00	[0.77; 1.00]
Random-effects model			0.99	[0.99; 0.99]

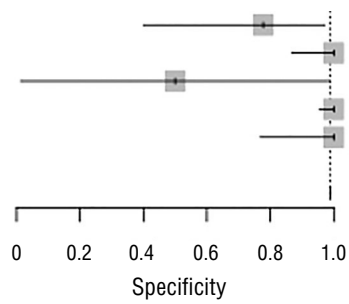


Fig. 4. Pooled sensitivity (*A*) and specificity (*B*) across the comparative studies for methods of detecting α-syn in SGs of PD patients and controls using anti-pS129-α-syn Abs and major SG specimens.

Conclusion

The meta-analysis results demonstrate a possibility of developing a diagnostic method of modified α-syn detection in major SG specimens and its implementation into clinical practice. The sensitivity and specificity were relatively high; however, comparative analysis with other methods of PD diagnosis is required. Further studies with quantitative data analysis are needed to gain greater insight into the method's role in verifying Parkinson's disease and informing of neurodegeneration severity and disease prognosis.

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Authors' contribution. *Khacheva K.K.* – selection of publications for the meta-analysis, participation in the statistical processing, analysis of the results, writing the text of the publication; *Karabanov A.V.* – selection of publications for the meta-analysis, participation in the analysis of the results; *Illarioshkin S.N.* – participation in the analysis of the results, editing the text of the publication. All authors took part in forming the idea of the study and developing the methodology for conducting the meta-analysis.

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