TISSUE BIOMARKERS IN PARKINSON'S DISEASE AND ATYPICAL PARKINSONISM.

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Abstract
Phosphorylated a-synuclein (phosaSYN), the pathological signature of Parkinson's disease (PD), is not confined to the central nervous system, but have also been reported in peripheral tissues. However, the usefulness of aSYN/phosaSYN detection in tissues accessible to biopsies as a reliable biomarker for prodromal PD remains unclear. A systematic review of studies using biopsies of skin, olfactory and gastrointestinal (GI) tissues was conducted to evaluate the sensitivity and specificity of both aSYN and phosaSYN staining in PD and related disorders. In total 128 post-mortem and in vivo studies were reviewed. Tissue was obtained from GI tract/salivary glands, skin and olfactory mucosa/bulb. We concluded that skin biopsy is an easy, minimum invasive approach which provides high specificity and good sensitivity for the detection and differential diagnosis of synucleinopathies. GI biopsies remain attractive in the detection of synucleinopathies. However, a standardized methodology is essential to increase their diagnostic value. The new promising assays could be incorporated in future cohorts, towards identifying the combinations and relative contributions of the sensitivity amongst peripheral tissues.

Key words: peripheral tissue biopsies, synucleuinopathies, gastrointestinal tract, skin, olfactory mucosa/bulb.

ΟΙ ΒΙΟΔΕΙΚΤΕΣ ΙΣΤΩΝ ΣΤΗ ΝΟΣΟ ΠΑΡΚΙΝΣΟΝ ΚΑΙ ΣΤΑ ΑΤΥΠΑ ΠΑΡΚΙΝΣΟΝΙΚΑ ΣΥΝΔΡΟΜΑ.

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Περίληψη
Η εντόπιση της φωσφορυλιωμένης α-συνουκλεΐνης (phosaSYN), της παθογνωμονικής πρωτεΐνης στη νόσο Πάρκινσον (PD), δεν περιορίζεται στο κεντρικό νευρικό σύστημα, αλλά επεκτείνεται και σε περιφερικούς ιστούς. Ωστόσο, η χρησιμότητα της ανίχνευσης aSYN/phosaSYN σε ιστούς (προσβάσιμους με βιοψίες) ως αξιόπιστου βιοδείκτη για τον προσδιορισμό του πρόδρομου σταδίου PD παραμένει ασαφής. Διεξήχθη μια συστηματική ανασκόπηση μελετών στις οποίες πραγματοποιήθηκαν βιοψίες δέρματος, οσφρητικού και εντερικού (GI) ιστού, ώστε να αξιολογηθεί η ευαισθησία και η ειδικότητα τόσο της χρώσης aSYN όσο και της phosaSYN στην PD και στις συναφείς διαταραχές. Συνολικά επανεξετάστηκαν 128 νεκροτομικές και in vivo μελέτες. Ο ιστός ελήφθη από τον γαστρεντερικό σωλήνα και σιελογόνους αδένες, το δέρμα και τον οσφρητικό βλεννογόνο/βολβό. Καταλήξαμε στο συμπέρασμα ότι η βιοψία δέρματος είναι μια εύκολη, ελάχιστη επεμβατική προσέγγιση, που παρέχει υψηλή ειδικότητα και καλή ευαισθησία για την ανίχνευση και τη διαφορική διάγνωση των συνουκλεϊνοπαθειών. Οι βιοψίες του γαστρεντερικού συστήματος παραμένουν ελκυστικές στην ανίχνευση των συνουκλεϊνοπαθειών. Ωστόσο, η κοινή αποδοχή μιας τυποποιημένης μεθοδολογίας είναι απαραίτητη για την αύξηση της διαγνωστικής τους αξίας. Οι νέες πολλά υποσχόμενες τεχνικές θα μπορούσαν να ενσωματωθούν σε μελλοντικές μελέτες. Ο προσδιορισμός ενός συνδυασμού διαφορετικών τεχνικών διαφορετικής ευαισθησίας στους περιφερικούς ιστούς, αποτελεί αντικείμενο μελλοντικής έρευνας.

Λέξεις κλειδιά: βιοψίες περιφερικών ιστών, συνουκλεϊνοπάθειες, γαστρεντερική οδός, δέρμα, οσφρητικός βολβός/βλεννογόνος

Introduction

In this review, we will present available studies suggesting reliable biomarkers in peripheral tissues for PD diagnosis and progression. We will focus on Lewy bodies (LB) and Lewy neurites (LN) pathology in skin, olfactory and GI tissues. Further, we will scrutinize the existing literature for various limitations of different studies on the potential of candidate biomarkers.

Parkinson's disease (PD) is a complex and progressive neurodegenerative disease, the second most common neurodegenerative disease of the elderly

population. The PD prevalence is 0.5 to 1% in the age group of 65–69 years, and it gradually rises with the increasing age.[1] The pathological hallmark of PD is the intraneuronal accumulation of abnormal -lamentous inclusions containing phosphorylated a-synuclein (phosaSYN) as the major component of Lewy bodies (LB) and Lewy neurites (LN).[2, 3] Besides CNS, LB/LN have also been detected in peripheral tissues, mainly in the autonomic nervous system. These findings allow in vivo minimally invasive procedures for PD based on peripheral tissue biopsies, towards providing a window to early potentially preclinical diagnosis of PD, differential diagnosis among parkinsonian syndromes and thus putative neuroprotective therapies during their prodromal phase.

Methods

We searched PubMed databases for publications published until August 2023 using the search terms Parkinson's disease (PD), Dementia with Lewy bodies (DLB), Multiple System Atrophy (MSA), Pure Autonomic Failure (PAF), isolated Rapid eye movement Behavior Disorder (iRBD), aSYN and phosaSYN pathology, peripheral biomarkers, biopsy, skin, olfactory system, salivary glands, gastric, enteric, esophagus, stomach, small intestine, colon, rectum. Both postmortem and in vivo studies were included. Studies which used techniques regarded as safe with an acceptable risk (i.e. absence of major adverse events), were selected. Only studies in English were considered. Case reports were also excluded.

Studies performed on bronchial/lung or pericardiac tissue were not included. The total number of cases analyzed, and the derived data are presented comprehensively in tables separately for each peripheral tissue system.

Results

Of 128 studies identified 34 were post-mortem, 89 in vivo investigations and 5 including both alive patients and cadavers. Whilst most of the studies included immunohistochemical detection of either aSYN or phosaSYN, a number of them were processed by other techniques (e.g. histological tinctorials, immunohisto- chemistry for nerve tissue epitopes/ neurotransmitters, transmission electron microscopy, seeding amplification assays).

Skin

55 studies on skin biopsies were identifed; 45 in vivo[4-48], 8 post-mortem[49-56] and 2 including both alive patients and cadavers [57, 58](table 1).

Detection of aSYN by western blot could not reveal any diferences between PD patients and controls aSYN.[4] It was confirmed by aSYN immunohistochemistry which showed immunoreactive signals both in PD patients and in controls, whilst specicity of phosaSYN was satisfactory, as there was absent staining in controls.[10] The same study demonstrated that PD patients with severe or longer disease duration or with autonomic dysfunction have a greater deposition. They also observed that the deposition was more in sympathetic adrenergic fibers than in cholinergic ones. Further studies confirmed that the deposition of phosaSYN was predominant in in the cutaneous autonomic nerve fibers.[7, 11, 12, 23, 25] Using the Proximity Ligation Assay (PLA) procedure Mazzetti et al., also detected the oligomeric form of a-synuclein in autonomic nerve terminals in skin biopsy for the first time.[53]

A number of studies evaluated the morphology and distributional pattern of different subtypes of cutaneous nerves (e.g. intraepidermal/dermal, sudomotor, pilomotor, vasomotor nerve bers).[5, 7-11, 15-17, 19, 21, 25, 26, 44, 52] Consistently, nerve ber density was decreased in PD patients suggesting that cutaneous nerve fiber loss may reflect both neuronal death and axonal degeneration, adjacent to neurodegenerative alterations observed in PD.[8, 14]. However, despite these structural deficits electrophysiological findings often appeared normal in these cases.[13] One of the afore mentioned studies was a 2- year longitudinal study, and estimated the progression of PD. The authors suggested the association of low intraepidermal nerve fibers density (IENFD) at baseline with an increased risk of developing a cognitive decline and motor impairment.[29, 41] Cervical cutaneous denervation has also been suggested as a potential biomarker of PD progression.[29]

Sleep disorders and dysautonomia are the most common non-motor features in synucleinopathies. 7 studies estimated Rapid eye movement Behavior Disorder (RBD)[19, 21, 22, 32, 34, 38, 42] and 5 pure autonomic failure (PAF) in skin respectively.[12, 16, 21, 35, 44] In the study of Doppler et al., patients with PD with or without RBD and individuals with isolated RBD (iRBD) were screened.[22] Dermal phosaSYN deposition was more frequently found (81.8% vs. 52.4%) in patients with PD and RBD compared to PD patients without RBD and was similar to patients with iRBD (79.1%). Two other studies which included iRBD population (without confirmed PD) showed that cutaneous phosaSYN aggregation was detected in most of them and was associated with greater autonomic dysfunction.[32, 34] Therefore, dermal phosaSYN can be considered a peripheral histopathological marker of synucleinopathy representing prodromal PD.

Table 1. Skin biopsy studies.

	Reference	PD (n)	DLB (n)	MSA (n)	PAF (n)	iRBD (n)	CTR (*n*)	Skin: lower limb	Skin: upper limb	Skin: trunk	Skin: finger	Scalp	Brain	Oth-er tis-sues	aSYNIR somata(LB) neurites (LN) %	phosaSYNIR somata(LB) neurites (LN) %	Other techniques: [1] H&E [2]Azan—Mallory [3]Bodian [4]Electron microscopy [5]VIP,TH (IHC) [6]Luxol fastblue [7]IHC [8]PET blot [9]PMCA [10]RT-QuIC [11]PLA	Comments
1	Ikemura et al. 2008[49]	*	*				194		•	•	•		•	•	n.d	PD& PDD; 40% DLB:70% CTR:0%	1,2	*Prospective study: 142 retro-spective cases, 279 prospective cases with LBD
2	Beach et al. 2010[50]	17	9				23			•			•	•	n.d	PD: 0% LBD: CTR: 0%		
3	Michell et al. 2005[4]	16					5			•						PD: 19%* CTR: 20%*		*Protein extraction from skin biopsy
4	Dabby et al. 2006[5]	17–21					15–19	•							n.d	n.d	3*	*Nerve fbers of blood vessels, sweat glands, erector pili muscles PD: decreased nerve fber density
5	Rossi et al. 2007[8]	n.s					n.s	*		*					n.d	n.d	3	PD: decreased nerve fber density
6	Nolano. et al. 2008[9]	18					30	*		*	*				n.d	n.d	3, 4*†	*Epidermal/ intrapapillar nerve fbers, Meissner corpuscles †Abnormal nerve sprouting/ altered Neurotransmitters PD: decreased nerve fber density increased nerve regeneration
7	Miki et al. 2010[6]	20					-	•		•					n.d	PD: 0%–10%*	?	*10% in skin biopsy from chest wall 0% in skin biopsyfrom lower limb
8	Wang et al. 2013[10]	20					14	•							PD: 100%* CTR: 100%	n.d	2,3,4 *	* PD: decreased nerve fiber density *Increased aSYN decreased nerve deposition and ratio fber density compared to CTR in Intraepidermal nerve fbers

9	Doppler et al. 2014[7]	31				35	●	●			n.d	PD: 52% CTR: 0%	2,3,4*	*Intraepidermal nerve decreased nerve fibers (length-fiber density dependent), SP-intraepidermal nerve fibers non-length-dependent) PD: decreased nerve fiber density
10	Donadio et al. 2014[11]	21				30	●	●			n.d	PD:14%–100%* CTR: 0%	2,3,4*	*14%–24% in leg, 42%–52% thigh, 75%–100% in trunk autonomic and somatic nerves PD: decreased nerve fiber density
11	Rodríguez-Leyva et al. 2014[27]	34	12	5		20		●*			PD:57,9%–62,1%	n.d	7	16 with Tauopathies were included in the study. *Retro auricular area
12	Gelpi et al. 2014[51]	10*	5			None	●				PD:0% DLB: 0%	PD: 0% DLB:0%	7	*Four with PD, six with PDD †Varying presence of LB/LN due to rostro-caudal gradient
13	Doppler et al. 2015[18]	30		12		39	●	●			n.d	PD: 67% MSA:67% CTR: 0%	7	Tauopathies were included in the study
14	Zange et al. 2015[23]	10		10		6		●			n.d	PD: 17–75% MSA: 0% CTR: 0%	7	
15	Haga et al. 2015[24]	38		13			●				n.d	PD: 5,3% MSA: 0	7	sensory IENFD was reduced in patients with PD compared with those with MSA
16	Gibbons et al. 2016[25]	28		23			●	●			n.d	n.d	7*	*PD: reduction in the sudomotor nerve and pilomotor fiber density a-Synuclein is deposited prominently in sympathetic adrenergic nerve fibers innervating the arrector pili muscles, but is also present in sudomotor (sympathetic 1cholinergic) nerve fibers, but is not detected in sensory fibers.
17	Rodríguez-Leyva et al. 2016[47]	17				17		●		●*	n.d†	n.d		10 PSP were included in the study *Occipital PD: co-occurrence of both tau and a-syn †Higher αSN immunopositivity in PD

18	Donadio et al. 2016[12]	16			14		15	•		•					n.d	*PD:31%-100% PAF:31-52% CTR:0%	7	*PD: 100% cervical, 75% thigh, 31% leg Differences in the innervation pattern and spatial distribution of neuritic p-syn inclusions in IPD and PAF
19	Gibbons et al. 2017[52]	11					5			•		•			*PD: 100% CTR:100%	n.d	7	*greater deposition of alpha-synuclein within pilomotor, sudomotor and vasomotor nerve fibers of PD compared to CTR
20	Donadio et al. 2017[45]		18				25	•		•					n.d	LBD:71%-100% CTR:0%	7	23 patients with non-synucleinopathy dementia were included in the study *LBD: p-syn 100%, 86% thigh, 71-94% leg
21	Donadio et al. 2017[28]	28								•*					n.d	†PD:100%	7	*Paravertebral C7&Th12 † C7: 100%, Th12: 62% Unilateral PD: 20% p-syn deposits in affected &non-affected site, 60% in both sites
22	Doppler et al. 2017[19]	25				18	20	•		•					n.d	PD: 80% iRBD:55,6% CTR:0%	7	
23	Doppler et al. 2018[20]	10*						•		•					n.d	PD: 60%†	7	*10 PD patients with 3 different GBA1 mutations †P-syn deposition was mainly detected in autonomic nerve fibers, but also in somatosensory fibers
24	Melli et al. 2018[29]	19					17	•		•					*PD:81,3% APtau:0% APsyn:57,1% CTR:14%	†PD:56% APtau:0% APsyn:0% CTR:0%	7	13 patients with atypical parkinsonism(AP) (7 syn, 6 tau) were included. immunofluorescence for: *5G4 †p-aSyn,
25	Donadio et al. 2018[13]	15	12	12	5		10	•		•					n.d	PD: 100% LBD:100% MSA:67% PAF:100% CTR:0%	7	localization and load differences of aggregates among synucleinopathies.
26	Donadio et al. 2019[14]	21	1	4		11		•		•					n.d	n.s*	7	Intra-laboratory analysis showed an excellent reproducibility in 2cemters. Inter-laboratory analysis showed reproducibility (90%; K = 0.8; P < 0.001). Different classification was mainly due to fragmented skin samples or weak fluorescent signals.
27	Donadio et al. 2018[46]	28*						•		•					n.d	n.s†	7	*14PD with & 14PD without neurogenic OH. †PD + OH showed a higher p-syn deposition

28	Kuzkinaet al. 2019[30]	27*		8			21	●		●					n.d	PD: 82% MSA:75% CTR:0%	7†	*Early PD †Immunostaining of nerve fibers with different conformation specific antibodies and digestion with PK gave comparable results.
29	Carmona-Abellan et al. 2019[44]	3*	3*	-	2*		3*			●					n.d	†PD: n.s LBD:n.s PAF:n.s CTR:0%	7	*PD:1E46K-SNCA, 2PARK2 LBD:3E46K-SNCA PAF:2E46K-SNCA CTR:1E46K-SNCA asymptomatic carrier &2 healthy controls †E46K-SNCA carriers: moderate to severe p-synuclein deposits-correlated with sudomotor dysfunction
30	Donadio et al. 2020[15]	25*		25*				●		●					n.d	†PD: 100% MSA:72%	7	*All patients with OH. †MSA-P: p-syn deposits mainly found in somatic fibers of subepidermal plexi
31	Giannoccaro et al. 2020[35]	21	7	13	13			●		●					n.d	*PD: 95,2% LBD:100% MSA:69,2% PAF:100%	7	*p-α-syn deposits rarely affected the autonomic fibers in MSA
32	Liu et al. 2020[39]	90				30		●	●	●					n.d	PD: 83,3% CTR:0%	7	
33	Wang et al. 2020[57]	47	7	3			43	●		●					n.d	*PD:71,6% LBD:53,1% MSA:51% PSP:17,8% CBD:13,3% AD:23,2% CTR:12,4%	10	30 patients with PSP(8), CBD(5),AD(17)were also included.
																*PD:48,2% LBD:51,6% MSA:54,6% PSP:26,2% CBD:1,5% AD: n.d CTR:11,9%	9	
		20					21	●		●					n.d	*PD:52,2% CTR:24,7%	10	
																*PD: 56,6% CTR:8,3%	9	
34	Wang et al. 2020[40]	29					21	●							n.d	*PD:100% in 50 μm 90% in 20 μm 73% in 10 μm CTR:0%	7*	*50-μm-thick tissue sections performed better than 20 or 10 μm tissue sections.
35	Chahine et al. 2020[48]	59					21	●		●				●*	n.d	PD; 24,1% CTR:0%	7	*Submandibular glands, colon

36	Yang et al. 2021[42]	59*					30	•		•					n.d	PD:79.7% CTR:0%	7	*LRRK2 G2385R carriers:12 LRRK2 G2385R non-carriers:47 †Different p-syn distribution pattern LRRK2 G2385R carrier vs non-carriers. LRRK2 G2385R carrier:increased prevalence of autonomic symptoms or RBD
37	Mazzetti et al. 2020[53]	57 (38+19) *					48 (39+19) *	•					•		PD:82% CTR:14% PD twins: 89% CTR twins: 47%	n.d	7,11	*Including 19 monozygotic twins discordant for PD. a-synuclein oligomers within synaptic terminals of autonomic fibres
38	Manne et al.2020[54]	25					25					•			n.d	PD:96% CTR:4%	10 (frozen skin tissues)	96% sensitivity and 96% specificity
		12					12									PD:75% CTR:16,7%	10 (formalin-fixed paraffin-embedded skin sections)	75% sensitivity and 83% specificity
39	Tanei et al. 2021[56]							•	•				•	•*	n.d	†	[7]	*Submandibular glands, esophagus, adrenal gland LB pathology in 34%(178/518) of autopsird cases. † LB pathology in skin: 18%
40	Vacchi et al. 2021[41]	30		12			22	•		•					n.d	n.s	7,11	2 years longitudinal study 11 patients with tauopathies were also included. *Immunofluorescence for αSyn-PLA, P-αSyn, αSyn-5G4 PD and MSA showed a significant reduction of IENFD compared to CTR. A linear discrimination analysis model of αSyn-PLA, P-αSyn, αSyn-5G4, and IENFD, stratified patients with accuracy (77.8%). discrimination between PD and MSA (84.6%).

41	Kuzkina et al. 2021[31]	34					30	•		•					n.d	†PD: 82,4% CTR:10%	10	Higher α-synuclein seeding activity was shown in PD with longer disease duration and more advanced disease
42	Donadio et al. 2021[16]	6	4	6	3		24*								n.d	†PD: 100% LBD:100% MSA:67% PAF:100% CTR:0%	7	†p-syn deposits mainly found inautonomic fibers of PD, DLB, and PAF, but detected in somatic fibers of the upper dermis in MSA
																††PD: 100% LBD:75% MSA:60% PAF:67% CTR:12,5%	10	††The mean skin α-synuclein ThT fluorescence reaction from patients with synucleinopathies was significantly higher than that from patients with non-synucleinopathies. 23,6% of patients with non-synucleinopathies showing positive α-synuclein ThT fluorescence reaction.
																		* CTR: patients with peripheral neuropathies Additionally, 23 non-synucleinopathieswere included in the study
43	Doppler et al. 2021[21]	0*				33*		•		•					n.d	iRBD:60,6%	7	*2-4year clinical and skin biopsy follow-up data of 33 iRBD (baseline)
		4*	1*			28*									n.d	PD: 100% (2/2) LBD:100% (1/1) iRBD:72,7%		*Phenoconvertion in 5/33 patients (follow-up)
44	Isonaka et al. 2021.	*19 iPD+ 25genetic PD					16+ 5with mutations			•					†iPD:95% SNCA:100% PRKN biallelic:0% LRRK2: 100% GBA:83% LRRK2/ GBA:95%	n.d	7	*SNCA:3 PRKN biallelic:7 PRKN monoallelic:3 LRRK2: 7 GBA:7 PARK7/DJ1 biallelic:1 PARK7/DJ1 monoallelic:2 †SNCA, DJ-1, LRRK2, GBA mutations have substantial intra-neuronal α-syn deposition in sympathetic noradrenergic nerves. Biallelic PRKN PD may have mildly increased α-synuclein deposition compared to CTR.
45	Mammana et al. 2021[58]	n.s*	n.s*				40*	•		•					n.d	PD+LBD:100% incidental Lewy body:85,7% CTR:2,5%	10	*Total: 49 (PD+LBD:2 incidental Lewy body:7 neurological CTR:40)
		13	15				41	•		•					n.d	PD: 76,9% LBD:100% CTR:4,9%	10	

46	Migliset al. 2021[34]	28				25	18	•		•					n.d	PD; 96% iRBD:64% CTR:0%		7	
47	Bargar et al. 2021	2					1	•				•		•		PD; + (n.s) CTR:0%		10	
48	Giannoc-caro et al. 2022[43]	26					26	•		•						*PD; 100% CTR:0%		7	26 patients fulfilling clinical diagnostic criteria of tauopathies (PSP, CBS) were included. *p-syn deposits found in 2/26 (7,7%) patients with tauopathies.
49	Oizumi et al. 2022	10					4		•	•			•			PD; 100% n.s		1, 7	PD: p-aSyn deposits in in dermal macrophages in skin
50	Doppler et al. 2022[22]	43*				43										PD with REM:81,8% PD withou-tREM:52,4% iRBD:79,1%		7	*PD with REM: PD without REM:
51	Nolano et al. 2022[37]	57		43				•			•							7	Higher p-α-syn deposits in autonomic nerves differentiated PD from MSA-p. p-α-syn
52	Gibbons et al. 2023[26]	54		31			24	•		•						*PD: 94,4% MSA:100% CTR:0%		7	*MSA: p-syn deposits in the subepidermal plexus region
53	Iranzo et al. 2023[38]					91	41	•		•						iRBD:76,9% CTR:2,4%		10	
54	Donadio et al. 2023[17]	34	46	16			50	•		•					n.d	PD: 100% LBD:100% MSA:787% CTR:0%		7	p-syn in RSCs, 74% in MSA 0% in PD, 0% in DLB patients.
55	Kuzkina et al. 2023[32]	27		3		18	30	•		•				•*	n.d	Olfactory	Skin		3 patients fulfilling clinical diagnostic criteria of PSP were also included. *Olfactory epithelium
																PD:48% MSA:67% iRBD:67% CTR:10%	PD:78,9% MSA:100% iRBD:100% CTR:n.d	10	
																n.d	n.d	7	

Post-mortem studies with accompanying brain pathology are shown in dark grey shaded cells, in vivo studies are shown in light grey shaded cells. aSYN, alpha synuclein; iPD, idiopathic PD; CTR, control; DLB, dementia with Lewy bodies; MSA, Multiple System Atrophy; PAF, pure autonomic failure; iRBD, Isolated Rapid eye movement Behavior Disorder; PSP, progressive supranuclear palsy; CBD, Corticobasal Degeneration; AD, Alzheimer disease; GBA1, glucocerebrosidase gene; GI, gastrointestinal; H&E, hematoxylin—eosin staining; IHC, immunohistochemistry; IR, immunoreactive; PMCA, protein misfolding cyclic amplification; RT-QuIC assay, real-time quaking induced conversion; PLA, Proximity Ligation Assay; LB, Lewy bodies; LN, Lewy neurites; n/a, not applicable; n.d., not determined; n.s., not stated; RSCs, Remak non-myelinating Schwann cells; PD, Parkinson's disease; PDD, PD dementia; IENF, intraepidermalnerve fibers; OH, orthostatic hypotension; phosaSYN, phosphorylated alpha synuclein; TH, tyrosine hydroxylase; VIP, vasoactive intestinal polypeptide; asterisks and daggers refer to the same table row. The percentages correspond to the sensitivity (PD%). The specifi city equals 100% – CTR%.

Donadio et al. were the first to publish that there are differences in the innervation pattern and spatial distribution of neuritic phosaSYN inclusions in idiopathic PD and PAF.[7] They further proved that besides the different pattern distribution there is higher phosaSYN load in PD patients with orthostatic hypotension.[14] The same authors who added patients suffering from DLB and MSA in later studies, stated that the distribution of phosaSYN deposits was more homogenous for PD patients with orthostatic hypotension compared to those without.[13, 15] The localization and load differences of aggregates led them to speculate that specific diagnostic traits identify different pathogenesis among synucleinopathies.[24] More specifically, phosaSYN positivity differed among patients with synucleinopathies, being mainly detected in autonomic fibers of PD, DLB, and PAF, but detected in somatic fibers of the upper dermis with relatively preserved autonomic innervation in MSA.[16, 26, 35] DLB-PAF showed the highest load of deposits among synucleinopathies with a widespread involvement of autonomic annexes.[13, 45] In MSA there was noticed a distal-to-proximal gradient of αSyn aggregates.[13, 41] A reliable clinical biomarker for MSA came up recently, by the detection of phosaSYN in skin Remak non-myelinating Schwann cells (RSCs) as Schwann cell cytoplasmic inclusions (SCCi), resembling brain and suggesting that non-myelinated glial cells are also involved in the MSA pathogenesis.[17]

5 in vivo studies which included people with tauopathies were identified.[18, 27, 29, 41, 43, 47]

Rodriguez et al. using immunohistochemical technique and the antibodies against p-tau (PHF and AT8) and a-syn reported that PHF values were similar among the PD, PSP, and controls.[47] AT8 was significantly higher in both PSP and PD groups as compared to controls whereas, a-syn values were significantly higher in the PD group as compared with both control and PSP groups.In line with their previous work,they found the presence of both a-syn and p-tau in the skin of PD patients not only in the nervous tissue, but also in the keratinocytes of the epidermis.[27] In a recent study, the use of PLA revealed that αSyn oligomers (αSyn-PLA) were more expressed in PD and MSA patients compared to Tau ones and controls.[41] Another study analyzed skin biopsies of patients with PD and atypical parkinsonism (synucleinopathies and tauopathies) by immunofluorescence for p-aSyn, 5G4. PD and atypical parkinsonism -synucleinopathies shared the features of marked cervical denervation and the presence of 5G4. In contrast atypical parkinsonism-tauopathies were normal.[29] Similar results were described by two other studies which described abundant phosaSYN deposition in patients with PD and MSA but 0% and 7,7% in patients with tauopathies respectively.[18, 43]

We found 4 studies dealing with genetic factors in PD.[20, 33, 42, 44] Doppler et al. screened 10 PD patients with 3 different glucocerebrosidase gene (GBA1) mutations (six N370S, three E326K, and one L444P).[20] phosaSYN deposition was mainly detected in autonomic nerve fibers, but also in somatosensory fibers with N370S and E326K mutations. Nevertheless, seems to offer the distribution and the frequency was the same observed in patients without a known mutation. Contrariwise, Isonaka et al. found that 83% of patients with GBA variants had higher total a-syn deposition (phosaSYN was not detected) in the skin noradrenergic nerves compared to controls.[33] In the same study he investigated the deposition of a-syn in PD patients with pathogenic mutations in SNCA, PRKN, LRRK2, and DJ1, in PD patients without known mutations and healthy controls. According to the researchers, SNCA, DJ-1, LRRK2, and GBA mutations had substantial intra-neuronal α-syn deposition in sympathetic noradrenergic nerves, but this finding was not observed in biallelic PRKN mutations. However, biallelic PRKN PD had mildly increased α-synuclein deposition compared to controls. The same year Yang et al. performed skin biopsy in 59 PD patients (12 LRRK2 G2385R carriers and 47 LRRK G2385R noncarriers) and 30 healthy controls.[42] He reported that that the distribution of skin phosaSYN in PD LRRK2 G2385R carriers had an homogeneous pattern and this variant was linked with increased prevalence of autonomic symptoms or RBD. PARK2 and SNCA E46K mutations were studied by Carmona-Abellan et al in cohort including people with PD, DLB, PAF, asymptomatic carriers and healthy controls. The results of the skin biopsies revealed moderate to severe phosaSYN deposits in E46K-SNCA carriers which were correlated with sudomotor dysfunction.

Interestingly, Oizumi et al. performing an immunohistological analysis of skin biopsy specimens from PD patients and controls suggested dermal macrophages with phosaSYN deposits as useful biomarkers for PD diagnosis. They also found that the total number of macrophages was significantly positively correlated with the number of macrophages with phosaSYN deposits.[36]

Gastrointestinal tract

Most studies (66 out of 129) have been performed in the gastrointestinal tract (GI) and it was the first peripheral tissue to be evaluated towards identifying PD pathology in 1960.[59] Amongst the 66 studies on the GI; 42 were in vivo, 21 post-mortem[59] and 3 including both alive patients and cadavers[60] respectively (table 2).

Salivary glands

Salivary glands appear as an attractive target for

biopsies as the highest amount of aSYN aggregates in the first autopsy studies was found in the submandibular gland.[50, 61] Incisional biopsy of the submandibular gland which is one of the major salivary glands (parotid, submandibular, sublingual gland)is associated with an increased risk of adverse events. Instead, numerous minor salivary glands are easily accessible at the vestibular site of the lower lip.[62]

After the introduction of phosaSYN immunohistochemistry techniques, Beach et al. reported submandibular specimen stained positive in 39% of all cases screened (i.e. dementia DLB, incidental LB disease (ILBD) and Alzheimer's disease with LB (ADLB)).[50] When the methodology of the process was improved with multiple sections phosaSYN staining rate raised to 93% in PD. When the same researchers replaced the needle core biopsies with large submandibular gland sections, phosaSYN immunoreactivity in nerve increased from 90% to 100% in PD.[63] In contrast, in an in vivo study, a better sensitivity was reported using needle core biopsies of the submandibular gland detecting phosaSYN immunoreactivity in 75% of PD (compared to 7% minor salivary gland biopsies).[64] Biopsies of minor salivary glands in alive patients demonstrate a great variability in phosaSYN staining rates (7% to 100%).[64-69] The in vivo and post mortem studies which explored the LB pathology in submandibular gland reported controversial results (sensitivity rates from 42% to 75%).[64, 70-73]

Table 2. GI tract biopsy studies.

	Reference	PD (n)	DLB (n)	MSA (n)	iRBD (n)	CTR (n)	Salivary glands	Esoph-agus	Stomach	Small intes-tine	Col. asc.	Col. desc. / Sigma	Rec-tum	Other tissues	Localization	aSYNIR somata(LB) neurites (LN) %	phosaSYNIR somata(LB) neurites (LN) %	Other techniques: [1] H&E [2]Azan—Mallory [3]Bodian [4]Electron microscopy [5]VIP;TH (IHC) [6]Luxol fastblue [7]IHC [8]PET blot [9]PMCA [10]RT-QuIC	Comments
1	DenHar-tog1960[59]	5				None		•	•	•	•			•		n.d.	n.d.	1 PD: 0%	
2	Qualman-*etal.*1984[74]	22				50		•	•	•	•	•	•	•		n.d.	n.d.	1,2,5,6 PD:9%LB* CTR:0%	*Esophagus, colon
3	Wakabayash-i*etal.* 1988[75]	7				24		•	•	•	•	•	•			n.d.	n.d.	1,2,3,4 PD:100%LB* CTR:33%LB*	*Across all GI tract segments
4	Wakabayashi et al. 1990[76]	3				3		•	•	•	•	•	•			n.d.	n.d.	PD: 100% LB+LN CTR: 0%LB+LN	
5	Bloch et al. 2006[78]	2				98		•						•		PD:50% CTR:6-14%	n.d.		
6	Braak*et-al.*2006[77]	5				5		•	•					•	Gastric ENS	PD:100% CTR:0%	n.d.		
7	DelTredici*etal.* 2010[61]	9		2		19	•							•		n.d.	PD: 100% MSA: 0% CTR: 0%		*Variable staining intensity
8	Beach *et al.* 2010[50]	17				23	•	•	•	•		•	•	•	submandibular gland (serial slides)	n.d.	PD:65%* n.d. (93%†) CTR: n.s.	7	*Across all GI tract segments †Esophagus,

9	Del Tredici and Duda 2011	3			None	•	•			•		PD:100%	n.d.	7	
10	Annerino et al. 2012	13			12		•	•		•	•	PD:100–25%† CTR: 0%	n.d.	7	*Transverse colon †Stomach to rectum (three slides)
11	Beach et al. 2013[63]	28		2	50	•						n.d.	PD: 0% (somata) PD:100%/ 90%* (neurites) MSA:0% CTR: 0%	7	AD, PSP, CBD needle core biopsy
12	Gold-*etal.*2013[93]	10			77				•	•	colon myenteric and submucosal ganglia	PD: 100% *AD: 0% CTR: 0% †	n.d	7	*8 patients with AD were also included †aSYN detection:PD: 100% AD: 0% CTR: 52%
13	Gelpi et al. 2014	10*	5		None	•	•	•		•	•	PD:80%† DLB: 100%	PD: 80%† DLB:100%	7	*Four with PD, six with PDD †Varying presence of LB/LN due to rostro-caudal gradient
14	Mu et al. 2015	10			4	•						n.s.	PD:100% CTR:0%	7	*tongue-pharynx-larynx-upper esophagus
15	Beach et al. 2016[119]	46	28	2	79	•					sigmoid colon mucosa and submucosa	PD: 91% DLB:71% MSA:0% CTR:0%	PD: 89% DLB:71% MSA:n.s CTR:0%	7	
16	Kupsky et al. 1987[141]	1			None				•	•		n.d.	n.d.	n.d.	1 PD: 100% LB*
17	Lebouvier et al. 2008[142]	5			5+3*				•		Submucosa of ascending colon (submucosal plexus)	n.d.	PD: 0% (somata) PD:80% (neurites) CTR: 0%	7	*Five healthy, three constipated subjects
18	Lebouvier et al. 2010[98]	29			10				•	•	Submucosa neurites	n.d.	PD: 0% (somata) PD:72% (neurites) CTR: 0%	7	

19	Forsyth et al. 2011[92]	9				10						•			n.a	PD: 100%* CTR: n.s.	n.d.		*Patients from study [41]
20	Cersosimo et al. 2011[124]	3				3	•								labial minor salivary	PD: 66% CTR: 0%	n.d.	n.d.	
21	Pouclet et al. 2012[91]	9				10					•	•	•		*Mucosa/ submucosa	PD: 0% CTR: 0%	PD: 33%/45%* CTR: 0%	7	
22	Pouclet et al. 2012[105]	9		6								•			submucosa and mucosa	n.d.	PD: 55,5% MSA:16,7%	7	
23	Shannon et al. 2012[95]	9				24						•			submucosa	PD: 100%* CTR: 8%†	n.d	7	*Variable staining intensity †Cells of unknown origin
24	Shannon et al. 2012[94]	3*				23						•			mucosa and two benign polyps	PD: 100% CTR: 0%	n.d	7	*prodromal PD
25	Devos et al. 2013[143]	18				14					•					n.d	PD: 67% CTR: n.s.		
26	Folgoas et al. 2013[69]	16				11	•								Labial salivary glands		PD: 19% CTR: 18%*	7	*Weak staining
27	Hilton et al. 2014[81]	62*				161		•	•	•	•	•	•	•†	mucosal and submucosal	PD: 0–13%** CTR: 0%.	PD: 0–13%** CTR: 0%	7	*62PD&Progromal PD †Gall bladder **Progromal PD 0% esophagus, 13% small/ large intestine, phosaSYN with better staining
28	Adler et al. 2014[64]	15				-	•								Submandibular gland*/labial salivary glands† (serial slides)	n.d	PD:75%*/7%†	7	
29	Sfi nchez-Ferro et al. 2015[85]	34*				23			•						Gastric and duodenal mucosa	PD:60,7% prodromal PD: 33.3% CTR:4,3%	†PD: 60.7% prodromal PD:16,6% CTR:4,3%	7	*28 PD &6 prodromal PD †67% agreement between phosaSYN and aSYN
30	Gao et al. 2015[65]	13				13	•								Minor salivary glands	n.d	PD: 69% CTR: 0	7	
31	Aldecoa et al. 2015[144]	6				8					•	•			Stomach, ileum, jejunum, and colon myenteric plexus	PD:100%	PD:67%	7	

32	Sprenger et al. 2015[104]	21			17	16					•	•	•		submucosa	PD:100% CTR:100% iRBD:100%	PD:5,3% CTR:0% iRBD:23,5%	7	
33	Visanji et al. 2015[126]	22*				11						•	•		mucosa	PD:100% CTR:81,8%	PD:100% CTR:90,1%	7	*15 early PD &7 late PD
														Early PD:80% Late PD:100% CTR:100%	Early PD:80% Late PD:100% CTR:100%	8			
34	Vilas et al. 2016[71]	24			9	26	•								Submandibular glands	n.d	PD:67% iRBD:89% CTR:0%		
35	Adler et al. 2016[70]	25*				10	•								Submandibular glands	n.d.	PD:74% CTR:22%	7	*early PD
36	Antunes et al. 2016[96]	19				8					•	•			mucosa	PD:94,7% CTR:100%	PD:100% CTR:100%	7	
37	Stokholm et al 2016[82]	57*				98	•	•	•	•	•	•			Gastrointestinal mucosa, submucosa and myenteric minor/submandibular glands	PD:61% CTR:59%	PD:44,4% Prodromal PD:56% CTR:25,5%	7	*18 PD & 39 prodromal PD
38	Chung et al.2015[87]	38		13		53			•		•	•			gastric and colonic mucosa	Stomach: PD:31.6% MSA:40% CTR:33.3% colon: PD:10.4% MSA:8% CTR:18.%	n.d	7	
39	Corbille et al. 2016[120]	9				3						•			mucosa-submucosa	n.s	n.s	7*	*Variable stainingmethods none of the tested method or staining pattern had a specificity and sensitivity more than 80 % regarding to PD
40	Iranzo et al. 2017[145]	-			20	32	•				•	•			Submandibular glands	n.d	iRBD: 50%* CTR: n.d	7	*colon: 2/4, salivary glands:1/2 †
41	Barrenschee et al. 2017[99]	12				11							•		submucosal ganglia	PD:100% CTR:100%	PD:25% CTR: 36%	7	
42	Shin et al. 2017[89]	33				21			•		•	•			Mucosa and myenteric plexus	n.d.	PD: 9.1% CTR: 18.2%	7	

43	Corbille et al. 2017[121] 1	17				13						•			n.s	n.s*	n.s*	7	*no differences between PD and for phosaSYN and aSYN levels
					1						•			n.s	n.s*	n.s*			
44	Iranzo et al. 2018[68]	13	10		62	33	•								minor salivary glands	n.d	PD:54% LBD: 50% iRBD:50% CTR:3%	7*	*phosaSYN 5G4 antibody. Only 1 patient with iRBD was positive with 5G4 and negative for phosaSYN
45	Yan et al. 2018[86]	31				32*			•		•	•	•		n.s	PD:54,8% CTR:21,8%	n.d	7	*CTR included patients with atypical parkinsonism
46	Carletti et al. 2018[123]	7				7									Labial minor salivary gland	n.s.	PD:71% CTR: 0%	7	
47	Chung et al. 2018[107]	38*				46					•	•			gastric and colonic mucosa	PD:92,3% CTR: 76,2%	n.d	7	*2 PD-SNCA-SNP rs11931074 variants were included. PD SNCA SNP-rs11931074 associated with aSYN staining
48	Ruffmann et al. 2018[83]	51*				21		•	•	•	•	•			mucosa and submucosa	PD: 35% Prodromal PD:42% CTR: 24%	PD: 15% Prodromal PD:13% CTR: 24%	7	*31 PD & 20 prodromal PD
49	Shin et al. 2018[146]	16				14	•								submandibular glands	n.d	PD:56.2% CTR:0%	7	
50	Ma et al. 2019[66]	8				7	•								minor salivary gland	n.d	PD:100% CTR:0%	7*	*Immunohistochemical 3-NT-Syn expression
51	Adler et al. 2019[147]	7*				-											PD: 100%†	7	*6 advanced PD& 1 early PD in previous study positive phos-a-syn patients (Adler et al. 2016) †increase density over time
52	Leclair-Visonneau et al. 2019[148]	43				-							•			n.s	PD: 47%		Correlation with dysautonomia with questionnaires and tests

53	Fenyi et al. 2019[88]	18				11			•			•	•			PD: 35,7% (5/14) CTR: 0% (0/3)	PD: 55,8% CTR: 09% *	9	Advanced PD
54	Punsoni et al. 2019[84]	16				298		•	•	•	•	•	•			PD:100% CTR:100%	PD:25% (somata) CTR: n.s	7	*15 pediatric and 14 adults
	17				10*											7	*6 pediatric and 4 adults		
55	Shin et al. 2020[106]	3*					•†							subman-dibular glands		n.d	PD:0%	7	*PRKN †core needle biopsy
56	Harapan et al. 2020[97]	25				20				•	•	•			submucosa and myenteric plexus	n.s*	n.s*	7	*similar rates of aSYN and phosaSYN in PD and CTR
57	Manne et al. 2020[73]	13				16	•								submandibular glands	n.s	*PD:100% CTR:0%	10	*paS seeding activity during the 24-h period
58	Beck et al. 2020[60]	7*				18			•		•	•			Mucosa, sub-mucosa, myenteric plexus	PD:57% prodromal PD:100% CTR:82-100%	PD:14% prodromal PD:100% CTR:0%	7	*3 prodromal PD were included
					4									n.a					
59	Chahine et al. 2020[48]	59				21	•				•	•		•*	submandibu-lary glands	†PD:56,1% CTR:7,1%	n.d	7	*skin †submandibulary glands
60	Fernfi ndez-Espejo et al. 2021[122]	6				6	•								submandibu-lary glands	n.d	PD:100% CTR:0%	7*	*Immunohisto-chemical 3-NT-Syn expression
61	Tanei et al. 2021[56]						•	•						•*	Mucosa, sub-mucosa, myenteric plexus	n.d	† PD: 75% PDD:92.1% LBD: 43.8% CTR:0%	7	*skin, adrenal gland, brain † LB pathology in esophagus LB pathology in 34%(178/518) of autopsird cases:

62	Sakashita et al. 2021[72]						•							•*	Submandibular glands	n.d	PD:89,1% DLB;75,4%	[7]	*brain Salivary gland biopsy in 64 autopsied patients who had prodromal or clinical LBD LB pathology in 32,8% (21/64) of cases LB pathology in 42,8% (9/21 of them. No CNS-LBD was found in all patients without LBD in submandibular glands
bo	Bargar et al. 2021[55]	2				1	•					•		•*	submandibular glands		PD; + (n.s) CTR:0%	[10]	*scalp skin, CSF
64	Jotanovic et al. 2022[103]	74*									•	•			Mucosa, submucosa	Subject with cerebral a-synucleinopathy: 81%†	n.d		*subjects with cerebral a-synucleinopathy †19% no GI a-syn 14,9%: GI a-syn , scarce a-syn in brain
65	Mangone et al. 2022[67]	27			16	18	•								Labial salivary glands	PD: 14.8% iRBD:18.8% CTR:0%	*PD:55.6% iRBD:43.8% CTR:38.9%	[7]	*anti-aggregated -Syn clone 5G4 antibody
66	Emmi et al. 2023[100]	18								•						n.d.	PD:100% CTR: + (n.s)	[7]	*14 advanced PD & 4 early PD

Post-mortem studies with accompanying brain pathology are shown in dark grey shaded cells, in vivo studies are shown in light grey shaded cells. aSYN, alpha synuclein; CTR, control; DLB, dementia with Lewy bodies; MSA, Multiple System Atrophy; LBD, Lewy body disease; PD, Parkinson's disease; PDD, PD dementia; iRBD, Isolated Rapid eye movement Behavior Disorder; UC, Ulcerative colitis; GI, gastrointestinal; H&E, hematoxylin—eosin staining; IHC,immunohistochemistry; IR, immunoreactive; PMCA, protein misfolding cyclic amplification; RT-QuIC assay, real-time quaking induced conversion; LB, Lewy bodies; LN, Lewy neurites; n/a, not applicable; n.d., not determined; n.s., not stated; phosaSYN, phosphorylated alpha synuclein; TH, tyrosine hydroxylase; SMP, submucosal plexus; VIP, vasoactive intestinal polypeptide; asterisks and daggers refer to the same table row. The percentages correspond to the sensitivity (PD%). The specificity equals 100% – CTR%. Col.desc, descending colon; col. asc, ascending colon.

Esophagus, Stomach, Small intestine, Colon and Rectum

The studies which evaluated the esophageal involvement in LB pathology are 15 and the 3 of them are post-mortem.[50, 59, 61, 63, 74-84] Qualman et al.in 1984 were the

first to report esophageal LB in 9% and in 25% of PD cadavers with dysphagia and achalasia respectively.[74] Staining for aSYN was positive in a range between 50% and 100% in 3 studies.[77-79] Comparing different studies of the last 4 decades, phosaSYN positivity reached 93% in PD, when multiple slides of paraffin-embedded and 80 lm frozen sections of esophagus were examined, in a survey which obtained different GI tract segments from patients with different synucleinopathies.[50] In the esophagus tissue most LB were found in neurons immunoreactive for vasoactive intestinal polypeptide (VIP).

The reports which explore stomach and small intestine are 19.[50, 51, 59, 60, 74-77, 79-89] aSYN staining was more abundant in stomach (from 80% to 100%) compared to other GI tract specimens.[51, 80, 90] The rostro-caudal gradient distribution in PD is confirmed by this finding.[50, 75, 91] However, the phosaSYN pathology ranged from 9,1% to 80%.[51, 80, 89]

It is out of any question that colon and rectum are the most studied segments of the GI tract.[59]

The accumulation and aggregation of aSYN in the gut mucosa of PD patient has been confirmed by several studies (table 2). Most of them reported that aSYN aggregates are more frequent in PD patients (54,8%-100%) compared to age-matched healthy controls (4,3%-21,8%).[85, 86, 92-95] In contrast, Harapan et al. and Antunes et al. argued that aSYN and phosaSYN rates did not differ between PD and controls.[96-97] In PD patients phosaSYN detection in the mucosa and submucosa of colon and rectum rates from 14%-100% in different studies.[56, 60, 98-100] Apparently, this variability could be partly attributed to the rostro-caudal gradient of the colorectal GI segment. A rather low sensitivity of rectal biopsies (23%) is noticed compared to biopsies taken from the ascending colon (65%).[101] It is noteworthy that deep submucosal biopsies increase the chance to discover phosaSYN neurites (45%) compared to conventional mucosal biopsies (33%).[91] Regardless the high heterogeneity, a systematic review and meta-analysis of 16 studies claimed a high degree of association between gut α-synuclein species and PD.[102]

5 in vivo studies estimated that LB pathology is present in the GI tissue of people up to 20 years prior to the onset of motor symptoms.[60, 81-83, 85, 94]. In contrast, one post-mortem study highlighted the absence of aSYN in 19% of people with LB pathology in brain.[103] GI phosaSYN deposition was frequently found in patients with iRBD, providing histopathological evidence that iRBD represents a synucleinopathy. Colonic and submandibular, revealed moderate sensitivity (23,5%-89%) to identify phosaSYN and very high specificity (97%-100%) to distinguish iRBD subjects from controls.[68, 71, 104] Nevertheless, Mangone et al. reported the that minor salivary gland biopsies lack sufficient accuracy to detect SYN species in salivary glands in PD and in iRBD. In a survey performed and using the anti-aggregated-Syn clone 5G4 antibody, they found oligomeric aSYN deposits in 55.6% in PD, 7 in iRBD, and 7 in 38.9% controls.[67]

In a post-mortem study included pathologically confirmed PD, MSA patients and controls, aSYN immunoreactivity was observed in tissue samples in nearly all cases of PD, but none in the control or MSA subjects.[61] aSYN immunoreactivity was less frequent in MSA (16,7%) than in PD (55,5%) in an in vivo study which examined submucosa and mucosa in colonic biopsies.[105] Chung et al. revealed similar rates of aSYN deposits when examined colon and stomach specimens of PD and MSA patients.[87] 2 studies explored the presence of phosaSYN in DLB. Iranzo et al. demonstrated deposits of deposits of phosaSYN in 50% of DLB patients (vs 54% of PD), and Gelpi et al. in 100% of cases with clinicopathological diagnoses of DLB (vs 54% of PD).[51, 68]

In a recent study it was described negative phosaSYN staining in the submandibular gland of patients carrying PRKN pathogenic variants.[106] In another survey in which gastric and colonic mucosa biopsies were obtained from PD patients and healthy controls, it was shown that PD SNCA variants (SNCA SNP-rs11931074) were associated with aSYN staining.[107]

Olfactory (mucosa & bulb)

Evaluation of the olfactory bulb is necessarily restricted to post-mortem examination in contrast to olfactory mucosa which can be investigated in vivo. We went through 11 post-mortem studies[78, 108-114] and three in vivo[115], which used immunochemistry techniques and seeding amplification assays (table 3). Post-mortem studies revealed a positive staining rate for aSYN between 0%-100%, and for phosaSYN between 75%-100%. In the only in vivo study using olfactory mucosa phosaSYN was not detectable in patients with PD.[115]

We also identified 5 recent studies which explored α-synuclein seeding activity using the RT-QuIC assay.[31, 55, 116-118] Herein, the findings were less heterogenous, as the positive aSYN RT-QuIC was from 46,5% to 67,4% for PD and 10% to 10,2% for controls in both in vivo and post-mortem studies. In another study was shown for the first time that RT-QuIC could detect aSYN aggregates in olfactory mucosa of DLB patients with sensitivity reaching 86,4%.[116] However, the rates of positive results were reduced in two other studies who included people with iRBD. The sensitivity for iRBD versus controls was between 44,4% and 67%, while the specificity was high (90%).[32, 117]

Table 3. Biopsy studies of olfactory mucosa and olfactory bulb.

	Reference	PD (n)	DLB (n)	MSA (n)	PAF (n)	iRBD (n)	CTR (*n*)	Olfactory mucosa	Olfactory bulb	Brain	Other tissues	aSYNIR somata(LB) neurites (LN) %	phosaSYNIR somata(LB) neurites (LN) %	Other techniques: [1] H&E [2]Azan—Mallory [3]Bodian [4]Electron microscopy [5]VIP,TH (IHC) [6]Luxol fastblue [7]IHC [8]PET blot [9]PMCA [10]RT-QuIC [11]PLA	Comments
1	Duda et al. 1999[108]							•		•		PD: 85%* CTR:90%*	PD: 85%* CTR:90%*	1,3	*In ORN
2	Bloch et al.2006[78]	2					98		•	•	•	PD: 100%* CTR:17%*, †	PD: 100%* CTR:17%*, †		*IR neurites predominate over IR somata †aSYN pathology was found in 17 (17.3%) out of 98 neurologically asymptomatic subjects. All of these had olfactory bulb involvement
3	Beach et al. 2009[109]	58					69		•	•		PD: 95%* CTR:8%	PD: 95%* CTR:8%		*LB-like perikaryal inclusions in ≈10%
4	Jellinger et al. 2009[110]	25					-	•	•	•		PD: 8%*	PD: 8%		*In olfacotry epithelium (exact site n.s.)
5	Witt et al. 2009[115]	7					25	•				PD: 0%* CTR: †	PD: 0%* CTR: 100%	1,4	*Loss of OMP IR in ORN †In some ORN
6	Arnold et al. 2010[111]	7					45	•		•		PD: 14% CTR:2%			Occasional intra-cytoplasmic inclusion
7	Funabe et al. 2013[112]	4					62	•	•	•	•	PD: 0% CTR: 0%	PD: 75%* CTR:0%	1,2	Nerve fibers in lamina propria
8	Toledo et al. 2016[149]	141	13						•	•	•*	PD:31,2%† PDD:30% DLB:38,4% AD/LB:22,7%	n.d	7	PDD:80, AD/LB pathology:308, were also included. *Submandibular gland, esophagus †LB pathology in AD/LB shows a distribution that differs from PD, with different patterns of spreading.
9	Saito et al. 2016[113]	8	2	2				•	•	•		n.d	PD:75%* iLB:9,1%	7	CBS:2, PSP:5, SCA:4, ALS:6, iLB:11 were .also included. *olfactory epithelium

10	Stevenson et al. 2020[114]	11					11		•			PD:100%*	PD:100%*	7	*AON
11	Perra et al. 2021[116]		37*					•				DLB:86,4%†		10	*Prodromal or propable DLB AD/LB pathology:6, non-LB related pathology:36 were also included †93.8% for CSF
12	Stefani et al. 2021[117]	41				63	59	•				PS: 46,5% iRBD:44,4% CTR:10,2%		10	
13	Bargar et al. 2021[55]	2					1			•	•*		PD; + (n.s) CTR:0%	10	*skin, submandibular glands
14	Bongianni et al. 2022[118]	66					29	•				PD:67,4% CTR:10%	PD:+ CTR:0%*	10, 7	*PhosaSYN: n.s
15	Kuzkina et al. 2023[32]	27		3		18	30	•			•*	Olfactory	Skin		3 patients fulfilling clinical diagnostic criteria of PSP. *skin
												PD:48% MSA:67% iRBD:67% CTR:10%	PD:78,9% MSA:100% iRBD:100% CTR:n.d	10	
												n.d	n.d	7	

Post-mortem studies with accompanying brain pathology are shown in dark grey shaded cells, in vivo studies are shown in light grey shaded cells. aSYN, alpha synuclein; iPD, idiopathic PD; PD, Parkinson's disease; PDD, PD dementia; CTR, control; DLB, dementia with Lewy bodies; MSA, Multiple System Atrophy; PAF, pure autonomic failure; iRBD, Isolated Rapid eye movement Behavior Disorder; ALS, Amyotrophic Lateral Sclerosis; PSP, progressive supranuclear palsy; CBD, Corticobasal Degeneration; AD, Alzheimer disease; SCA: Spinocerebellar Ataxia; GBA1, glucocerebrosidase gene; GI, gastrointestinal; H&E, hematoxylin—eosin staining; IHC, immunohistochemistry; IR, immunoreactive; PMCA, protein misfolding cyclic amplification; RT-QuIC assay, real-time quaking induced conversion; PLA, Proximity Ligation Assay; LB, Lewy bodies; LN, Lewy neurites; n/a, not applicable; n.d., not determined; n.s., not stated; RSCs, Remak non-myelinating Schwann cells;; orthostatic hypotension; phosaSYN, phosphorylated alpha synuclein; OMP, olfactory marker protein; ORN, olfactory receptor neurons; AON, anterior olfactory nucleus, TH, tyrosine hydroxylase; VIP, vasoactive intestinal polypeptide; asterisks and daggers refer to the same table row. The percentages correspond to the sensitivity (PD%). The specificity equals 100% – CTR%.

Discussion

Technical Issues: Which process? Which immunohistochemical marker?

In the majority of the skin biopsy studies, samples were derived from the trunk (C7-C8 C8 paravertebral area) and lower limb (i.e. thigh;15 cm above the patella and distal leg;10 cm above the lateral malleolus) which are considered the optimal biopsy-taking sites. But what happens with people with prodromal disease or unilateral motor symptoms? An unsolved question concerns phosaSYN aggregates and their preferential side of distribution. Does deposition reflect the site of motor dysfunction? It was found that in PD patients with unilateral disease 20% had abnormal deposits only in the affected motor side, 60% in both sides and 20% only in the non-affected side respectively. Regarding the spine topographical distribution of skin phosaSYN, it seems that deposits displayed a uniform distribution between both sides (and not following the motor dysfunction) in unilateral patients. It was also demonstrated a spine gradient with the cervical site expressing the highest positivity compared to Th12.[28] Furthermore, study findings on phosaSYN in skin biopsies revealed that the range of sensitivity depends on the biopsy site (ranging from 31% in distal leg, to 100% in cervical site).[12] However, according to other authors, the biopsy site does not affect the potency of total aSYN detection (90% sensitivity and specificity).[25]

Obviously, the detection rate of phosaSYN depends not only on the exact biopsy site taken but also on methodological differences using sections of different tissue thickness. It was demonstrated that double-immunostained 50 μm skin biopsy tissue sections are superior to 20 and 10 μm tissue sections for the detection of phosaSYN. Apparently, the greater volume of tissue analyzed and the improved visualization of nerve fiber architecture increases the sensitivity of the procedure.[39, 40]

Similarly, the amount of nervous tissue is usually insufficient, in conventional colonic biopsies. Therefore, the discovery of LB pathology is increased by obtaining full-thickness sections of colon. Beach et al. reported that the submucosa has the highest prevalence of pathological LB staining, followed by the muscularis and mucosa.[119] Notably, the distribution of aSYN/phosaSYN varies between different gut tissues, following a rostro-caudal gradient pattern, resembling skin topographical allocation. aSYN/phosaSYN burden shows highest levels in the esophagus and lowest involvement of the distal colon and rectum.[50, 51-77] Most of the in vivo studies which obtained tissue from the gastrointestinal tract have used immunohistochemistry techniques for the detection of LB pathology. The vast majority of them had a specificity and sensitivity less than 80 % regarding to PD. Moreover, the biochemical methods tested were not adequate for the prediction of PD.[120, 121] More specifically, salivary glands, studies showed higher sensitivity for needle core biopsies obtained from the submandibular gland (56,2%-100%)[73, 89, 122], whereas biopsies from minor salivary glands resulted in largely varying rates of positive phosaSYN/aSYN staining (7%–100%) in PD.[64, 66, 69, 123, 124] The heterogeneity of findings obtained from these studies is complicated not only by differences in immunocytochemical staining techniques, dissection protocols, and subjects included (accuracy of clinical diagnosis), but also by study design (cohort sampling size and stratification, retrospective vs. longitudinal, in vivo vs post-mortem).

The early and persistent accumulation of phosaSYN/aSYN in the GI of patients with prodromal PD supports the hypothesis that disease originates from the colon. On the other hand, LB pathology is present in colon in people who never developed the disease when alive.[103] Borghammer et al. conducted a focused re-analysis of two postmortem datasets, which included large numbers of mild LB disease cases. They observed that the pathologic process starts in either the olfactory bulb or the ENS, but rarely in the olfactory bulb and GI simultaneously. The above findings revise the dual-hit hypothesis of PD which postulates that the pathologic process starts from the olfactory bulb and dorsal motor nucleus of the vagus nerve.[125]

The first neuropathological attempts towards identifying a reliable biomarker in synucleinopathies in peripheral tissues started with the use of antibodies against aSYN. We already know that aSYN is also detectable in healthy people. Several studies have shown that the frequency of positive aSYN staining is varies reaching even 100% in healthy subjects and is seems to be unlikely that all controls included were affected by synuclein associated disease. (Tables 1–3).[10, 52, 60, 78, 82-85, 93, 96, 99, 104, 126] It was underlined by Beach et al. in 2013 that aSYN is one of the most abundant proteins in neural tissue, and therefore positive aSYN staining cannot be abnormal.[63] They suggested the use of antibodies against phosaSYN. In the same research work, they also proposed proteinase K pretreatment towards digesting normal aSYN and allowing affected pathological phosaSYN inclusions to be revealed.[127] The most reliable immunohistochemistry marker which distinguishes pathological deposits from physiological aSYN is phosphorylated αSyn (phosaSYN) at serine 129.Amongst the phosaSYN antibodies tested, many researchers retrieved the best results with the use of the monoclonal antibody directed against peptide 124–134 including phosphorylated Ser129 (Wako Pure Chemical Industries Ltd., Neuss, Germany).[128, 129]

The significance of aSYN phosphorylation is a mat-

ter of debate. In vitro studies reported that phosaSYN impels the formation of inclusions. Only a small amount of aSYN is phosphorylated in healthy human brain and aSYN appears to be phosphorylated as disease progresses.[130] More interestingly, aSYN oligomerization has been described as an early event in the pathologic process, independent of the phosphorylation.[131] Recently, aSYN targeting antibody, αSyn-5G4 showed high conformational specificity and strong immunoreactivity for all forms of αSyn aggregates, reliability in identifying aSYN deposits and was also able to detect astrocytic and oligodendroglial aSYN inclusions across synucleinopathies.[100, 132, 133] It is suggested that 5G4 deposits appear at an early stage of the disease and they are less detectable after the spread of neurodegeneration. Additionally, they have a different distribution among skin biopsy sites, compared to phosaSYN.[29] Another marker of the early stage of the pathology is PLA which recognizes the oligomeric form of aSYN. PLA does not reveal physiological aSYN and detects pathology in the form of extensive diffuse deposition of aSYN oligomers which are often localized, in the absence of Lewy bodies.[134] With the use of PLA, Mazzetti et al. first described, that aSYN oligomers accumulate within synaptic terminals of autonomic fibers of the skin in PD.[53] A combination of tests run with phosaSYN, aSYN, aSYN-5G4A, aSYN-PLA, and IENFD, will increase the diagnostic yield and open new windows in understanding the temporal events of aSYN spread.[41]

Seeding amplification assays (SAAs) as the Protein misfolding cyclic amplification (PMCA) and the real-time quaking-induced conversion (RT-QuIC), were originally developed to mimic prion replication.[135, 136] A meta-analysis study revealed that skin aSYN-SAAs exhibited the highest sensitivity (0.92), which was not different from that of cerebrospinal fluid (CSF) (0.90),[137] and therefore, skin biopsies could represent a valid alternative to CSF analysis.[58] Olfactory mucosa aSYN-SAAs exhibited a lower sensitivity compared to CSF and skin.[137] However, RT-QuIC sensitivity is significantly increased when nasal swab is performed at different areas covered by olfactory epithelium indicating that aSYN aggregates are preferentially detected in olfactory areas with higher concentration of olfactory neurons.[118] Additionally, applying the method in diverse tissues (i.e. olfactory as part of the central nervous system and skin as peripheral nervous system), diagnostic accuracy could increase.[32] The high sensitivity, specificity of RT-QuIC assay in skin specimens was confirmed by isolated in vivo and post-mortem studies.[31, 32, 38, 53, 57] Higher α-synuclein seeding activity in RT-QuIC was shown in patients with longer disease duration and more advanced stage of disease and was correlated with non-motor symptoms (i.e. RBD, cognitive decline, constipation).[31, 38] Therefore, the method could be useful not only for diagnostic reasons, but also for monitoring disease progression.[54, 57]

Classical LB are defined as round eosinophilic inclusions located in neuronal somata with hyaline appearance.[138] The pathological signature of LB diseases has broaden with the advent of immunohistochemistry. Additional morphological features have been described for LB/LN in the CNS (i.e. diffuse, granular or pleomorphic intraneuronal structures or intra-neuritic dot-like structures and axonal spheroids).[139] The question which arises is if positive aSYN/phosaSYN staining of neuronal somata or processes in peripheral tissues can be regarded as and termed LB or LN, respectively. Thus, studies on peripheral tissues should not only describe the absence or presence of aSYN/phosaSYN immunoreactivity but also precisely depict the morphological features resembling LB/LN-like structures.

Conclusions and future perspectives

This review of a combination of postmortem and in vivo studies redefines the remarks of previous evaluations regarding optimal tissue source, technique and immunohistochemical marker.[140] Skin biopsy is an easy, minimum invasive approach which provides high specificity and good sensitivity for the detection and differential diagnosis of synucleinopathies. GI biopsies remain attractive in the detection of synucleinopathies. However, a standardized methodology is essential to increase their diagnostic value. The new promising assays could be incorporated in future cohorts, towards identifying the combinations and relative contributions of the sensitivity amongst peripheral tissues.

Review highlights.

• Phosphorylated a-synuclein (phosaSYN) is the pathological signature of Parkinson's disease.
• phosaSYN is confined to the central nervous system, but also to peripheral tissues.
• Studies on peripheral tissues should not only describe the absence or presence of aSYN/phosaSYN immunoreactivity but also depict the morphological features resembling LB/LN-like structures.
• The most reliable immunohistochemistry marker which distinguishes pathological deposits from physiological aSYN is phosphorylated αSyn (phosaSYN) at serine 129.
• Cutaneous phosaSYN aggregation is detected in most of iRBD population.

• Dermal phosaSYN can be considered a peripheral histopathological marker of synucleinopathy representing prodromal PD.
• phosaSYN is mainly detected in autonomic fibers of PD and DLB.
• In MSA phosaSYN is detected in skin Remak non-myelinating Schwann cells (RSCs).
• The in vivo and postmortem studies in submandibular gland report controversial results.
• The distribution of aSYN/phosaSYN varies between different gut tissues, following a rostrocaudal gradient pattern.
• In GI tract, the submucosa has the highest prevalence of pathological staining, followed by the muscularis and mucosa.
• RT-QuIC can detect aSYN aggregates in olfactory mucosa in synucleinopathies with high sensitivity.
• A combination of tests run will increase the diagnostic yield.

Useful points to clinical practice

		PD diagnosis	Differential diagnosis among synucleinopathies	Early diagnosis	Disease progression
Tissues	skin	+	+	-	-
	GI			?	
	Olfactory mucosa	-	-	-	-
Techniques & markers	aSYN-5G4A			+	
	aSYN-PLA			+	
	aSYN-SAAs (RT-QuIC)	+			+

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