

## INTRODUCTION

Isolated REM sleep behavior disorder (IRBD) is a parasomnia that confers a 91% risk at 15-years of follow up of developing a sinucleinopathy (approximately 45% Parkinson's disease, 45% dementia with Lewy bodies and 5% Multisystem Atrophy)<sup>1</sup>, characterized by brain alpha-synuclein (a-Syn) deposits.

Recent studies in IRBD have shown the presence of pathological a-Syn also in the cerebrospinal fluid with high sensitivity (90.4%) and specificity (90%)<sup>2</sup>. In addition, immunohistochemistry with antibodies against phosphorylated synuclein (p-Syn), targeting pathologic a-Syn, shows inclusions in the peripheral autonomic nervous system (pANS) of IRBD, but with conflicting results. Prospective studies in IRBD collecting samples from submandibular gland, parotid, labial salivary glands, colon, stomach and skin have shown high specificity (94-100%) but a wide range of sensitivity (24-82%)<sup>3,4,5,6,7,8</sup>. In a retrospective study with p-Syn using previous surplus samples of the gastrointestinal tract from the clinical practise, 56% of prodromic Parkinson's disease, 50% of Parkinson's disease and up to 26% of controls had pANS pathological inclusions<sup>9</sup>. This study had the advantage to use previous samples and avoid new interventions with potential side effects, but the samples were not obtained with the purpose to assess a-Syn in the autonomic nervous tissue, and this could explain the worse results. Recently, a novel antibody (5G4) against oligomeric pathologic forms of a-Syn (o-Syn) has been used in brain tissues with excellent results but to our knowledge there is no experience in peripheral tissue in IRBD.

Our aim was to assess the presence of phosphorylated a-Syn (p-Syn) and oligomeric a-Syn (o-Syn) in previous surplus gastrointestinal, skin, and urological biopsies/surgical resections of IRBD patients and matched controls.

## METHODS

A total of 164 samples (either biopsies- small size- or surgical resections –bigger size-) from 49 polysomnogram (PSG) confirmed RBD patients were retrospectively collected from the Pathology Department archives of the last 25 years (1998-2023) and matched with 161 samples from 122 neurological controls by organ, pathology age and gender (i.e., prostate adenocarcinoma in a 70-year-old man). Immunohistochemistry with antibodies against phosphorylated a-Syn (p-Syn, pSyn64, Wako) and oligomeric a-Syn (o-Syn, 5G4, Roboscreen), were used.

## RESULTS

### A. IRBD Samples (case biopsies or surgical resections found)

Peripheral nerve tissue	IRBD (n=164)	Surgical resection (%) Samples with bigger area/size, otherwise are smaller biopsies	Genitourinary tract	57 (34.8%)
<b>Gastrointestinal tract</b>	<b>83 (50.6%)</b>		<b>Prostate</b>	<b>49</b>
Esophagus	12	44.7% →	Bladder	7
Stomach	6		Kidney	1
Duodenum	3			
Small intestine	1		<b>Skin</b>	<b>24 (14.6%)</b>
Blind	1		Face	8
Colon	25	29.8% →	Thorax	6
Sigma	23		Arms	1
Straight	6		Legs	5
Anus	4	← 25.5%	Pelvis	3
Gall bladder	3		Unknown	1

### B. Analysis by matched samples

#### General analysis\*

	IRBD (n=164)	Controls (n=161)	P-value
Age at sample (years)	70.6 ± 7.7	70.3 ± 8.1	0.7
Gender (male)	90.9%	89.4%	0.7
Area (mm <sup>2</sup> )	53.7 ± 81.2	64.8 ± 92.5	0.3
p-Syn (+) lax	<b>23.8%</b>	<b>9.3%</b>	<b>&lt;0.001</b>
p-Syn (+) strict	<b>6.7%</b>	<b>0.6%</b>	<b>0.004</b>
o-Syn (+) lax*	<b>14%</b>	<b>1%</b>	<b>&lt;0.001</b>
o-Syn (+) strict*	<b>10%</b>	<b>1%</b>	<b>&lt;0.001</b>

#### Analysis of IRBD (+) samples by clinical status\*

	Pre RBD symptoms	Symptoms pre v-PSG	IRBD	PD or DLB
n° of samples	34	38	77	15
P-Syn (+) lax	20%	18.9%	29.9%	13.3%
P-Syn (+) strict	<b>2.4%</b>	<b>5.4%</b>	<b>9.1%</b>	6.7%
o-Syn (+) lax*	<b>6%</b>	<b>11%</b>	<b>18%</b>	<b>20%</b>
o-Syn (+) strict*	<b>5%</b>	<b>8%</b>	<b>10%</b>	<b>20%</b>

\*o-Syn: Only 80 IRBD and 74 control samples have been assessed with 5G4. These results are provisional and shown without decimals

### C. Analysis by subjects

	TCSRA (n=49)	Controls(n=122)	P-value
Number of samples	3.3 ± 2.5 (1-12)	1.3 ± 0.8 (1-8)	<b>&lt;0.001</b>
p-Syn (+) lax	22 (44.9%)	13 (10.7%)	
p-Syn (+) strict	9 ( <b>18.4%</b> )	1 (0.8%)	
p-Syn and/or o-Syn (+) lax*	24 ( <b>49%</b> )	14 ( <b>11.5%</b> )	<b>&lt;0.001</b>
p-Syn and/or o-Syn (+) strict*	15 ( <b>30.6%</b> )	2 ( <b>1.6%</b> )	<b>&lt;0.001</b>

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## CONCLUSIONS

**Pathological a-Syn can be found in surplus biopsies/surgical resections of patients with IRBD even years before the onset of the parasomnia symptoms with low sensitivity and variable specificity.**

The heterogeneity of the tissue (organ, pathology-inflammation) and its processing (fixation, dehydration, paraffin, years from acquisition) **hinders to avoid the nonspecific background in the samples and this limits their interpretation.**

Strict criteria for positivity of α-synuclein deposits according to the background offers high specificity, but very low sensitivity.

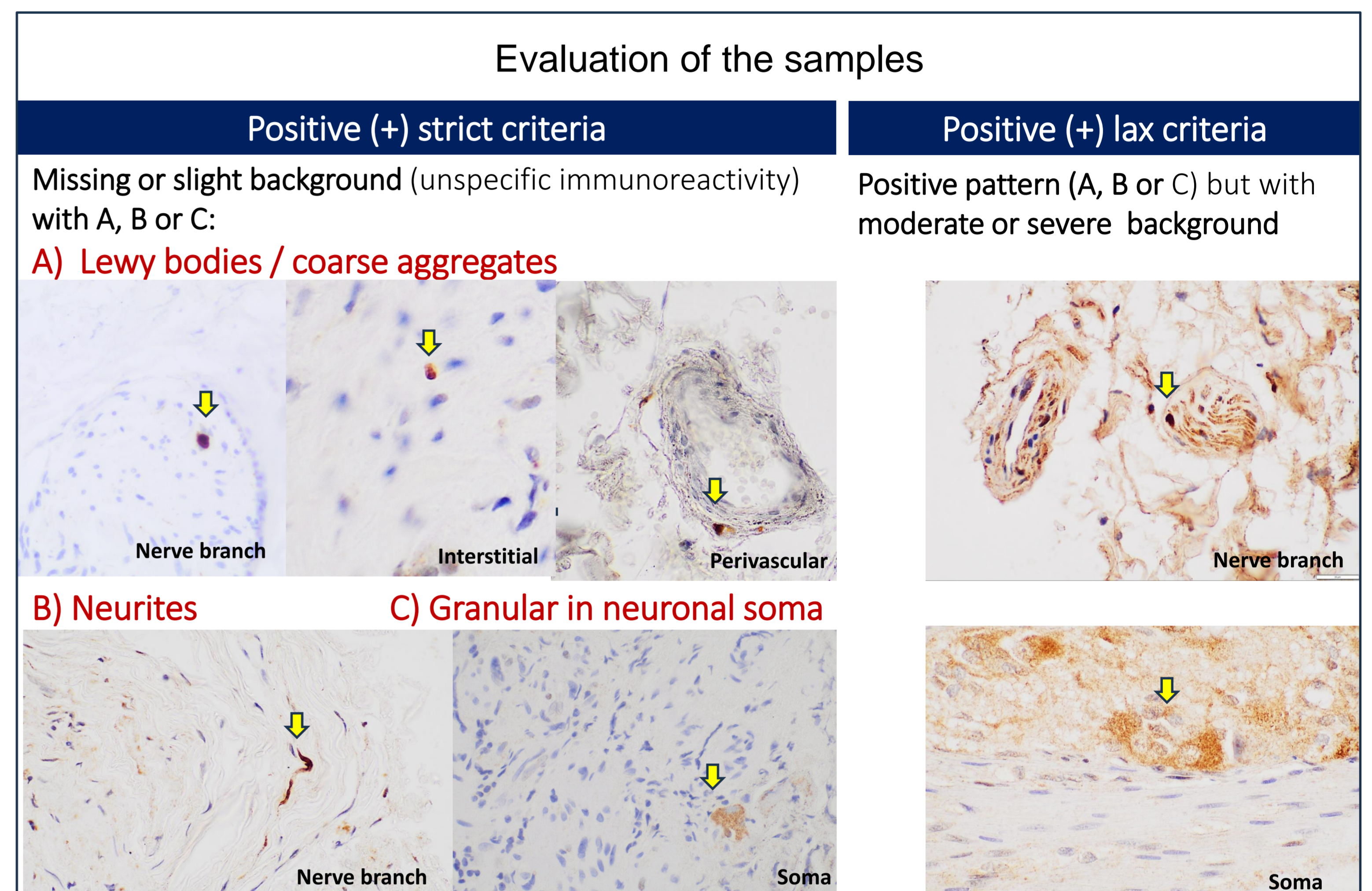
The 5G4-SYN antibody against oligomeric a-Syn has less background (non-specific immunoreactivity) and could be more specific with a similar low sensitivity.

RT-QuIC techniques are more sensitive and specific for the diagnosis of Synucleinopathies.

**The use of antibodies against p-Syn and o-Syn with surplus peripheral tissue for the diagnosis of a synucleinopathy in IRBD probably has not clinical utility.**

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#### Analysis of IRBD (+) samples by anatomical origin\*

	o-Syn (+)*		p-Syn (+)	
	Lax	Strict	Lax	Strict
<b>IRBD</b>	15%	8%	26.5%	3.6%
<b>Control</b>	2%	2%	6.3%	0%
Background+	7%		42%	
<b>IRBD</b>	16%	<b>14%</b>	21.1%	<b>14%</b>
<b>Control</b>	2%	0%	7%	1.8%
Background+	9%		51%	
<b>IRBD</b>	25%	8%	<b>20.8%</b>	<b>0%</b>
<b>Control</b>	4%	0%	<b>25%</b>	<b>0%</b>
Background+	6%		<b>79%</b>	

Urologic tissue was more frequently (+) as there were a higher percentage of surgical resections, which had a higher positive ratio.  
p-Syn (+) in the skin was similar in case and controls  
**+Moderate to severe background**