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Establishing On-Site Reference Values for ^{123}I-FP-CIT SPECT (DaTSCAN®) Using a Cohort of Individuals with Non-Degenerative Conditions

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Abstract

Purpose: To overcome the issue of reference values for DaTSCAN® requiring healthy controls, we propose an original approach using scans from individuals with non-degenerative conditions performed at one single center following the same acquisition protocol.

Procedures: From a cohort of 970 consecutive patients, we identified 182 patients with a clinical diagnosis of non-degenerative parkinsonism or tremor and a visually normal DATSCAN®. Caudate nucleus (C), putamen (P), and striatum (S) uptake values, C/P ratios, and asymmetry indexes (AI) were calculated using semi-quantitative methods. Outcomes were assessed according to age and gender, and reference limits were established using the percentile approach.

Results: A significant negative linear effect of age was found upon striatal nuclei uptake of 0.21–0.22 per decade (6.8 %/decade for striatum), whereas a potential gender influence proved unclear. Inferior reference limits were established at the 5th percentile. C/P ratios and AIs were not influenced by age or gender, and superior reference limits were set at the 95th percentile.

Conclusions: We here propose a convenient approach to calculate site-specific reference limits for DaTSCAN® outcomes not requiring scanning healthy controls. The method appears to yield robust values that range within nearly identical limits as those obtained in healthy subjects.

Key words: SPECT, Reference values, Dopaminergic uptake, Gender, Age

Introduction

[^{123}I]FP-CIT ([^{123}I]ioflupane, DaTSCAN®, GE Healthcare, Glattbrugg, Switzerland) single-photon emission computed tomography (SPECT) is one of the most widely used nuclear medicine methods to assess the integrity of the nigrostriatal pathway [1, 2]. It has proved highly valuable to distinguish presynaptic degenerative forms of parkinsonism, notably Parkinson's disease (PD) and dementia with Lewy bodies (DLB), from non-degenerative parkinsonian syndromes, such as drug-induced parkinsonism (DIP) or psychogenic parkinsonisms (PP), or from essential tremor and other atypical tremors [3–7]. However, the validity and clinical usefulness of this test rely heavily on the method used to assess scan images and, thus far, two approaches have been proposed, sometimes in combination. First, a visual assessment using a grade 0 to 3 staging system [8] is simple and does not involve specific software, yet this

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approach requires some expertise, may suffer from significant intrarater and interrater variability, and may be inconclusive in the not-so-rare cases with mild or questionable uptake deficits [9]. Second, a more objective, semi-quantitative assessment method providing handmade, region-of-interest (ROI)-based or automated data has the advantage of well-defined and comparative values [10–12]. Until recently, the validity of this quantitative approach was mitigated by the lack of reference limits obtained from healthy controls and by standardization issues related to methodologies varying from one center to another involving machine or protocol differences [9, 13–15].

A few years ago, a European multicenter group generated a [^{123}I]FP-CIT SPECT database obtained from healthy controls, and results from this study were published two years ago [16, 17]. A total of 139 scans performed in 13 different centers and covering a large range of age and both genders equally were analyzed. Using a stringent methodology, various outcomes were calculated and authors were able to show, as other groups previously, that dopamine transporters (DAT) availability seems to be increased in women compared to men and that there is an age-related decline of ligand uptake of 5.5 % per decade. While very valuable, this approach showed also some limitations. First, despite efforts to limit center-to-center variability, the use of 17 different SPECT imaging systems of 11 different models in 13 different centers led to some inconsistencies when comparing data. Second, this study enrolled a relatively small sample of subjects per center, so that the validity of data at the single center level might be problematic. Finally, while this work was not specifically aimed at providing reference limits that could separate normal and abnormal uptake values, authors proposed formulas to calculate them.

We therefore decided to reappraise the issue of reference values for [^{123}I]FP-CIT SPECT using an original approach designed to minimize intercenter variability. According to these constraints, image acquisition was performed at one single nuclear medicine center only, the same machine and protocol were used for all subjects and we included a high number of individuals with non-degenerative parkinsonism and non-parkinsonian tremor, all conditions well known to be associated with an unaltered [^{123}I]FP-CIT SPECT [1, 3, 4, 18–20]. The purpose of the present study was then to establish reference values and limits for the caudate nucleus (C), putamen (P), and striatum (S) and to address the influence of age and gender upon DAT availability. We also analyzed other parameters that might be useful to discriminate between normal scans and the presence of degenerative parkinsonism at an early stage, including the C/P ratio and the asymmetry indexes (AIs) between right and left striatal structures.

Materials and Methods

Patients

As shown on Fig. 1 detailing the inclusion process, we collected data from 970 consecutive patients who underwent a total of 1005 [^{123}I]FP-CIT SPECT at our hospital from May 2003 to September 2013. Thirty-three subjects had two [^{123}I]FP-CIT SPECT, and one patient had three scans; in these cases, only the first scan was considered for our study. Every patient was evaluated at least once by a neurologist trained in movement disorders, except for a few cases whose SPECT had been requested by a general neurologist. A detailed chart review was conducted for each case, including all available neuroimaging data. The following clinical information was assessed and computed: age at the time of scan, gender, disease onset, age at disease onset, symptoms, body side predominance, and final diagnosis. Diagnoses of non-degenerative parkinsonism or tremor were established using specific and validated sets of criteria: the Consensus statement of the Movement Disorder Society on tremor for essential tremor (ET) [21], the criteria proposed by

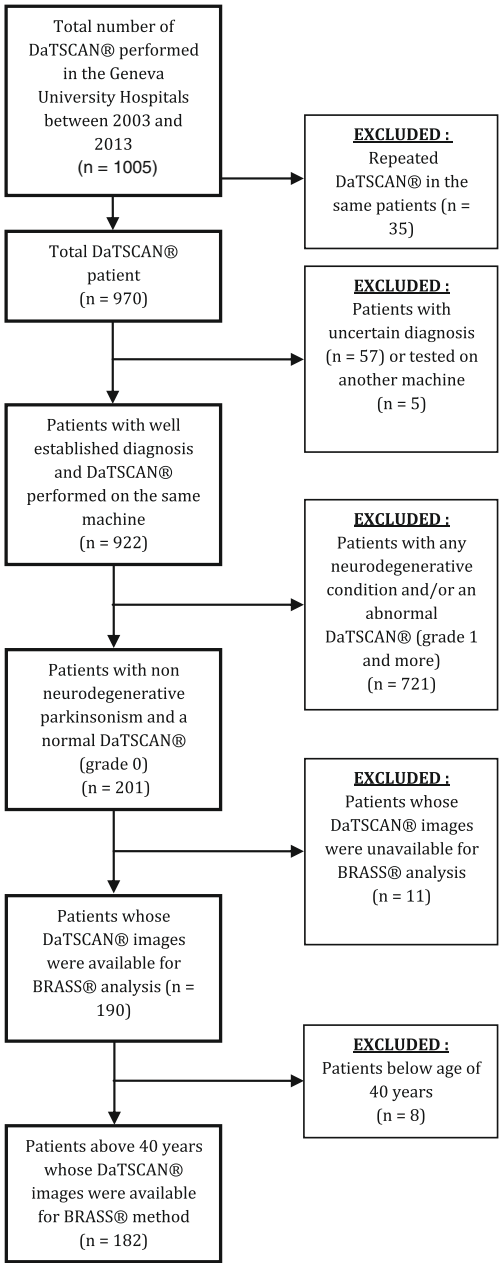

Fig. 1 Diagram showing the multiple-step procedure for patients and DaTSCAN® inclusion in the present study

Morgante et al. for psychogenic movement disorders [22], the EFNS guidelines for primary dystonia [23], the clinical criteria proposed by Zijlmans et al. [24] for vascular parkinsonism, and, for DIP, the development of parkinsonism, preferably symmetric, in temporal relation (use of antidopaminergic drugs in the 6 months preceding the onset of symptoms, along with a previously negative history for parkinsonian signs) to exposure to neuroleptics or other drugs with antagonistic properties to dopamine receptors, was required the more so as parkinsonian symptoms disappeared or subsided 6 months after withdrawing the offending drug. For degenerative parkinsonisms, clinical diagnosis was established based on the most widely used criteria for PD [25], DLB [26], multiple system atrophy (MSA) [27], progressive supranuclear palsy (PSP) [28], and corticobasal degeneration (CBD) [29]. Sixty-two patients were classified in the "Undefined" category and thus excluded because of incomplete data or unclear diagnosis (n=57) or because another machine was used (n=5). A total of 908 [^{123}I]FP-CIT SPECT from 908 different patients were eventually included in our database.

Among these patients, we identified 201 subjects with a diagnosis of non-degenerative condition and a normal [^{123}I]ioflupane SPECT defined as a grade 0 visual staging. Eight patients were younger than 40 years old and were excluded because this small population of young individuals was considered irrelevant given the usually higher age range of ^{123}I-FP-CIT SPECT candidates. In addition, raw scan images were unavailable for 11 patients, precluding the automatic BRASS® analysis to be performed. By the end of the inclusion process, we were able to collect and analyze data from a total of 182 patients older than 40 years (range 40–93) harboring a clinical diagnosis of non-degenerative condition associated with a grade 0 SPECT, a cohort of individuals that could be considered, from a dopaminergic point of view, equivalent to healthy controls and therefore useful to establish reference values.

Imaging: Data Acquisition and Reconstruction

[^{123}I]FP-CIT SPECT was performed according to the manufacturer's instructions. Patients received about 185 MBq of [^{123}I]FP-CIT in slow intravenous injection and were administered Lugol solution (5 drops 5 % KI before and 4 h after injection) for thyroid blockade. SPECT data acquisition started 4 h after administration of the DAT tracer. Medications in general, and dopaminergic agents in particular, whenever used, were not discontinued.

All scans were acquired on the same triple-head gamma camera (GCA-9300A/UI Toshiba Medical Systems AG, Oetwil am See, Switzerland) equipped with fan beam, low-energy, high-resolution collimators. In all cases, the head was fixed in a head-holder to minimize motion artifacts. Acquisition parameters included step-and-shoot mode over 30 min. Sixty projection angles were taken over 360° and a 128×128 matrix was used. Reconstruction was done using filtered back projection by means of the manufacturer's software, with a Shepp and Logan filter cutoff at the Nyquist frequency.

Uniform Chang attenuation correction was performed to compensate for photon attenuation, using the theoretical mean attenuation coefficient of soft tissues for 159 keV 123I photons (0.15 cm^2) and a triple-energy window method for scatter correction [30].

Image Processing and Analyses

[^{123}I]FP-CIT SPECT images were analyzed visually using a previously validated semi-quantitative four-point scale, classifying the study as normal (grade 0) or abnormal grades 1, 2, and 3 [3]. We added an in-house intermediate grade (between normal and grade 1) to this classification indicating doubtful findings, a questionable asymmetry, despite a good visualization of both striata, or a lower signal-to-background contrast with preserved "comma-shaped" and symmetrical uptake.

Quantification of uptake was based on six striatal volumes of interest (VOIs), including the right and left C, P, and S, respectively. The regions were manually drawn on a summed image including the whole striatum, providing measures of mean count concentration in each striatal region (Cs). On the same image, a VOI including the occipital cortex was drawn, providing quantification for non-specific background mean counts (Cb). We used these values to calculate striatal uptake ratios defined as [(Cs−Cb)/Cb].

The regions were manually drawn over the expected C and P distribution, even when the uptake was confined to the C. Figure 2a provides an example of the manual drawing in a normal and in a grade 2 (Fig. 2b) study.

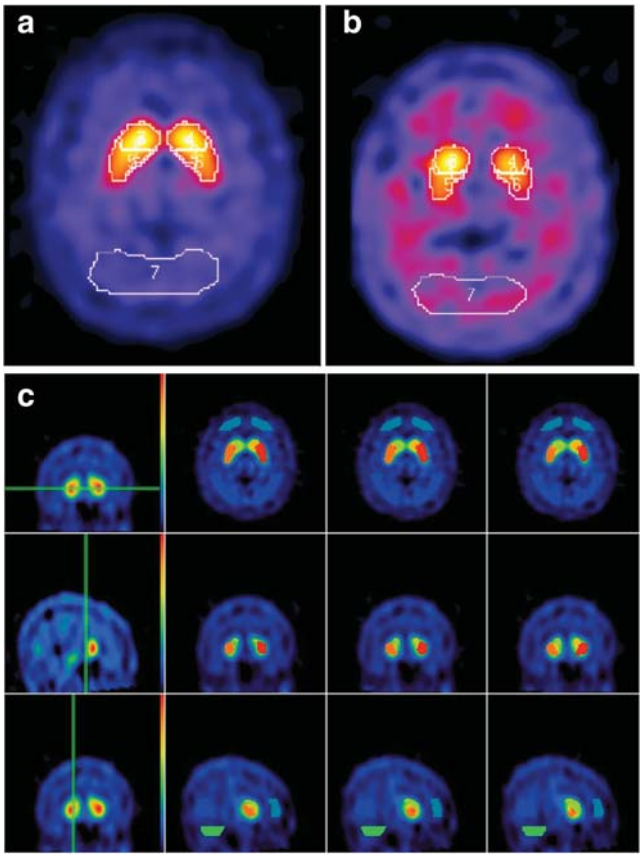

Fig. 2 Selected DaTSCAN® images from two individual studied using the manually delineated ROIs method. **a** Patient with ET and a normal (grade 0) scan; **b** patient with PD and an abnormal scan (grade 2). **c** Example of an automated BRASS® analysis of a grade scan (same patient as in **a**).

In addition, DaTSCAN® images were quantitatively analyzed using BRASS™ automated functional brain analysis software (Hermes *BRASS* software, Nuclear Diagnostics AB, Sweden) [31]. Briefly, BRASS® software spatially coregisters the patients' tomographic data to a 3D reference template in a standard space, and then applies a predefined template VOI set [10, 32]. Regional mean counts in striatal and background occipital VOIs are used to calculate striatal uptake values [31, 33] (Fig. 2c).

Outcomes

We aimed at evaluating reference values for the following outcomes: (1) C, (2) P, and (3) S uptake on both sides, (4) the C/P ratio, and the asymmetry index (AI) for right and left C, P, and S.

The three VOI uptakes were defined as the mean value of the left and right side measures, the C/P ratio as the mean of the two ratios determined in the right and left side, and the three asymmetry indices were determined according to a slightly modified version of Zijlmans [24] formula $[(R-L)/(R+L)]\times 2\times 100$, where R determined the side with the higher uptake and L the side with the lower uptake, in order to obtain a positive value.

Finally, the reference limits were defined as the 5th percentile for the C, P, and S uptakes, and the 95th percentile for the C/P ratio and the three AIs.

Statistical Analyses

Reliability and Agreement Analyses Reliability and agreement between manual and BRASS® methods were assessed for the C and P outcomes using intraclass correlation coefficient (ICC (3, 1) of Shrout and Fleiss), percentage of agreement, and Bland-Altman plot. Both reliability and agreement were not satisfactory, and we therefore decided to use the BRASS method measures to define reference values. The reliability and agreement of the S was not assessed because its delineation overlapped that of the C and P.

Age and Gender Effect A possible relationship between patient demographic parameters (age and gender) and outcomes of interest was evaluated using graphical representation, Student's *t* tests, and linear regression analyses. Normality of outcomes was assessed using Normal Q-Q plots. It was satisfactory for the C, P, and S distribution whereas a box-cox power transformation method was applied to normalize the distribution of the C/P ratio and asymmetry indexes.

Reference Values For C, P, and S outcomes, reference values were estimated so that 5 % of the sample has lower values (5th percentile); for the C/P ratio and the three AIs, reference values were estimated so that 5 % of the sample has higher values (95th percentile). All reference values are reported with their corresponding 95 % confidence interval (95 %CI). Hence, we considered that any scan performed in our center following the same protocol should raise the possibility of nigrostriatal dopaminergic dysfunction when VOIs uptake values are below the 5th percentile and/or when C/P ratio and AIs are above the 95th percentile.

As gender effect was not supported by our data, we decided to establish reference values without distinction of gender. When a linear effect of age could not be justified, as was the case for C/P ratios and AIs, we estimated the reference value and 95 % CI as the 5th or 95th (depending on the outcome) percentile calculated on the sample mean and standard deviation, assuming normality. The estimates obtained on the normalized distributions were then back-transformed on their original scale. When a linear effect was justified by our data, as was the case for the other outcomes, we verified that the standard deviation of the linear regression residuals were constant and do not depend on age (homoscedasticity). The reference values were finally set as the regression line vertically shifted by the value of the estimated 5th (or 95th as according to outcome) percentile of the residuals; percentile (and 95 % CI) was calculated based on the residuals mean and standard deviation, assuming normality.

All analyses were performed on R-3.1.0 (R foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org), except the percentile estimation which was performed on Stata 13.0 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Results

DaTSCAN® data from 182 patients (109 (60 %) women, mean age 69.1±11.2 years, range 40 to 93 years) with a normal (grade 0) visual dopaminergic uptake and a non-degenerative condition were collected. Final clinical diagnoses included ET (*N*=38), DIP (*N*=80), PP (*N*=31), primary focal and generalized dystonia (*N*=8), and various conditions unassociated with parkinsonism (*N*=25). Medications were not discontinued with the exception of DIP cases where the offending agent, such as neuroleptics or calcium-channel blockers, was stopped several weeks prior to the scan. Twelve patients were deceased by the end of 2013, among which only one had an autopsy, confirming the absence of any neurodegenerative process.

Comparison Reliability and Agreement of Manual and BRASS Methods Analysis

Mean values for C, P, S, C/P ratios and AIs are available in Table 1. When comparing the C, P, and S values provided by the two quantification methods, ICC reliability coefficients were 0.37 (95 % CI [0.24–0.49]), 0.38 (95 % CI [0.25–0.50]), and 0.40 (95 % CI [0.27–0.51]), respectively (Fig. 3a–c). Moreover, Bland-Altman plots (Fig. 3d, e, f) reveal important bias with 64, 70, and 69 % of the absolute differences between the two methods higher than 0.5 and 30, 36, and 27 % of the absolute differences even higher than 1 for the C, P, and S, respectively. The manual method showed many disadvantages: it was time-consuming, required specific expertise and the manual drawing of ROIs may have led to problematic repeatability. For example, mean C values were similar using both methods, possibly because this particular structure is easier to draw compared to the more complex shape of the P. Indeed, mean putaminal values show significant differences between the two methods (2.39±0.78 for the manual method vs 3.04±0.51 with BRASS, $p<0.001$), corresponding to a 27 %

Table 1. Uptake values for right and left striatum, caudate nucleus, and putamen, C/P ratios, asymmetry indexes, calculated using the manual method based on VOIs and the automated BRASS method, according to gender

		Manual				BRASS			
		All (n=182)	Female (n=109)	Male (n=73)	Pval	All (n=182)	Female (n=109)	Male (n=73)	Pval
Striatum	Right	2.66±0.77	2.69±0.84	2.60±0.65	0.413	3.19±0.52	3.25±0.56	3.09±0.45	0.035
	Left	2.65±0.76	2.68±0.82	2.60±0.67	0.467	3.18±0.51	3.22±0.54	3.11±0.46	0.151
	Average	2.65±0.76	2.69±0.82	2.60±0.66	0.437	3.18±0.51	3.24±0.54	3.10±0.45	0.072
Caudate nucleus	Right	3.21±0.98	3.26±1.03	3.13±0.91	0.393	3.44±0.57	3.52±0.60	3.32±0.51	0.016
	Left	3.18±0.95	3.22±1.00	3.12±0.89	0.485	3.30±0.57	3.36±0.60	3.22±0.51	0.092
	Average	3.20±0.96	3.24±1.01	3.13±0.89	0.433	3.37±0.55	3.44±0.58	3.27±0.48	0.033
Putamen	Right	2.40±0.81	2.43±0.88	2.36±0.70	0.576	3.00±0.54	3.05±0.57	2.92±0.47	0.102
	Left	2.38±0.77	2.39±0.80	2.35±0.73	0.698	3.08±0.52	3.12±0.55	3.03±0.47	0.275
	Average	2.39±0.78	2.41±0.83	2.36±0.70	0.629	3.04±0.51	3.08±0.54	2.98±0.45	0.155
C/P ratio	Right	1.36±0.20	1.37±0.20	1.34±0.21	0.310	1.16±0.14	1.16±0.13	1.15±0.15	0.423
	Left	1.36±0.18	1.36±0.17	1.35±0.19	0.661	1.08±0.13	1.09±0.14	1.07±0.11	0.298
	Average	1.36±0.17	1.37±0.16	1.35±0.18	0.401	1.12±0.10	1.12±0.11	1.11±0.10	0.227
Assymetry (%)	Striatum	4.66±4.19	4.89±4.39	4.33±3.86	0.368	4.65±3.84	4.88±4.06	4.31±3.50	0.314
	Caudate	6.31±4.58	6.51±4.88	6.01±4.10	0.463	7.93±6.92	8.15±6.78	7.59±7.16	0.601
	Putamen	7.58±6.94	7.72±7.22	7.36±6.55	0.724	7.46±7.12	7.31±7.06	7.69±7.27	0.726

Pval indicates the statistical significance between both methods. Values are mean±SD

difference. Consequently, striatal values, which are superimposed to those from C and P, may also suffer from this examiner-dependant method. In addition, standard deviations obtained with the manual method are higher than with the BRASS method, which is in line with a potentially poorer repeatability of the former. For all these reasons, the

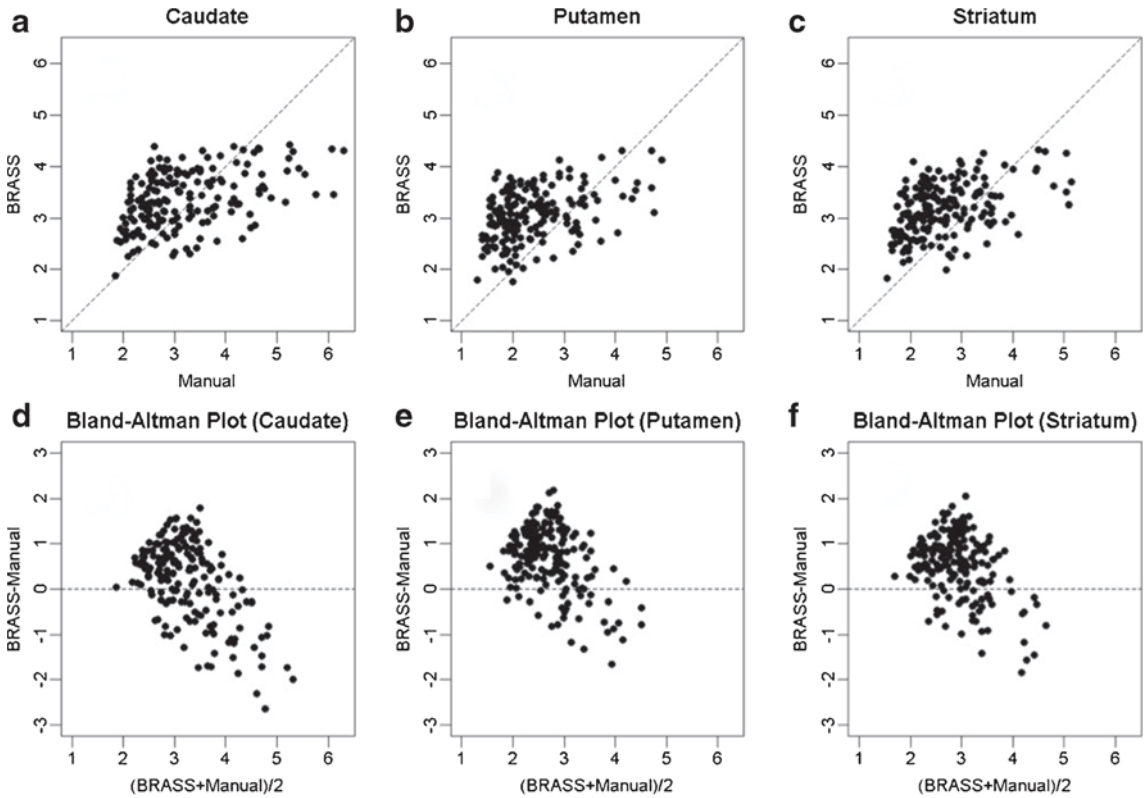

Fig. 3 Comparisons of BRASS® and manual methods. **a** Caudate nucleus, **b** putamen, and **c** striatum BRASS® values are shown as a function of manually obtained values. For each parameter, differences between the two methods as a function of the mean value of the two methods are represented in **d**–**f**, respectively (Bland-Altman plot). Differences are large and not symmetrically distributed around 0, indicating disagreement between the two methods. Data are the average of right and left sides.

BRASS method was here considered more convenient and suitable to estimate our reference values.

Uptake Values, C/P Ratio, and AIs

As detailed in Table 1, we found a BRASS-derived mean uptake value of 3.18±0.51 for the S, 3.37±0.55 for the C, and 3.04±0.51 for the P. In addition, we calculated a mean C/P ratio of 1.12±0.10. The AI varied depending on the structure considered: 4.65±3.84 for the whole S, 7.93±6.92 for the C, and 7.46±7.12 for the P. Values obtained with the manual method are detailed in Table 1.

Analysis of diagnostic subgroups of non-degenerative parkinsonisms shows similar results for mean C, P, and S uptake values ($p>0.05$), as well as AIs ($p=0.45$) and C/P ratio ($p=0.80$). Results are available in Table 2.

Gender Effect

When comparing uptake values, C/P ratio and AIs between men and women, most of the differences were not statistically significant (Table 1). Considering the mean of right and left sides, only the mean difference of the C between men and women was statistically significant (3.27±0.48 vs 3.44±0.58, respectively, $p=0.033$ (Student's t test)). Figure 4 represents the uptake values for men and women as a function of age. No interaction was found between age and gender (results not shown).

Age Effect

We found a consistent decrease of uptake for all VOIs with age, at least in the age range considered, i.e., 40 to 93 years, and this trend was statistically significant ($p<0.001$). This decrease appeared linear with a loss per decade estimate of 0.22 (SE=0.03) for the P and S, and 0.21 (SE=0.03) for the C. We failed to find any age effect for C/P ratio and AIs, as shown in Table 3.

Reference Values

We estimated reference values without distinction of gender as our data showed limited evidence of a gender effect. Reference values were estimated as a function of age for all VOIs, and the resulting lines above which a value is considered normal are presented in Fig. 5. A single reference value was estimated for the C/P ratio and AIs as we had no evidence of an age effect on these parameters. The resulting reference values of the C/P ratio and striatum AI, back-transformed on their original scale, are presented in Fig. 5.

Discussion

To overcome the lack of reference values available for routine DaTSCAN® interpretation, we propose in this study an original approach that can be easily replicated and used in any individual nuclear medicine center. Rather than relying on values calculated at other centers using different SPECT machines and protocols or on data obtained from brain phantoms or from healthy controls, we examined a large cohort of patients with various non-degenerative conditions known and confirmed to be associated with a normal scan. All were scanned on the same machine following the same protocol, and images were analyzed using two distinct quantitative methods, one based on manually drawn VOIs and another, automated one, the BRASS® method. Because the automated approach is rapid and more convenient and because we suspect it having a better repeatability, we used this method to establish reference limits of common parameters, including uptake values of striatal nuclei, C/P ratios, and AIs. Values outside these limits strongly support an altered nigrostriatal system.

We analyzed the effects of age and gender on all outcomes, and our results showed a significant linear effect of age on C, P, and S uptake values, as already reported by others [16, 17, 34–37]. Based on this assessment, it can be estimated that a DaTSCAN® can be labeled as normal in our center when the uptake is above $3.78883-(0.02156\times\text{age})$

Table 2. Mean uptake values, C/P ratio, and striatum AI (expressed mean±SD) in the different non-degenerative parkinsonisms

Group	Mean striatum uptake	Mean caudate nucleus uptake	Mean putamen uptake	Mean C/P ratio	Mean striatum AI %
Drug-induced parkinsonism ($n=80$, M/F ratio 0.60, age 69.9±11.1, range 41–86)	3.10±0.50 (range 1.83–4.26)	3.28±0.53 (1.88–4.43)	2.97±0.50 (1.8–4.18)	1.10±0.13 (0.69–1.50)	5.1±3.9 (0.1–19.3)
Essential tremor ($n=38$, M/F ratio 1.38, age 71.1±9.7, range 44–86)	3.29±0.46 (2.58–4.30)	3.49±0.47 (2.68–4.39)	3.14±0.48 (2.28–4.30)	1.12±0.14 (0.91–1.49)	4.2±2.8 (0.5–10.3)
Psychogenic parkinsonism ($n=31$, M/F ratio 0.34, age 63.7±12.1, range 42–93)	3.35±0.52 (2.28–4.09)	3.55±0.59 (2.41–4.39)	3.21±0.49 (2.19–3.94)	1.11±0.13 (0.87–1.51)	4.1±2.5 (0.1–10.8)
Dystonic tremor ($n=8$, M/F ratio 0.60, age 62.3±14.2, range 42–79)	3.34±0.64 (2.35–4.32)	3.53±0.57 (2.61–4.34)	3.20±0.70 (2.16–4.30)	1.12±0.11 (0.95–1.27)	2.8±2.4 (0–7.4)
Others ($n=25$, M/F ratio 0.67, age 72.2±8.6, range 49–86)	3.01±0.49 (1.99–3.89)	3.22±0.59 (2.30–4.35)	2.86±0.46 (1.77–3.65)	1.14±0.16 (0.91–1.54)	5.3±6.1 (0–22.3)
p Value[a]	0.057	0.054	0.096	0.808	0.451

[a] p Values are adjusted for age and gender

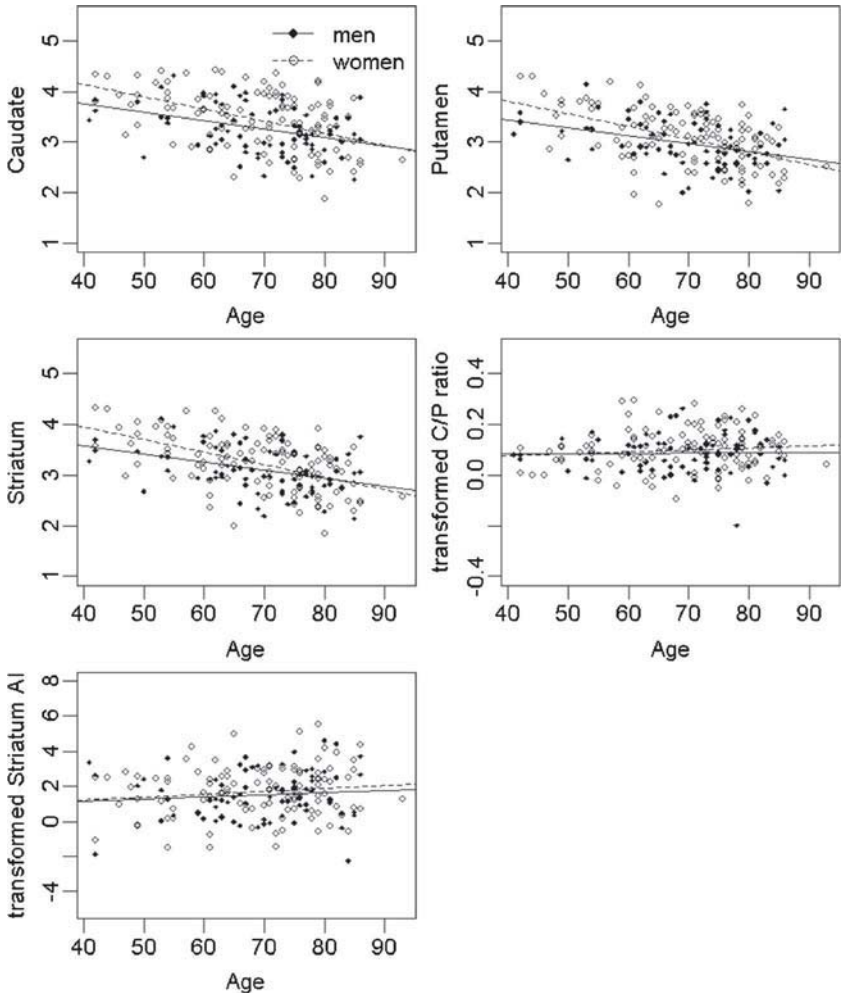

Fig. 4 Effects of age and gender upon uptake values of caudate nucleus, putamen, striatum, caudate nucleus to putamen ratio, and striatum asymmetry index. Data are the average of right and left sides.

for the S, 3.87465−(0.02141×age) for the C, and 3.65557 −(0.02168×age) for the P. For example, a healthy 70-year-old individual is expected to exhibit values above 2.27 for the S, 2.37 for the C, and 2.13 for the P on each side. Linear regression analysis showed an age-related decline that was consistent and very similar across the three structures, with a mean decline of −0.21 per decade for the C and of −0.22 for the P and C. Of note and at variance with other authors, we estimated a constant decline per decade rather than a decreasing decline per decade. Indeed, when other authors report a percent decline per decade, they do the assumption that the loss will be non-linear, smaller between 60 and 70 than between 50 and 60 years old. We found that our data (with an age range from 40 to 93 years) do not support such a relation but rather a linear relation. Nonetheless, we calculated a mean decline over our whole population of 6.8 % (striatum), 6.5 % (caudate nucleus), and 7 % (putamen) per decade, which is consistent with what has been reported in the currently available literature on healthy controls (between 4 and 9 % per decade) [16, 37–40].

Table 3. Linear regression analyses of all outcomes according to age

		Coef	SE	*p* Value
Caudate nucleus	Intercept	4.85	0.23	
	Slope	−0.21	0.03	<0.001
Putamen	Intercept	4.54	0.21	
	Slope	−0.22	0.03	<0.001
Striatum	Intercept	4.67	0.21	
	Slope	−0.22	0.03	<0.001
C/P ratio[a]	Intercept	0.059	0.036	
	Slope	0.005	0.005	0.313
Caudate nucleus asymmetry index[a]	Intercept	1.57	0.83	
	Slope	0.14	0.12	0.248
Putamen asymmetry index[a]	Intercept	1.38	0.85	
	Slope	0.11	0.12	0.365
Striatum asymmetry index[a]	Intercept	0.60	0.66	
	Slope	0.15	0.09	0.111

[a]Box-cox transformed data

With respect to a potential gender effect, our study yielded inconclusive results, women showing slightly higher mean values than men for all VOIs, some of them (right S, right and mean C) being statistically significant. In previous studies [16, 36, 41–43], some hypotheses have been put forward to explain

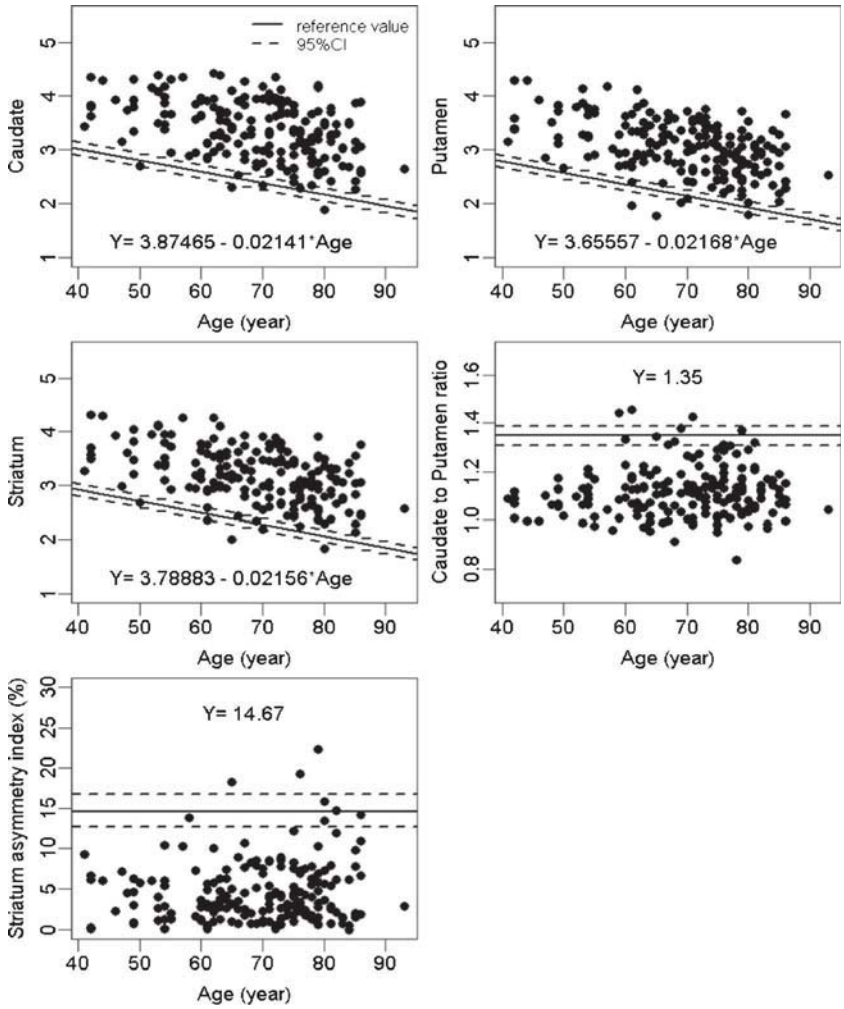

Fig. 5 Calculated reference values for the whole population, irrespective of gender. The *solid line* represents the reference value(s) defined as the 5th percentile for caudate nucleus, putamen and striatum, and 95th percentile for C/P ratio and striatum AI; the *dotted lines* represent the 95 %CI. Reference values depend on age for the caudate nucleus, putamen, and striatum parameters; as data do not support an age effect on C/P ratio and striatum AI, a general reference value is given for these two parameters.

why women have sometimes been found to have a higher striatal uptake. The most likely explanation relies on an artifact whereby a similar number of neurons—than men—project a higher density of nerve terminals because of a relative smaller brain volume [34, 43]. However, in most previous studies, small samples of healthy control patients were used and this difference tended to disappear with ageing. In fact, we observed the same trend in our study, with an age-related decline of all three VOIs found greater in women than men, yet none of these differences in slope was statistically significant. While it is possible that our study lacked power to detect differences in slope, reasons for a stronger decline of striatal uptake in women remain unclear. In conclusion, a gender effect could not be excluded. However, with respect to the purpose of our study, this led us to establish reference values identical for both genders, the more so as other studies have failed to show significant differences of striatal uptake in relation to gender [44].

We also took advantage of this study to examine other parameters that might be of interest to detect very early parkinsonism and to differentiate between the various forms of degenerative parkinsonism. For example, it has been proposed that the beginning of PD may be associated with a higher than normal asymmetry of uptake between both sides, and PSP has been shown to exhibit lower AI and C/P ratio than MSA or PD [45, 46]. Also, some studies have emphasized the usefulness of AI to distinguish PD from vascular parkinsonism [47, 48]. It therefore appeared useful to establish reference values for these two outcomes in our population. Regarding the C/P ratio, we found no evidence that this parameter does change with age or gender. We estimated the upper reference limit to be 1.35 on both sides. Mean values are similar to that reported previously [16, 38] and show usually clear-cut differences with PD patients, who have a much higher C/P ratio due to early putaminal decline [49, 50]. Similarly, we found no evidence for all

three AIs that they may vary according to age or gender. Mean AIs ranged from 4 to 7 % according to the region studied and striatum AI was here considered normal when lower than 14.37 %. It can be therefore suggested that a normal DaTSCAN® is associated with low AIs and relatively low C/P ratios, irrespective of age and gender, and values above the proposed limits potentially suggest subtle structural abnormalities in the nigrostriatal pathway.

Comparing uptake values across studies is usually difficult, given the many, sometimes subtle methodological differences between centers. Previous attempts by others have included small samples of healthy volunteers [41] or pooled data from different centers, leading to significant differences of mean VOIs values [16]. Besides, reference values have never been clearly provided in previous studies. In comparison with the results of Varrone et al. in the ENC-DAT study [16], we found slightly higher intercepts and greater slopes (for example, 95 % CI mean slope for striatum is −0.22 (range −0.27 to −0.16) per decade in our study, whereas in the ENC-DAT study, −0.15 (−0.21 to −0.09) is found. Importantly, however, mean values for caudate and putamen (male and female) were similar if not identical, as shown in Table 1 where our data can be compared with uncalibrated ACSC mean values from the ENC-DAT study. This finding somewhat supports the validity of the methodological approach proposed here.

Our study has some limitations. First, as it is the case for most studies on this topic, diagnoses were based on clinical criteria and final neuropathological assessment was not available for the vast majority of included subjects. Therefore, it may be possible that the study population was contaminated by cases with pre-clinical degenerative conditions affecting the nigrostriatal system, yet this issue is also true for any study using healthy controls of this age category. In our study, however, much effort was done to minimize this problem. Nearly all patients were examined by trained movement disorders specialists, stringent and well accepted diagnostic criteria were applied and, perhaps more importantly, the long duration of the study allowed most cases to be followed over time, with those developing PD or any other forms of degenerative parkinsonism being excluded along the study duration. In fact, the retrospective nature of this study might be considered an advantage for the issue of diagnostic accuracy as it may allow uncertain cases to be reassessed over a period of time during which clinical diagnosis may have become clearer. Second, a normal scan was here defined as a grade 0 [^{123}I]FP-CIT SPECT according to the visual scale proposed by Catafau et al. [8] and used by most authors. While the validity of this scale has been demonstrated, there have been some controversies regarding intrarater and interrater reproducibility, leaving some room for interpretation and possibly some grading misattribution in doubtful cases [51]. However, in our study, borderline scans were clearly identified, separated from normal scans (between grade 0 and 1) and excluded. In addition, a normal scan was only a secondary criterion for enrolment, the primary one being a clear clinical diagnosis of non-degenerative condition to be fulfilled. Cases with a questionable diagnosis and a normal scan were not included in the study. Third, to achieve enough statistical power, a relatively large study population is required. In our case, with a mean recruitment of 100 scans per year, it took about 10 years to collect nearly 200 subjects for the present study. Thus, for the model proposed here to be replicated elsewhere, a minimal number of scans should be performed at the center, yet preliminary reference limits can likely be calculated already with about 100 well-documented cases of grade 0 SPECT. Importantly, the purpose of our study was not to provide data applicable to other centers, but rather to propose a new method allowing defining on-site reference values established following the local methodological specificities.

Finally, the very usefulness of establishing precise reference values for [^{123}I]FP-CIT SPECT in the clinic might be a matter of controversy, as visual interpretation is likely sufficient in many cases to separate normal from abnormal scans. It is however our experience that a significant percentage of examined scans is variably interpreted, likely depending on the expertise level of the nuclear medicine and/or movement disorders specialists. In fact, we propose that semi-quantitative methods of assessment complement visual interpretation rather than substituting for it. It may even become mandatory when assessing difficult clinical situations such as aging-related pseudo-parkinsonism, very early degenerative conditions with borderline scans, or patients with scan without evidence of dopamine deficiency (SWEDD).

Conclusion

To the best of our knowledge, this is the first study to address the issue of quantitative reference values for DaTSCAN® parameters that can be used at the site level, including uptake values for the C, P and S, the C/P ratios, and AIs between both sides. We propose to analyze grade 0 scans of patients with clinically clear non-degenerative conditions using the BRASS® method, or any validated software, and to establish age-dependent, reference limits based on the percentile approach. Although our results appear robust, they now need to be tested against a cohort of patients with degenerative forms of parkinsonism. This next step is under way in our institution. Moreover, replication and confirmation by independent groups are still required before this model can be applied on a routine basis.

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Conflict of Interest. The authors state that they have no conflict of interest.

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