International PhD School In Neuropharmacology

-XXIII Cycle-

ASYMMETRY OF NIGRAL DOPAMINERGIC NEURONS' DEPLETION IN PARKINSON'S DISEASE

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1. PREFACE

Parkinson's Disease (PD) is a progressive neurological disorder associated with neuronal loss of the substantia nigra and other brain structures. It is the second most common neurodegenerative disorder after Alzheimer's disease and is expected to impose an increasing social and economic burden on societies as populations age. Despite all recent advances derived from preclinical and clinical researches, the exact pathogenetic mechanisms underlying the selective dopaminergic neuronal depletion in PD are still not understood. Current thinking is that sporadic PD could be the result of the interaction between environmental factors and susceptibility genes **(de Lau and Breteler, Lancet Neurol 2006**).

The diagnosis of PD is essentially clinical, based on the results of clinical assessment; however the differential diagnosis versus other forms of parkinsonism can be challenging, especially early in the disease, when signs and symptoms of different forms of parkinsonism have greater overlap; error rates in clinico-pathological studies draw attention to the need for the identification of early predictors of diagnosis.

Increasing our knowledge in underlying causes of progressive neuronal loss in nigrostriatal and extranigral structures and improving the diagnostic accuracy of this condition could be crucial to the development of future novel dopaminergic therapeutic strategies in PD.

2. GENERAL INTRODUCTION

2.1 THE DIAGNOSTIC CHALLENGE OF PARKINSON'S DISEASE

Parkinson's Disease (PD) is characterized by tremor, rigidity, and slowness of movements. In the absence of reliable diagnostic markers, diagnosis currently relies upon the presence of clinical features and diagnostic confirmation depends on the finding of depletion of brain stem pigmented neurones with Lewy bodies at necropsy.

Several sets of clinical diagnostic criteria for PD have been proposed, most of which are based on the authors' experience or on a literature review. The most commonly used are the UK Parkinson's Disease Society Brain Bank criteria (**Table 1**) (**Gibb and Lees, J Neurol Neurosurg Psychiatry 1988)** and those proposed by Gelb et al. (clinically possible and clinically probable categories, **Table 2**) (**Gelb et al, Arch Neurol 1999**), both of which are based on the presence of the cardinal features akinesia, rigidity and resting tremor in the absence of any exclusion criteria.

Misdiagnosis is especially common during the early stages of disease, even among movement disorder specialists, the commonest alternative diagnoses being essential tremor, vascular parkinsonism, dementia, and drug induced parkinsonism.

In a clinicopathologic study in the early 1990s, of 43 patients with a clinical diagnosis of PD the rate of correct diagnosis at the initial visit was only 65% compared with the pathological diagnosis, but rose to 76% at the final visit (**Rajput et al, Can J Neurol Sci 1991).**

Another study found the accuracy of clinical diagnosis in a series of 100 patients who had died with a diagnosis of PD to be only 76% **(Hughes et al, J Neurol Neurosurg Psychiatry 1992**). In a subsequent study the same group reported that the rate of misdiagnosis had fallen to 10%, demonstrating that the clinical diagnostic accuracy of PD could be improved by using stringent criteria (**Hughes et al, Neurology 2001**).

In a population based study, the authors found results corresponding with those deriving from pathological studies, indicating that PD is often confused with other disorders (**Schrag et al, J Neurol Neurosurg Psychiatry 2002**). In this study, at least 15% of patients with a diagnosis of PD in the population did not fulfil strict clinical criteria for the disease, and approximately 20% of patients who did have the disease and who had already come to medical attention were not diagnosed as such. As may have been expected, PD was more often recognised in patients with greater disease severity, who had had the disease for longer, and had already developed complications such as depression and dyskinesias, and were more likely to live alone or with their family than in a nursing home (**Schrag et al, J Neurol Neurosurg Psychiatry 2002**).

Some studies tried to identify the early clinical features that would allow to improve the accuracy of clinical diagnosis. In a large clinico-pathologic study, the clinical variables of patients with clinically diagnosed PD confirmed by autopsy were reviewed; the best predictors of diagnosis resulted asymmetrical onset and the absence of atypical features (**Hughes et al, Neurology 1992**). This finding was confirmed in another study were the features that best distinguished cases of PD from non-PD through autopsy findings were asymmetrical onset of

parkinsonism (bradykinesia, rigidity and above all rest tremor), levodopa response (moderate to excellent benefit and levodopa-induced dyskinesias) and absence of pyramidal and oculomotor symptoms. (**Litvan et al, Arch Neurol 1998).**

All the mentioned studies highlights the problem of assuming that PD is a specific morbid entity that can be diagnosed reliable on clinical grounds. Until biologic markers are developed, we must accept that diverse neuropathologic disorders may produce clinical syndromes indistinguishable from Lewy body PD; the use of strict inclusion and exclusion criteria can help reduce misdiagnosis but will also exclude a proportion of genuine cases. **(Hughes et al, Neurology 1992).**

TABLE 1. UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria

Inclusion criteria	**Exclusion criteria**	**Supportive criteria**
- *Bradykinesia* (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions) And at least one of the following*:* - *Muscular rigidity* - *4–6 Hz rest tremor* - *Postural instability*	- History of repeated strokes with stepwise progression of parkinsonian features - History of repeated head injury - History of definite encephalitis - Oculogyric crises - Neuroleptic treatment at onset of symptoms - More than one affected relative - Sustained remission - Strictly unilateral features after 3 yr - Supranuclear gaze palsy - Cerebellar signs - Early severe autonomic involvement - Early severe dementia with disturbances of memory, language, and praxis - Babinski sign - Presence of cerebral tumour or communicating hydrocephalus on CT scan - Negative response to large doses of LD (if malabsorption excluded) - MPTP exposure	(*Three or more required for diagnosis of definite PD*) - Unilateral onset - Rest tremor present - Progressive disorder - Persistent asymmetry affecting side of onset most - Excellent response (70–100%) to LD - Severe LD-induced chorea - LD response for 5 yr or more - Clinical course of 10 yr or more

UK, United Kingdom; PD, Parkinson's disease; CT, computed tomography; LD, levodopa

TABLE 2. Gelb's Proposed Diagnostic Criteria for PD.

POSSIBLE Parkinson disease: At least 2 of the 4 features in Group A* are present; at least 1 of these is tremor or bradykinesia And Either None of the features in Group B* is present Or Symptoms have been present for less than 3 years, and none of the features in Group B* is present to date And Either Substantial and sustained response to levodopa or a dopamine agonist has been documented Or Patient has not had an adequate trial of levodopa or dopamine agonist
PROBABLE Parkinson disease: At least 3 of the 4 features in Group A* are present And None of the features in Group B* is present (note: symptom duration of at least 3 years is necessary to meet this requirement) And Substantial and sustained response to levodopa or a dopamine agonist has been documented
DEFINITE Parkinson disease: All criteria for POSSIBLE Parkinson disease are met And Histopathologic confirmation of the diagnosis is obtained at autopsy
***Group A features**: characteristic of PD Resting tremor Bradykinesia Rigidity Asymmetric onset ***Group B features**: suggestive of alternative diagnoses Features unusual early in the clinical course Prominent postural instability in the first 3 yrs Freezing phenomena in the first 3 yrs Hallucinations unrelated to medications in the first 3 yrs Dementia preceding motor symptoms or in the first yr Supranuclear gaze palsy or slowing of vertical saccades Severe, symptomatic dysautonomia unrelated to medications Documentation of a condition known to produce parkinsonism and plausibly connected to the patient's symptoms .

2.2 **THE DOPAMINERGIC RESPONSIVENESS**

Even though a robust motor response to dopaminergic drugs is one of the most important discriminative features suggestive of PD, since the introduction of levodopa there have been reports of patients thought to have PD who had poor responses to the drug.

In 1969, Yahr et al. reported that 15 of 60 patients (56 diagnosed with PD, 4 with parkinsonism) treated with levodopa experienced less than 20% clinical improvement (18% initial; 7% after chronic therapy) (**Yahr et al, Arch Neurol 1969**). Tolosa reported 16 clinically diagnosed PD patients (mean disease duration of 10 years) including 2 who had shown only minimal response to levodopa despite documentation of high plasma levodopa levels (**Tolosa et al, Neurology 1975**). In a case series of 44 clinically diagnosed PD patients, 18% had less than 25% improvement with initial levodopa therapy and 40% were considered poorly responsive after 3 years (**Lees and Stern, Adv Neurol 1983**). Additional evidence comes from studies in which the diagnosis of PD was confirmed by necropsy. Hughes et al. reported that the initial levodopa response was “nil to poor” in 6%, “moderate” in 17%, “good” in 53%, and “excellent” in 24% (**Hughes et al, Arch Neurol 1993**).

In a recent study, that tried to further characterize levodopa response in early PD, although in most patients levodopa treatment significantly improved motor signs when compared with placebo, a wide range and considerable overlap in clinical responses to levodopa and placebo was found; a substantial proportion of subjects with early PD did not experience a significant response to levodopa. (**Hauser et al, Mov Disord 2009**).

Those differences of clinical response to levodopa can be evident also after several years of treatment, when initial stable response is gradually replaced by a fluctuating motor response and dyskinesia. In fact, some patients with advanced disease manifest wild and unpredictable swings in motor function, whereas others are severely disabled, do not fluctuate and respond poorly to drug treatment. The reason for the observed differences in the pattern of responsiveness are still unknown. It could be hypothesized that they would reflect the variability in the pathological process or in the neurochemical changes subsequent to the cell death. Irrespective to this hypothesis, a clinico-pathological study on fluctuating versus non fluctuating PD patients failed to reveal any significant difference in the severity or in the distribution of Lewy bodies; nevertheless the authors underline that differences may be present in early stages, but they could be no longer detectable at post-mortem examination. (**Kempster et al, Brain 2007**).
The pattern of response to pharmacological treatment has important influences on the course of PD. A longitudinal study on PD patients followed up at 3 yearly interval over a mean period of 11.4 years from the point of commencement of levodopa treatment, demonstrated that a good initial response predicted the development of motor fluctuations and dyskinesia, so that patients with the best initial response and the largest test-dose response amplitude tended to develop more symptomatic motor fluctuations and dyskinesia (**Clissold et al, Mov disord 2006**). However, a strongest response appeared a favourable prognostic indicator, since the magnitude of the response tended to be conserved over time (**McColl et al, Mov disord 2002; Clissold et al, Mov disord 2006).**

2.2.1 THE SHORT TERM LEVODOPA TEST

Since the initial description by Esteguy and colleagues (**Esteguy et al, Rev Neurol (Paris) 1985**), the single-dose acute challenge with levodopa or apomorphine is commonly performed in clinical practice or in clinical research either to demonstrate levodopa response or to characterize the response in de novo or previously treated PD patients with motor fluctuations and it has been incorporated into guidelines for experimental studies on parkinsonian subjects.

Evidence on the practice of acute pharmacological challenge tests in parkinsonian patients was reviewed by a committee of experts, which achieved a general consensus that with the appropriate indication and setting, acute challenge tests are useful in diagnosis and therapy of PD (**Albanese et al, Mov Disord 2001**).

A study had evaluated the motor responses to the acute administration of levodopa and apomorphine in a series of 134 patients with a clinical diagnosis of idiopathic PD, multiple-system atrophy (MSA), progressive supranuclear palsy (PSP), and unclassified parkinsonian syndrome and the authors demonstrated that acute pharmacological challenges can be considered supportive for the diagnosis of PD (**Rossi et al, Eur Neurol 2000).**

It has been also demonstrated that the short term levodopa test accurately predict sustained long-term levodopa responsiveness (**Zappia et al, Mov Disord 1997; Merello et al, Mov Disord 2002; Hughes et al, Neurology 1991).**

However, lack of motor improvement following an acute challenge is possible in PD patients and demands caution on certain occasions. In fact a negative response in a drug-naive patient or in a patient at the beginning of treatment does not always exclude a positive chronic response; the false negative rate in drug-naive PD patients for dopaminergic challenge tests may be as high as 40% (**Hughes et al, Lancet 1990; Hughes et al, Neurology 1991; Gasser et al, Arch Neurol 1992**).

Moreover, in non-fluctuating Parkinson's disease patients, who have an unclear response to chronic dopaminergic treatment, the short-duration response to an acute challenge may be masked by the long-duration response to the chronic treatment (**Zappia et al, Ann Neurol 1997**) that may last many hours or days after discontinuation of treatment. However, in most clinical investigations, the response to dopaminergic drugs is evaluated after only an overnight without levodopa, ie, a period ranging from 8 to 12 hours from the last dose intake, that may not ensure determination of true baseline untreated status.

Furthermore, it should be noted that the acute administration of dopaminergic drugs may cause significant adverse events due to the peripheral dopaminergic stimulation, such as nausea, vomiting, or orthostatic hypotension, thereby the coadministration of domperidone is often necessary.

2.3 **THE MOTOR ASYMMETRY**

Also asymmetric onset is considered a major criterion for diagnosis (**Hughes et al, Neurology 1992**) and has been suggested as one of the strongest supportive features for diagnosis of PD rather than other variants of parkinsonian syndromes.

In fact, PD is characterised by a predominantly unilateral appearance of the three main features rest tremor, rigidity and bradykinesia, and the asymmetry of major features persists throughout the course of the illness.

In a cross-sectional study of 198 patients with PD, it was found that with increasing duration of symptoms, the focal asymmetry of bradykinesia is sustained without any significant changes, in spite of the progressively accumulating deficits that appear with advancing pathology; the authors explained this unexpected finding with the hypothesis that neurons already damaged by an event ultimately undergo a premature death, leaving unaffected neurons with a normal expectation of survival (**Lee et al, Neurology 1995**).

A postmortem study of 21 patients with PD assessed asymmetry of the extent of degeneration in the substantia nigra, showing lower number of surviving nigral neurons contralateral to the initially more affected side and thus providing pathological confirmation for the predominantly unilateral motor manifestations. Asymmetry was of the order of a 25% reduction in the substantia nigra contralateral with respect to the ipsilateral to the less affected side (**Kempster et al, J Neurol Neurosurg Psychiatry 1989**).

The observation that one side tends to be affected predominantly and persistently also in more advanced stages of disease was also demonstrated in a recent study with single-photon emission computed tomography (SPECT), in which asymmetry of radioligand uptake between the ipsilateral and contralateral sides of the caudate and putamen was still maintained many years after motor symptom onset, although less striking than known in early disease (**Djaldetti et al, Mov Disord. 2010**).

The reason for the symptom-side predominance remains unclear. There are a few potential mechanisms that could be responsible for the asymmetric appearance of motor symptoms in Parkinson's disease. In twins studies, side concordance was not demonstrated, so that it could be concluded that side of symptoms predominance is random and not genetically determined. However, it is possible that a genetic predisposition lead to a greater vulnerability to damage in one substantia nigra over the other, so that once the event causing the disease strikes this side will degenerate first and reach the critical level of cell loss for symptoms to emerge; another possibility is an inborn unequal number of dopaminergic neurons on both sides of the substantia nigra that brings the side with the lower number of neurons earlier to the vulnerable point of cell loss (**Djaldetti et al, Lancet Neurol 2006**).

Nevertheless, none of these suggested mechanisms have been explored in relation to side of asymmetry and since there is no convincing evidence for the above mentioned causative or contributory pathogenetic mechanisms, it is possible to conclude that asymmetry in the disease could be down to pure chance.

2.4 THE ROLE OF FUNCTIONAL IMAGING: SPECT WITH DaT-SCAN

Functional neuroimaging methods, such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), are capable of demonstrating in vivo at a very early stage the degeneration of presynaptic dopaminergic nigrostriatal neurons, which underlay PD and other neurodegenerative parkinsonian syndromes. In particular, SPECT imaging of striatal Dopamine Transporters (DaT) has more recently been introduced as a valuable, more accessible tool than [18F]fluorodopa PET, being less expensive and readily available for routine clinical use.

DaT are Na+/Cl−-dependent presynaptic proteins located on the membrane of dopaminergic neuron terminals and control dopamine levels by active reuptake of dopamine from the synaptic cleft after the termination of its interaction with the dopamine receptors on the postsynaptic neurons. PD is characterized by degeneration of nigral dopaminergic cells and their striatal terminals, resulting in decreased striatal dopamine and a parallel loss of the dopamine transporters. This makes the dopamine transporter a potential surrogate marker for dopaminergic nigrostriatal neurons.

Several SPECT tracers binding presynaptic DaT, that are [123I]-labeled cocaine analogues, have been developed; however [123I]-FP-CIT ([123I]-Ioflupane, DaTSCAN), owing to its fast kinetics that allows for scanning 3–6 hours after administration, resulted the most convenient for outpatient evaluation. Moreover, it has high specific affinity in binding to dopamine transporters and has a better dopamine to 5-HT transporter selectivity (**Gunther et al, Nucl Med Biol 1997**).

In agreement with human post-mortem study showing fallout of dopaminergic neurons with advancing age in the substantia nigra pars compacta at a rate of 5–7% per decade (**Fearnley et al, Brain 1991**), striatal DaTSCAN binding decline of 4% per decade has been observed in healthy controls (**Ishikawa et al, J Nucl Med 1996; Booij et al, Synapse 2001**), so the effect of advanced age on radiotracer uptake appear to be negligible.

In PD a significant relationship between global severity and SPECT imaging, with radioligand uptake correlated with severity and duration of disease, have been demonstrated (**Benamer et al, Mov Disord 2000**).

DaTSCAN SPECT may help in diagnosis of clinically uncertain parkinsonian syndromes patients, having a significant impact on their clinical management (**Catafau et al, Mov Disord 2004**), and offers significant potential in defining the nigrostriatal changes in PD.

In clinical practice, DAT imaging assists mainly in patients in whom there is a diagnostic doubt between true parkinsonism and other movement disorders without presynaptic dopaminergic loss. However, it do not offer diagnostic support to a diagnosis of PD versus other neurodegenerative presinaptic parkinsonism (**Marshall et al, *Mov Disord* 2003; Scherfler et al, *Mov Disord*. 2007**).

Moreover, even if it is expected to be helpful in differential diagnosis with Vascular Parkinsonism (VP), where the DaTSCAN uptake is usually normal or slightly reduced, in some cases a significantly reduced striatal binding compared to controls was found (**Zijlmans et al, Mov Disord 2007**). It has been suggested that a rather symmetrical localization could be suggestive of VP since such a pattern is rarely

encountered in neurodegenerative Parkinsonism (**Scherfler et al, *Mov Disord*. 2007).**

2.4.1 FUNCTIONAL IMAGING AND LEVODOPA RESPONSIVENESS

Some studies employed neurofunctional imaging to investigate the role of dopamine terminal loss in the pattern of levodopa response.

It is well established that the response magnitude to acute challenge test increases as nigrostriatal degeneration progresses **(Rodriguez et al, JNNP 1994; Contin et al, J Neurol 2003**).

In a study with a levodopa pharmacokinetic-dynamic modelling and SPECT, it was observed that the levodopa equilibration half-life ($t1/2_{eq}$) between plasma and effect-site concentrations, expressing the lag time between changes in drug plasma concentrations and changes in the effect, was positively correlated with FP-CIT controlateral putamen uptake ratio **(Contin et al, J Neurol 2003**). Since t1/2eq is positively related to duration of response and inversely to the magnitude of peak response (**Nutt and Holford, Ann Neurol 1996**), it is possible to conclude that the controlateral radioligand uptake is lower in those patients who had a larger response to levodopa.

At variance with this result, a PET study investigating a possible correlation between variables describing the time-response curve to levodopa and the putamen binding potential of radioligand, failed to detect any significant correlation of asymmetry (defined as the difference between the more affected and the less affected side) of dopaminergic depletion with asymmetry of the duration and the magnitude of levodopa response, supporting the hypothesis that the

summentioned parameters cannot be accounted for solely by dopaminergic terminal density (**Kumar A et al, Brain 2003**).

Similar conclusions have been reached by a recent SPECT study, conducted on dyskinetic versus non dyskinetic Parkinson's disease patients, where any such hypothesized relationship with pattern of levodopa response (in term of latency, magnitude and duration of motor response) or radioligand uptake were not found, suggesting that additional factors beyond nigrostriatal denervation should be sought. (**Linazasoro et al, Clin Neuropharmacol 2009**).

3. MANUSCRIPT 1

USEFULNESS OF [^{123}I] FP-CIT SPECT STRIATAL ASYMMETRY INDEX AS SPECIFIC TOOL TO DIFFERENTIATE PARKINSON'S DISEASE FROM VASCULAR PARKINSONISM.

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ABSTRACT

The differential diagnosis between Vascular Parkinsonism (VP) and Parkinson's Disease (PD) is difficult, due to the overlap in clinical presentation and the lack of specificity at neuroimaging. We studied 20 PD, 20 VP and 20 Essential Tremor (ET) patients as control group. All the patients underwent a cerebral [123I] FP-CIT SPECT. The binding of the ligand in the most affected side resulted significantly lower in VP than in ET patients but higher compared to PD patients. Striatal Asymmetry Index (SAI) was significantly higher in PD compared to VP ($p<0.001$) and ET ($p<0.001$) groups. We found that a cut-off of SAI greater than 14.08 could differentiate PD from VP with a 100% specificity and a 60% sensitivity. SAI detected using [^{123}I]FP-CIT SPECT can be used to differentiate VP and PD with a good degree of certainty.

INTRODUCTION

The concept of Vascular Parkinsonism (VP) was first introduced in 1929, when Critchley described a syndrome characterized by a hypokinetic-rigid parkinsonism due to cerebrovascular disease, and considered this condition, named "Arteriosclerotic parkinsonism", independent from Parkinson's Disease (PD).[1] Since then, many studies have been performed in order to describe the defining clinical features and to identify some instrumental tools useful to distinguish VP from PD. In fact, although in retrospective descriptive studies some prominent clinical features of VP have been described, such as symmetrical lower-body parkinsonism associated with gait difficulty, history of stroke, presence of cardiovascular risk factors and poorly responsiveness to l-dopa therapy, [2] the differential diagnosis could be very difficult also in a movement disorders' specialist setting, due to the great heterogeneity of the clinical presentation. Indeed, at least a 3% of patients diagnosed as PD presented lacunar infarcts and no Lewy bodies. [3] Neuroimaging could help physicians in the diagnostic approach; patients with suspected VP seem to present more subcortical vascular lesions detected by MRI brain imaging compared to PD patients. [4] However, vascular lesions are also a common incidental finding in PD patients and in elderly control subjects. Hence, cerebrovascular disease and Lewy body pathology may coexist and this is not surprising as both disease processes increase with age. Also the results obtained at [^{123}I] FP-CIT SPECT studies are not conclusive. Despite in VP patients the ligand uptake is usually normal or slightly reduced, in some cases a significantly reduced striatal binding compared to controls was found, and a true degeneration of

nigrostriatal pathways has been proposed to explain this finding. [5] Zijlman and colleagues found that there were not significative differences in the ligand uptake between VP and PD, except for the striatal asymmetry index (SAI) that in VP could be lower. [6] On these grounds, it has been suggested that the presence of a rather symmetrical FP-CIT uptake in the basal ganglia could be useful in distinguishing VP from PD. [6] We performed a SPECT study in order to replicate Zijlmans's results and to investigate if a possible SAI cut-off value could be useful in the differential diagnosis also in a clinical setting.

MATERIALS AND METHODS

Study Population

We retrospectively studied from our database 20 PD patients and 20 VP patients who consecutively attended the Movement Disorders' Clinic of University of Catania. PD patients (13 men and 7 women, aged 60.3±7.5 years, mean±SD), fulfilled the U.K. Parkinson's Disease Society Brain Bank criteria, [7] while VP patients (16 men and 4 women, aged 73.5±6.9 years), fulfilled Zijlmans' proposed diagnostic criteria. [8] VP patients presented a weak response to dopaminergic chronic therapy evaluated at follow-up visits. We also studied as control group 20 patients with a clinical diagnosis of Essential Tremor (ET) (8 men and 12 women, aged 57.6±11.8 years). The retrospective study was approved by the Local Ethics Committee. Clinical features at onset, cardiovascular risk factors and presence of vascular lesions at brain MRI (extended subcortical white matter lesions or lacunar infarcts in the region of basal-ganglia circuit) have been collected in both PD and VP patients. Patients with unilateral lesions of basal ganglia were excluded. Clinical evaluation of motor status was made using the Unified Parkinson's Disease Rating Scale-Motor Examination section (UPDRS-ME) [9] in practically defined "*off*" condition, i.e. at least after 12 hours of withdrawal of anti-parkinsonian medication.

SPECT procedure

The [^{123}I]FP-CIT (DaTSCAN; Amersham Health–GE Healthcare) SPECT study was performed in "off" motor status. Patients were

pretreated with potassium perchlorate orally to block thyroid uptake of free radioactive iodide.

All subjects received 110–185 MBq (3–5 mCi) of 123I-FP-CIT in slow intravenous injection and imaging was performed 3-5 h after injection. The SPECT study was performed using a dual-head-camera (G.E. Millenium V.G.) equipped with a low-energy, high-resolution (LEHR) collimator. Data was acquired in a 128x128 matrix, 40 seconds per view, with 64 views in total acquired. Energy discrimination was centered in 158 keV with a 15% window. Image filtering (Butterworth order 10; cut-off 0.5) was followed by attenuation correction (Chang 0.11/cm).

SPECT semi-quantitative analysis

Regular regions of interest (ROI) were constructed around the areas corresponding to both right and left caudate and putamen. The absolute value of SAI was estimated using the following standardized formula: [6]

[(Y-Z)/(Y+Z)]x2x100, where Y and Z are the two different sides *Striatal-binding Indexes*, calculated from ipsilateral caudate and putamen ROI radioactivity counts using the algorithm: [(ROI caudate+ROI putamen)-O]/O, with O = mean counts per pixel in the occipital cortex (background). Bilateral *Caudate/Putamen ratios* were also calculated as specific counts in the caudate nucleus divided by specific counts in the putamen.

Statistical Analysis

Scalar measures were presented as mean ± SD, categorical variables as frequency (percent). Normal distribution of continuous variables was

tested using Kolmogorov-Smirnov test. Parametric t-test was used for means comparison between groups. Chi-square test was used for proportions comparison. Sensitivity and specificity of SAI for differentiating PD from VP were calculated using the optimal cut-off values determined by ROC (receiver operating characteristic) curve analysis.

RESULTS

Gait disorders were more common in the VP group, while motor score at baseline did not significantly differ between PD and VP patients. Evidence of cerebrovascular disease detected by MRI as well as the presence of cardiovascular risk factors were more frequent among VP patients. Clinical features and MRI findings are shown in **table 1.**

As expected, Striatal-Binding Indexes were significantly lower in PD and VP patients compared to ET patients. Considering the most affected side as the side with lower striatal ligand uptake, Striatal Binding Index of VP patients resulted significantly lower than those of ET subjects (6.5±2.4 versus 9.3±1.6, $p<0.001$), but higher compared to the striatal radiotracer uptake of PD patients (5.1±1.2, $p=0.02$). The Caudate/Putamen Ratio of the most affected side in VP subjects was higher compared to ET subjects (1.4±0.3 versus 1.2±0.1, $p=0.007$), but it was lower when compared to PD patients (1.7±0.3, $p=0.009$).

SAI was significantly higher in PD compared to VP (18.7±9.7 versus 4.9±4.2, $p<0.001$) and ET (4.2±3.2, $p<0.001$) (**fig.1**). A cut-off of SAI greater than 14.08, estimated using the ROC curve analysis method (Accuracy of SAI: Area under the ROC curve = 0.912; $p<0.001$), differentiated PD from VP patients with the maximal specificity (100%; 95% CI: 83-100) and a sensitivity of 60% (95% CI: 36.1-80.8). Using a SAI cut-off value of 6.4 we obtained the maximal sensitivity (90%; 95% CI: 68.3-98.5) according to the lowest reduction of specificity (75%; 95% CI: 50.9-91.2).

DISCUSSION

The present study showed that SAI, detected using the [^{123}I] FP-CIT SPECT, could distinguish patients with VP from those with PD, thus confirming the result of a previous study; [6] moreover we found that a SAI cut-off value greater than 14 could specifically identify only patients with PD, thus being useful for the differential diagnosis between PD and VP.

Our study population is representative of the true populations of VP and PD patients, indeed we found that an unilateral onset was a prominent feature of PD patients, whereas VP patients showed some peculiar characteristics. Gait abnormality, described as reduced steppage or shuffling associated to posture abnormality more pronounced during walking, [10] was more prominent at onset in VP patients as compared to PD, probably due to the ischemic damages of the basal ganglia connections with the motor or the frontal cortex. [11]

Using a [^{123}I] FP-CIT SPECT semiquantitative analysis method, we found that patients with VP presented a reduction of striatal ligand uptake compared to ET subjects. Besides, caudate to putamen ratio resulted higher among VP patients compared to ET subjects, demonstrating a possible involvement of the nigrostriatal pathway. Our results are consistent with the study by Lorberboym and coll., [5] who described a decreased putamen to caudate ratio on one side in 11 of 20 VP screened patients, and by Zijlmans and coll., [6] whose VP patients showed a caudate to putamen ratio significantly higher compared to 14 normal subjects. [6] We found also a significant difference of the caudate to putamen ratio between VP and PD groups, underlying, as expected, a greater involvement of the nigrostriatal pathway in the

idiopathic process. Anyway, in our study population, we found that caudate to putamen ratio cannot be used to distinguish VP from PD considering the overlap of the values between groups.

On the other hand, according to the result previously obtained by Zijlmans and coll., [6] we found that SAI was significantly higher in patients with PD compared to VP subjects. Using the receiver operating characteristics (ROC) curve analysis, we obtained a cut-off value that in our sample allowed to differentiate the two conditions with a specificity of 100% and a sensibility of 60%. Indeed, in our study, none of the patients with a SAI greater than 14 belonged to the VP and ET groups, while lower values were obtained in all the groups.

Our work has provided an important glimpse about the possible role of functional imaging in differentiating with a good degree of certainty VP from PD, suggesting that SAI detected using [^{123}I]FP-CIT SPECT could be used to this purpose.

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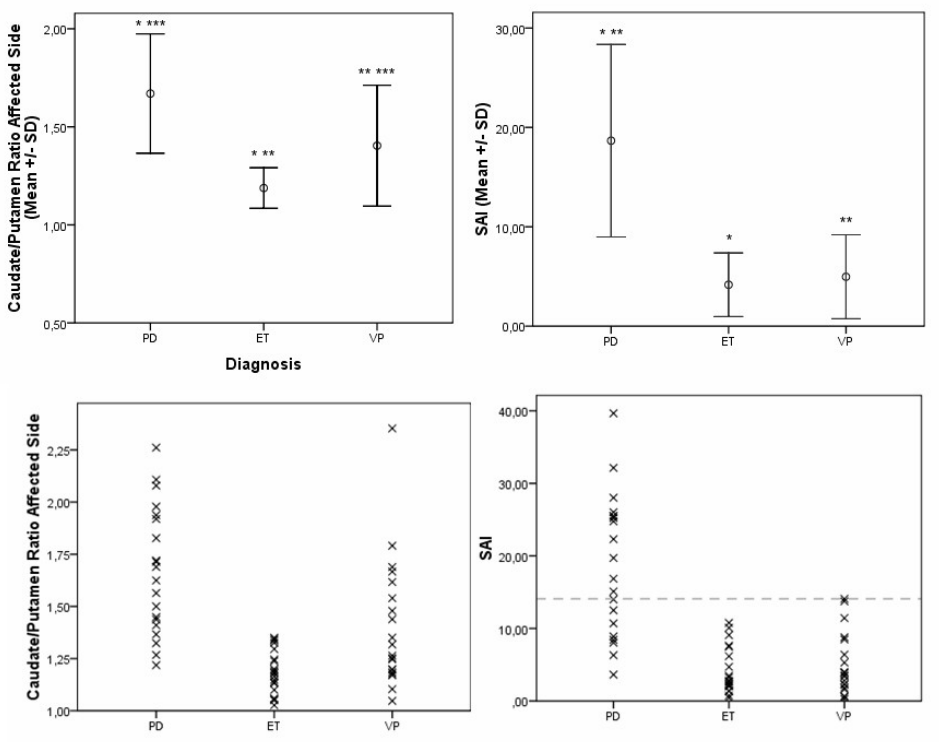
Table 1 title: Clinical characteristics, cardiovascular risk factors and MRI findings

	Parkinson Disease ***N=20***	***Vascular Parkinsonism*** ***N=20***	***p-value***
Clinical features at Onset:			
Unilateral impairment (n, %)	17 (85)	10 (50)	0.018
Bradykinesia (n, %)	9 (45)	15 (75)	ns
Rigidity (n, %)	7 (35)	3 (15)	ns
Tremor (n, %)	12 (60)	13 (65)	ns
Gait Disturbance (n, %)	3 (15)	13 (65)	0.001
UPDRS-ME score (mean ± SD)	24.6 ± 9.8	27.8 ± 9.6	ns
Cardiovascular risk factors (n, %)**:**	5 (25)	16 (80)	<0.001
Hypertension (n, %)	4 (20)	14 (70)	0.001
Diabetes (n, %)	1 (5)	6 (30)	0.037
Heart disease (n, %)	1 (5)	8 (40)	0.008
Vascular lesions detected by MRI (n, %)**:**	3 (15)	20 (100)	<0.001
Exstensive subcortical W.M. lesions (n, %)	2 (10)	12 (60)	<0.001
Lacunar infarcts (n, %)	1 (5)	8 (40)	0.008

Legend: ns= not significant; UPDRS-ME= Unified Parkinson's Disease Rating Scale; W.M.= White Matter.

Figure 1 Title: Striatal Asymmetry Index in patients with Parkinson's disease, Vascular Parkinsonism and Essential Tremor



Legend to the figure

A: Striatal Asymmetry Index (SAI) in different groups (*p<0.001; **p=0.001)

B: Scatter Plot of individual SAI valuesn and cut-off value separating Parkinson's Disease (PD), Vascular Parkinsonism (VP) and Essential Tremor (ET).

4. MANUSCRIPT 2

SPECT STRIATAL ASYMMETRY INDEX MAY PREDICT DOPAMINERGIC RESPONSIVENESS IN PARKINSON'S DISEASE.

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KEYWORDS: Parkinson's Disease, dopaminergic responsiveness, levodopa, [^{123}I] FP-CIT SPECT, Striatal Asymmetry Index.

ABSTRACT

Objectives: Parkinson's Disease (PD) is a neurodegenerative disorder, characterized by the excellent response to levodopa and by asymmetry of neurological signs. Aim of the present study is to investigate a possible relationship between responsiveness to levodopa in PD patients and asymmetry detected by Single Photon Emission Computed Tomography (SPECT) with 123I-FP-CIT (DaTSCAN).

Methods: we performed a retrospective study in 20 PD patients never previously exposed to levodopa, who had undergone i) a short term levodopa test with levodopa/carbidopa 250/25 mg to quantify dopaminergic responsiveness and ii) a SPECT with DaTSCAN to assess the degree of nigrostriatal neuronal degeneration. We estimated the magnitude and the duration of the response to levodopa test as well as the Striatal Asimmetry Index (SAI) detected by SPECT with DaTSCAN.

Results: At levodopa short term test, most patients showed at least a mild response to the drug and only 3 patients presented no response. Overall the UPDRS-ME score at baseline was 24.9±8.2 and at peak was 21.2±8 with a magnitude of the response scoring 16±13.9%; the duration was 254± 91.2 minutes. The caudate and putamen uptakes of DaTSCAN were lower contralaterally to the most affected side. A significant positive correlation between the SAI and the magnitude of the response to levodopa was found ($r=0.64$, $p=0.002$). Linear regression model provided an increase of 0.76 units of magnitude of levodopa response every SAI unit.

Conclusions: Asymmetry resulted positively related to the magnitude of the response to levodopa short term test and may be usefully employed to predict dopaminergic responsiveness in patients with PD.

INTRODUCTION

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the lateralization of motor manifestations, that is still evident late in the course of disease.[1-3] The diagnosis is essentially clinical, based on the identification of the core features of the disease bradikinesia, rigidity and tremor, and supported by the evidence of a good clinical dopaminergic response. Using stringent criteria the probability of misdiagnosis is as high as 10 %[4,5] and the evidence of a good clinical dopaminergic response together with the asymmetry in clinical symptoms represent the more important supportive features to the diagnosis since the early stages of disease.[6]

Up to 40% of PD patients, however, could not have a good response to levodopa in the early phases of disease[7-12] and the availability of predictors of dopaminergic responsiveness could be crucial for an early diagnosis of PD. Moreover, it has been demonstrated that initial response to levodopa could predict motor fluctuations after some years of chronic treatment;[13,14] hence, characterizing levodopa responsiveness since the early stages of the disease could influence medication treatment strategies.

On these grounds, we performed a retrospective study to investigate whether the asymmetry of dopaminergic depletion and the response to levodopa in drug-naïve PD patients could be linked to each other as well as to establish whether an index of dopaminergic asymmetry could predict dopaminergic responsiveness.

MATERIALS AND METHODS

Population Study

We performed a retrospective study in twenty PD patients with a diagnosis of PD according to the diagnostic criteria of the U.K. Parkinson's Disease Society Brain Bank.[15] The patients were retrospectively chosen on the basis of 1) their known positive response to chronic dopaminergic treatment; 2) they should had undergone an acute challenge with levodopa before starting chronic treatment; 3) they should had been studied by mean of Single Photon Emission Computed Tomography (SPECT) with [123I]FP-CIT. Acute levodopa test and SPECT with [123I]FP-CIT were done as normal diagnostic workup to support clinical diagnosis of PD. Patients who at the time of levodopa test were previously exposed to levodopa were excluded from the study. The study was approved by the local Ethic Committee.

Levodopa acute challenge procedure

The acute challenge with levodopa had been performed following a standardized procedure.[16,17] Briefly, patients were pretreated for at least three days with domperidone, in order to avoid the peripheral dopaminergic effects. On the day of the test, after an overnight fast, at 8 am they received an oral dose of levodopa/carbidopa 250/25 mg. The clinical evaluation was made by mean of "Unified Parkinson's Disease Rating Scale-Motor Examination section" (UPDRS-ME)[18] scale, performed prior to the test and 1, 2, 4, 6, 8, 10, and 12 hours after drug intake, until the return to the baseline condition. The magnitude of the response was considered the percentage of the maximal improvement (the difference between patient basal values and peak values of

UPDRS-ME score). The duration of the response was considered the time in minutes when the response decreased to 50% of its maximal improvement.

SPECT procedure

In order to avoid methodological differences, we enrolled only patients who underwent a brain [123I]FP-CIT (DaTSCAN; Amersham Health–GE Healthcare) SPECT study in the same centre.

After the oral administration of potassium perchlorate to block thyroid uptake of free radioactive iodide, the subjects received 110–185 MBq (3–5 mCi) of 123I-FP-CIT in slow intravenous injection and imaging was performed from 3 to 5 h after injection. The SPECT was performed using a dual-head-camera (G.E. Millenium V.G.) equipped with a low-energy, high-resolution (LEHR) collimator. Data was acquired in a 128x128 matrix, 40 seconds per view, with 64 views in total acquired. Energy discrimination was centered in 158 keV with a 15% window. Image filtering (Butterworth order 10; cutoff 0.5) was followed by attenuation correction (Chang 0.11/cm).

Regular regions of interest (ROI) were constructed around the areas corresponding to both right and left caudate and putamen. To establish the degree of asymmetry of the ligand uptake we used the Striatal Asymmetry Index (SAI), proposed by Zijlman and colleagues (**19**). The absolute value of SAI was therefore estimated using the following standardized formula: [(Y-Z)/(Y+Z)]x2x100, where Y and Z are the two different sides Striatal Binding Indexes (SBI), calculated from ipsilateral caudate and putamen ROI radioactivity counts using the algorithm: [(ROI caudate+ROI putamen)-O]/O, with O = mean counts per pixel in the occipital cortex (background).

Statistical Analysis

Scalar measures were presented as mean ± SD, categorical variables as frequency (percentage). Normal distribution of continuous variables was tested using Kolmogorov-Smirnov test. Parametric t-test was used for means' comparison between groups. Correlations were evaluated using Pearson and, in case of positive correlation, linear regression model was carried out.

RESULTS

The age of PD patients (13 men, 65%) was 60±11 years, the age at onset was 57.5±10.9 years, the duration of disease was 2.4±1.5 years. The Hoehn-Yahr stage was between 1 and 3 (1.9±0.4).

At levodopa short term test, most patients showed at least a mild response and only 3 patients presented no response to the drug. Overall, the UPDRS-ME score at baseline was 24.9±8.2 and at peak was 21.2±8 (magnitude 16±13.9%). The duration of the response was 254±91.2 minutes.

Semiquantitative analysis of SPECT with DaTSCAN study are shown in **Table 1**; as expected mean caudate and putamen uptake were lower contralaterally to the most affected side as well as mean SBI even if not statistically significant. The mean absolute value of SAI was 19.1±11.8.

SAI score resulted significantly correlated to the magnitude of the dopaminergic response (r=0,64, p=0,002). A linear regression model (y=1.48+0.76x) accounted for an increase of 0.76 units (95% CI: 0.3-1.2) of magnitude of levodopa response every SAI unit (**Figure 1**).

No correlation was found between SAI and duration of levodopa response (r=0,12). Moreover, we did not find any correlation between SAI and clinical features, including those indicating disease severity, i.e., Hoehn-Yahr stage, UPDRS-ME score at baseline and duration of disease.

DISCUSSION

In our study, SAI resulted directly related to the magnitude of dopaminergic response and could be considered a good predictor of levodopa responsiveness; on the contrary, we found no correlation with duration of the response nor with clinical features. It is well known that asymmetry of neuronal loss in dopaminergic projections to motor striatum is a hallmark of PD[1] and we used the SAI to estimate the degree of asymmetry in nigrostriatal degeneration.[19]

In parkinsonian patients, the unilateral onset and the asymmetry, together with a good responsiveness to levodopa, resulted the best predictors of a correct diagnosis.[6] However, even if levodopa responsiveness represent one of the key features of the disease, a substantial proportion of early PD patients does not experience a significant improvement after the initial administration of dopaminergic drugs;[8-12] therefore, a scarce or no response to levodopa early in the disease course could not exclude a diagnosis of PD. Nevertheless, a definite therapeutic response to levodopa increases the likelihood of PD, so that the availability of predictors of levodopa responsiveness could be crucial to the diagnosis.[20,21]

Moreover, a good initial dopaminergic response may predict the development of motor fluctuations and dyskinesia, so that patients with the best initial response and the largest test-dose response magnitude tend to develop more symptomatic motor fluctuations and dyskinesia.[13,22-24] Consequently, the initial response to levodopa could influence the initial choice of the best pharmacological therapy to minimize problems with or retard the appearance of motor complications.

Acute challenge with levodopa or apomorphine is a current practice in the clinical assessment of PD patients, supporting the diagnosis and providing useful clues for the prediction of response to chronic treatment.[17,20,21,25] Our study suggests that SAI, detected by SPECT with DaTSCAN, could be used beside to short term levodopa test to predict levodopa responsiveness.

We are aware that this study has some important limitations, first of all the retrospective nature and the lack of post-mortem pathological confirmation of diagnosis, so that the results may be spurious and the significant association purely due to chance. However, just the retrospective design helped us to identify those patients who, even if initially not responsive to levodopa, eventually developed a significant improvement after dopaminergic treatment, major criterion for a correct diagnosis of PD.

In summary, SAI can be used to predict future dopaminergic response, not just supporting the diagnosis of Parkinson's Disease but also helping in the choice of the best treatment strategies since the early stages of disease, to reduce the emergence of motor fluctuation and subsequently to improve quality of life of PD patients.

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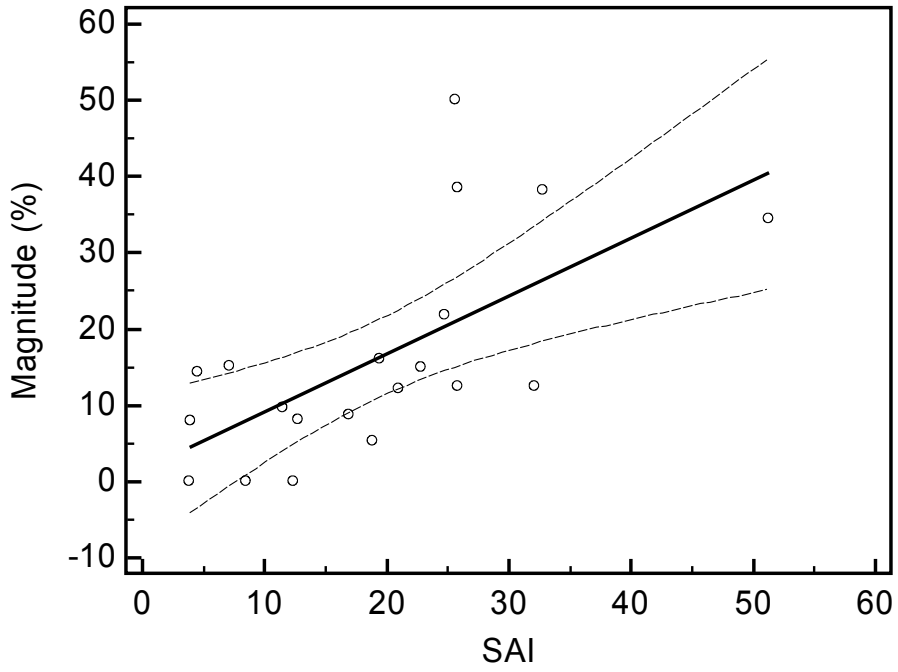
Table 1: Mean Putamen and Caudate specific/non-specific (occipital) uptake ratio and Striatal Binding Index in the two sides (contralateral and ipsilateral to the most affected side of the body).

	Contralateral	**Ipsilateral**	***p* value**
Caudate/occipital	3.9±0.7	4.4±0.7	**0.03**
Putamen/occipital	2.4±0.5	2.9±0.6	**0.007**
Striatal Binding Index	257.3±87.5	310.9±110.8	0.1

Figure 1: Correlation of Striatal Asymmetry Index (SAI) with magnitude of levodopa response at acute levodopa challenge test. Regression line shown with 95% confidence limits.



5. GENERAL DISCUSSION

To quantify the asymmetry of depletion of nigrostriatal dopaminergic neurons, we used the Striatal Asymmetry Index, that was calculated on the DaTSCAN uptake semiquantitative values at SPECT Study. This index was previously described by Zijlmans and colleagues (**Zijlmans et al, Mov Disord 2007**), and it was demonstrated to be significantly lower in Vascular Parkinsonism (VP) patients respect to Parkinson's disease (PD) patients.

The work we carried out has moved in to directions: first of all we tried to verify the practical usefulness of the index on a clinical setting, possibly identifying a cut-off value that could distinguish PD from VP, on the other hand we looked for a possible relationship between the asymmetry index and the prophile of levodopa response, as come out at acute challenge levodopa test, in PD patients.

We used SPECT with a radiotracer, the [123I]FP-CIT or DaTSCAN, that binds specifically and with high affinity to presynaptic dopamine transporters. SPECT with DaTSCAN can be considered, to date, the best technique for in vivo assessment of depletion of pre-synaptic dopaminergic projections to the motor striatum.

DaTSCAN-SPECT is commonly used to support or reject a diagnosis of PD, allowing to detect the reduction of radiotracer uptake since the early stages of disease. However it cannot always reliably help in distinguishing PD towards other neurodegenerative parkinsonism. Contrariwise, theoretically FP-CIT binding is expected to be normal in VP patients when clear vascular lesions in the basal ganglia have been excluded. In fact, presynaptic dopaminergic circuitry should be

preserved in VP, so that in the absence of postsynaptic causes of Parkinsonism and the presence of vascular lesions on structural neuroimaging and considering the clinical context, normal striatal ligand uptake is confirmatory of VP. Also unilateral abnormal decreased binding in a distribution delineating a vascular lesion in the basal ganglia with normal contralateral uptake can confirm VP when this pattern matches unilateral clinical features (**Scherfler et al, *Mov Disord*. 2007).**

A diagnosis of VP nevertheless may be considered also in the presence of a bilateral reduction of striatal DAT signal loss, thereby mimicking the pattern of dopaminergic dysfunction in idiopathic PD. In a recent SPECT study, Zijlman and colleagues found that there were not significative differences in the ligand uptake between VP and PD, except for the Striatal Asymmetry Index and the author concluded that a symmetrical uptake could help in the differential diagnosis (**Zijlmans et al, Mov Disord 2007**). However, to date, no studies had been performed in order to find out clear cut-off values to separate the two condition according to striatal DAT availability and the management of particular patients remained subjective.

In our study we found a cut-off value of DaTSCAN-SPECT Striatal Asymmetry Index, that would allow to separate VP from PD with a good sensitivity and the maximal specificity; if confirmed by further ad hoc studies, this could be of relevance in routine clinical practice, since the differential diagnosis can be challenging as vascular lesions and white matter basal ganglia ischemia are frequent in the elderly people.

In the second part of our work, we aimed to investigate if there could be a relationship between the two most important distinctive features

of PD, the asymmetry and the levodopa responsiveness. It is well known that the magnitude of the response to levodopa test doses is the critical factor determining if patients will notice and report motor fluctuation related to levodopa medication (**McColl et al, Mov disord 2002; Clissold et al, Mov disord 2006)**; hence the availability of predictors of dopaminergic responsiveness could be useful not just to support a diagnosis of idiopathic PD but also in the management of the therapy since the early stages.

The single-dose short term test with levodopa is the most widely used test either to demonstrate levodopa response or to characterize the response in PD patients. It is an inexpensive, easy to perform, and universally accessible test but suffer from a significant incidence of adverse events, the need for domperidone pretreatment, a lack of agreement on what assessment methods to use and on threshold responses, the need for day case admission (**Clarke et al, J Neurol Neurosurg Psychiatry 2000**).

We found that Striatal Asymmetry Index significantly correlated with the magnitude of dopaminergic response at levodopa tests; thereby it is possible to postulate that this index could be used in clinical practice to predict levodopa responsiveness, helping physicians in handling the therapy of PD.

6. **CONCLUSIONS**

The Striatal Asymmetry Index evaluated on DaTSCAN-SPECT imaging may provide valuable information in patients presenting with parkinsonian symptoms and cerebrovascular disease, where diagnosis could be challenging. Moreover, it could be of usefulness in PD patients to predict the magnitude of dopaminergic responsiveness. This appears to be fundamental particularly early in the disease when correct treatment decisions may have far reaching implications in the development of motor fluctuation and dyskinesia, that are some of the variables that most affect the quality of life of PD patients.

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