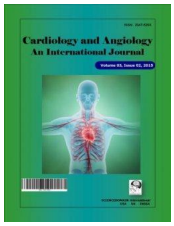

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Non-linear Analysis of Heart Rate Variability Improves Differential Diagnosis between Parkinson Disease and Multiple System Atrophy

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DB and RF designed the study, wrote the protocol. Authors DB and EI managed the literature searches, performed patient's recordings and compiled clinical database. Authors EI, CC, FF, KE and AV performed signal analyses and statistical evaluation of the results of the study. Author DB wrote the first draft of the manuscript. Author RF edited the final version of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Parkinson's disease (PD) and multiple system atrophy (MSA) are neurodegenerative disorders characterized by motor "parkinsonian" symptoms and non-motor symptoms related to autonomic nervous system (ANS) dysfunction. The latter can be quantified with the analysis of Heart Rate Variability (HRVa) and of its complexity. In this study nonlinear (NL) HRV complexity parameters were calculated to assess their predictive accuracy as markers of "disease" useful for early differentiation between PD and MSA in parkinsonian syndromes of uncertain diagnosis.
Study Design: Observational study.
Place and Duration of Study: Clinical Physiology-Biomagnetism Center, Policlinico A. Gemelli, Rome Italy. Patients enrolled from January 2010 to October 2013.
Methodology: 51 patients [25 with "certain" diagnosis of PD, 9 with a "highly probable" diagnosis of MSA and 17 with parkinsonian syndromes of uncertain neurological definition (6 with "undefined

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parkinsonism" and 11 with "suspected MSA")] and 40 age-matched healthy control subjects were studied. Short-term NL HRVa was performed during daily activity and during REM/NREM sleep from 24 h ECG recordings. Discriminant analysis (DA) was used to identify which NL HRV parameters (or their combination) were efficient to differentiate between PD and MSA in cases of uncertain diagnosis.

Results: Compared with healthy controls, most NL HRV parameters were significantly altered in patients ($p<0.05$), during both active and passive awakeness and during sleep. Most evident HRV abnormalities were found during active awakeness in MSA. DA of recurrence plot parameters provided the best predictive accuracy (76.5%) for the classification of parkinsonian patients with uncertain diagnosis.

Conclusion: NL HRVa is efficient in differentiating MSA from PD and may improve earlier diagnosis in patients with parkinsonian symptoms of uncertain nature, useful to address second level diagnostic steps and to guide more individualized drug treatment.

Keywords: Autonomic nervous system; Parkinson disease; multiple system atrophy; non linear HRV analysis; discriminant analysis.

1. INTRODUCTION

Parkinson's disease (PD) and multiple system atrophy (MSA) are neurodegenerative disorders belonging to the family of alpha-synucleinopaties [1], characterized by symptoms of "parkinsonism", such as bradykinesia, tremor at rest, muscle rigidity, postural instability, ataxia, which can occur in different combinations and gravity.

PD is the most common movement disorders and affects about 1% of people over the age of sixty. In most cases the etiology is unknown (idiopathic form), but are described both familiar [1] and genetically determined forms [2]. MSA is a sporadic neurodegenerative disease of unknown etiology that predominantly affects males between fifty and sixty years, with a prevalence of 4.6 cases per 100,000 people [1,3]. Both diseases are characterized by the combination of motor and non-motor symptoms. Among the latter, undoubtedly the dysfunction of the autonomic nervous system (ANS) is one of the main determinants of the altered quality of life of patients [4-8].

In PD autonomic dysfunction may precede even many years the typical motor signs, may be evident already in the first phase or dominate throughout the entire course of the disease [5,9].

The dominance of autonomic symptoms (orthostatic hypotension, urinary dysfunction, impaired intestinal motility, body temperature dysregulation) is a hallmark of MSA, in variable combination with typical signs of "parkinsonism", cerebellar ataxia and/or pyramidal signs [3]. Although it is known that ANS dysfunction is a consequence of the degenerative phenomena occurring in both central nervous system and in peripheral ganglia [10-14], the mechanisms responsible for motor and non-motor symptoms are somehow different and not fully understood [15-17]. Along the last 30 years the diagnosis of ANS derangement, relevant for prognostic judgment and therapeutic decisions, has been one of the major challenges for neurologists dealing with "parkinsonism" of uncertain nature. For this reason, several methods, including the Ewing Protocol [3,18,19], thermoregulation assessment [20], myocardial scintigraphy with iodine-123 meta-Iodobenzylguanidine (123I MIBG) [21] and Heart Rate Variability (HRV) analysis [8,10,14,22-25], have been proposed to attempt quantification of ANS derangement and to provide early differentiation between PD and MSA [26-29].

In most recent studies, time-domain (TD) and frequency-domain (FD) HRV parameters were efficient to assess cardiovascular dysautonomia in parkinsonian syndromes [23-25]. In particular, TD parameters were sensitive for the assessment of early stage of the PD whereas alteration of FD parameters was associated with the disease's duration [24,25].

It was also shown that HRVa in combination with the Ewing protocol provides a better assessment of cardiovascular dysautonomia in parkinsonian syndromes, useful to differentiate PD from MSA [23].

Although recent literature has shown that the Discriminant analysis (DA) of nonlinear (NL) HRV parameters can be more efficient that linear HRV parameters to investigate certain ANS conditions [30,31], their diagnostic accuracy to distinguish

parkinsonian syndromes of uncertain origin has been little investigated [32,33]. These nonlinear techniques are expected to provide additional information about the nonlinearity and complexity of autonomic cardiovascular control which cannot be reflected by linear HRV analysis. The goal is not that NL HRV techniques would replace the linear analysis, but they have to be considered as an addition, yielding information about a specific aspect of scaling behavior, complexity or chaos in the underlying system [34].

The aim of this study was to evaluate the predictive accuracy of NL HRV parameters to identify markers of dysautonomia sensitive to differentiate PD from MSA in early stage of parkinsonian syndromes of uncertain diagnosis.

2. MATERIALS AND METHODS

2.1 Patients Population

51 consecutive ambulatory patients (35 males and 16 females) (mean age 63±10 years) presenting with parkinsonian symptoms (Table 1) and 40 age-matched healthy subjects (20 males and 20 females), as case control, were included in the study.

Preliminarily patients were clinically classified according to standardized diagnostic criteria [17,27,28]. 25 patients (49%) had a “certain” diagnosis of PD, 9 (18%) a “highly probable” diagnosis of MSA (7 MSA-p, 2 MSA-c). 17 patients (33%) had an uncertain neurological definition, (6 “undefined parkinsonism” and 11 “suspected MSA”) (Table 1). 62,8% of patients were under pharmacological treatment with a mean LED (Levodopa dose equivalent) of 495.35, not significantly different in PD (485.6) from MSA (497.2), ($p = 0.95$), which was not discontinued the day of Holter recording. Patients treated with medications that could interfere with the sympatho-vagal balance (e.g. β -blockers, calcium channel blockers and vasodilators), were excluded.

After careful clinical history, physical examination and ECG recording in basal conditions, to verify the possible presence of spontaneous arrhythmias, twenty-four hours 12-lead Holter ECG was recorded (*H-Scribe -Mortara-Rangoni Instruments*).

Since factors, such as circadian rhythm, body position, activity level prior to recording, medication, verbalization, and breathing condition may influence the HRV, special precautions were taken to maintain similar condition in all patients, such as starting Holter session approximately at the same time of day (usually in the mid-late morning, after a light breakfast). Moreover all patients were instructed to perform some moderate physical activity at least twice during daytime before and after lunch, and to note accurately the timing of resting in bed, sleep, and eventual awakeness intervals during the night.

The study was approved by the local ethics committee and was performed in accordance with the ethical standards of the 1964 declaration of Helsinki. All patients gave informed consent to all clinical examinations and to the possible anonym inclusion of their data in scientific reports.

2.2 Heart Rate Variability Analysis

Quantitative HRV analysis was performed according to the European Society of Cardiology and the North American Society of Pacing and Electrophysiology guidelines [22] as follows. First raw ECG data were extracted from the Holter recordings with a custom software routine and edited to manually remove technical artifacts and/or physiological artifacts. The fraction of total RR intervals labeled as normal-to-normal (NN) intervals was used as a measure of data reliability, with the purpose to exclude records with a ratio less than a 95% threshold. Then a further editing was performed by visual analysis of the tachogram and of corresponding ECG, with manual correction of possible residual artifacts. Finally, HRV parameters were calculated in the TD, FD and with NL methods [30-41], using the Kubios HRV software (version 2.1) [42]. The "Smoothness Priors regularization" (lambda value: 500) was used to remove “non-stationary” low-frequency components [43].

The following methods were chosen for Non Linear Heart Rate Variability analysis (NL HRVa):

2.2.1 Poincare' plot

The Poincare' plot is a common graphical representation of the correlation between successive RR intervals, which analysis consists in fitting an ellipse oriented according to the line-of-identity and computing the standard deviation of the points perpendicular to and along the line-of-identity referred as *sd1* and *sd2*, respectively (*sd1* describes short-term variability; *sd2* describes long-term variability; *sd1/sd2 ratio* that is a measure of the interaction between short-

term and long- term variability) [30,35,36]. In a study investigating correlation amongst TD, FD and NL HRV parameters SD1 was highly correlated to RMSSD (r=0.99) (thus parasympathetic modulation) and SD2 to SDNN (r=0.95) [44].

Table 1. Demographic of the 51 investigated patients

Disease	Age	Therapy	UPDRS III: 0-68	HOEN & YAHR
MSA-c	65	no	30	3
MSA-c	67	no	n.a.	n.a.
MSA-c	69	no	55	5
MSA-p	45	Carbidopa, Levodopa/Benserazide	47	5
MSA-p	63	Levodopa/Carbidopa, Pramipexole	41	3
MSA-p	63	no	38	4
MSA-p	64	Levodopa/Carbidopa	62	5
MSA-p	69	Levodopa/Carbidopa, Ropirinol	n.a.	n.a.
MSA-p	77	Levodopa/Carbidopa	41	4
MSA-p	77	Levodopa/Carbidopa	n.a.	n.a.
PD	38	no	27	2
PD	48	Levodopa/carbidopa, Selegiline	41	n.a.
PD	51	Rasagiline	n.a.	n.a.
PD	53	Levodopa/Benserazide, Selegiline	13	2
PD	56	Rasagiline	25	2
PD	57	Rasagiline, Mevelodopa/Carbidopa, Ropirinol	16	2
PD	58	Rasagiline	9	1
PD	60	Rasagilina, Mevelodopa/Carbidopa, Ropirinol	26	2
PD	61	no	26	2
PD	62	Levodopa/benserazide, Ropirinol, Selegiline	n.a.	n.a.
PD	62	Rasagiline	26	2
PD	63	Levodopa/Carbidopa, Ropirinol	22	2
PD	65	Ropirinol	20	2
PD	65	Levodopa/Carbidopa, Entecapone	17	2
PD	65	Pramipexole, Levodopa/Carbidopa, Selegiline	n.a.	n.a.
PD	69	Levodopa/Carbidopa	32	2.5
PD	70	no	22	2
PD	71	Levodopa/Benserazide	33	2
PD	72	Levodopa/Benserazide, Ropirinolo	n.a.	n.a.
PD	72	Levodopa/Benserazide	18	2
PD	76	Levodopa/carbidopa, Ropirinol	31	3
PD	77	Levodopa/Benserazide	20	2
PD	77	Levodopa/Benserazide	n.d.	n.d.
PD	85	no	19	2
susp MSA-c	41	no	n.a.	n.a.
susp MSA-c	51	no	n.a.	n.a.
susp MSA-c	57	no	n.a.	n.a.
susp MSA-c	63	no	n.a.	n.a.
susp MSA-c	64	no	n.a.	n.a.
susp MSA-c	66	no	15	n.a.
susp MSA-c	69	no	n.a.	n.a.
susp MSA-p	57	Pramipexole, Carbidopa, Rasagiline	n.a.	n.a.
susp MSA-p	65	Levodopa/Benserazide	n.a.	n.a.
susp MSA-p	66	Levodopa/Benserazide	30	4
susp MSA-p	67	no	n.a.	n.a.
Und park	43	Pramipexole, Selegiline	n.a.	n.a.
Und park	57	Rotigotine	24	2
Und park	67	no	n.a.	n.a.
Und park	68	Selegiline	n.a.	n.a.
Und park	71	no	n.a.	n.a.
Und park	74	no	n.a.	n.a.

PD: Parkinson Disease, MSA-c: Multiple System Atrophy- cerebellar type; MSA-p: Multiple System Atrophy- Parkinsonian type; und: Undefined; susp: Suspected; n.a: Not available

2.2.2 Recurrence plot

The Recurrence plot analyzes the complexity of a given time series [30,37] divided in several parameters [mean line length (*lmean*), max line length (*lmax*), recurrence rate (*rec*), determinism (*det*), Shannon Entropy (*shanen*)].

2.2.3 Entropy

a) Approximate entropy (*apen*) is a measurement of the irregularity or complexity of the signal [30,38,39], b) Sample entropy (*sampen*) similar to *apen* [39,40], measures regularity or randomness of heart rate variations. Higher values indicate greater irregularity and are commonly a feature of health. Sample entropy decreases by moving from supine to orthostatic posture, thus with an increase of sympathetic modulation [45].

2.2.4 Detrended fluctuation analysis

Detrended fluctuation analysis (DFA), quantifies the fractal correlation properties of physiological signals [41,42]. DFA detects self-similarity and its variables are *dfα1* (short term scaling component: 4-11 beats) and *dfα2* (intermediate term scaling component: >11 beats). An α value of 0.5 suggests that the signal is truly random (white-noise) with larger values suggesting less noise. In previous studies, it was shown that DFA values rise with vagal blockade and decrease with sympathetic blockade [46,47].

2.2.5 The correlation dimension

The correlation dimension (*d2*) another way to measure the complexity of a time series. It gives information on the minimum number of dynamic variables needed to model the underlying system [30].

Quantitative HRV analysis was carried out from 5-minutes (Standard Short-Term, SST) time intervals selected during daytime, at rest (passive awakeness), during moderate physical activity (active awakeness) and during physiological sleep, identifying whenever possible NREM and REM stages. Given their short duration and transient variations, only 1-minute time-segments were used for HRV analysis during REM phases. For comparison and to evaluate the effect of shorter segment duration on quantitative assessment of NL parameters, HRV was also calculated from 2-minutes and 1-minute time-segments within the 5-minutes intervals during awakeness and NREM sleep.

The criteria chosen to validate the selection of the time-segments used for HRV calculation within each explored condition were: 1) the highest possible “stationarity” of the RR signal (defined as the absence of arrhythmias and of any kind of artifacts at visual analysis of corresponding ECG recordings) and 2) the best coherence among spectral output obtained with the Fast Fourier Transform (FFT) and autoregressive (AR) methods.

2.3 Statistical Analysis

All statistical calculations were performed with SPSS software, version 13.0 (*SPSS Inc., Chicago, Illinois*) [48]. Results are expressed as mean value ± standard deviation (SD). The significance between different groups was assessed by the chi-square test for discrete variables and by unpaired Student t-test for continuous variables. A probability level of $P<.05$ was chosen as the least significant difference. Factors differentiating between PD and MSA were tested by univariate and multivariate analyses. Independent variables for entry into the multivariate analysis were selected according to their weight on univariate testing (p values and shorter 95% confidence intervals) [49].

Discriminant Analysis (performed also with *Addinsoft XLSTAT, Version 2013.4.07*) was used to evaluate if HRV parameters were adequate to provide a separation between PD and MSA patients. DA search for linear combinations of the input features that can provide an adequate separation between the investigated subjects, in this study [31]. The discriminant functions used by linear DA are built up as a linear combination of the variables that seek to maximize the differences between the investigated groups. The classification accuracy of the method is defined as the ability to discriminate between the investigated groups.

The formula (F1, see section 3.2) obtained with DA of HRV data of patients with “certain PD" and “highly probable MSA” [31], was applied to classify the subpopulation of patients with parkinsonian symptoms of uncertain diagnosis.

3. RESULTS

3.1 Comparison between Patients (PD + MSA) and Controls

Independently from the length of the time segments explored, the majority of NL HRV parameters were significantly altered in patients as compared with healthy controls, especially during active awakeness. Several parameters were also altered during passive awakeness and during NREM sleep. Only sd1, sd2, dfa2 and d2 were abnormal during REM sleep (Table 2).

DA applied to NL HRV parameters had high predictive accuracy (above 80%) in differentiating patients (PD+MSA) from healthy controls in all conditions, reaching 91.3% with parameters calculated during the REM sleep (Table 3).

3.2 Comparison between PD and MSA Patients

When confronting patients with "certain" PD and "high probable" MSA, only recurrence plot parameters calculated during active awakeness and dfa2 during passive awakeness were significantly different (Table 4).

At DA (Table 5), the classification accuracy of single parameters ranged between 58,8 and 73.5%. The best accuracy (76.5%) was obtained with a combination of parameters *rplmean*, *rpadet* and *rpshen*, in formula (F1):

F1= -0.61 × *rplmean* + 0.15 x *rpadet* + 6.51 × *rpshen* - 27.53

In which: -0.61, 0.15, and 6.51 are the coefficients derived from the discriminant function of the DA for each parameter, while -27.53 is the constant derived from the same function.

When the formula F1 (if <0, the patient was classified as MSA, otherwise as PD) was applied to reclassify the seventeen patients with an uncertain clinical diagnosis, nine of them were classified as PD and six as MSA, only 2 remain unclassified.

Out of them, when comparing the HRV results with the definitive neurological diagnosis during the outcome during follow-up, five of the six patients with initial diagnosis of "undefined parkinsonism" were confirmed correctly classified as PD and one as MSA. Out of the eleven patients initially considered "suspected MSA", in five of the six patients classified as MSA on the basis of HRV criteria the diagnosis was confirmed by the clinical evolution during the follow-up. In the remaining six patients with shorter follow-up a definitive diagnosis is still uncertain.

4. DISCUSSION

The evaluation of autonomic dysfunction from heart rate pattern has proven useful for the stratification of risk associated with numerous diseases [50] including heart failure, hypertension, ischemic heart disease, diabetes, sepsis and neurological diseases linked to brain damage, especially patients with parkinsonism syndromes, to attempt early differentiation between PD and MSA and to define prognosis [3,18-20,22,23,26].

In spite of the increasing number of clinical studies, univocal criteria for the use of HRV a as a diagnostic tool in parkinsonian syndromes are still lacking [6,8,51,52]. This may be due to differences study protocols and/or experimental conditions not taking uniformly into account variables related to physiological variation of sympatho/vagal modulation due to circadian rhythm, physical activity, different phase of sleep etc.

Furthermore, quantitative HRV analysis can be affected by eventual non-stationarity of physiological conditions even during standard short-term analysis. Moreover, linear HRV analysis (in the TD and FD) may not be adequate to highlight the complexity of the autonomic cardiovascular modulation even in physiological conditions [42,43].

In this view, the use of methods based on NL mathematical models and on chaos theory (*Poincaré plot analysis, Recurrence Plot analysis, Correlation Dimension, Entropy*, etc.) which have proven efficient in improving the predictive value of HRVa in several other diseases [31,53,54], could be more efficient than linear HRV analysis also in the study of parkinsonian dysautomia.

Table 2. Comparison between NL HRV of 51 patients (PD + MSA) and 40 healthy controls

A	Passive awakeness (2 min)							Active awakeness (2 min)						
	Controls			PD+MSA			P	Controls			PD+MSA			P
	Mean		SD	Mean		SD		Mean		SD	Mean		SD	
sd1/sd2	0.47	±	0.16	0.67	±	0.25	<.05	0.45	±	0.17	0.66	±	0.26	<.05
sd1	16.96	±	10.49	10.56	±	8.26	<.05	17.52	±	10.06	8.99	±	7.63	<.05
sd2	37.53	±	20.64	18.25	±	16.09	<.05	40.94	±	20.76	15.73	±	13.38	<.05
rplmean	9.24	±	2.51	8.24	±	4.77	n.s.	9.62	±	3.15	8.03	±	3.84	<.05
rplmax	91.70	±	48.03	52.18	±	35.14	<.05	85.60	±	42.44	55.57	±	32.63	<.05
rprec	27.43	±	7.65	23.68	±	14.64	n.s.	29.06	±	8.33	24.20	±	12.87	<.05
rpadet	97.03	±	2.03	94.93	±	2.84	<.05	97.39	±	1.88	95.20	±	2.93	<.05
rpshen	2.89	±	0.30	2.64	±	0.43	<.05	2.90	±	0.33	2.66	±	0.36	<.05
dfa1	1.28	±	0.27	0.99	±	0.30	<.05	1.31	±	0.27	1.02	±	0.33	<.05
dfa2	0.38	±	0.14	0.44	±	0.14	n.s.	0.38	±	0.13	0.45	±	0.14	<.05
apen	0.82	±	0.10	0.78	±	0.10	n.s.	0.83	±	0.09	0.84	±	0.11	n.s.
sampen	1.63	±	0.34	1.75	±	0.40	n.s.	1.66	±	0.37	1.58	±	0.30	n.s.
d2	1.20	±	1.41	0.25	±	0.67	<.05	1.30	±	1.31	0.15	±	0.52	<.05
B	**NREM sleep (2 min)**							**REM sleep (1 min)**						
	Controls			PD+MSA			P	Controls			PD+MSA			P
	Mean		SD	Mean		SD		Mean		SD	Mean		SD	
sd1/sd2	0.85	±	0.25	0.89	±	0.29	n.s.	0.5	±	0.2	0.5	±	0.2	n.s.
sd1	28.75	±	17.57	14.25	±	9.54	n.s.	27.6	±	17.9	13.1	±	7.9	<.05
sd2	33.24	±	15.89	17.27	±	12.19	n.s.	55.5	±	26.7	27.1	±	16.6	<.05
rplmean	8.42	±	2.89	7.29	±	2.43	<.05	9.9	±	5.6	8.1	±	2.7	n.s.
rplmax	41.85	±	15.83	35.47	±	15.17	n.s.	43.9	±	12.9	41.3	±	18.4	n.s.
rprec	19.49	±	4.07	16.22	±	4.19	<.05	30.2	±	13.2	25.7	±	11.1	n.s.
rpadet	95.85	±	1.62	94.17	±	3.11	<.05	97.3	±	2.1	96.0	±	2.7	n.s.
rpshen	2.69	±	0.27	2.53	±	0.28	<.05	2.6	±	0.3	2.5	±	0.3	n.s.
dfa1	0.69	±	0.27	0.69	±	0.25	n.s.	1.2	±	0.2	1.3	±	0.3	n.s.
dfa2	0.20	±	0.11	0.29	±	0.11	<.05	0.4	±	0.2	0.5	±	0.2	<.05
apen	0.66	±	0.09	0.71	±	0.09	<.05	0.5	±	0.1	0.5	±	0.1	n.s.
sampen	1.84	±	0.32	1.87	±	0.41	n.s.	1.4	±	0.4	1.6	±	0.4	n.s.
d2	1.72	±	1.48	0.40	±	0.95	<.05	1.5	±	0.9	0.6	±	0.9	<.05

Table 3. Predictive accuracy of NL parameters in differentiating patients from healthy controls

Condition	Sensitivity	Specificity	Predictive accuracy
Passive awakeness	77.8%	89.7%	84.9%
Active awakeness	66.7%	89.5%	81.4%
REM sleep	91.3%	91.3%	91.3%

Table 4. Comparison between NL HRV parameters of patients with certain PD and highly probable MSA diagnosis

	Passive awakeness (2 min)					Active awakeness (2 min)					NREM sleep (2 min)				
	certain PD		highly probable MSA		P	certain PD		highly probable MSA		P	certain PD		highly probable MSA		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
sd1/sd2	0.6	± 0.2	0.7	± 0.2	n.s.	0.6	± 0.2	0.8	± 0.3	n.s.	0.9	± 0.2	1.0	± 0.4	n.s.
sd1	10.7	± 7.3	9.2	± 7.5	n.s.	8.4	± 3.6	7.4	± 4.5	n.s.	14.8	± 9.1	11.0	± 8.4	n.s.
sd2	18.4	± 12.9	14.1	± 10.8	n.s.	15.6	± 9.5	10.9	± 8.4	n.s.	17.9	± 10.4	10.9	± 6.2	n.s.
rplmean	7.8	± 2.4	8.2	± 3.7	n.s.	8.0	± 2.3	6.0	± 1.4	< .05	7.3	± 1.5	7.3	± 3.1	n.s.
rplmax	57.7	± 35.6	44.9	± 32.1	n.s.	57.4	± 30.2	34.6	± 18.7	< .05	34.4	± 9.5	35.4	± 18.3	n.s.
rprec	23.6	± 10.1	23.8	± 12.6	n.s.	23.7	± 8.5	16.6	± 6.9	< .05	16.9	± 3.9	15.1	± 3.9	n.s.
rpadet	95.3	± 2.8	94.8	± 2.7	n.s.	95.8	± 2.2	93.0	± 2.4	< .05	94.6	± 3.4	94.0	± 2.9	n.s.
rpshen	2.6	± 0.3	2.7	± 0.4	n.s.	2.7	± 0.3	2.4	± 0.2	< .05	2.6	± 0.2	2.5	± 0.4	n.s.
dfa1	1.0	± 0.3	1.0	± 0.2	n.s.	1.1	± 0.3	0.9	± 0.4	n.s.	0.7	± 0.2	0.6	± 0.3	n.s.
dfa2	0.4	± 0.1	0.6	± 0.2	< .05	0.5	± 0.1	0.4	± 0.1	n.s.	0.3	± 0.1	0.3	± 0.1	n.s.
apen	0.8	± 0.1	0.8	± 0.1	n.s.	0.8	± 0.1	0.9	± 0.1	n.s.	0.7	± 0.1	0.7	± 0.1	n.s.
sampen	1.7	± 0.3	1.8	± 0.4	n.s.	1.6	± 0.3	1.6	± 0.2	n.s.	1.9	± 0.5	1.7	± 0.3	n.s.
d2	0.3	± 0.7	0.2	± 0.6	n.s.	0.1	± 0.4	0.0	± 0.1	n.s.	0.5	± 1.1	0.1	± 0.3	n.s.

PD: Parkinson Disease, MSA: Multiple System Atrophy

Table 5. Performance of the classification rules (PD vs MSA) based on single NL HRV parameter and combination of parameters

	Classified as PD if			Sens	Spec	PPV	NPV	PA
dfa2	passive awakeness	<	0.440	80.00%	55.56%	83.33%	50.00%	73.53%
rprec	active awakeness	>	18.744	60.87%	75.00%	87.50%	40.00%	64.52%
rplmean	active awakeness	>	7.471	48.00%	88.89%	92.31%	38.10%	58.82%
rpadet	active awakeness	>	95.039	76.00%	66.67%	86.36%	50.00%	73.53%
rpshen	active awakeness	>	2.622	72.00%	77.78%	90.00%	50.00%	73.53%
F1	active awakeness	>	0	80.00%	66.67%	86.96%	54.55%	76.47%

F1 = -0.61 × rplmean + 0.15 × rpadet + 6.51 × rpshen – 27.53

PD: Parkinson Disease, MSA: Multiple System Atrophy, Sens: Sensitivity, Spec: Specificicty, PPV: Positive Predictive Value; NPV: Negative Predictive Value

The present study focused on the application of NL HRVa from short-term time segments of different lengths (5-2-1 min), taking into account also different phases of daily activity (both passive and active awakeness) and of sleep (NREM and REM), to attempt a more comprehensive quantification of different degrees of cardiovascular autonomic dysfunction detected in patients with "parkinsonian" movement disorders, but due to illness with very different prognosis and outcome.

In a first phase of the study we compared HRV parameters of patients (PD + MSA) with those of age-matched control group. As expected, the majority of NL HRV parameters were significantly abnormal in parkinsonian patients (*P<.05*) compared to healthy subjects, during both active and passive awakeness as well as during NREM sleep, thus confirming the well-known altered autonomic control of RR variability and complexity (Table 2) [23-25,32,33]. Such decreased value of complexity measures reflects a change towards more stable and periodic behavior of the heart rate in patients, which may be associated with "decoupling of multimodal integrated networks and deactivation of control-loops within the cardiovascular system" [31].

However the real clinical challenge is to provide the neurologist with additional tools improving non-invasive early differentiation between PD and MSA, especially in patients with uncertain clinical patterns.

Whereas previous studies questioned the value of comprehensive autonomic nervous system testing for risk assessment of patients with parkinsonism [50-52], in the present study NL HRV parameters were significantly more altered in MSA compared to PD, especially during active awakeness. This result indicate a greater impairment of sympathetic autonomic response during daily activity, which could be responsible for the increased prevalence and severity of orthostatic hypotension in MSA compared to PD.

The performance of the classification rules based on DA of NL parameters distinguished between the two diseases with accuracy provided by single NL parameters ranging between 58.8% and 73.5%.

Since clinical experience with the analysis of HRV complexity and regularity in parkinsonian patients is still limited, it is difficult at the moment to speculate about their physiological meaning in different abnormal conditions and about possible reasons why only few NL parameters were significant in differentiating between MSA and PD in this study. However it may be interesting to note that the combination of NL parameters *rplmean*, *rpadet*, *rpshen*, in the formula F1 (Table 5), improved the predictive accuracy of evolution in MSA to a 76.5%, which may be a reasonably good additional information to attempt an early differentiation between parkinsonian syndromes with different prognosis. In fact, by applying F1 to attempt a better classification of the seventeen patients with a uncertain diagnosis, more than half of them were properly classified as demonstrated by the evolution of the clinical picture during the follow-up.

4.1 Limitations of the Study

A first obvious limitation of the study is certainly the limited number of enrolled patients, especially patients with highly probable MSA, but we must consider that the disease is very disabling and often limits the "patient's compliance" to participate to clinical studies.

Second, for accurate evaluation of ANS balance, one should study patients either before the beginning of pharmacological treatment or after an appropriate period of drug washout. Unfortunately, this is often impossible, particularly in cases with severe motor impairment and/or marked dysautonomia, since, in the absence of therapy, the patients could not be evaluated in terms of mobility and of daily life activities.

Finally, as there are now sensitive second-level diagnostic tools that allow very accurate and precise diagnosis of alpha-synucleinopatie diseases, such as *Datscan* and brain *Pet* [55,56] as well as myocardial scintigraphy with meta-iodobenzylguanidine [21] for studying cardiac sympathetic innervation, the lack of these data in some of our patients with uncertain diagnosis is still limiting the evaluation of the results of this paper.

On the other hand HRVa is a non-invasive tool, that requires only a good quality electrocardiographic recording which is applicable to most patients, even in uncomfortable clinical conditions. Thus it may be an optimal first level additional tool for a better early diagnostic classification at very low cost and with no need to expose the patient to ionizing radiation.

5. CONCLUSION

NL HRVa might be a simple and quick method to improve the quantization of the degree of derangement of cardiovascular autonomic modulation in patients with parkinsonian neurodegenerative syndromes associated with signs of dysautonomia [3,23]. Furthermore the assessment of the degree of ANS impairment is certainly more pronounced in MSA compared to PD and this element seems better highlighted by investigating the complexity of HRV with NL methods which seem to have a greater stability, probably because less affected by non-stationarity conditions [29-35].

In this study NL analysis provided satisfactory differentiation between patients with a "certain or highly probable" diagnosis of PD or MSA with a good (76.5) predictive accuracy. The classification rules could be useful for an earlier definition of prognostic evolution toward one or other type of disease in patients with parkinsonian symptoms of uncertain nature, to address second level assessment and to guide the choice of early and more individualized drug treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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