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Titolo tesi

Molecular imaging in management of Parkinsonian Syndrome: accuracy of semi-quantified [^{123}I]FP-CIT SPECT in a multi center setting.

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Introduction

Parkinson's Disease (PD) is the second most frequent neurodegenerative disease in the world, caused by the degeneration of dopaminergic neurons in the nigro-striatal pathway and its incidence will double in the next fifteen years[1,2]. Its clinical onset, characterized by, rigidity, bradichinesia, resting tremor and postural instability, is shared with other atypical parkinsonian disorders included in parkinsonian neurodegenerative syndrome such as Multiple System Atrophy (MSA), Progressive Supranuclear Palsy (PSP) and Cortico-Basal Degeneration (CBD). The most involved sub-cortical nucleus of all these disorders are caudates and putamina (Basal Ganglia).

Neuropathology

Parkinson's Disease

Braak *et al* described recently the neuropathologic staging system of Parkinson's Disease according to 6 stages[3].

In the first stages of disease brains, in particular an area extending from ala cinerea to the ventrolateral surface of the lower brain stem, demonstrate inclusion Lewy Neurites (LN) within the spindle-shaped projection cells of the dorsal IX/X motor nucleus and intermediate reticular zone. In more advanced cases, LNs initially outnumber the small and inconspicuous Lewy Bodies (LB). Small cells of subnuclei on the solitary tract, area postrema and the subnucleus gelatinosus are involved as well, although less. On the other hand, cells containing neuromelanin (the most frequents in substantia nigra area) and presents in the zone outlined above do not presents these alterations. At stage 2, the affection is accentuated of both the dorsal IX/X motor nucleus and the intermediate reticular zone. However in this stage appears also LNs and LBs in projection neurons of the caudal raphe nuclei and the reticular formation. The first cells with neuromelanin in the brain

to develop LNs and LBs are neurons of the coeruleus–subcoeruleus complex, with LNs that appear before LBs. However in this complex, cells without neuromelanin remain exempt from the formation of LNs and LBs. Finally, the substantia nigra, remain uninvolved during stages 1 and 2.

In stages 3 and 4 the severity of lesions increases gradually in the higher stages, in particular melanin-laden nerve cells of the dorsal IX/X motor nucleus and of the intermediate zone develop LBs. However in stage 3, begins to appear lesions in a subset of melano-neurons in the substantia nigra and the involvement of lipofuscin-laden projection neurons in the magnocellular nuclei of the basal forebrain. On the other hand no lesions are observed in non-olfactory cortical areas. In fact, in this stage the lesions in substantia nigra are detectable only microscopically, particularly restricted to melanized projection neurons of the posterolateral and posteromedial subnuclei. Such as in the first stages, LNs appears in a considerable amount in the vulnerable nigral subnuclei before the development of LBs in melanocites, while in non melanocites no development of LNs/LBs is observed so far. The melanized neurons usually develop LBs within the boundaries of their neuromelanin accumulations, and, a group of small LBs occurs within a single perikaryon. Therefore, important hallmark of stage 3 is the involvement of melanized cells of substantia nigra by the accumulation of LN and LB. These cells project their axons in the putamen and caudate nucleus of basal ganglia. On the other hand, at this stage no inclusion body are detected on extranigral melano-neurons in the mesencephalic tegmentum while, a network of lengthy LNs begins to appear on the compact portion of the pedunculopontine tegmental nucleus as well as on magnocellular nuclei of the basal forebrain and on the hypothalamic tuberomamillary nucleus. In stage 3 neither the anteromedial temporal mesocortex nor areas of mature neocortex are involved by the accumulation of LNs, by contrast, anterior olfactory nucleus generally begin to be affected. Long LNs extending throughout the second sector of the Ammon's horn are detectable at this stage. At stage 4, the involvement of substantia nigra become more accentuated, in particular in vulnerable

subnuclei where melano-neurons are stored. In this stage a loss of neurons is observed with local accumulations of extraneuronal neuromelanin granules, especially in the posterior regions of the pars compacta, and the depigmentation is detectable macroscopically. The nerve cells of posterior regions of pars compacta project their axons in putamina, and this is the reason why the [^{123}I]FP-CIT SCAN shows firsts alterations on the putamina. The paranigral nucleus and the pigmented parabrachial nucleus of mesencephale are involved by destruction, in particular neuromelanin-containing projection neurons. In this stage LBs and LNs are present on lipofuscin-laden projection neurons of the oral raphe nuclei as well as the compact portion of the pedunculopontine tegmental and even become detectable in portions of the tectum and central gray; severe involvement of the magnocellular nuclei of the basal forebrain and on hypothalamic tuberomamillary nucleus are further observed.

In stage 4, other areas are involved, in particular stria terminalis as well as cortical and central nuclei of the amygdala, the ventral claustrum begin to develop LBs and LNs as well as in subnuclei of the thalamus. In stage 4, anterior olfactory nucleus present a significant damage whereas in cortical areas lesions are observed only in some cases, in particular in the anteromedial temporal mesocortex, and the plexus of LNs in the Ammon's horn increases in thickness. In the anteromedial temporal mesocortex the outer cellular layers develop a network of filiform LNs, while the inner ones show spherical LBs. Furthermore, the white matter below the temporal mesocortex sustain also heavy damage. The lesions at stage 4 presents a declining gradient from the anteromedial temporal mesocortex following the cortex in the direction of the medially adjoining entorhinal region and laterally adjoining high order sensory association areas of the temporal neocortex.

In the two last stages the damages described in subcortical and mesocortical areas are significantly increased. In substantia nigra melanin-laden neurons are almost disappeared with pallor upon macroscopic inspection. In these stages, the amount of LN and LB begin to decrease while

neuromelanine aggregations increase at a extraneuronal level. Neuronal loss of melanized cells is observed also within the dorsal IX/X motor nucleus, intermediate reticular zone, reticular formation, and coeruleus–subcoeruleus complex. Olfactory areas are severely damaged in this stage. The second Ammon's horn sector exclusively damaged until this stage, is associated with lesions from long LNs extended into adjoining portions of the first and the third sectors. In the last stages the destructive process involving the neocortex, temporal mesocortex as its point of departure extended into the adjoining sensory association areas of the neocortex, insular fields, the anterior cingulate cortex, and prefrontal areas. Several pyramidal cells with LBs are present in the infragranular layers of the neocortex, whereas the net of supragranular LNs is less dense. A gradient is observed on the density of LB-bearing pyramidal cells with reduction in the direction of the more distant sensory association areas and prefrontal fields. In stage 5, sensory association and premotor areas as well as the primary sensory and motor areas are free of LNs and LBs. While in stage 6 is involved nearly the entire neocortex, premotor areas, primary motor field, sensory association and primary sensory areas are mildly involved by Parkinson related pathology.

Lewy Body Dementia (DLB) and Parkinson's Disease Dementia (PDD), are probably the same neuropathologic entity but with different clinical onset. The staging pathological scheme proposed by Braak et al for Parkinson's Disease, is more or less applicable in Lewy Body Dementia and Parkinson's Disease Dementia, however some differences can be observed, in particular in DLB, at least in the first stages, the substantia nigra neuronal loss is lesser while alpha synuclein pathology is more accentuated in the striatal neurons than in PD[4].

The other disorders are definitively less frequent and therefore, it is harder to establish canonical stages, however they has been neuropathologically described as below.

Multiple System Atrophy

Depending on the predominant signs and symptoms, MSA is subdivided into MSA-C, for those with predominant degeneration in cerebellar circuitry and ataxia, and MSA-P for those with predominant degeneration in the basal ganglia with parkinsonism[5]. Usually, autonomic dysfunction is associated in MSA. Coated filaments similar to the filaments in Lewy bodies from 10 to 20 nm diameter are detected at glial cytoplasmic on the ultrastructural level. Furthermore, within certain neuronal populations, neuronal cytoplasmic and intranuclear inclusions, particularly those in the pontine base, inferior olive, and putamen, are observed. Pathologically in MSA, neuronal loss of dopaminergic neurons in the substantia nigra and noradrenergic neurons in the locus ceruleus is associated with pontine atrophy, in particular in MSA-C when it is evident also gray discoloration of the cerebellar white matter. In MSA-P atrophy of the posterolateral putamen is associated with brown color correlating with increased iron pigment. More subtle atrophy is noted in the medulla and the cerebellar cortex[6].

Progressive Supranuclear Palsy

PSP is a neurodegenerative taupathy with accumulation of tau filamentous neurons. Tau is a microtubule protein biochemically composed of six major isoforms. In addition to neurofibrillary tangles, tau pathology of PSP is characterized by inclusions in astrocytes and in oligodendroglia. The latter glial lesions are distinct from the glial cytoplasmic inclusions of MSA and PD due to their immunoreactivity with tau and to their morphology. PSP has marked atrophy of the midbrain and mild frontal cortical atrophy as well as cerebellar dentate nucleus associated to discoloration of the white matter in the dentate hilus, and of the superior cerebellar peduncle. Subthalamic nucleus is almost always atrophied and often discolored while basal ganglia and thalamus are near to normal from a macroscopic point of view. Recently, greater cortical pathology has been noted, particularly affecting motor and premotor cortices of the frontal lobe. The nuclei affected in PSP are: globus

pallidus, subthalamic nucleus, substantia nigra, midbrain tectum, periaqueductal gray, locus ceruleus, and the cerebellar dentate nucleus. Other regions affected include corpus striatum, ventrolateral thalamus, red nucleus, pontine and medullary tegmentum, pontine base, and inferior olivary nucleus. Intermediolateral cell columns of spinal cord are frequently involved also[6].

Cortico-basal Degeneration

Cortico-basal degeneration is a parkinsonism-plus disorder with cortical signs in addition to atypical levodopa-nonresponsive parkinsonism. Clinical features are progressive asymmetrical rigidity and apraxia (the cortico-basal syndrome), less frequently are observed progressive aphasia and progressive frontal lobe dementia. Parkinsonism is characterized by bradykinesia, rigidity, and dystonia usually without tremor. Neuropathologic alterations are characterized by focal cortical atrophy, uncommon in PD, MSA, and PD. This cortical atrophy is more accentuated in the superior frontal gyrus, and the motor cortex. On the contrary of others parkinsonism the midbrain does not present atrophy, however depigmentation of substantia nigra is a hallmark of this disease also. Microscopically, neuronal loss, spongiosis, and gliosis with swollen achromatic or ballooned neurons is observed in the involved cortical areas. Cortical neurons in gray as well as in white matter and in basal ganglia also presents pleomorphic tau-immunoreactive inclusions. The characteristic lesion is the “astrocytic plaque” a tau accumulation in distal processes of astrocytes. Astrocytic plaques are definitively different from the tufted astrocytes seen in PSP. In substantia nigra is observed moderate to severe neuronal loss with extraneuronal neuromelanin, gliosis and tau immunoreactive neuronal lesions. Neuronal loss and gliosis is noted within globus pallidus, putamen and thalamic nuclei[6].

Molecular Imaging

Molecular imaging has been one of the most important contributors in the diagnosis of PD and other neurodegenerative parkinsonian syndromes since the '90[7]. In the past, radiopharmaceuticals such as ^{18}F-Fluoro DOPA, ^{123}I- Beta CIT, and ^{99m}Tc TRODAT were used in the differential diagnosis between PD and Essential Tremor (ET) or between Lewy Body Dementia (LBD) and Alzheimer Dementia (AD)[7]. Subsequently, it has been observed that even in other degenerative parkinsonian syndromes such as, Progressive Supranuclear Palsy (PSP), Cortico-Basal Degeneration (CBD) or Multi-Systemic Atrophy (MSA) the nigrostriatal pathway was altered with degeneration of dopaminergic axons and neurons soma[8]. Therefore, molecular imaging with ^{18}F-Fluoro DOPA, ^{123}I- Beta CIT, ^{99m}Tc TRODAT, ^{123}I-IBZM and nowadays, particularly with [^{123}I]FP-CIT has became crucial in the clinical setting in order to distinguish neurodegenerative parkinsonian syndrome from other syndromes and/or diseases with similar clinical appearance, but with different treatment and prognosis, such as ET, dystonia, vascular parkinsonism or iatrogenic parkinsonism, etc[9].

The Dopamine Transporter (DAT), a protein on the presynaptic dopaminergic nerve terminals mediates the reuptake of free dopamine intrasynaptic space. Several SPECT radioligands for DAT are available, but 2, [^{123} I]2 β -carboxymethoxy-3 β -(4-iodophenyl) tropane ([^{123}I]2 β -CIT) and [^{123}I]N-w-fl uoropropyl-2 β -carbomethoxy-3 β -(4-iodophenyl) nortropane ([^{123}I]FP-CIT) are commercially available and frequently used in clinical routine. These tracers have high binding potentials to DAT and to serotonin transporters, however the last one are less expressed in the striatum. Other [^{123}I]-labeled DAT radioligands include [^{123}I]-2 β -carbomethoxy-3 β -(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane ([^{123}I]-Altropane), [^{123}I]-N-(3-iodopropen-2-yl)-2 β -carbomethoxy-3 β -(4-chlorophenyl)tropane ([^{123}I]-IPT), and [^{123}I]-labeled N-(3-iodoprop-(2E)-enyl)-2 β -carboxymethoxy- 3 β -(4-methylphenyl)nortropane ([^{123}I]-PE2I) and finally [99m Tc]-TRODAT-1 ([^{99m}Tc]-[2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2 0.1]oct-2-

yl]methyl](2- mercaptoethyl)amino]ethyl]amino]ethanethiolato(3-)-N2,N2,' S2,S2']oxo-[1R-(exo-exo)]), the only available technetium labeled radioligand. [^{123}I] β -CIT present an optimal image acquisition at 18 to 24 hours after injection due to ligand-to-receptor equilibrium, while 3 to 6 hours after injection of [^{123}I]FP-CIT. This is the principal reason why [^{123}I]FP-CIT is already the most used radiotracer. Signal acquisition is then achieved by multiple detectors system with appropriate collimators (LEHR, LEUHR) and scanning times approximately 30-45 minutes. Filtered backprojection or iterative algorithms are used for image reconstruction of entire brain volume. Low-pass filtering is used to perform attenuation correction to minimize signal alterations from variably deep brain structures such as the striatum. In the routine clinical setting, visual assessment of DAT is sometimes associated to semiquantitative analysis using regions of interest (ROI) and compared to absent background DAT expression. So the assessment is focused on the striatum because it is the brain area with highest DAT density, however some authors estimate DAT density in brain regions with somewhat lower but still detectable signal[10]. In clinical routine, dopaminergic therapy does not need to be discontinued because it has not been demonstrated definitively a significant impact of dopaminergic therapy on striatum DAT density, while some CNS stimulants, serotonin reuptake inhibitors and others have a significant influence on radioligand binding, hence are stopped before the exam. In the third and fourth stage of Parkinson' s Disease it has been described a significant loss of dopamine neurons on substantia nigra that project on the striatum, more than 50% of loss is necessary in order to provoke clinical appearance of disease. Several studies have shown that DAT ligand uptake mirrors the decline in levels of striatal dopamine in the early stage of PD, then it has been included in the clinical routine of PD diagnosis in patients presenting with mild symptoms[11-17]. On the contrary, patients with normal striatal DAT availability and clinical signs of parkinsonism or tremor disorders are unlikely to have early degeneration of the nigrostriatal dopaminergic pathway.

On the other hand, presynaptic DAT imaging is normal or near to normal in patients fulfilling essential tremor criteria without overlapping clinical features. Also, patients with adult-onset dystonic tremor were reported to have normal striatal DAT availability[18].

Atypical parkinsonian disorders

Differentiation of PD from atypical parkinsonian disorders like Multisystem Atrophy (MSA), Progressive Sopranuclear Palsy (PSP) or Cortico-Basal Degeneration (CBD) is not easy, in particular in the first stages of disease. In general atypical parkinsonian disorders present poor levodopa responsiveness and more rapid clinical deterioration are, and involve pre- and postsynaptic dopaminergic degeneration. Usually, the reduction of DAT density is observed symmetrically on putamina and then in caudate nuclei in patients with atypical parkinsonian disorders, however differential diagnosis between PD and atypical parkinsonian disorders is not always feasible[19]. Striatal DAT loss in CBD is in the same range as it is in PD and other forms of atypical parkinsonism although it is much more asymmetrical and less pronounced than seen in MSA and PSP[11,19].

Psychogenic parkinsonism

Psychogenic parkinsonism (PsyP) is hard to differentiate from PD, however early and accurate diagnosis is important to offer an adequate prognosis, to provide an adequate treatment, and to avoid unnecessary diagnostic procedures. It presents a sudden onset and static course of motor symptoms, variability of tremor and limb rigidity with distraction or during activation of movements in opposite limbs, deliberate slowness, bizarre gait disorders and active resistance to limb movements[20]. DAT-SPECT in patients with PsyP have found striatal DAT availability in the range of normal controls[21]. Hence, DAT-SPECT is useful in the differentiation of psychogenic from degenerative parkinsonism, while clinical hints alone, are difficult to rule out organic parkinsonism.

On the other hand, psychogenic movement disorders can coexist with an underlying organic disease, in this case DAT SPECT can be altered.

Vascular parkinsonism

Vascular parkinsonism has a clinical presentations similar with degenerative forms and difficulties are raised for the clinician even in presence of CT or MRI brain scans. Furthermore, vascular lesions are not rare in pathologically confirmed PD, and a huge amount of patients with late onset PD have white-matter changes observed on CT/MRI scans. Proposed diagnostic criteria for vascular parkinsonism, includes bradykinesia, cerebrovascular disease visualized by CT or MRI, and a temporal relationship between the location of vascular lesions and the appearance of parkinsonian symptoms or the presence of extensive subcortical white-matter lesions and bilateral symptoms at onset[22]. It is clear that in order to diagnose vascular parkinsonism, other causes as space occupying lesions, pharmacological and toxic effects, head trauma, or encephalitis should be excluded. Usually, presynaptic dopaminergic neurons are preserved in VP, and striatal DAT binding, the putamen/caudate ratio are normal or only mildly reduced in patients with VP, excepted in cases of basal ganglia infarct. The true value is in cases with clinical parkinsonism and vascular lesions on MRI or CT in whom DAT-SPECT is normal. These would then be classified as vascular parkinsonism using the criteria by Zijlmans et al. A proportion of those fulfilling these criteria may, however, also have abnormal tracer binding, which would not be incompatible with a diagnosis of vascular parkinsonism.

DAT-SPECT in drug-induced parkinsonism

Neuroleptics interfering with the postsynaptic D2 receptors and calcium-channel blockers and tetrabenazine, interfering on presynaptic level can lead to drug-induced parkinsonism (DIP) which manifests usually similar to degenerative parkinsonism on clinical grounds. Although commonly associated with symmetric symptoms, but unilateral parkinsonian features are not rare. The affinity

of neuroleptics to the DAT and its influence toward changes of DAT density is negligible, therefore DAT SPECT has become an important instrument to distinguish DIP from degenerative parkinsonism[11]. In this scenario, however, attention should be paid in drugs that should be refrained before the scan. It can be concluded that DAT-SPECT is likely to be normal in DIP unless presymptomatic degenerative parkinsonism is present and hence is important to guide appropriate therapeutic management.

Dementia with Lewy bodies

DLB is one of the commonest forms of degenerative dementia after Alzheimer's disease (AD) but patients with AD do not show severe nigrostriatal degeneration, while DLB patients do. Abnormal DAT imaging was therefore also included as a suggestive feature in the DLB consensus criteria in 2005[23]. It is important to distinguish AD from DLB in terms of prognosis and appropriate treatment.

A tracer of disease progression

[^{123}I]FP-CIT SCAN presents several advantages as a biomarker of disease progression in PD: it is objective and reproducible in early PD patients, it has a significant sensitivity to deterioration of the nigrostriatal dopaminergic system, it is well related with disability, and it is not affected by therapy[11]. However there may be different effects of dopamine agonists and levodopa on the regulation of DAT expression, thus serial SPECT assessments of striatal DAT binding is not used routinely, to date, such as in the diagnostic setting.

DAT-SPECT in clinical practice

The role of DAT-SPECT is universally recognized when clinical signs are inconclusive or vague and a PD diagnosis is not definitively confident. The accuracy of DAT-SPECT to identify neurodegenerative parkinsonian syndrome in patients with onset of symptoms has been evaluated in different studies and in the last years a meta-analysis has definitively pointed out the good accuracy of this exam in the clinical setting[24]. Other trials have confirmed its positive contribution on the

global economy of the patients and of the healthcare system[25]. Sensitivity varies in two studies between 78 % and 92 % , specificity between 96 % and 100 % for DAT-SPECT to correctly classify neurodegenerative parkinsonian syndrome. Almost always the diagnostic accuracy and reliability of DAT-SPECT have invariably been evaluated against the clinical examination. However, to determine most accurately the discriminant power of DAT-SPECT separating neurodegenerative from other causes of parkinsonism, correlations of imaging with results from autopsy are needed. This approach is certainly the most convincing, but even the less useful for the single patient.

The good accuracy of this radiopharmaceutical seems to be related to its intrinsic high affinity for the pre-synaptic dopamine transporters, but even to the ability to do semi-quantitative analysis. In fact, during the onset of disease, a qualitative evaluation of this exam could have a reduced sensibility and is highly dependent on the nuclear medicine physician's experience. Recently different ways of semi-quantitative evaluations have been proposed such as voxel-based methods or Region Of Interest (ROI)-based methods in order to optimize sensibility[26]. The Italian Association of Nuclear Imaging and Molecular Imaging proposed few years ago a freely PC-based software, BasGan V2, with the objective to uniform the semi-quantitative evaluation of DaTSCAN acquired in different centers with different equipments and independently from nuclear medicine physician's experience. BasGan performs a semi-quantitative evaluation, in addition a comparison with a database of semi-quantitative data acquired from normal subjects is enabled[27].

However, to date only few studies have assessed the role of this kind of evaluation in the clinical setting among patients with suspected neurodegenerative parkinsonian syndrome[28]. During the PhD course our research raised different aims which are treated in this dissertation and also published in peer review journals: 1) to assess accuracy of a semi-quantitative analysis (by the mean of BasGan) of DaTSCAN, acquired in a unique center; 2) the assessment of accuracy of a semi-quantitative

analysis of DaTSCAN, acquired in three centers which use different equipments; 3) to evaluate the accuracy of a voxel-based analysis of DaTSCAN, acquired in three centers with different equipments.

The studied population included patients acquired between 2006-2010 in order to have a sufficient follow-up, given that the clinical diagnosis was used as gold standard.

Finally, the last findings of these researches, in particular voxel-based analysis have not been published yet, but I confidently think, they will be soon.

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FP-CIT SPECT evaluation: time to go beyond visual assessment!

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Extended Summary

Nowadays, [^{123}I]FP-CIT SPECT is used in the assessment of patients with extra-pyramidal syndrome, and several semi-quantitative methods have been proposed: methods with manual positioning of anatomical ROIs, with expedients to avoid partial volume effect (PVE), methods with advanced automated quantification data (in particular BASGAN), with voxel-based statistical methods (such as SPM and NEUROSTAT). However, despite both the need of experience for a correct visual assessment and a strong encouragement for semi-quantitative evaluation from the "European Association of Nuclear Medicine and Molecular Imaging", no semi-quantitative method has been accepted as standard.

BasGan is a PC-based software freely available on the net. It is not dependent on a specific gamma-camera and performs a comparison with normal results database. The utility of BasGan needs to be confirmed in multicenter trials. These should validate normal references and demonstrate their usefulness to correctly establish the presence of disease.

In our institution we evaluated the role of BASGAN in 59 consecutive patients (mean age 68 years, 32M and 27F). FP-CIT SPECTs obtained in 2006 and 2007 were analyzed with the above mentioned software and then correlated with final diagnosis. Disease (if any) was established by the neurologists in light of all relevant data. Results from this software showed good correlation with final diagnosis. Logistic regression demonstrated a significant correlation between final diagnosis and both caudate values ($p<0.001$) as well as putamen values ($p<0.001$). ROC curves (accuracies

for both caudate and putamen semi-quantitative values) showed high AUC values (range 0.82-0.87, $p \leq 0.0001$ for all occurrences). In conclusion, in our center, BasGan allowed good accuracy in [^{123}I]FP-CIT SPECT reporting.

Original Article

Nowadays, FP-CIT SPECT is used in the assessment of patients with extra-pyramidal syndrome. Meta-analysis confirmed the role of radionuclide imaging in this setting [1] and further cost-effectiveness studies showed the economical benefits of this diagnostic procedure [2, 3]. Recently, we read with great interest the paper of Badiavas and colleagues [4] published in your journal. The authors presented a stimulating review about SPECT imaging in movement disorders and focused in the semiquantitative evaluation, after a hint on tracer kinetic models. They reviewed several semiquantitative methods: with manual positioning of anatomical ROIs, with expedients to avoid partial volume effect (PVE), with advanced automated quantification methods (in particular BASGAN), with voxel-based statistical methods (such as SPM and NEUROSTAT). In conclusion was remarked that, despite both the need of experience for a correct visual assessment and a strong EANM encouragement for semiquantitative evaluation, no semi-quantitative method has been accepted as a standard. Consequently, qualitative evaluation is still the standard. In authors' view, a perfect software should be PC-based, automated (i.e. would analyze raw or mildly processed data), should give reproducible output and should have no dependence on a specific gamma-camera. An ideal software will also perform a comparison with a database of values from normal subjects.

BASGAN is a PC-based software freely available from the AIMN site. It is not dependent on a specific gamma-camera, (it analyzes DICOM images after a transaxial reconstruction) and performs a comparison with normal results database. Furthermore, the BASGAN creators announced at the last EANM congress the final phase of an European project aiming to realize a database with values from healthy subjects acquired all around the European continent [5]. On the other hand, to the best of our knowledge, no published study evaluated BASGAN in a patient population, except for a small population study performed by the above mentioned group. So, the utility of BASGAN needs

to be confirmed in larger population from multicenter trials. These should validate normal references and demonstrate their usefulness to correctly establish the presence of disease. Indeed, BASGAN should be useful to improve FP-CIT SPECT report in different centers, from several countries, acquired with distinct gamma-camera models and reconstructed with diverse processing applications.

In our institution we evaluated the role of BASGAN in 59 consecutive patients (mean age 68 years, 32M and 27F). FP-CIT SPECTs obtained in 2006 and 2007 were analysed with the above mentioned software and then correlated with final diagnosis. Disease (if any) was established by the neurologists in light of all relevant data; however, given the long follow up (at least three years), we think that the diagnosis was independent from FP-CIT scan report. Results from this software showed good correlation with final diagnosis. Logistic regression demonstrated a significant correlation between final diagnosis and both caudate values ($p<0.001$) and putamen values ($p<0.001$). ROC curves (accuracies for both caudate and putamen semi-quantitative values) showed high AUC values (range 0.82-0.87, $p\leq0.0001$ for all occurrences) [6].

In conclusion, in our center, BASGAN allows good accuracy in FP-CIT SPECT reporting. This accuracy was not dependent from the operator experience and showed a good reproducibility.

In the near future, we will study accuracy and reproducibility of FP-CIT SPECT evaluated by BASGAN in a multicenter trial. This could be the proof of concept for a successful computer-aided report of FP-CIT SPECT.

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Assessing the accuracy and reproducibility of computer-assisted analysis of 123I-FP-CIT SPECT using BasGan (V2)

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Extended Summary

Background and Purpose

It is well known that treatment strategies for patients with Parkinson's disease (PD) are ineffective and even contraindicated when applied to other movement disorders, such as essential tremor (ET), on account of their severe side-effects; hence the need for high–impact diagnostic tools. [^{123}I]FP-CIT-SPECT (DAT-SPECT) has increasingly been used over the last 12-15 years to help discriminate PD from ET in patients with movement disorders, with results rating favorably on both accuracy and cost-effectiveness. BasGan is a freely available software that assists [^{123}I]FP-CIT SPECT evaluation by estimating semi-quantitative values for each basal nucleus and compares the results to a database of healthy subjects.

The aims of this study were: 1) to assess the accuracy of qualitative analysis and of semi-quantitative, BasGan-assisted evaluations of [^{123}I]FP-CIT SPECT; 2) to compare the accuracy of both methods when applied to 'doubtful' cases; 3) to appreciate the reproducibility of the BasGan-assisted evaluations.

Materials and Methods

Seventy-eight patients were included in this 4-year follow-up study. At a four-year interval, the initial diagnosis was reviewed for fulfillment of Step 1 of UK Brain Bank criteria for PD and of Findley & Koller criteria for ET, independently from DAT-SPECT report. A definitive diagnosis was established in 78 patients (33 males, 45 females, mean age at the time of SPECT acquisition, 68 ± 9 years). Fifty-four patients (69%) were diagnosed with neurodegenerative parkinsonian

syndrome (NPS). Twenty-four (31%) were found NPS-free and were diagnosed as follow: 12 ET, 3 Dystonia, 2 Cerebral vasculopathy and 1 for Restless Leg syndrome, Cerebellar Ataxia, ET in thyreopathy, Primary Progressive Aphasia, Multiple Sclerosis, Huntington Chorea and Semantic Dementia. Brain SPECTs were acquired as per standard procedures. Patients were imaged 4 hours post-injection using a dual-head gamma-camera. SPECT images were visually stratified into normal, abnormal and equivocal by the three nuclear medicine physicians, based on striatal-to-background uptake ratio, symmetry in uptake reduction and patients' age. Cases in which full diagnostic agreement was not reached were classed as 'doubtful'.

In addition, images were analyzed using BasGan, and semi-quantitative data were obtained for each basal nucleus. Accuracy of the analysis was checked against semi-quantitative putaminal and caudate uptake values in the most severely affected hemisphere. Data from each of the 4 nuclei were then adjusted by computing the ratio to the corresponding values reported in the software database for normal subjects of similar age (age-adjusted values).

Results

The overall sensitivity, specificity and accuracy scores were 92% (44/48), 60% (3/5), and 89% (47/53), respectively, in cases in which an agreement was acquired among three nuclear medicine physicians. In the 25 cases that were given different diagnoses by one or more of the investigators were classed as 'doubtful'.

BasGan-assisted analysis of DAT-SPECT showed a high accuracy on predicting disease. Further, on doubtful cases, accuracy was significantly higher when using BasGan as opposed to relying solely on the investigators' visual assessment.

Conclusion

BasGan-based analyses of DAT-SPECTs were remarkably accurate and reproducible, especially with regard to data for the most severely affected putamen. The software proved particularly useful in the analysis of 'doubtful' cases, thus demonstrating its potential to assist in the diagnosis of PD.

Original Article

Introduction

Parkinson's disease (PD) is a common neurodegenerative condition. At the time of writing, its prevalence rate ranges from 100 to 300 cases per 100,000 persons [1] and is estimated to double by 2030 [2]. For this reason, the scientific community recognises the need for improved patient care management. Early disease recognition is followed by a timely and appropriate multi-agent therapy based on dopaminergic drugs in combination with exercise therapy and other treatments currently being implemented. Drugs such as rasagiline seem to have neuroprotective action [3], therefore an early diagnosis is all the more important for a successful management of the disease.

It is well known that treatment strategies for patients with PD are ineffective and even contraindicated when applied to other movement disorders, such as essential tremor (ET), on account of their severe side-effects; hence the need for high–impact diagnostic tools. ^{123}I-FP-CIT-SPECT (DAT-SPECT) has increasingly been used over the last 12-15 years to help discriminate PD from ET in patients with movement disorders [4], with results rating favourably on both accuracy [5] and cost-effectiveness [6, 7].

DAT-SPECT is usually assessed through qualitative analysis, which raises questions as to the reproducibility of the technique [8]. Several authors, as well as the European Association of Nuclear Medicine (EANM), encourage both collegial discussion and the adoption of standardized interpretation criteria based on automated, semi-quantitative evaluations [9, 10] with a view to ensuring uniformity among different institutions. This, in turn is a prerequisite for multi-centre studies.

BasGan is a PC-based software freely available from the Italian association of nuclear medicine website (http://www.aimn.it/struttura/gruppi/gs_neuro.php). This software may be suitable for widespread employment, in that: 1) it analyses DICOM images after transaxial reconstruction; 2) it automatically draws a 3D volume-of-interest (VOI) over each basal nucleus and provides a background VOI; 3) it determines the uptake/binding-potential (BP) values in the basal ganglia; 4) it automatically compares the values from each nucleus to a reference database of 96 healthy subjects of various ages, thus helping determine the age-adjusted values for each basal ganglium [11].

With the exception of the original method description and experimental validation, also including a limited sample of patients and controls [11], no research has been carried out as yet on the accuracy and reproducibility of the software-based analyses of a given patient population, nor have normal subject values been validated in any way. The aims of this study were: 1) to assess the accuracy of qualitative analysis and semi-quantitative software-assisted evaluations of DAT-SPECT; 2) to compare the accuracy of both methods when applied to 'doubtful' cases (i.e. cases in which full diagnostic agreement was not reached after a visual stratification by three nuclear medicine physicians); 3) to appreciate the reproducibility of the BasGan-based evaluations as performed by adequately trained junior technologists.

Materials and Methods

Patient selection

From January 2006 to December 2007, two hundred nine patients underwent DAT-SPECT at our institution, all having been referred from neurologists with clinical expertise in movement disorders. All patients showed symptoms suggesting initial onset of the disease. Interfering drugs, were discontinued. These included psychostimulants, antidepressants, muscarinic receptor antagonists and anorexic drugs.

At a four-year interval, the initial diagnosis was reviewed for fulfilment of Step 1 of UK Brain Bank criteria for PD and of Findley & Koller criteria for ET, independently from DAT-SPECT report. A definitive diagnosis was established in 78 patients (33 males, 45 females, mean age at the time of SPECT acquisition, 68 ± 9 years). Fifty-four patients (69%) were diagnosed with neurodegenerative parkinsonian syndrome (NPS). Twenty-four (31%) were found NPS-free and were diagnosed as follow: 12 ET, 3 Dystonia, 2 Cerebral vasculopathy and 1 for Restless Leg syndrome, Cerebellar Ataxia, ET in thyreopathy, Primary Progressive Aphasia, Multiple Sclerosis, Huntington Chorea and Semantic Dementia. The study was carried out in accordance with the ethical guidelines of the local Ethics Committee for Clinical Investigation. All patients gave their informed consent prior to DAT-SPECT.

SPECT acquisition

Brain SPECTs were acquired as per standard procedures: 140-180 MBq of ^{123}I-FP-CIT (DaTSCAN®, GE Healthcare Ltd, Little Chalfont, UK) were injected intravenously 40-60 minutes after administration of $KClO_4$ 400 mg to block free iodine uptake into the thyroid. Patients were imaged 4 hours post-injection using a dual-head gamma-camera (Philips Axis) equipped with low-energy high resolution parallel hole collimators. In order to optimize the spatial resolution of reconstructed images is mandatory to reduce as possible rotation radius. For this purpose, the

patient is positioned so that the whole neurocranium is included in the field of view while salivary glands could be excluded.

One hundred twenty views (40 sec/view) were acquired using a step-and-shoot protocol at 3° interval (matrix 128 x 128; zoom 1.6, circular orbit, peak: 159 ± 10% keV). Total counts ranged 1.4-2.6 million; pixel size was 2.92 mm. All exams, both acquired and reconstructed transaxial images, were visually assessed by three nuclear physicians (A.S., T.A., V.P.). No motion artefacts were identified and quality resulted suitable for diagnostic purposes (comma-shaped striata clearly distinguished from background activity). In order to meet software requirements, all sets were reconstructed by filtered back-projection (order = 7.0, cut-off = 0.45). The Chang algorithm was used for attenuation correction ($\mu = 0.10\ cm^{-1}$). Transaxial images were reconstructed with 2.92 mm slice thickness and reoriented on the orbitomeatal line.

Qualitative classification and semi-quantitative BasGan-assisted evaluation

SPECT images were visually stratified into normal, abnormal and equivocal by the three nuclear medicine physicians, based on striatal-to-background uptake ratio, symmetry in uptake reduction and patients' age. Cases in which full diagnostic agreement was not reached were classed as 'doubtful'.

Reoriented transaxial images were analysed using BasGan, and semi-quantitative data were obtained for each basal nucleus (raw data). Accuracy of the analysis was checked against semi-quantitative putaminal and caudate uptake values in the most severely affected hemisphere. Data from each of the 4 nuclei were then adjusted by computing the ratio to the corresponding values reported in the software database for normal subjects of similar age (age-adjusted values).

Finally, intra- and inter-operator reproducibility of semi-quantitative raw data for each basal nucleus was assessed. Three junior technologists, adequately trained carried out the analyses, which were repeated at a minimum interval of one week.

Statistical Analysis

Receiver operating characteristic (ROC) curve analysis was applied to assess the accuracy of the software-assisted evaluation of DAT-SPECT. The Chi Square test and the Student's *t* test were also used as appropriate.

The Spearman test was used to assess the intra-operator reproducibility of semi-quantitative values. Intra- and inter-operator reproducibility was calculated with reference to the intra-operator (k) and to the inter-class (ICC) correlation coefficients. Scores were rated as 'high' when > 0.8 and 'very high' when > 0.9. A p value < 0.05 was considered statistically significant. SPSS vers. 19 (IBM Corporation, Armonk, NY, USA) and MedCalc vers. 12 (MedCalc Software, Mariakerke, Belgium) were used for all statistical purposes.

Results

Accuracy of qualitative evaluation of DAT-SPECT

A full diagnostic agreement was achieved in 53 (68%) out of 78 patients: of these, 48 were diagnosed with NPS, while the remaining 5 were NPS-free. The overall sensitivity, specificity and accuracy scores were 92% (44/48), 60% (3/5), and 89% (47/53), respectively. The 25 cases that were given different diagnoses by one or more of the investigators were classed as 'doubtful'. Further information on these patients is presented below.

Accuracy of the semi-quantitative evaluation of DAT-SPECT

The characteristics of the patient population are showed in Table 1. Accuracy, areas under the ROC curves (AUCs), cut-off and *p*-values were calculated with reference to the semi-quantitative data (both raw and age-adjusted) for the most severely affected putamen and caudate. Scores are listed in Table 2. All four AUCs were large and statistically significant, thus corroborating the high accuracy of the BasGan-assisted analysis of DAT-SPECT, as well as the overall efficacy of the software as a diagnostic tool (Figure 1 shows the ROC curve computed with raw data from the most affected putamen).

No significant difference (p = ns) was found in raw versus age-adjusted data from both the most severely affected putamen and caudate.

Accuracy of semi-quantitative evaluation in 'doubtful' cases

'Doubtful' SPECTs were analysed using BasGan. Six out of 25 subjects were diagnosed with NPS while 19 were not (10 ET, 3 Dystonia, 2 Cerebral vasculopathy and 1 for Restless Leg syndrome, Cerebellar Ataxia, ET in thyreopathy, Primary Progressive Aphasia). Demographic data and software-derived outcomes of 'doubtful' patients are showed in Table 3. Based on ROC curve analysis (Figure 2), good accuracy was achieved in the semi-quantitative evaluation of data for the most severely affected putamen (AUC 0.789, p = 0.036). Sensitivity, specificity, positive and

negative predictive as well as accuracy values were calculated using the same cut-off point as that applied to the whole patient population. Total scores were 50%, 95%, 75%, 86% and 84%, respectively. Accuracy was significantly higher when using BasGan as opposed to relying solely on the investigators' visual assessment (Table 4).

On the other hand, software-assisted evaluation of data for the most severely affected caudate shows no significant results (AUC 0.667, p = ns).

Reproducibility of the BasGan-assisted evaluations

Intra-operator reproducibility of semi-quantitative values for each basal ganglium was rated good to excellent. *K* scores for the three trained junior technologists involved were 0.95-0.99, 0.85-0.95 and 0.97-0.98, respectively. All results were significant at $p < 0.001$ (Table 5). Inter-operator reproducibility of semi-quantitative values for each basal nucleus was rated very high, with ICC ranging 0.87-0.94. Again, all results were significant at $p < 0.001$ (Table 5).

Discussion

Parkinson's disease has been known and investigated for centuries but not until 1817 was it given a comprehensive clinical description by James Parkinson. Two centuries later many patients with movement disorders are still being denied a correct diagnosis even several years after the initial onset of symptoms [12]. Nuclear medicine plays a major role in this respect, due to its reliance on dopamine receptors/transporters imaging. As of late, different evaluation techniques and a number of radiotracers have been proposed [9], with ^{123}I-FP-CIT being the most widely used radiopharmaceutical on account of its high binding affinity for presynaptic dopamine transporters, its high specific-to-non specific ratio and the relatively short injection-to-scan interval. Qualitative evaluation of DAT-SPECT imaging has recently come under scrutiny with regard to reproducibility [8]. The latter could depend, *inter alia*, on 3 main factors, namely observer's experience, imaging equipment and prevalence of the disease in the patient population.

In our study, we assessed the accuracy of the BasGan-assisted analysis of DAT-SPECT and evaluated the software as a diagnostic tool. In our opinion, two of its main plus points are the following: 1) it calculates semi-quantitative uptake values for each basal nucleus based on automatically drawn 3D-shaped VOIs and 2) it compares the calculated values to a reference database of 96 healthy subjects (Fig 3 and Fig 4). We assessed the accuracy of several software-derived data (both raw and age-adjusted) for the most severely affected putamen and caudate. The ROC AUC for the putaminal results was found to be the largest, which is in keeping with several other studies [9, 14] showing that data from the most severely affected putamen are indicative of disease. In our patient population, accuracy of the cut-off for the putamen rated very high. However, no significant difference was found between raw and age-adjusted semi-quantitative data (cut-off 3.3 and 0.635, respectively). The accuracy score for age-adjusted data was found to be

slightly below expectations – possibly due to the not-large-enough database (96 subjects from 40 to 90 years old). Naseri Shoustari et al are currently working on creating a larger database of normal subjects, which is expected to be available in the near future [15, 16]. It is salient to remark, however, that software-assisted SPECT analysis consistently achieved a significant level of accuracy (84%) when applied to 'doubtful' cases, i.e. exams in which qualitative analysis alone was not conducive to reliable evaluations (Fig 5). Accuracy for semi-quantitatively-assessed data was in fact remarkably higher than that achieved by each of the three nuclear medicine physicians, which could be taken as evidence of the appropriate classification of these exams as doubtful. The result was due, in our opinion, to the higher specificity of the BasGan-assisted analyses in what must be considered a low-prevalence cluster (6 NPS; 19 ET). In this context, BasGan was of major diagnostic value for the analysis of DAT-SPECT, as it helped reach a definitive and accurate conclusion.

Finally, the reproducibility of the software-assisted analyses was assessed. Three junior technologists, adequately trained, performed DAT-SPECT analysis with the help of BasGan. The calculated intra- and inter- operator correlation coefficients were remarkably high, showing that no significant experience is required to use the software and that the results obtained are by no means operator-dependent.

These results must be tempered in light of the limitations of our study design, namely its retrospective approach and the relatively small sample size. The risk of a possible selection bias that may have been introduced due to low response rate should also be taken into account, given that roughly only 1 patient out of 3 returned to the same neurologist for follow-up. In our opinion, larger population studies (and possibly multi-centre studies) are needed to confirm our findings.

Conclusions

To the best of our knowledge, this is the first study to assess the feasibility and usefulness of BasGan, which is a free software, outwith the centre/institution in which it was originally implemented.

Our results show that the BasGan-based analyses of DAT-SPECTs were remarkably accurate and reproducible, especially with regard to data for the most severely affected putamen. The software proved particularly useful in the analysis of 'doubtful' cases, thus demonstrating its potential to assist in the diagnosis of PD. We can therefore conclude that BasGan is suitable for widespread employment in different centres. The ability of the software to overcome the differences of imaging equipment and processing application, although to be verified in in-course multi-centre studies, makes it a good tool towards the standardization of DAT-SPECT evaluation.

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Tables

Table 1.

Sex, age, raw and age-adjusted semi-quantitative values for the most severely affected putamen and caudate (BasGan-based analyses).

CAUD: caudate, PUT: putamen, NPS: neurodegenerative parkinsonian syndrome, M/F: male / female, m±sd: mean ± standard deviation, r: raw, a: age-adjusted.

	NPS+	NPS-	p-value
#	54	24	
sex M/F	32/22	12/12	0.6074
age m±sd	69±9	67±10	0.384
rCAUD m±sd	2.8±1.1	4.3±1.0	<0.0001
rPUT m±sd	2.0±1.2	4.1±0.9	<0.0001
aCAUD m±sd	0.7±0.3	1.0±0.2	<0.0001
aPUT m±sd	0.5±0.3	0.9±0.2	<0.0001

Table 2.

Area under the curve, *p*-value, cut-off, sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the most severely affected putamen and caudate (raw and age-adjusted data; BasGan-based analyses).

AUC: Area under the curve, CAUD: caudate, PUT: putamen, SE: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value, ACC: accuracy r: raw, a: age-adjusted.

	AUC	p-value	cut-off	SE (%)	SP (%)	PPV (%)	NPV (%)	ACC (%)
rPUT	0.909	0.0001	3.3	83	92	96	71	86
rCAUD	0.845	0.0001	3.4	76	83	91	61	78
aPUT	0.898	0.0001	0.635	76	96	98	64	82
aCAUD	0.836	0.0001	0.797	70	88	93	57	76

Table 3.

Sex, age, raw and age-adjusted semi-quantitative values for the most severely affected putamen and caudate in doubtful patients (BasGan-based analyses).

CAUD: caudate, PUT: putamen, NPS: neurodegenerative parkinsonian syndrome, n: number, M/F: male / female, m±sd: mean ± standard deviation r: raw, a: age-adjusted.

	NPS+	NPS-	p-value
N	6	19	
sex M/F	6/0	9/10	0.0693
age m±sd	76±4	67±8	0.0151
rCAUD m±sd	3.8±0.7	4.3±0.8	0.1839
rPUT m±sd	3.4±0.6	4.1±0.7	0.0381
aCAUD m±sd	1±0.1	1±0.2	1
aPUT m±sd	0.8±0.1	1±0.2	0.0287

Table 4.

Accuracy of visual assessment by each observer and of the BasGan-based evaluations of DAT-SPECTs, with *p* value of the differences.

R: report, NPS: neurodegenerative parkinsonian syndrome, ACC: accuracy, n: normal, e: equivocal and a: abnormal.

Nucl. Phy	R	NPS- (19)	NPS+ (6)	ACC	p-value
# 1	N E A	4 9 6	0 2 4	32%	0.0006
# 2	N E A	8 7 4	1 1 4	48%	0.0169
# 3	N E A	12 6 1	0 5 1	52%	0.0338
BASGAN	N A	18 1	3 3	84%	NA

Table 5.

Reproducibility coefficients for each basal ganglium: Intra-operator correlation coefficient (k) for each technologist and Intra-Class Correlation coefficient (ICC).

R: right, L: left, CAUD: caudate, PUT: putamen and Op : operator.

	R CAUD	L CAUD	R PUT	L PUT
Op 1	0.948	0.966	0.985	0.980
Op 2	0.847	0.906	0.903	0.919
Op 3	0.967	0.982	0.984	0.974
ICC	0.871	0.894	0.941	0.943

Figures

Fig 1

ROC curve for the whole patient population based on raw semi-quantitative values for the most severely affected putamen (AUC 0.909, $p = 0.0001$).

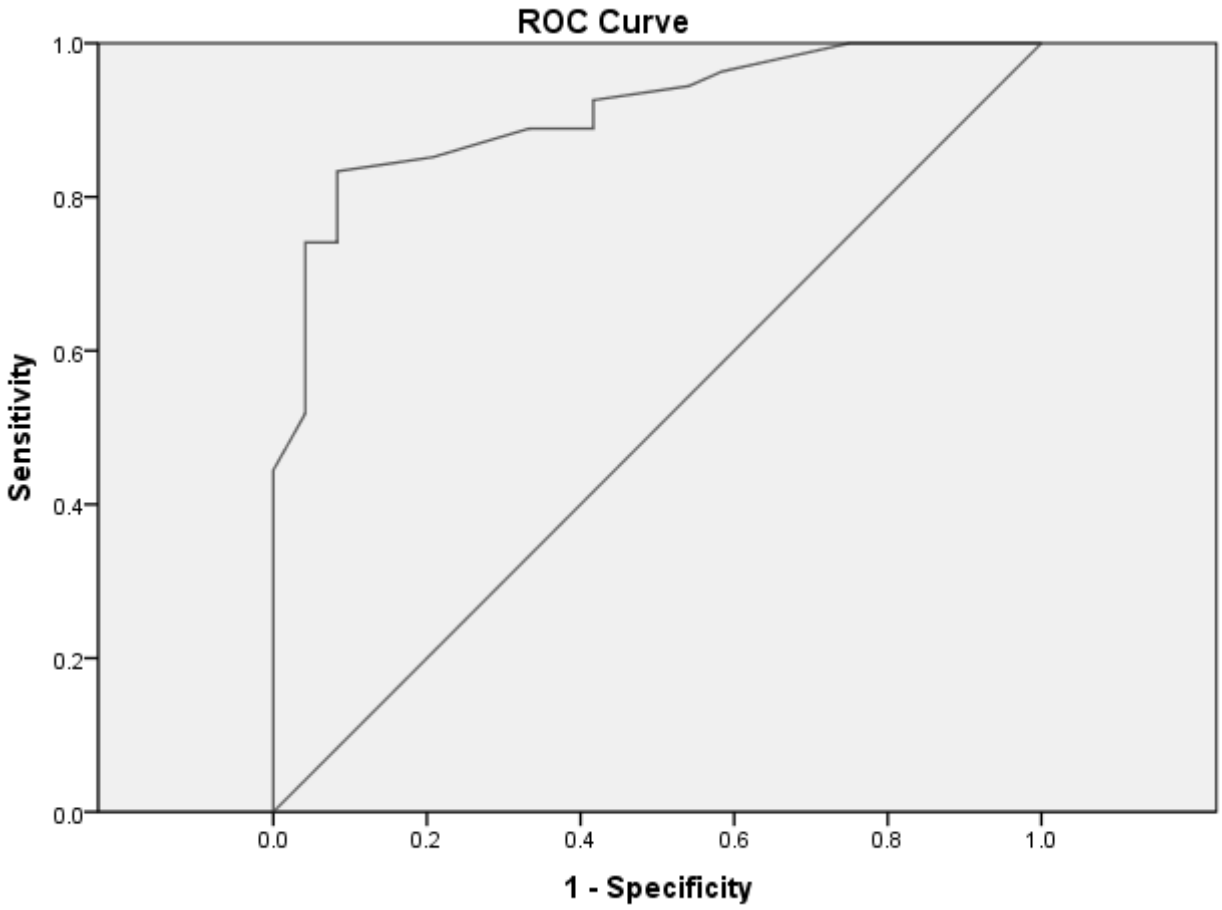


Fig 2

ROC curve for doubtful patients based on raw semi-quantitative values for the most severely affected putamen (AUC 0.789, $p = 0.036$).

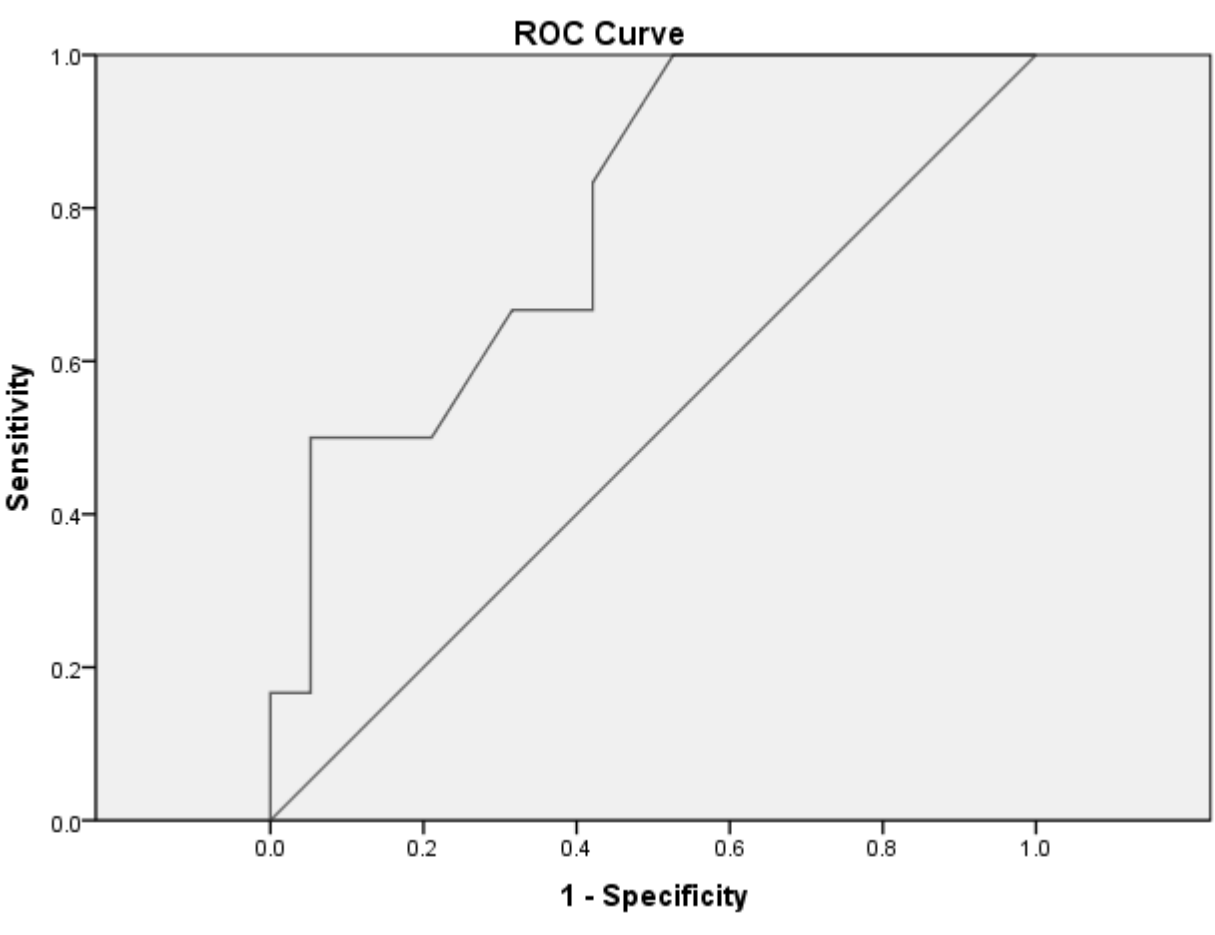
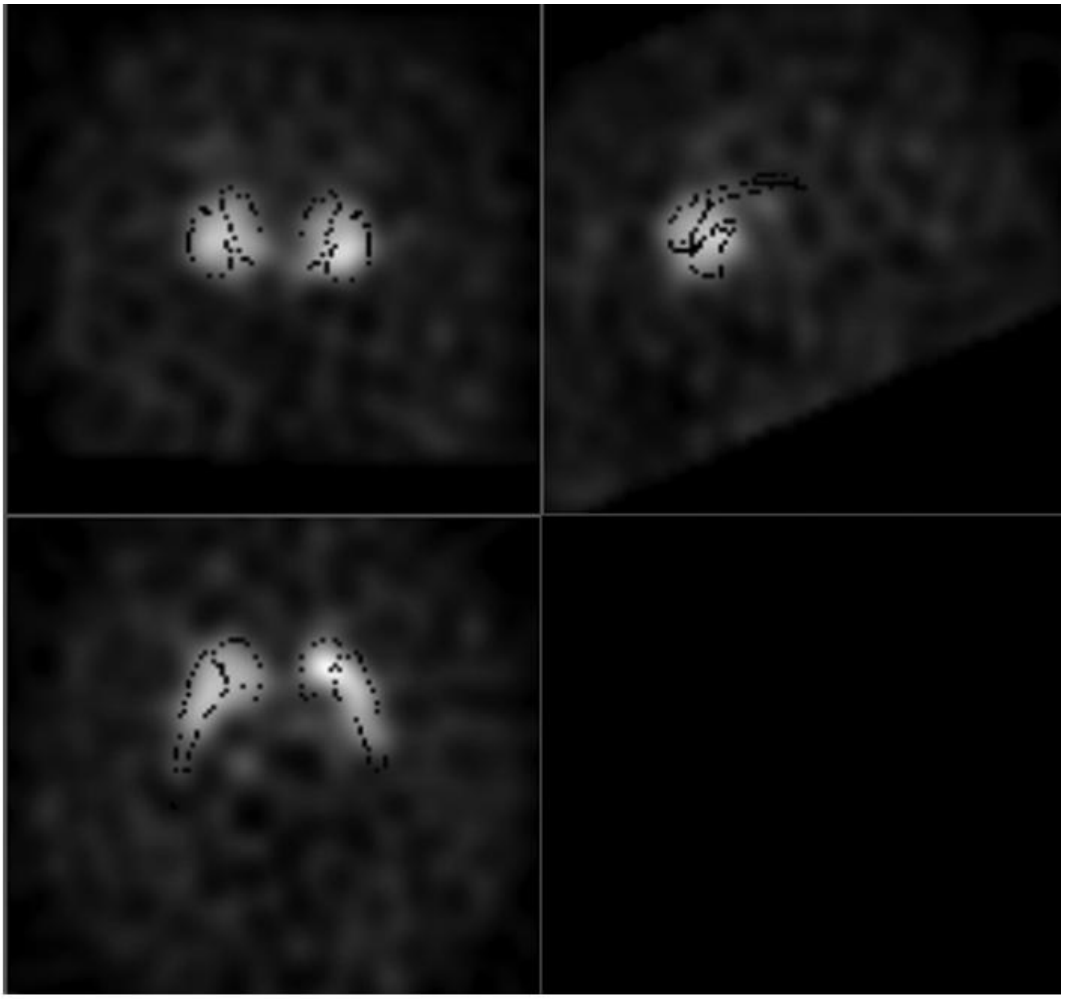


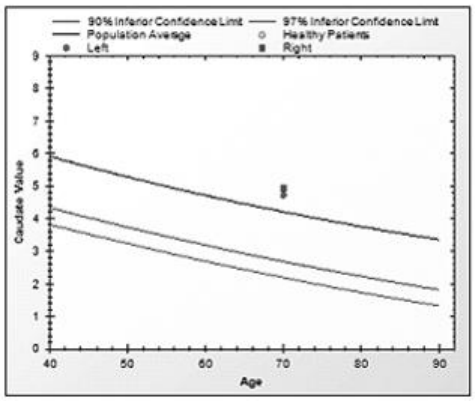
Fig 3

Coronal, sagittal, transaxial images and BasGan output of a 70 year-old patient. He was correctly classified by all three nuclear physicians as normal. Huntington Chorea was finally diagnosed.



	Left	Right
Caudate Counting	327.71	340.32
Caudate - Background/Background	4.72	4.94
Putamen Counting	327.71	334.02
Putamen - Background/Background	4.72	4.83
Putamen/Caudate	1.00	0.98

	Background
Total Number of Voxels	9169.70
Total Counting	525355.60
Counting Mean in Background Area	57.29



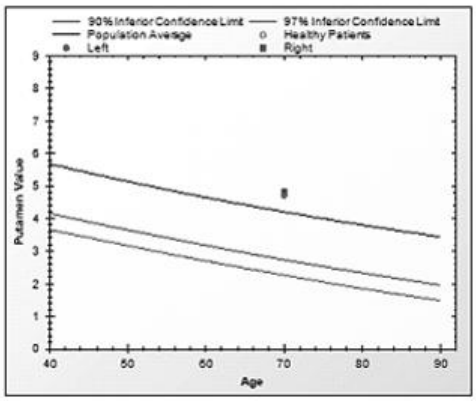
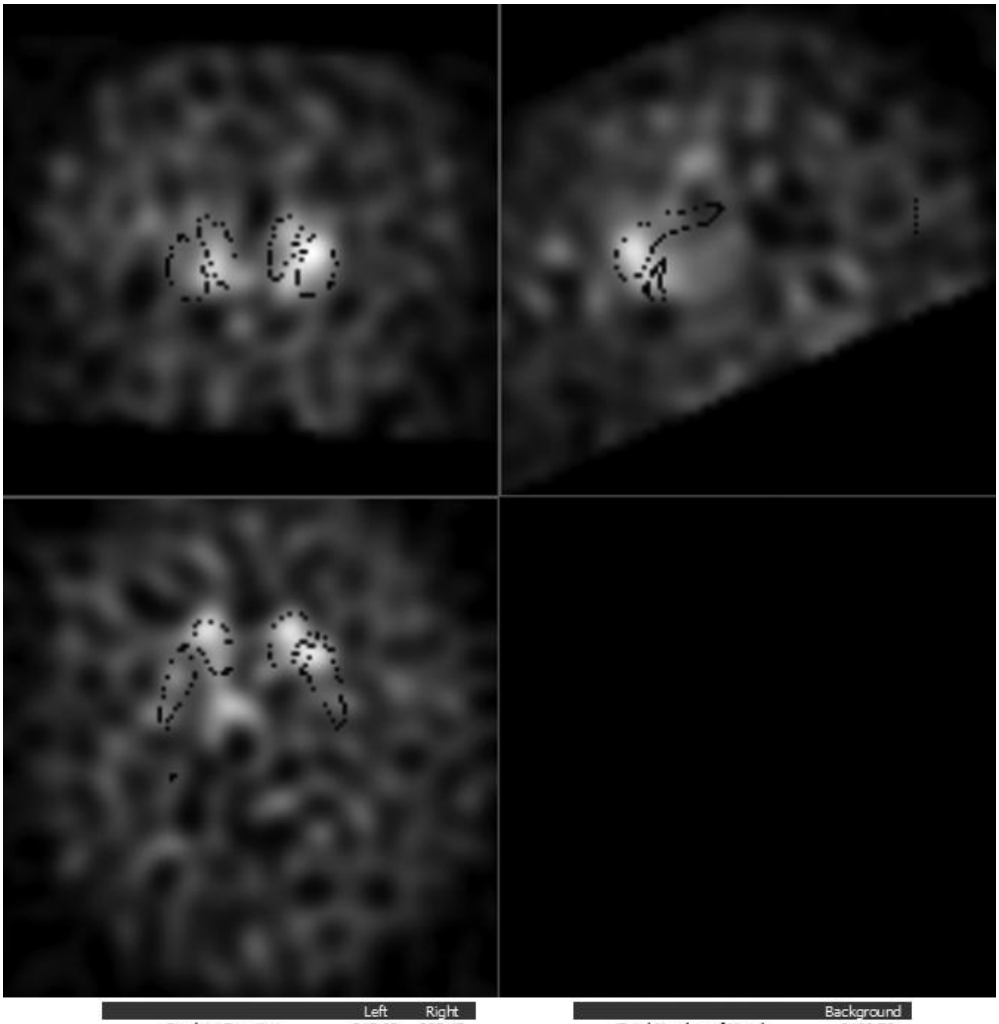


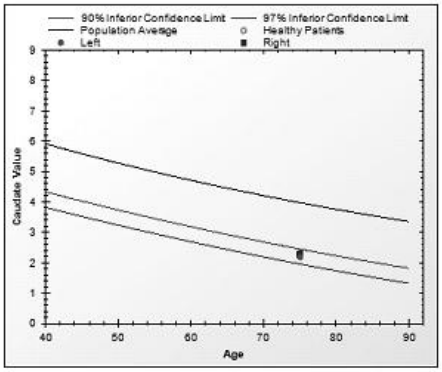
Fig 4

Coronal, sagittal, transaxial images and BasGan output of a 75 year-old patient. She was correctly classified by all three nuclear physicians as abnormal. Parkinson's disease was finally diagnosed.



	Left	Right
Caudate Counting	215.03	222.45
Caudate - Background/Background	2.19	2.30
Putamen Counting	163.13	148.30
Putamen - Background/Background	1.42	1.20
Putamen/Caudate	0.65	0.52

	Background
Total Number of Voxels	9169.70
Total Counting	618107.80
Counting Mean in Background Area	67.41



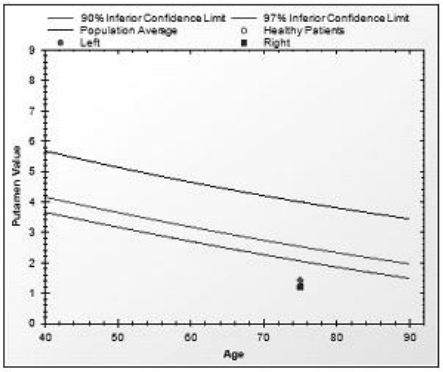
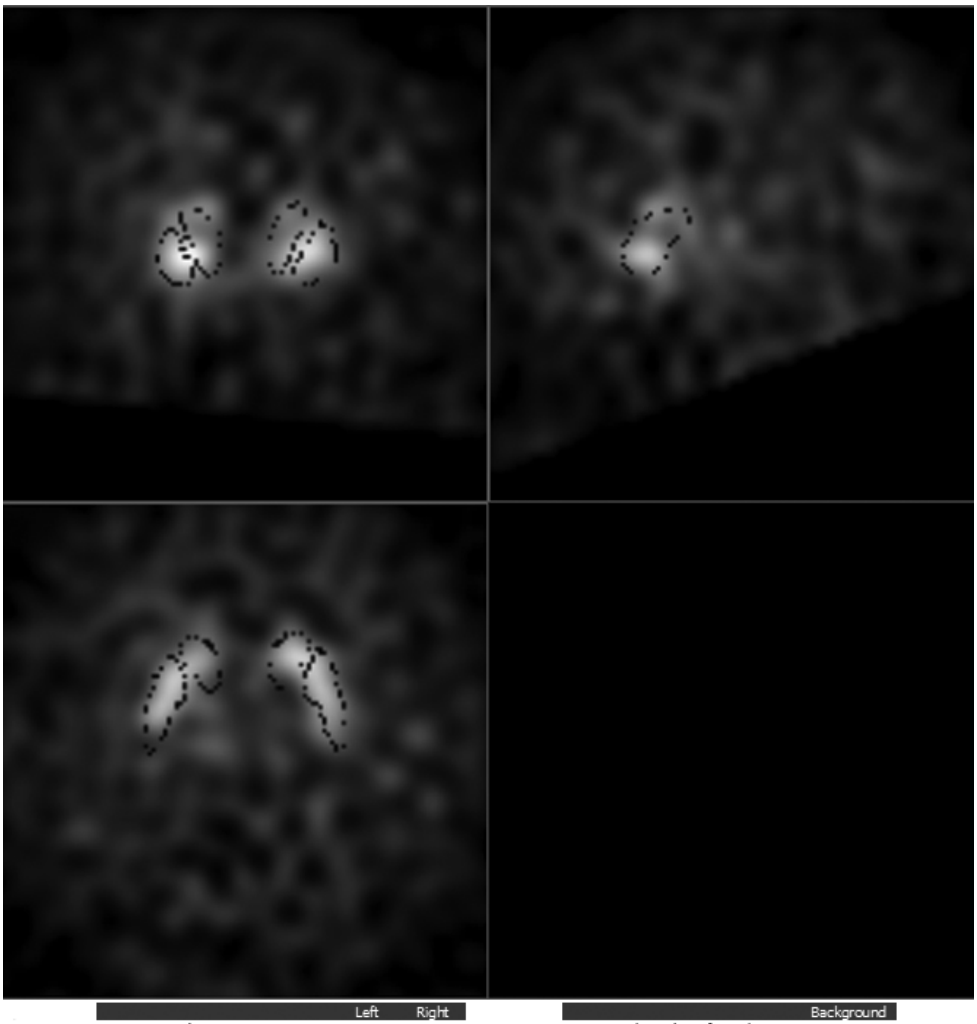


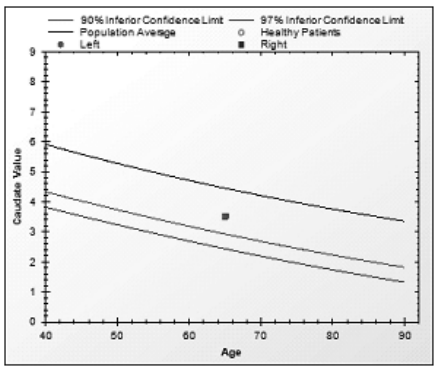
Fig 5

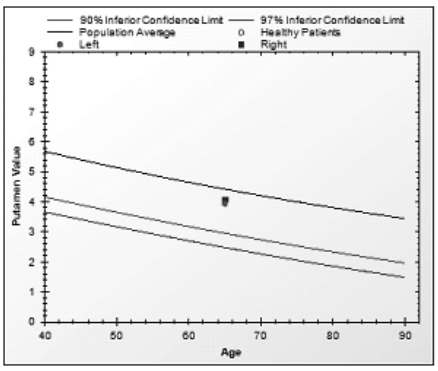
Coronal, sagittal, transaxial images and BasGan output [11] of a 65 year-old patient. No agreement was reached among all three nuclear physicians with regard to classification. Essential tremor was finally diagnosed.



	Left	Right
Caudate Counting	288.46	288.46
Caudate - Background/Background	3.51	3.51
Putamen Counting	316.60	323.63
Putamen - Background/Background	3.95	4.06
Putamen/Caudate	1.13	1.16

	Background
Total Number of Voxels	9169.70
Total Counting	586489.00
Counting Mean in Background Area	63.96





Multicenter semiquantitative evaluation of ^{123}I-FP-CIT brain SPECT

In press on Journal of Neuroimaging

Extended Summary

Background and Purpose

BasGan V2 is a PC-based software, freely available from the Italian association of Nuclear Medicine website. It has been already used in a multicenter study of healthy volunteers and in some uni-centric studies of suspected parkinsonian patients or with *de novo* Parkinson's Disease. However, to the best of our knowledge, no multicenter studies so far have assessed the accuracy of semi-quantitative, BasGan-assisted evaluations of [^{123}I]FP-CIT SPECT (DAT SPECT), to predict Neurodegenerative Parkinsonian Syndrome (NPS). This study was carried out at three different centers, and it involved the use of different equipments. BasGan V2 software was run to obtain [^{123}I]FP-CIT binding values at basal ganglia. Our aims were: 1) to cross-compare data from semi-quantitative, software-assisted and phantom corrected evaluations of DAT SPECT acquired in three different centers; 2) to assess the accuracy of semi-quantitative data; and 3) to identify the threshold with the best accuracy, sensitivity, and specificity in a patient population with suspected Parkinsonian Syndrome.

Material and methods

Two-hundred-twenty consecutive patients from three different centers were included in this trial. Patients underwent DAT-SPECT from January 2006 to July 2010, all having been referred by neurologists with clinical expertise in movement disorders for initial onset of movement disorders. The gold standard, dichotomized in Neurodegenerative Parkinsonian Syndrome (NPS) or NPS-free, was established as the diagnosis formulated by a neurologist, experienced in movement disorders,

based on clinical criteria, evolution of disease and treatments response 2-4 years post-scan. The anthropomorphic striatal phantom consists of symmetrical chambers for each "striatum" and of a large chamber around those, which simulates the rest of the brain and its SPECT acquisition was used in order to adjust data from patients acquired in three centers. At center A, 78 patients were imaged using a dual-head gamma camera (Philips Axis) equipped with low-energy, high-resolution, parallel hole collimators. At center B, we used a dual-head GE Millennium camera, equipped with a fan-beam collimator and a circular orbit with patient-tailored radius in order to acquire 71 patients. At center C, a Siemens "e.cam 2002" dual head gamma camera, equipped with low energy, high resolution, parallel hole collimators was used to acquire 71 patients. In all cases, a BasGan evaluation was performed, which computes fine adjustments in positioning a 3D Volume of Interest (VOI) template to better match radioactive counts, and also locates an occipital VOI for background evaluation. To obtain BasGan scintigraphic specific binding ratio for each nucleus, non-specific background binding is subtracted from the putamen and caudate nucleus uptake.

Results

No significant difference resulted across centers, neither among data of NPS nor among data of NPS-free patients (Anova: p= ns). Significant differences were observed between data of NPS and data of NPS-free patients both in single center as well as in a multi-center setting (*t* test: $p<0.0001$). Areas under the ROC curves (AUCs), p-values, best cut-off, sensitivities and specificities were calculated for pooled data with reference to caudate and putamen. Best cut-off were 1.53 and 1.56 for putamen and caudate, respectively.

Conclusion

This work shows the feasibility of semi-quantitative, phantom-corrected analysis of [^{123}I]FP-CIT SPECT. We identified a unique, accurate threshold for all centers, with high (and similar) sensitivities and specificities. Standardized DAT SPECT assessed with BasGan, age-normalized

and corrected for equipment, shows high levels of accuracy and yields similar results regardless of the center where it was performed.

Original Article

Introduction

Single photon emission computed tomography (SPECT) imaging of dopamine transporter (DAT) is a well-established diagnostic approach to evaluate the integrity of the nigro-striatal system and to define differential diagnoses in patients with extra-pyramidal symptoms[1–4]. ^{123}I-FP-CIT (^{123}I-ioflupane, DaTSCAN; GE Healthcare) is the most widely used radiopharmaceutical for DAT evaluations in both Europe and the USA. A multicenter study by Catafau *et al*. showed that ^{123}I-FP-CIT SPECT contributed to define diagnosis in 52% of patients and to change the clinical management in 72% of patients[5]. In a review of two multicenter trials, high accuracy for this tracer was reported[6].

In routine clinical settings, qualitative analysis of striatal uptake is probably the most common approach for scan reporting. The majority of scans can be easily reported after a simple qualitative evaluation by an expert reader[3,4,7]. However, in "borderline" scans, characterized by a high inter-observer discrepancy, or when an assessment of disease progression is required, a quantification of imaging data is often useful[8]. Quantitative analysis can also provide a reference value to express the wide clinical variability which is more suitable for correlative analysis with other disease markers, such as those expressing severity of motor impairment, or monitoring of neuro-protective treatments[9]. For all these reasons, (semi)quantitative evaluation has also been encouraged by EANM[10]. Several quantification methods have been proposed in recent years[11], such as manual or semi-automatic positioning of anatomical regions of interest (ROIs), automated quantification methods, or voxel-based statistical methods.

Furthermore, in order to perform a multicenter trial, different approaches have been proposed[12–14]. Based on the revised version of the EANM procedure guidelines[15], Dickson *et al*. presented a proposal for quality assurance standardization of imaging devices and protocols, and for the characterization of the imaging equipment to obtain good, comparable imaging outputs[16]. Tossici-

Bolt *et al*. published a quantitative analysis of anthropomorphic phantom measurements acquired in different centers based on the same protocol, aiming to derive gamma camera-specific recovery coefficients across a wide range of commonly used SPECT systems[17]. Lastly, a recent multicenter study published semi-quantitative data of DAT SPECT in healthy volunteers[18].

BasGan V2 is a PC-based software, freely available from the Italian association of Nuclear Medicine website (http://www.aimn.it/?page_id=340)[19]. It has been already used in a multicenter study of healthy volunteers[20] and in some uni-centric studies of suspected parkinsonian patients or with *de novo* Parkinson's Disease[8,21,22]. However, to the best of our knowledge, no multicenter studies so far have assessed the accuracy of semi-quantitative, BasGan-assisted evaluations of DAT SPECT, to predict Neurodegenerative Parkinsonian Syndrome (NPS). This study was carried out at three different centers, and it involved the use of different equipments. BasGan V2 software was run to obtain DAT binding values at basal ganglia. Our aims were: 1) to cross-compare data from semi-quantitative, software-assisted and phantom corrected evaluations of DAT SPECT; 2) to assess the accuracy of semi-quantitative data; and 3) to identify the threshold with the best accuracy, sensitivity, and specificity in a patient population with suspected Parkinsonian Syndrome.

Materials and methods

Patient selection

Two-hundred-twenty consecutive patients (mean age at the time of SPECT acquisition, 67 ± 10 years) from three different centers (San Luigi Gonzaga Hospital, "center A" n=78; San Giovanni Battista Molinette Hospital, "center B" n=71; Mauriziano Umberto I Hospital, "center C" n=71) were included in this trial. Patients underwent DAT-SPECT from January 2006 to July 2010, all having been referred by neurologists with clinical expertise in movement disorders. All patients showed symptoms suggesting initial onset of movement disorders. Drugs interfering with DAT-SPECT were discontinued, as previously indicated[23]. These included: psychostimulants, antidepressants, muscarinic receptor antagonists, and anorexic drugs.

The gold standard, dichotomized in Neurodegenerative Parkinsonian Syndrome (NPS) or NPS-free, was established as the diagnosis formulated by a neurologist, experienced in movement disorders, based on clinical criteria, evolution of disease and treatments response 2-4 years post-scan. This time period was chosen in order to have the most objective diagnostic definition without significant interferences from scan report. Among NPS patients, UK Brain Bank Criteria Step 1 was used to identify Parkinson's disease (PD) and among NPS-free patients, Findley & Koller criteria were used to identify Essential Tremor (ET). One-hundred-thirty-eight patients (63%) were diagnosed with NPS. Eighty-two (37%) were found NPS-free and were diagnosed as described in Table 1; in Table 2 has been reported the exact diagnosis as established by the neurologist in charge. A cluster analysis was performed also between PD patients and ET patients, the two most representative categories of diagnosis.

Seventy-eight patients were scanned at the first center (mean age 68 years), and 71 at each of the other two centers (mean age 65 and 69 years, respectively). The study has been approved by Ethical

Committees of the three institutions involved in this work and all procedures were performed in compliance with local laws and institutional guidelines.

Phantom description and acquisition

The anthropomorphic striatal phantom consists of symmetrical chambers for each "striatum" and of a large chamber around those, which simulates the rest of the brain. The entire phantom is shaped to mimic the anatomy of human brain. The above-described chambers are surrounded by an artificial "skull" and by a soft material, with attenuation similar to that of human tissues (Fig. 1).

The widest chamber (1300 mL) was filled with saline. Then, 1 mL of ^{123}I-iodine-containing solution (6.5 MBq/mL) was added to serve as background activity after being measured at well counter. This resulted in an activity concentration of approximately 5 kBq/mL. Each "putamen" and each "caudate" were filled with 6 mL and 5 mL, respectively, of a solution containing ^{123}I-Iodine (≈38 KBq/mL), measured at well counter, and methylene blue. From these data, BG true specific binding of the phantom was calculated (see below).

An experienced person filled the phantom once, subsequently it was transported in each center to be acquired. SPECT images of the striatal phantom were acquired on the same day at the three centers, using the same phantom content. Acquisition, reconstruction and methods for semi-quantitative evaluation were the same as those used for patients. The purpose of the phantom study was to obtain a reference DAT SPECT for subsequent equipment normalization, by calibrating patient's uptake values against striatal phantom data.

SPECT imaging and semi-quantification

Brain SPECTs were acquired following standard procedures: 150-185 MBq of ^{123}I-FP-CIT (DaTSCAN®, GE Healthcare Ltd, Little Chalfont, UK) were i.v. injected 40-60 minutes after administration of $KClO_4$ 400 mg to block iodide uptake by the thyroid. Acquired counts ranged 1.8-

2.5 million for each exam acquired 3-4 hours post-injection. In all centers, according to Italian Law and European guidelines[16], quality control of nuclear medicine imaging devices, were performed by experienced medical physicists.

At center A, patients were imaged using a dual-head gamma camera (Philips Axis) equipped with low-energy, high-resolution, parallel hole collimators. One hundred twenty views (40 sec/view) were acquired using a step-and-shoot protocol at 3° interval (matrix 128 x 128; zoom 1.6, circular orbit, peak: 159 ± 10% keV). Pixel size was 2.92 mm.

At center B, we used a dual-head GE Millennium camera, equipped with a fan-beam collimator and a circular orbit with patient-tailored radius. One hundred twenty views (30 sec/view) were acquired using a step-and-shoot protocol at 3° interval (matrix 128 x 128; peak: 159 ± 10% keV). In this case, pixel size was variable (mainly due to the collimator geometry), however in all cases a voxel size of roughly 3 mm was obtained.

At center C, a Siemens e.cam 2002 dual head gamma camera, equipped with low energy, high resolution, parallel hole collimators was used. One-hundred-twenty views (40 sec/view) were acquired using a step-and-shoot protocol at 3° interval (matrix 128 x 128; zoom 1.45, circular orbit, peak: 159 ± 10% keV). Pixel size was 3.3 mm.

All exams – both acquired views and reconstructed transaxial images – were visually assessed by a nuclear physician. No motion artifacts were identified and quality resulted suitable for diagnostic purposes. In order to meet BASGAN requirements, all sets were reconstructed, in a single workstation with Xeleris version 1.0.10.0, by filtered back-projection (Butterworth, order = 10.0, cut-off = 0.45). The Chang algorithm was used for attenuation correction (μ = 0.10 cm^{-1}). Transaxial images were reconstructed with a voxel size in the range required by BasGan (2.5-3.5 mm). In all cases, a BasGan evaluation was performed. The software automatically performs fine adjustments in positioning a 3D Volume of Interest (VOI) template to better match radioactive counts, and also locates an occipital VOI for background evaluation. To obtain BasGan

scintigraphic specific binding ratio for each nucleus, non-specific background binding is subtracted from the putamen and caudate nucleus uptake, according to:

$$\text{BasGan scintigraphic Specific Binding} = \frac{\text{Counts (striatum)/voxel - Counts (occipital cortex)/voxel}}{\text{Counts (occipital cortex)/voxel}}$$

In addition, BasGan V2 automatically compares the values from each nucleus to a reference database from 96 healthy subjects of various ages, determining age-adjusted values for each basal ganglion [19]. In particular, BasGan before the evaluation of each patient needs sex and age of the subject. Then, after the evaluation, BasGan shows in a graph subject's data and mean data for each age of normal subjects. These intrinsic data of BasGan were used for age correction. . After age-correction, data from NPS-free subjects appeared significantly different across centers. In order to correct differences due to different equipments, mean data from the phantom's "putamen" and "caudate," acquired at the respective centers, were used to normalize all patients data acquired at that particular center as follows:

$$\text{BG binding}_{\text{(patient)}} = \frac{\text{age corrected BG scintigraphic specific binding}_{\text{(patient)}}}{\text{CF}_{\text{of the respective centre}}}$$

Where CF signifies Correction Factor and is equal to:

$$\text{Correction Factor (CF)} = \frac{\text{BG scintigraphic specific binding}_{\text{(phantom)}}}{\text{BG true specific binding}_{\text{(phantom)}}}$$

And specific binding is equal to:

$$\text{Specific Binding} = \frac{\text{Counts (striatum)/voxel - Counts (background)/voxel}}{\text{Counts (background)/voxel}}$$

for putamen and caudate data respectively.

Subsequent analyses were performed based on $\text{BG binding}_{\text{(patient)}}$ data for the most deteriorated putamen and caudate ganglion.

Statistics

Receiver operating characteristic (ROC) curve analysis was applied to assess the accuracy of the software-assisted evaluation of DAT-SPECT, by estimating the area under the curve (AUC). The best cut-off (the cut-off that allowed to obtain the highest accuracy) was then defined. Chi Square test, Student's *t* test, and ANOVA were also used as appropriate. A p-value < 0.05 was considered statistically significant. SPSS vers. 19 (IBM Corporation, Armonk, NY, USA) and MedCalc vers. 12 (MedCalc Software, Mariakerke, Belgium) were used for all statistical purposes.

Results

Age- and center-corrected mean values, clustered for each center, from both NPS and NPS-free subjects are illustrated in Table 3. No significant difference resulted across centers, neither among data of NPS nor among data of NPS-free patients (Anova: p= ns). On the other hand, significant differences were observed between data of NPS and data of NPS-free patients both in single center as well as in a multi-center setting (*t* test: $p<0.0001$). Similar results are observed in the cluster analysis of PD versus ET patients (Table 4).

Areas under the ROC curves (AUCs), p-values, best cut-off, sensitivities and specificities were calculated for pooled data (220 patients) with reference to caudate and putamen (Table 5). Best cut-off were 1.53 and 1.56 for putamen and caudate, respectively.

All AUCs were large, statistically significant and without significant differences across centers (Fig. 2). In addition, no significant differences were observed among sensitivities and specificities from each center, or in the entire population, neither for putamen, nor for caudate data. Based on semi-quantitative data for the putamen, accuracy results were 83% (center A), 85% (center B), and 90% (center C), yielding an overall accuracy score of 86%. For caudate data, accuracy results were 76% (center A), 76% (center B), 82% (center C) and 78% (overall).

Finally, a cluster analysis between PD patients and ET patients was performed. PD was the most representative category of patients with NPS and ET was the same among NPS-free patients (see Table 2). The results of this analysis are synthesized in Tables 4 and 6. Based on semi-quantitative data for the putamen, accuracy results were 85% (center A), 85% (center B), and 92% (center C), yielding an overall accuracy score of 88%. For caudate data, accuracy results were 73% (center A), 78% (center B), 80% (center C) and 78% (overall).

Discussion

In clinical routine, qualitative visual analysis of striatal uptake is probably the most common approach for DAT SPECT interpretation[3,4,7]. The emphasis on qualitative analysis, however, has currently overturned and semi-quantification of DAT scans has progressively emerged as a viable alternative, especially in borderline cases or when an assessment of disease progression is required [9,10]. In a recent study doubts were raised about the reproducibility of visual assessment [24]. The authors reported a suboptimal inter-observer agreement in the interpretation of DAT SPECT with a three-point scale (normal, abnormal and equivocal). Another paper shows discrepancies in decisions taken by nuclear physicians on ^{123}I FP-CIT reporting, albeit limited to few cases[25]. Finally, a quantitative approach can also assist junior nuclear medicine physicians when interpreting scans, until more experience is acquired[8].

The BasGan software is easy to use, allows a higher degree of standardization compared to individual ROI manipulation, facilitates the creation of large databases and can therefore be employed in studies leading to higher statistical power. In this study it was performed a standardized semi-quantitative evaluation of DAT SPECT in a multicenter setting, showing the feasibility of this type of image analysis. To the best of our knowledge, this is the first paper that focuses on semi-quantitative, automated analysis (BasGan V2) of DAT uptake in a multicenter setting. We estimated the diagnostic accuracy of this method in differentiating NPS from NPS-free subjects in a multicenter patient database. First, results were normalized for age, according to data from normal subjects of the same age, previously entered in the BasGan code. Significant differences persisted among uptake values obtained in NPS-free patients from different centers (data not shown) despite similar patients' age, population size, patients' preparation, administered activity, injection-to-scan time interval, duration of acquisition, reconstruction, and semi-quantitative evaluation. Therefore, we pointed out that different equipments yield different semi-quantitative uptake values. On the other hand, data from a single striatum phantom allow

calibration of patients uptake values and also a comparison across different centers. We showed that normalization for age and for equipment improves comparability of results across centers.

The standardization of acquisition, reconstruction and evaluation protocols plays a major role in delivering results that might be comparable across centers[16,17,26]. Standardization is primarily required to improve diagnostic quality and secondly to allow adequate sharing of information among colleagues. Additionally, standardization is also useful to overcome many of the problems associated with the use of different equipments.

In a study involving repetition of DAT SPECT after a three-year interval, Marshall et al. showed an overall accuracy of 84%, while McKeith et al. reported an overall accuracy of 86%[27,28]. Similar data were also observed in the present work (86% and 78% for putamen and caudate values, respectively). In our case, however, once standardization was performed, semi-quantification in a multicenter setting appears to work towards avoiding discrepancies in intra- and inter- observer evaluations. In our view, this probably represents the main asset of our work. Finally, as multicenter settings are often the favorite environments for drug evaluation trials, DAT SPECT evaluated in this way could be used, in our view, to establish disease involvement, before introducing the patient in the study, and also to assess patient's response to that treatment.

A cluster analysis was performed in patients with PD versus patients with ET, given that these subjects were the most representatives among NPS and NPS-free categories, respectively. This analysis showed results similar (without statistically significant differences) to the global analysis (Tables 3-6) with high accuracy to predict disease, implying also a good homogeneity of the global population. The other clusters of disease were insufficient to allow inferential statistics (see Table 2).

While in a single-center setting, the use of BasGan allowed operators to achieve good reproducibility and accuracy[8], this study shows that in a multicenter setting, the level of accuracy is high, once the procedure has been appropriately standardized.

The higher accuracy of putamen values is likely explained by earlier and bigger affection of pigmented neurons from the ventrolateral substantia nigra pars compacta. These dopaminergic neurons, in fact, project axons in the dorsal putamen. Subsequently, dorso-medial nigral neurons with projections on the head of the caudate are affected[29,30].

Finally, this work shows some limitations, the most important of which is his retrospective nature. This aspect impacts particularly in the diagnose definition done by the clinician. However, the first aim of this work was the assessment of the feasibility of a semi-quantitative multicenter study, and not just the assessment of DAT SPECT, it has been already accepted in the diagnostic iter of patients with suspected parkinsonian syndrome. Furthermore, the long-lasting follow-up allowed the diagnostic definition quite confidently, such as in other papers[28,31].

Conclusions

Ensuring high diagnostic accuracy for each patient independently from the site of exam acquisition is mandatory in nuclear medicine. Multicenter trials drawing on a large database of patients allow carrying out studies with higher statistical power and having a serious impact on diagnostic work-up as well as treatment. Comparability of semi-quantitative SPECT results across centers before patients' recruitment should be ensured by standardized procedures.

This work shows the feasibility of semi-quantitative, phantom-corrected analysis of ^{123}I-FP-CIT SPECT. We identified a unique, accurate threshold for all centers, with high (and similar) sensitivities and specificities. *In varietate concordia* was reached in this study. Standardized DAT SPECT assessed with BasGan, age-normalized and corrected for equipment, shows high levels of accuracy and yields similar results regardless of the center where it was performed. Therefore, this kind of image analysis should be available in each center.

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Tables

	Center A			**Center B**			**Center C**			**Multicenter**		
	#	age	♂	#	age	♂	#	age	♂	#	age	♂
NPS	54	68.7	32	44	63.6	27	40	70.6	21	138	67.6	80
NPS free	24	66.8	12	27	67.9	16	31	66.2	15	82	66.9	43
Total	78	68.1	44	71	65.2	43	71	68.7	36	220	67.4	123

Table 1

Number (#) of NPS (Neurodegenerative Parkinsonian Syndrome) and NPS-free patients, mean age and number of men (♂), for each center and total.

	Center A	Center B	Center C	TOT
NPS	44 PD 3 CBD 3 MSA 2 LBD 2 PSP	27 PD 2 CBD 4 MSA 3 LBD 4 PSP 2 FTD 2 WD	26 PD 4 CBD 2 MSA 4 LBD 4 PSP	97 PD 9 CBD 9 MSA 9 LBD 10 PSP 2 FTD 2 WD
NPS free	13 ET 2 VASC 3 DYS 1 Cereb AT 1 REST 1 MS 1 PPA 1 SD 1 HC	18 ET 4 VASC 2 DYS 1 DEP 1 Cereb AT 1 IATR	20 ET 2 VASC 2 DYS 2 DEP 1 REST 1 MS 1 LAB 1 AD 1 HYDROC	51 ET 8 VASC 7 DYS 3 DEP 2 Cereb AT 2 REST 2 MS 1 PPA 1 SD 1 HC 1 IATR 1 LAB 1 AD 1 HYDROC

Table 2

Number of NPS and NPS-free patients classified by diagnosis, for each center and total.

PD, Parkinson Disease; CBD, Cortico-Basal Degeneration; MSA, Multi-System Atrophy; LBD, Lewy Body Dementia; PSP, Progressive Supranuclear Palsy; FTD, Fronto-Temporal Dementia; WD, Wilson Disease; ET Essential Tremor, VASC, Vascular Parkinsonism; DYS, Dystonia; DEP, Depression; Cereb AT, Cerebellar Ataxia; REST, Restless Legs Syndrome; MS, Multiple Sclerosis PPA, Primary Progressive Aphasia; SD, Semantic Dementia; HC, Huntington Chorea; IATR, Iatrogenic Parkinsonism; LAB, Labyrinthitis; AD, Alzheimer Disease; HYDROC, Hydrocephalus.

	NPS				NPS-free			
	Center A	**Center B**	**Center C**	**Overall**	**Overall**	**Center A**	**Center B**	**Center C**
Putamen								
Mean	**1.096**	**1.014**	**1.061**	**1.060**	**2.082**	**2.227**	**1.937**	**2.096**
95% CI	0.90-1.29	0.87-1.16	0.89-1.24	0.96-1.16	1.97-2.20	2.03-2.43	1.69-2.18	1.94-2.25
p (Anova)	ns					ns		
p (*t*-test)				<0.0001				
Caudate								
Mean	**1.298**	**1.350**	**1.221**	**1.292**	**1.883**	**1.966**	**1.951**	**1.760**
95% CI	1.15-1.45	1.21-1.49	1.08-1.36	1.21-1.38	1.79-1.97	1.79-2.15	1.77-2.13	1.64-1.88
p (Anova)	ns					ns		
p (*t*-test)				<0.0001				

Table 3

Mean data obtained after age- and center-correction for the most affected putamen and caudate. The table also reports the 95% confidence interval (95% CI) and the p-values from ANOVA (statistics across centers) and from t-tests (statistics between Neurodegenerative Parkinsonian Syndrome "NPS" and NPS-free patients); ns, not significant.

	PD				ET			
	Center A	**Center B**	**Center C**	**Overall**	**Overall**	**Center A**	**Center B**	**Center C**
Putamen								
Mean	**1.049**	**1.063**	**1.073**	**1.059**	**2.168**	**2.301**	**2.03**	**2.206**
95% CI	0.85-1.25	0.86-1.27	0.83-1.32	0.94-1.18	2.07-2.26	2.1-2.50	1.87-2.2	2.06-2.35
p (Anova)	ns					ns		
p (*t*-test)				<0.0001				
Caudate								
Mean	**1.281**	**1.485**	**1.254**	**1.331**	**1.947**	**2.02**	**2.011**	**1.839**
95% CI	1.12-1.44	1.3-1.67	1.07-1.44	1.23-1.43	1.86-2.04	1.79-2.26	1.87-2.16	1.72-1.96
p (Anova)	ns					ns		
p (*t*-test)				<0.0001				

Table 4

Mean data obtained after age- and center-correction for the most affected putamen and caudate. The table also reports the 95% confidence interval (95% CI) and the p-values from ANOVA (statistics across centers) and from t-tests (statistics between Parkinson's Disease "PD" and Essential Tremor "ET" patients); ns, not significant.

PUTAMEN	Center A	Center B	Center C	**Multicenter**
AUC	0.880	0.866	0.920	**0.882**
p-value	0.0001	0.0001	0.0001	**0.0001**
cut-off	1.53			
Sensitivity % (95% C.I.)	78 (65-88)	86 (73-95)	88 (73-96)	**83 (76-89)**
Specificity % (95% C.I.)	96 (79-99)	81 (62-94)	94 (79-99)	**91 (83-97)**
CAUDATE				
AUC	0.826	0.827	0.854	**0.827**
p-value	0.0001	0.0001	0.0001	**0.0001**
cut-off	1.56			
Sensitivity % (95% C.I.)	67 (53-79)	68 (52-81)	80 (64-91)	**71 (63-78)**
Specificity % (95% C.I.)	96 (79-99)	89 (71-98)	84 (66-95)	**89 (80-95)**

Table 5

Area Under the Curve (AUC), p-value for ROC analysis, best cut-off for the multicenter setting, sensitivities and specificities for the best cut-off value, and 95% confidence interval (95% CI) for the most affected putamen and caudate, respectively (results are given for single-center and multicenter settings).

PUTAMEN	Center A	Center B	Center C	**Multicenter**
AUC	0.932	0.94	0.937	**0.923**
p-value	0.0001	0.0001	0.0001	**0.0001**
cut-off	1.53			
Sensitivity % (95% C.I.)	80 (65-90)	82 (62-94)	89 (70-97)	**83 (73-89)**
Specificity % (95% C.I.)	100 (75-100)	89 (65-98)	95 (75-99)	**98 (90-99.7)**
CAUDATE				
AUC	0.867	0.837	0.879	**0.85**
p-value	0.0001	0.0001	0.0001	**0.0001**
cut-off	1.60			
Sensitivity % (95% C.I.)	68 (52-81)	63 (42-81)	77 (56-91)	**69 (59-78)**
Specificity % (95% C.I.)	92 (64-99)	100 (81-100)	85 (62-97)	**94 (84-99)**

Table 6

Cluster analysis of Parkinson Disease vs Essential Tremor: Area Under the Curve (AUC), p-value for ROC analysis, best cut-off for the multicenter setting, sensitivities and specificities for the best cut-off value, and 95% confidence interval (95% CI) for the most affected putamen and caudate, respectively (results are given for single-center and multicenter settings).

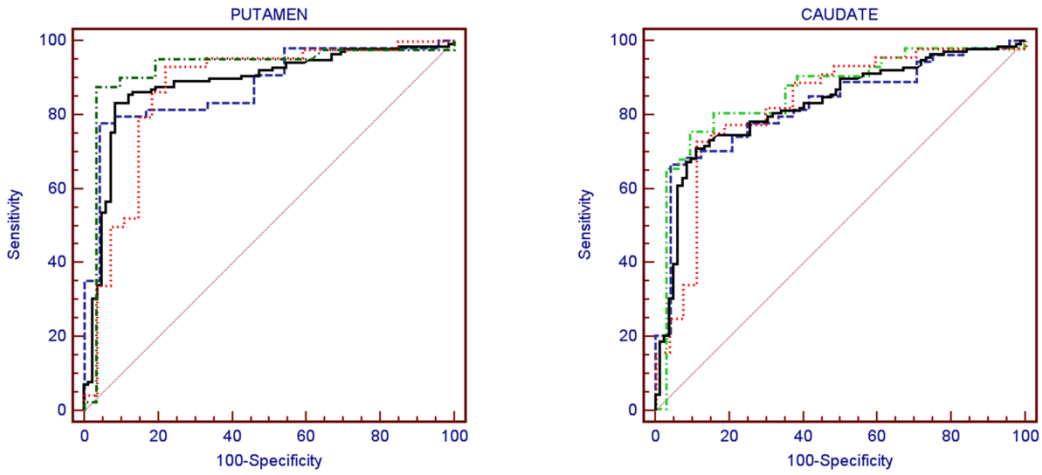
Figures

Figure 1

Anterior, oblique and top-to-anterior view of the anthropomorphic phantom used in this study.

Figure 2

ROC curve for both putamen (left hand side) and caudate (right hand side) in each center (center A, dashed line; center B, dotted line; center C, dot-dashed line) and for the entire population (full line).



Conclusion

Neurodegenerative diseases are one of the most important medical challenges of the new century. New drugs are continuingly developed in order to reduce neuronal degeneration rate, and especially to prevent neurodegeneration[1]. According to this approach, diagnosis should be the prompter possible in order to offer the best standard of cure.

Different signs and symptoms are manifest on the onset of disease, in particular in Parkinson's Disease, however, to date, the accuracy of a clinical diagnosis is definitely insufficient[2], considering overall secondary effects of the treatment on one hand, and the risk of retarded treatment on the other hand. Therefore, objective diagnosis by the mean of imaging has became essential in this context. In the case of Parkinson's Disease and others neurodegenerative parkinsonian syndromes, such as Multi-System Atrophy, Lewy Body Dementia, Cortico-Basal Degeneration or Progressive Supranuclear Palsy, [^{123}I]FP-CIT SCAN (DAT SCAN) is the most wide spread imaging technique of Molecular Imaging and Nuclear Medicine field. Its facility to produce, the wide spread of gamma camera, its biodistribution that allow fast scan acquisition and its good diagnostic accuracy associated to good cost-effectiveness leaded a rapid diffusion of the scan among nuclear physicians and to kind acceptance among neurologists[3].

Furthermore, it is well known that multicenter trials have acquired significant importance thanks to the larger data pools and higher statistical power reached; moreover they led to standardization with improvement of patients management. However, in nuclear medicine and molecular imaging field, where different equipments, acquisition protocols, data analysis and interpretation/presentation of clinical results have been observed[4], comparability of semi-quantified results is still to be assured[5].

Standardization in Nuclear Medicine is a trend followed with success for years. This is required primarily in order to ensure diagnostic quality for patients and secondly to permit an adequate sharing of information between colleagues. Furthermore, standardization is useful to correct errors associated with various instrumentation as well. However, in the case of dopaminergic system imaging, Morton et al observed that all exams should be acquired in the same camera type to have homogenous data and phantom data should be used to normalize the database accordingly[6]. On the other hand, both Meyer and coll and Koch et al demonstrated that homogenous data could be obtained from different equipments, although corrections factors were mandatory[7,8]. However, to the best of our knowledge, no multicenter studies evaluated the accuracy of semi-quantitative data to predict neurodegenerative parkinsonian syndrome.

Moreover, the semi-quantitative evaluation of [^{123}I] FP-CIT SCAN presents many facets[9].

Drawing irregular Regions of Interest (ROI) covering the striatum namely caudate nucleus and putamen and a reference region in order to calculate the specific binding activity is used in many centers. Anatomical atlas is useful to draw in an individualized way or to built ROI template. However, this approach is subjective and imply important intra- and inter-observer variability. The numerical outcome of such a manual interpretation involves the Signal to Background Ratio (SBR) for the striatum, caudate nucleus and putamen, and differences of the unilateral structures .
Very often the patients with suspected NPS perform a MRI scan before or just after DAT SPECT and multimodal image fusion of structural and functional images lead to easy ROI positioning. Lee and coworkers originally developed an automated SPECT and MRI image registration algorithm based on anatomical characteristics using image intensities for improved precision. This automated co-registration is useful in order to perform a computer-aided evaluation of SPECT[10,11].
In order correct for partial volume effect a quantification method that calculates uptake ratios from two large rectangular ROIs covering striata and one rectangular ROI that is placed on the occipital

lobe has been developed[12]. PVE is the phenomenon due to which counts are blurred out of the structure's physical volume, underestimating and preventing accurate evaluation of uptake, when very high count small areas are next to very low count areas, as it happens in striatal borders where the high uptake striatum gives its place to the nonspecific uptake brain parenchyma[9]

Specific uptake size index (SUSI) has been proposed by Fleming and coworkers, this index measures total uptake of striatum related to the specific activity of a reference region independently of the resolution of the imaging process[13]. With their method, operator intervention is limited to the placement of two large trapezoidal ROIs separately covering striata, while the reference region is automatically defined as the whole of the brain parenchyma excluding the striatal regions. Another resolution-independent quantification method was described by Dobbeleir and coworkers[14].

QuantiSPECT, from Mirada Solutions, a PC-based automated software package offers three semi-quantification methods. The "two box method" is founded on the above described technique for partial volume correction, the "three box method" perform the ration with the background on the occipital lobe and the "crescent method" based on a paper of Løkkegaard and coworkers[15]. The last method provides uptake values for the striatum and putamen, as well as the putamen to caudate ratio.

EXINI DAT is another PC-based commercially available software for computer-assisted diagnosis of DAT SPECT (EXINI Diagnostics AB, Lund, Sweden). Transverse slices after reconstruction are inputted via DICOM file, then automatic quantitative analysis of DAT images is performed. The report shows the specific uptake of striatum, caudate and putamen[9].

BRASS (Nuclear Diagnostics, Stockholm, Sweden) performs a multistep registration of the single studies to a template of healthy controls. Subsequently it is applied a fine adjustment of a standardized 3-D VOI map to estimate semi-quantitative values. The template and 3-D VOI map could be built for each centre, however they are also available by default in BRASS. In this case

too, it is calculated mean counts per voxel values for each VOI as well as ratios of striatum, caudate and putamen to the occipital cortex[9].

BasGan is a freely available PC based application. It automatically performs fine adjustments in positioning a 3D Volume of Interest (VOI) template to better match radioactive counts, and also locates an occipital VOI for background evaluation. To obtain BasGan scintigraphic specific binding ratio for each nucleus, non-specific background binding is subtracted from the putamen and caudate nucleus uptake, according to:

$$\text{BasGan scintigraphic Specific Binding} = \frac{\text{Counts (striatum)/voxel - Counts (occipital cortex)/voxel}}{\text{Counts (occipital cortex)/voxel}}$$

In addition, BasGan V2 automatically compares the values from each nucleus to a reference database from 96 healthy subjects of various ages, determining age-adjusted values for each basal ganglion [1calvini]. In particular, BasGan before the evaluation of each patient needs sex and age of the subject. Then, after the evaluation, BasGan shows in a graph subject's data and mean data for each age of normal subjects. These intrinsic data of BasGan can be used for age correction[16].

Voxel-based statistical analysis methods

These methods use firstly a normalization, i.e. manipulation of each voxel geometry in order to overlap the single study on a template space. Then data of each voxel are calculated based on the new geometry and finally a statistical analysis comparison is performed for each voxel, followed by the correction for family wise errors. Statistical Parametric Mapping or SPM (Wellcome Department of Cognitive Neurology, University College London, UK) SPM is one of the most diffused software of this kind that allows comparison between groups of patients and a group of healthy subjects. Built-in templates are derived from the Montreal Neurological Institute (MNI). In fact this software can be used in order to compute several other scripts such as IBZM tool tested on clinically well-documented patients and managed to differentiate MSA subjects from controls with

100% sensitivity and 86% specificity providing improved statistical power compared with manual VOI analysis[17].

NEUROSTAT was developed for assessment of functional brain scans by Minoshima and coworkers[18]. After a first standardization it works on a pixel-by-pixel basis. It was validated and found to be as effective as SPM in PET studies of normal brains with[18]F-fluorodeoxyglucose (FDG)[19]. It was also successfully used to evaluate [^{123}I]FP-CIT SCAN[20].

Habraken and coworkers developed a fully 3-D automatic, observer-independent technique for the quantification of the neuronal radiotracer binding on a voxel-by-voxel basis, which they call voxel-based automatic neuronal quantification (VANQ)[21]. This software automatically calculates the specific to nonspecific ratio for the patient's striatum, an apparent limitation of the method that uses the whole striatum (not its substructures), which compares it to a database of healthy volunteers. They found that this automatic method produces equally good results to visual assessment[9].

Different aims were raised during this work: 1) to assess accuracy of a semi-quantitative analysis (by the mean of BasGan) of DaTSCAN, acquired in a unique center; 2) to assess accuracy of a semi-quantitative analysis of DaTSCAN, acquired in three centers which use different equipments; 3) to evaluate accuracy of voxel-based analysis of DaTSCAN, acquired in three centers with different equipments.

The first aim was evaluated in 78 patients, the data showed the high accuracy of this kind of assessment, the very high reproducibility and its usefulness in "borderline" patients. These results were a good background for the development of the second aim. In the second study, 220 patients assessed, technical complexity was higher and the use of a phantom was mandatory in order to overcome it. However, after a homogenous acquisition of the DAT SCAN and homogenous treatment of the information, we showed that a semi-quantitative assessment, was not only feasible,

but was highly accurate, at least as other authors has describe in single center evaluation[22,23]. This result is not negligible, because it opens the door to the use of this scan in multicenter trials, as well as to its interpretation in a "grayscale" rather than in a "white-black" mode.

Finally, the third aim was faced with the background of the first two. This set of data has not been published yet in peer-review journals, for reasons of time, however the interest aroused has permit to present these results in international congresses. In fact, in order to perform a voxel-based analysis the technical complexity is greater, and collaboration with other subjects such as Montreal Neurological Institute was necessary, in particular for template construction. Overall, the preliminary data showed the good accuracy of this kind of analysis, but also allowed to make visible unseen areas in unicentric studies, enhancing the understanding of parkinsonian syndromes pathophysiology, in particular in extrastriatal areas. Voxel-based studies in standardized nuclear medicine could open new scenarios of diagnosis as well in treatment of neurodegenerative parkinsonian syndromes.

In conclusion, I think that the most important message of this research is the showed usefulness of semi-quantitative data in image treatment on the molecular imaging and nuclear medicine field in order to increase our contribute for the patients and the health care system.

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