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BRIEF COMMUNICATION

Biomarkers for REM sleep behavior disorder in idiopathic and narcoleptic patients

Elena Antelmi[1,2], Fabio Pizza[1,2], Vincenzo Donadio[1,2], Marco Filardi[1], Yuri L. Sosero[1], Alex Incensi[2], Stefano Vandi[1,2], Monica Moresco[1,2], Raffaele Ferri[3], Sara Marelli[4,5], Luigi Ferini-Strambi[4,5], Rocco Liguori[1,2] & Giuseppe Plazzi[1,2]

[1]Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy
[2]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy
[3]Sleep Research Centre, Oasi Research Institute - IRCCS, Troina, Italy
[4]"Vita-Salute" San Raffaele University, Milan, Italy
[5]Department of Clinical Neurosciences, IRCCS San Raffaele Scientific Institute, Neurology - Sleep Disorders Centre, Milan, Italy

Correspondence
Giuseppe Plazzi, Department of Biomedical and Neuromotor Sciences, University of Bologna IRCCS Istituto delle Scienze Neurologiche di Bologna, Bellaria Hospital, Via Altura 3, Bologna, Italy.
Tel: +39.051.4966964;
Fax: +39.051.4966176;
E-mail: giuseppe.plazzi@unibo.it

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Abstract

To search for discriminating biomarkers, 30 patients with idiopathic rapid-eye-movements sleep behavior disorder (iRBD) were compared with 17 patients with RBD within narcolepsy type 1. Both groups underwent extensive examinations, including skin biopsy searching for phosphorylated α-synuclein deposits and whole-night video-polysomnography. Skin biopsy was positive for phosphorylated α-synuclein deposits in 86.7% of iRBD patients and in none of narcoleptic patients. The analysis of video-polysomnographic motor events showed differences in their occurrence throughout the night in the two groups. iRBD and RBD due to narcolepsy do have different clinical and pathological findings, confirming a different pathophysiology.

Introduction

Rapid-eye-movements (REM) sleep behavior disorder (RBD) is a parasomnia characterized by repeated episodes of dream enactment associated with loss of muscle atonia during REM sleep.[1] Idiopathic (or isolated) RBD (iRBD) has the highest positive predictive value for impending synucleinopathies.[2,3] After neurodegenerative diseases, the second most common cause of secondary RBD is narcolepsy type 1 (NT1), a central hypersomnia, linked to loss of hypocretin 1 (Hcrt-1) neurons.[1]

RBD is reported to occur in NT1 with a frequency ranging between 7% and 63% and may rarely be the heralding symptom of NT1.[1,2]

iRBD and RBD secondary to narcolepsy share both discrete and overlapping features.[1,2,4–7] While iRBD has been consistently linked to an underlying synucleinopathy and positive synucleinopathy at skin biopsy has been consistently reported to be a biomarker of iRBD,[3,8] RBD within narcolepsy has been linked to the Hcrt-1 loss. However, not all NT1 patients do have RBD and RBD does not occur only in narcoleptic patients with Hcrt-1 deficiency.[2] The pathophysiology of RBD within narcolepsy is therefore a still controversial issue.

In order to advance the understanding of these ambiguities and to possibly identify a discriminating marker, we recorded clinical, neurophysiological, and pathological biomarkers in NT1 patients with a complaint of RBD and compared the findings with those obtained in adult patients with iRBD.

Methods

Among 72 adult consecutive patients referred to our clinic within the last two years (October 2016- October 2018) and receiving a final diagnosis of NT1, according to the current criteria,[1] we selected 17 patients who subjectively reported a complaint of RBD, confirmed by

video-polysomnography (vPSG).[1] NT1 patients with RBD where then compared with 30 age-matched consecutive patients who received a final diagnosis of vPSG-confirmed iRBD, according to the current criteria.[1]

Patients with alcohol-use disorder, taking antidepressants, or beta-blockers or with signs of motor or cognitive dysfunctions were excluded.

Both groups underwent extensive examinations, with complete clinical and neurological examination. All the motor and nonmotor symptoms were assessed by means of history taking, except for orthostatic hypotension for which we performed blood pressure measurement while lying-down and after 1–3 min of standing. We also performed neuropsychological testing, including the Brief Mental De terioration Battery,[9] brain MRI, nigrostriatal dopamine transporter ligand [123I]ioflupane-DaTscan and skin biopsy looking for phosphorylated α-synuclein (p-α-syn) deposits. For skin biopsy, 3-mm punch biopsy was performed bilaterally, proximally (i.e., C8 paravertebral area) and distally (10 cm above the lateral malleolus of legs). Samples were then incubated and analyzed, as previously reported.[3]

In order to exclude motor symptoms, in patients with iRBD we used the Unifying Parkinson Disease Rating Scale Part III – UPDRS-III.

Whole-night vPSG was performed, which included conventional electroencephalogram (at least three channels, including frontal, central, and occipital leads, referred to the contralateral mastoid), bilateral electrooculogram, submentalis and anterior tibialis electromyography, respiratory parameters, and electrocardiogram. Sleep was scored according to standard criteria.[10] Files were exported in European Data Format and the REM Atonia Index was computed, using the validated automatic analysis implemented in Hypnolab v. 1.2 software.[11]

Time synchronized vPSGs were reviewed offline and separately by two sleep experts, blinded to clinical diagnosis (EA and FP). All sleep stages were considered, except for wakefulness before and after sleep onset. Movements were scored in both NREM and REM sleep stages and classified as elementary (if brief and nonpurposeful, as, e.g., stereotypies, facial grimacing, pelvic movements) or complex (if longer and purposeful, hence mimicking a scenic behavior, likely linked to a dream mentation).[12] Motor events were judged as having a stereotyped pattern if they tended to occur with the same/similar pattern on different occasions. The mean interscorer agreement was 0.94, with kappa values ranging from 0.85 for the label "stereotyped pattern of simple motor activity in NREM" to 1 for the label "presence of simple gesturing during REM sleep." Group differences between NT1 and iRBD in clinical, neurophysiological, and biochemical data were analyzed by means of chi-squared test and Mann–Whitney *U* Test, as appropriate. False discovery rate (FDR) was used to correct for multiple comparisons; *P* values < 0.05 were considered to be statistically significant.[13]

This study has been approved by the internal ethics committee, and all the participants signed an informed consent form.

Results

The final sample included 17 patients with NT1 (mean age 64.24 years, standard deviation 12.86; 70.6 % males), compared to 30 patients with iRBD (mean age 69.90 years, standard deviation 8.38; 83.3% males).

All patients with iRBD had a score of less than 5 at the UDRS, Part III (mean 1.35, standard deviation 1.99).

As expected, NT1 patients had pathological values at the Epworth Sleepiness Scale.

Patients with iRBD showed older age for RBD onset, reported more frequently violent episodes and complained more frequently of an every-night occurrence of RBD episodes (Table 1), while all the other clinical data were comparable between the two groups. Neurophysiological data are reported in Table 1.

Motor activity/behavior during sleep

When looking at the motor activity during REM sleep, we found that both groups had the same occurrence of simple motor activity, which, however, in NT1 patients tended to show more frequently an almost intra-individual stereotyped pattern, that is, to recur showing the same/similar pattern whenever they occurred (although this finding did not pass the correction for multiple comparisons). Complex motor activity in NT1 patients occurred similarly in the first and second half on the night, when compared with iRBD patients (in whom it was mainly confined to the second half of the night) (Table 2).

Moreover, compared to iRBD, more NT1 patients presented simple motor episodes also during NREM sleep. In NT1 patients, simple motor episodes tended also to have more frequently an almost intraindividual stereotyped pattern and occurred in all NREM sleep stages, whereas in iRBD simple motor episodes occurred mainly in lighter sleep (Table 2). Complex motor activity during NREM, configuring a NREM parasomnia, was captured only in narcoleptic patients.

Pathological biomarkers and neurophysiological tests

Intraneural p-α-syn deposits at skin biopsy were found in 86.7% of iRBD patients, but in none of NT1 patients. No differences were found for the frequency of abnormalities at DaTscan or neuropsychological testing (Table 2).

Table 1. Comparison of clinical and neurophysiological data obtained in the two groups of patients.

	iRBD (n = 30) mean ± SD or %	RBD-NT1 (n = 17) mean ± SD or %	P	q
Clinical data				
Age, years	69.9 ± 8.38	64.2 ± 12.86	0.094	
Males	83.3%	70.6%	0.305	
Urinary urgency	23.3%	5.9%	0.126	
Orthostatic hypotension	3.3%	–	0.447	
Reduction of olfaction	23.3%	5.9%	0.126	
Motor symptoms	23.3%	11.8%	0.333	
Cognitive symptoms	23.3%	11.8%	0.333	
Psychiatric symptoms	20.0%	17.6%	0.844	
Constipation	43.3%	29.40%	0.345	
RBD onset, years	57.8 ± 9.56	43.8 ± 17.69	**<0.01**	<0.05
Violent RBD episodes	53.30%	–	**<0.0005**	<0.01
Everyday occurrence of RBD episodes	33.30%	–	**<0.01**	<0.05
Epworth Sleepiness Scale score	7.1 ± 2.89	17.3 ± 3.08	**<0.0001**	<0.0001
Neurophysiological data				
Sleep latency, min	34.6 ± 52.40	5.3 ± 6.07	**<0.0001**	<0.0005
REM latency, min	120.0 ± 76.34	76.5 ± 105.20	**<0.01**	<0.05
Total sleep time, min	304.7 ± 88.79	318.2 ± 55.10	0.673	
Sleep efficiency	68.9% ± 17.60	68.0% ± 12.27	0.289	
N1	16.3% ± 9.28	11.12% ± 8.33	0.057	
N2	46.9% ± 10.51	34.9% ± 11.34	**<0.005**	<0.05
N3	19.2% ± 8.84	29.2% ± 15.24	**<0.05**	<0.05
REM	15.7% ± 6.62	22.6% ± 9.93	**<0.05**	<0.05
ODI	4.9 ± 6.49	2.9 ± 3.52	0.335	
PLMS index	24.8 ± 28.15	24.9 ± 21.66	0.568	
Atonia index	0.53 ± 0.21	0.63 ± 0.21	0.153	

Significant P values are in bold. min, minutes; N1-N2-N3-REM (stage 1,2,3 of NREM sleep; REM sleep); ODI, oxygen desaturation index; SD, standard deviation; PLMS, periodic limb movements during sleep; q = FDR-adjusted P-values.

Discussion

Two "clear-cut" signatures were found in this study, helpful for the differential diagnosis between iRBD and RBD due to NT1. First, positive skin biopsy confirmed to be the biological fingerprint of iRBD, whereas it turned to be always negative in NT1 patients with RBD, corroborating the hypothesis of a complete different pathophysiology between these two conditions.

The second main finding is that while in iRBD complex motor activity is confined to REM sleep, in NT1 patients with RBD, nocturnal motor activity/behaviors recurred throughout the whole night, in both NREM and REM sleep, and showed frequently an almost stereotyped pattern in the same subject.

The occurrence of RBD in patients with narcolepsy has been hypothesized to be linked to Hcrt-1 loss,[9] but no consensus has been reached for the mechanisms involved.[1,2,14] Indeed, not all patients with NT1 present RBD and, contrary to cataplexy, RBD has not been consistently associated with a deficit of Hcrt-1.[2]

iRBD/RBD within Parkinson disease (PD) and RBD within NT1 share some overlapping features, from both a clinical (i.e., hyposmia, autonomic changes, cognitive and neuropsychiatric symptoms, sleep attacks) and a biological (i.e., reduction of CSF-Hcrt-1 levels) standpoint.[1,2,4,15] Moreover, ictal single-photon emission tomography studies exploring the RBD pathways in vivo showed similar cerebral activation in patients with iRBD and in those with RBD linked to PD or NT1.[4] However, solid evidence supports a completely different etiopathogenesis of the underlying diseases.[2,5–7]

To this regard, 86.7% of our iRBD patients, but none of patients with NT1-RBD had positive skin biopsy for p-α-syn deposits. This finding confirms, in this relatively large cohort of patients, the notion that iRBD is a synucleinopathy,[3,8] but also corroborates the hypothesis of different pathophysiological processes in iRBD and NT1-associated RBD, implying differences in the risk of conversion to neurodegenerative disorders.

The finding of increased motor activity throughout the whole night supports previous observations,[2] and

Table 2. Comparison of data on motor episodes during REM sleep and markers of degeneration obtained in the two groups of patients.

	iRBD (n = 30) Mean ± SD or %	RBD-NT1 (n = 17) Mean ± SD or %	P	q
Motor episodes in REM sleep				
Presence of simple motor episodes	93.1%	94.1%	0.893	
Number of simple episodes	9.3 ± 7.35	12.4 ± 12.65	0.659	
Intraindividual stereotypy of events	40.7%	75%	**<0.05**	
Presence of complex motor episodes	58.6%	70.6%	0.417	
Number of complex episodes	5.2 ± 3.91	5.6 ± 4.98	0.929	
Mean duration of complex episodes, seconds	20.7 ± 16.30	15.1 ± 18.91	0.071	
Intraindividual stereotypy of events	37.5%	66.7%	0.127	
Violent/energetic pattern	27.6%	5.9%	0.073	
Episodes occurring in the 2nd half of the night	93.8%	42.3%	**<0.05**	**<0.05**
Episodes occurring in the 1st and 2nd half of the night	6.2%	57.7%		
Motor episodes in NREM sleep				
Simple motor episodes	44.8%	76.5%	**<0.05**	
Number of episodes	6.8 ± 6.40	21.5 ± 15.52	**<0.005**	**<0.05**
Intraindividual stereotypy of events	33.3%	76.9%	**<0.05**	
Distribution of motor episodes				
N1	46.6%	7.7%	**<0.001**	**<0.005**
N1-N2	46.2%	7.7%		
N2-N3	7.7%	3.8%		
N1-N2-N3	0	53.8%		
Presence of complex motor episodes	0	15%	0.054	
Markers of neurodegeneration				
Pathological DaTscan[§]	36.7%	13.3%	0.104	
Pathological neuropsychological tests	3.3%	0	0.475	
Single deficits at neuropsychological tests	50%	20%	0.053	
Positive skin biopsy for p-α-syn	86.7%	0	**<0.0001**	**<0.0001**

N1, N2, N3, stage 1, 2 and 3 of NREM sleep; EF, executive function; VF, verbal fluency; VL, verbal logic; VM, verbal memory; VSA, visuo-spatial abilities, p-α-syn, phosphorylated alpha synuclein; q, FDR-adjusted P-values. Significant P values are in bold. [§] = 2 NT1 patients = putamen one-side; 11 iRBD patients = # 5 putamen one-side; # 1 caudate one side; # 1 putamen and caudate = # 3 = putamen bilaterally; # 1 caudate bilaterally.

confirms a broader abnormal motor control than that found in iRBD, where motor activity is mainly restricted to REM sleep and occurs mostly in the second half of the night, with a motor pattern of the episodes that is usually more energetic-violent than that observed in RBD of NT1 patients.

In NT1 patients, elementary and complex motor activity not only recurred throughout the whole night but had also an almost stereotyped intra-individual pattern, probably reflecting the typical instability of sleep, with subcontinuous sleep-transitionns[16,17] and occurrence of dissociated states[1,2] that brings to a mentation reflecting this "twilight" state.

To conclude, this study indicates that even if iRBD and RBD-NT1 may show some similarities, they do have different clinical and pathological findings.

Both vPSG and skin biopsy can discriminate between these two conditions.

A notable finding is the much greater rate of positive synuclein skin biopsy (86.7%) compared to the rate of abnormal DaTscan (36.7%) in the 30 iRBD patients, indicating a much greater sensitivity of the former test if used as a biomarker in patients with iRBD.

This study confirms that skin biopsy confirms is a feasible, reliable, specific, and sensitive marker of the underlying synucleinopathy in iRBD patients.

Authors Contributions

E.A. involved in conception of the study, acquisition of data, analysis and interpretation of data, drafting the manuscript, revising it critically for important intellectual content, and final approval of the version to be published. F.P. and M.F involved in acquisition of data, analysis of data, statistical analysis, and final approval of the version to be published. V.D. participated in acquisition of data, analysis and interpretation of data, and final approval of the version to be published. Y.L.S., A.I., S.V., S.M., and M.M involved in acquisition of data, analysis of data, and final approval of the version to be published. R.F. and L. F-S. involved in analysis of data, revising it critically for important intellectual content and final approval of the

version to be published. R.L. and G.P. involved in conception of the study, interpretation of data, revising it critically for important intellectual content and final approval of the version to be published.

Conflict of Interest

G.P. participated in advisory board of UCB pharma, Jazz pharmaceuticals and Bioproject. All the other authors have nothing to disclose.

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