Survival in Parkinson's disease in relation to striatal dopamine transporter binding

Elina Mäkinen [a], Juho Joutsa [a, b c, d], Tero Vahlberg [e], Valtteri Kaasinen [a, d]

[a] Division of Clinical Neurosciences, University of Turku and Turku University Hospital, Turku, Finland
[b] Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[c] Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA
[d] Turku PET Centre, University of Turku, Turku, Finland
[e] Department of Biostatistics, University of Turku and Turku University Hospital, Turku, Finland

Corresponding author:
Dr. Elina Mäkinen
Division of Clinical Neurosciences,
University of Turku and Turku University Hospital
POB 52, FIN-20521
Turku, Finland
Tel. +358-2-3130000
Email: elmama@utu.fi

Key words: Parkinson's disease; SPECT; dopamine; survival; mortality

Abstract

Objective

To investigate whether dopamine transporter (DAT) binding, as measured with single photon emission computed tomography (SPECT), can be used to predict mortality in patients with Parkinson's disease (PD).

Methods

A total of 162 patients with PD and abnormal [I-123]FP-CIT SPECT were clinically followed for a median of 5.8 years. A multivariate Cox regression model was used to investigate survival with the independent predictors of age, gender, severity of motor impairment, levodopa-equivalent daily dose of medication, presence of cognitive defects, and putaminal specific binding ratio (SBR) of [I-123]FP-CIT. In addition, associations between striatal and extrastriatal SBRs and survival were investigated using voxel-based analyses.

Results

The overall mortality was 25.9%, and the Kaplan-Meier estimate for mortality was 36%. Older age ($P < 0.001$), presence of cognitive defects ($P = 0.001$), and more severe motor symptom severity ($P = 0.002$) were significantly associated with increased mortality. No associations were found between putaminal DAT binding and survival ($P = 0.99$). There were no significant differences in SBRs in any striatal or extrastriatal region between survivors and non-survivors, and no associations were found between SBRs and scan-to-death intervals among non-survivors.

Conclusions

Unlike the severity of motor and cognitive symptoms, the level of striatal dopaminergic defect in DAT SPECT does not predict mortality in PD. Although presynaptic dopaminergic functional imaging may have value as a diagnostic tool, the clinical symptom-based characteristics are superior for predicting lifespan.

Introduction

Parkinson's disease (PD) is associated with increased mortality as the mortality hazard ratio in PD patients is generally between 1.2 and 2.4 compared to in individuals without PD [1]. There is evidence of factors that are associated with the increased mortality in PD, such as cognitive impairment and an older age at the disease onset [1-3] with mixed results concerning the influence of gender on mortality [1, 4, 5]. A recent meta-analysis found no evidence to suggest that the use of levodopa reduces mortality [6]. From a clinical perspective, increased mortality in PD appears to be connected to the phenotype of postural instability and gait disorder (PIGD), symmetricity of motor signs and the general severity of the motor symptoms [5, 7]. Although demographic and clinical factors impact survival in PD [8], identification of the primary neurobiological survival-related mechanisms is important for the development of treatments that aim to increase lifespan in patients with PD.

Brain dopamine transporter (DAT) imaging is mainly used during the early diagnostic phase of clinically uncertain parkinsonism, with [I-123]FP-CIT SPECT being the most widely used method [9]. DAT imaging has been shown to separate PD patients from healthy controls with high precision [9], but the exact mechanism and relevance of the striatal DAT binding defect is unclear. For instance, although correlations have been reported in mixed patient populations [10, 11], striatal DAT does not seem to correlate with the loss of dopamine-producing tyrosine hydroxylase (TH)-positive neurons in the substantia nigra pars compacta of PD patients, at least after some years of disease progression [12]. In addition, even though lower DAT binding at the early stages of PD has been reported to be associated with a higher UPDRS motor score later on [13], and the Hoehn & Yahr stage appears to be associated with DAT binding [14], dopaminergic imaging parameters do not strongly correlate with all clinical symptom-based ratings such as tremor [15, 16]. A recent study on patients with dementia with Lewy bodies (DLB) even suggested that DAT binding may show no correlation with the clinical disease severity of DLB as neither the H&Y stage nor the MMSE is correlated with DAT [17]. The mixed results suggest that correlations between DAT binding and clinical symptoms are generally modest and could even be absent in Lewy body spectrum diseases.

Our earlier results in PD patients scanned with 6-[18F]fluoro-L-DOPA (FDOPA) PET suggested that striatal [18-F]FDOPA uptake does not predict mortality [18]. The results of the 88 unmedicated PD patients scanned in the early stages of the disease showed that, while age and motor severity (Unified Parkinson's Disease Rating Scale Part III) were associated with survival, FDOPA uptake was not. It is important to acknowledge that marked compensatory upregulation of FDOPA uptake has been described in the early stages of PD [19-21], which could worsen the predictive value of FDOPA-PET. On the other hand, DAT reuptake does not seem to be associated with upregulation but rather with downregulation in PD, which could make DAT SPECT more sensitive for finding clinical correlates compared to FDOPA [19]. While FDOPA uptake reflects central aromatic L-amino acid decarboxylase (AADC) enzyme activity and dopamine storage in nerve terminals of the nigrostriatal dopaminergic pathway, [I-123]FP-CIT is a tracer that binds to DAT proteins in dopaminergic axons involved in the reuptake of synaptic dopamine [21]. In light of the questions of the cellular mechanisms related to increased mortality in PD, as well as the different functions of FDOPA and [I-123]FP-CIT in the measurement of the presynaptic dopaminergic system, and the possibility of up- and downregulation affecting these functions, we sought to investigate whether the level of DAT binding in [I-123]FP-CIT SPECT can predict survival in Parkinson’s disease in a large clinical sample of patients.

Patients and Methods

Study population

The study sample was part of a larger sample that included 559 patients consecutively scanned with [I-123]FP-CIT SPECT in the years 2007-2012 at Turku University Hospital, Finland due to clinically uncertain parkinsonism [16]. In this retrospective cohort study, one-hundred and sixty-two patients with clinically confirmed diagnoses of idiopathic PD at the end of the follow-up, abnormal DAT SPECT scans, and sufficient clinical information were selected as the final study sample (Figure 1). These patients were followed until death or the end of the follow-up, and the survivors were followed for a minimum of 3.8 years. The date of exit for the follow-up was set at October 4th 2016, whereas the entry date of the survival analysis was the date of imaging. Thus, the follow-up interval for the survival analysis ranged from 1 to 115 months (0.1 to 9.6 years), and the median follow-up interval was 69 months (5.8 years).

Clinical and demographic details at the time of imaging were collected, including gender, age at scan, anti-parkinsonian medication comprising levodopa daily dose and levodopa-equivalent daily dose (LEDD) [22], presence of cognitive impairment, and modified Hoehn & Yahr (H&Y) stage [23]. Out of the 162 patients, 96 (59.3%) were male and 66 (40.7%) female, and the mean (SD) age at the scan was 66.4 (10.4) years. Fifty-seven patients (35.2%) were treated with levodopa and/or other antiparkinsonian medications at the time of imaging with a median LEDD of 200 mg. Thirty-two patients (19.8%) were receiving levodopa, with a median daily dose of 300 mg, whereas 26 patients (16.0%) were receiving other antiparkinsonian drug(s), e.g., selegiline, ropinirole and pramipexole, and nine patients (5.6%) were receiving both. Thirty-three patients (20.4%) had a cognitive defect at the time of imaging on the basis of MMSE ($\leq$ 24) and/or clinical medical records of cognitive impairment. The clinical characteristics of the study sample are shown in Table 1. The original study, including its subparts, was approved by the ethical committee of the local hospital district and was conducted according to the principles of the Declaration of Helsinki.

Image analysis

The scans were analysed using an automated semi-quantitative method (BRASS, version 3.6, Hermes Medical Solutions, Stockholm, Sweden) as previously described [16]. The specific binding

ratio (SBR = [region-occipital]/occipital) of tracer binding in the posterior putamen of the most severely affected hemisphere was used as the marker of the level of dopaminergic defect. The median, bottom and upper SBR quartiles were calculated, and according to the quartiles, the level of putaminal dopaminergic defect was categorized under three categories. Category 1 included patients in the bottom quartile of SBR with the most severe dopaminergic defect (SBR < 1.37), category 2 included patients with an average DAT binding (SBR = 1.37-2.23), and category 3 included patients in the top quartile of SBR with a less severe dopaminergic defect (SBR > 2.23).

Survival analysis

Initially, Kaplan-Meier analyses were performed to investigate the effect of the following factors on survival: gender, modified H&Y stage, use of antiparkinsonian medication other than levodopa, levodopa daily dose in three categories (1. no levodopa, 2. levodopa dose < 300 mg, 3. levodopa dose ≥ 300 mg), LEDD in three categories (1. no medication, 2. LEDD < 200 mg, 3. LEDD ≥ 200 mg) and presence of cognitive defects at the time of imaging (Table 1). The modified H&Y scale was combined into the following three clinical categories: category 1 (H&Y 1, 1.5 and 2) including patients without impairment of balance, category 2 (H&Y 2.5 and 3) including patients with bilateral symptoms and (slight) postural impairment, and category 3 (H&Y 4 and 5) including patients with severe motor disability and postural impairment. First, univariate Cox regression was used to investigate the effect of factors on survival. Finally, factors significantly associated with survival in the univariate model as well as on the basis of clinical interest were included in multivariate Cox proportional hazard model. The multivariate model was also separately used to investigate the effect of these factors on survival in less advanced patients (H&Y stages 1, 1.5 and 2, n = 120). Results were expressed using hazard ratios (HR) with 95% confidence intervals (CI). IBM SPSS Statistics Version 24 (IBM Corp., New York, USA) was used in the survival analyses.

The association between survival and striatal and extrastriatal SBRs were also investigated voxel-by-voxel over the entire brain using Statistical Parametric Mapping software (SPM8; running in MatlabR2015a (Mathworks Inc., Natick, MA, USA). Out of the 162 patients, 14 were excluded from the SPM analysis because of an inappropriate field of view (i.e., midbrain cut out from the image), leaving 148 patients for the final sample. The images were warped to the Montreal Neurological Institute (MNI) space using an in-house [I-123]FP-CIT SPECT template as described earlier [24, 25]. The reference region uptake was extracted from the occipital cortex and SBR images were created

by dividing the original image voxel values by the reference region average uptake. Finally, the SBR images were smoothed using an 8 mm isotropic Gaussian kernel to improve the signal-to-noise ratio. The SBR images were then analysed using a general linear model (GLM). Subjects who had died by the 5-year follow-up were compared to subjects who were alive at 5 years after the scan date. In addition, an association between SBRs and scan-to-death intervals was analysed in non-surviving patients similarly using a GLM. Both analyses were conducted with and without covariates (age, H&Y category and cognitive defect) in the model. An analysis mask was used which limited the search volume to regions of the cerebral cortex, midbrain and pons where the tracer uptake exceeded the uptake in the reference region, as described earlier [25]. Family-wise error (FWE) correction was applied at the voxel- or cluster-level, and *P* values less than 0.05 were considered significant.

Results

The overall mortality was 25.9% (42 out of 162 patients). Kaplan-Meier estimate for mortality, which takes into account the censored observations, was 36%. The main demographics of the study population are shown in Table 1.

The use of antiparkinsonian drug(s) other than levodopa showed no significant association with survival in the univariate Cox regression analysis ($P = 0.11$), and this factor was left out of the multivariate Cox regression analysis. In the other univariate analyses, age at scan ($P < 0.001$), LEDD ($P = 0.01$), levodopa daily dose ($P < 0.001$), presence of cognitive defects ($P < 0.001$), modified H&Y combined into three categories ($P < 0.001$), and the categorical putaminal SBR of DAT binding ($P = 0.03$) were associated with survival, except gender ($P = 0.17$). LEDD and levodopa daily dose were highly correlated, and LEDD was chosen for the multivariate Cox regression model. The results of the multivariate Cox regression analysis are shown in Table 2, and the graphical survival functions are shown in Figure 2. No significant associations were found between the level of putaminal DAT binding and survival (Table 2, Figure 2a). Gender and LEDD categories were not associated with survival (Table 2). The mortality risk increased with age, and was clearly increased in patients with cognitive defects at the time of imaging (Table 2, Figure 2b). H&Y stage was associated with survival (Table 2, Figure 2c) as the mortality risk was increased in H&Y category 2 (stages 2.5 and 3) and category 3 (H&Y 4 and 5) compared to patients in H&Y category 1 (H&Y 1, 2.5 and 2) (Table 2, Figure 2c). Also in a separate analysis of 120 less advanced patients (H&Y 1, 1.5 and 2), no associations were found between the level of putaminal DAT binding and survival ($P = 0.76$). When including the disease duration to the multivariate model, none of the significant results changed to non-significant, or vice versa, and the symptom duration had no associations with survival ($P = 0.61$).

In the SPM analysis, there were no significant differences between any striatal or extrastriatal regional SBR between survivors and non-survivors at the 5-year time point. In addition, no statistically significant association was found between regional SBR and the scan-to-death interval in patients who died during the follow-up. The significance of the results did not change when adjusting for age, H&Y category and cognitive defect.

Discussion

The results of this study show no relationship between striatal or extrastriatal DAT binding and survival in PD. However, a higher mortality risk was associated with older age, cognitive defects and a greater motor symptom severity at the time of imaging, and these effects, supported by other studies [1-3, 5, 7], serve as positive controls verifying that the sample was quantitatively and qualitatively sufficient to demonstrate possible associations with DAT. Nevertheless, the independent significance of DAT in predicting mortality was clearly not significant ($P = 0.99$), which suggests that even a major increase in sample size would not have changed the essential result. It would therefore appear that, unlike age and clinical symptoms, striatal dopaminergic defects do not predict mortality in PD patients.

The results of this study are in line with our earlier findings of survival in a smaller sample of PD patients scanned with FDOPA-PET [18]. Thus, the different presynaptic dopaminergic target molecular mechanisms of [I-123]FP-CIT and FDOPA demonstrated no differences in the outcome. Although there may be a correlation between the clinical severity and dopaminergic tracer binding in the early stages of the disease in PD [13], these factors may separate when the disease progresses. The clinical symptom severity is tightly connected with clinical disability and death, whereas brain dopamine function may be influenced by numerous coexisting pathological and compensatory mechanisms complicating the interpretation. The exact mechanism of the decrease in striatal DAT function in PD detected with DAT SPECT remains unclear as it may reflect either lower endogenous dopamine levels, dysfunctional DAT expression or the degeneration of dopaminergic nigrostriatal nerve endings [19, 21]. There is recent evidence to suggest that DAT binding does not correlate with post mortem substantia nigra neuron counts [12], although it remains possible that the DAT tracer uptake reflects neuronal function or activity and not the number of neurons. The loss of dopamine neurons in the substantia nigra may lead to an increased synthesis and release of dopamine and increased dopaminergic activation in the surviving dopaminergic nerve endings, and the dopamine reuptake by DAT may correspondingly be downregulated in the striatum [19]. In addition, a recent study on mice models suggested that besides dopaminergic neurons, there are also striatal non-dopaminergic monoenzymatic neurons containing TH and AADC, the dopamine synthesis enzymes, and the production of dopamine in

these neurons increases as the dopamine deficiency rises in the degraded parkinsonian striatum [26]. Finally, although striatal DAT function loss is approximately 50% in the contralateral posterior putamen at the onset of clinical parkinsonism [27, 28], this varies between patients with equal mild hemi-parkinsonian symptoms [17, 28]. Likely, this variable individual dopaminergic threshold obscures later correlations with clinical end-points such as disability and death.

Mortality in PD is not necessarily directly associated with the underlying loss in the number of dopamine-producing neurons, but non-dopaminergic mechanisms may be more relevant. PD and other Lewy body diseases are associated with intraneuronal inclusions of alpha-synuclein protein [17, 29], and the rate in the accumulation of toxic alpha-synuclein aggregates could be a more important neuropathological event in predicting mortality. Indeed, recent neuropathological findings suggest that alpha-synuclein pathology, as well as the burden of neurofibrillary tangles and amyloid plaque pathology are the strongest pathological predictors of survival in synucleinopathies [29]. In PD associated with mixed Alzheimer's disease neuropathology, the increasing level of cerebral tau neurofibrillary tangles may shorten the lifespan [29]. There is also evidence that the deposition of nigral neurofibrillary tangles may induce parkinsonian symptoms [17]. The proteinopathic cascade to cortical regions could forecast disability and death more accurately than binding characteristics of striatal dopaminergic tracers.
In our study, cognitive impairment was related to a decreased survival as the mortality risk was over three-fold higher in patients with cognitive disturbances compared to those without cognitive impairment. Indeed, the presence of cognitive defects have previously been shown to be the greatest risk factors of higher mortality in PD [1, 2]. Although the cognitive decline may be hard to predict, it seems that even a mild cognitive dysfunction, with an incidence of up to 25% in de novo PD patients, is associated with a high risk of progression to dementia [29, 30]. The connection between cognition and mortality in PD suggests that cortical biomarkers, such as alpha-synuclein– and tau–sensitive PET-ligands, may be more accurate in predicting advancing disability than basal ganglia–specific dopamine tracers.

There are limitations in this study including the retrospective study design and the lack of neuropathological confirmation of PD diagnoses. However, the clinical PD diagnoses were confirmed both by abnormal DAT SPECT scans and clinically relevant follow-up periods. It should be noted that the mortality risk was higher in patients with H&Y stages 2.5 and 3 than in patients

with H&Y stages 4 and 5 (n = 5) probably due to the low number of patients with the highest severity stages. However, the differences between these categories was not significant, and the differences between severe and mild (H&Y 1, 1.5 and 2) patients were clear. Importantly, the correlation between DAT binding and survival remained non-significant when advanced patients (H&Y > 2) were excluded from the analysis, indicating that the results were not affected by the higher mortality probability of patients with the highest motor disability. Survival was not related to either dopaminergic medication or gender. The findings on the medication effect are generally in accordance with earlier studies [6], although there are discrepant results concerning the effect of gender on mortality in PD [1, 4, 5]. It should be noted that, as the patients in our study sample were scanned with DAT SPECT to aid in the clinical diagnostic workup, they may not be a representative sample of idiopathic PD in general population. It should also be noted that the evaluation of cognitive defects or dementia was not based on published validated criteria but solely on the documented cognitive disturbances as evaluated and/or tested by the treating clinicians using variable methods. Finally, mortality in PD may be affected by several other factors, such as presence of other systemic diseases, that were not included in the present model.

To conclude, we have shown that DAT SPECT imaging cannot be used as a clinical predictor of mortality in Parkinson's disease as the level of striatal dopaminergic defect in DAT SPECT does not influence long-term survival unlike older age, higher clinical motor symptom severity and presence of cognitive defects. Notably, even large differences in DAT SPECT findings (mild to severe abnormality in DAT binding) do not seem to be associated with future lifespan in PD patients. We must underline, however, that the results of the present study do not imply that the binding characteristics of dopaminergic radiotracers are irrelevant in diagnostics or clinical monitoring but, rather, that there may be compensatory changes and confounding factors in the nigrostriatal dopamine transmission in patients with PD, which lessen the value of functional dopaminergic imaging in predicting survival. The proteinopathic cascade to cortical regions might forecast disability and death more accurately than the nigrostriatal dopaminergic degeneration as measured with functional imaging.

Conflicts of interest

None.

Sources of Funding

This study was supported in part by the Finnish Parkinson Foundation, Turku University Foundation and Turku University Hospital (ERVA-funds).

Author contributions

E.M. was in charge of writing the first draft of the manuscript, took part in the organization and execution of the study as well as in the design, execution and review of the statistical analyses. V.K. carried out the conception of the study, took part in the organization and the execution of the study as well as in the design, execution, review and critique of the statistical analyses. J.J. and T.V. took part in the execution, review and critique of the statistical analyses. All of the authors took part in the review and critique of the manuscript and have approved the final version of the manuscript.

Table 1. The clinical characteristics of the study population at the time of the follow-up onset (the time of imaging).

	All	Survivors	Non-Survivors
N (%)	162	120 (74.1)	42 (25.9)
Age at scan, mean (SD), years	66.4 (10.4)	63.1 (9.3)	75.9 (7.1)
Gender (male/female)	96/66	68/52	28/14
Cognitive defect, n (%)	33 (20.4)	19 (15.8)	14 (33.3)
H&Y, median (range)	1.5 (4.0)	1.5 (3.0)	2.8 (4.0)
1, 1.5 and 2, n (%)	120 (74.1)	104 (86.7)	16 (38.1)
2.5 and 3, n (%)	25 (15.4)	11 (9.2)	14 (33.3)
4 and 5, n (%)	17 (10.5)	5 (4.2)	12 (28.6)
Levodopa, median (range), mg	300 (750)	300 (700)	300 (700)
No levodopa, n (%)	130 (80.2)	105 (87.5)	25 (59.5)
Levodopa daily dose < 300* mg, n (%)	10 (6.2)	4 (3.3)	6 (14.3)
Levodopa daily dose ≥ 300 mg, n (%)	22 (13.6)	11 (9.2)	11 (26.2)
LEDD, median (range), mg	200 (893)	142 (893)	300 (723)
No antiparkinsonian medication, n (%)	105 (64.8)	82 (68.3)	23 (54.8)
LEDD < 200** mg, n (%)	27 (16.7)	21 (17.5)	6 (14.3)
LEDD ≥ 200 mg, n (%)	30 (18.5)	16 (11.3)	13 (31.0)
Putamen SBR, median (range)	1.76 (2.83)	1.80 (2.79)	1.67 (2.13)
SBR < 1.37, n (%)	39 (24.1)	25 (20.8)	14 (33.3)
SBR = 1.37-2.23, n (%)	82 (50.6)	61 (50.8)	21 (50.0)
SBR >2.23, n (%)	41 (25.3)	34 (28.3)	7 (16.7)

SD = Standard deviation
n = number of patients
H&Y = Hoehn & Yahr stage. Category 1: modified H&Y stages 1, 1.5 and 2; category 2: modified H&Y stages 2.5 and 3; and category 3: modified H&Y stages 4 and 5.
LEDD = Levodopa equivalent daily dose.
SBR = Specific binding ratio of tracer [I-123]FP-CIT in the posterior putamen of the most severely affected hemisphere in the automated analysis (BRASS). Category 1: SBR < 1.37 (bottom quartile), category 2: SBR = 1.37-2.23, category 3: SBR > 2.23 (upper quartile).
*Median levodopa dose = Median daily dose of levodopa in patients that were receiving levodopa at the time of imaging.
**Median LEDD = Median levodopa equivalent daily dose of all antiparkinsonian medications in patients that were medicated with antiparkinsonian medications (levodopa and/or other antiparkinsonian medications) at the time of imaging.

Table 2. The association of the factors on survival in Parkinson's disease (n=162).

	Adjusted HR (95% CI)	*P*-value
Age at scan (years)	1.14* (1.09-1.20)	< 0.001
Male gender	1.84 (0.90-3.84)	0.10
Cognitive defect	3.35 (1.67-6.72)	0.001
Hoehn & Yahr		0.002
H&Y category 2 vs. 1	3.83 (1.75-8.36)	0.001
H&Y category 3 vs. 1	2.67 (1.11-6.39)	0.03
LEDD		0.10
LEDD <200 vs. no medication	0.60 (0.22-1.64)	0.32
LEDD ≥200 vs. no medication	1.79 (0.83-3.89)	0.14
Putamen SBR		0.99
Putamen SBR category 2 vs. 1	1.00 (0.49-2.05)	1.00
Putamen SBR category 3 vs. 1	0.95 (0.35-2.58)	0.91

HR = Hazard ratio for mortality after adjustment for other factors in the model
CI = Confidence interval
*HR for one-year increase in age
H&Y category 1: modified H&Y stages 1, 1.5 and 2; category 2: modified H&Y stages 2.5 and 3; category 3: modified H&Y stages 4 and 5.
LEDD = Levodopa equivalent daily dose.
SBR = Specific binding ratio of tracer [I-123]FP-CIT in the posterior putamen of the most severely affected hemisphere in the automated analysis (BRASS). Category 1: SBR < 1.37, category 2: SBR = 1.37-2.23, category 3: SBR > 2.23.

Figure 1. Study flow chart. One-hundred and sixty-two patients with clinically confirmed diagnoses of idiopathic Parkinson's disease (PD), abnormal DAT SPECT scans and sufficient clinical information were selected as the study sample from a larger sample of patients scanned with [I-123]FP-CIT SPECT due to clinically uncertain parkinsonism. DAT = dopamine transporter, DLB = dementia with Lewy bodies, PSP = progressive supranuclear palsy, MSA = multiple system atrophy, CBD = corticobasal degeneration, BRASS = automated semi-quantitative analysis method of scans.

Figure 2. Survival function graphs showing the independent impact of three factors on survival in patients with PD, after adjusting each factor for other variables in the multivariate Cox regression model. Cumulative survival is plotted against the clinical follow-up time. A = Specific binding ratio (SBR) of tracer [I-123]FP-CIT in the posterior putamen of the most severely affected hemisphere in the automated analysis (BRASS) (note that the three curves are nearly identical); B = Patients with and without cognitive impairment at the time of imaging; C = Hoehn & Yahr stage combined into three clinical categories at the time of imaging.

References

[1] Macleod AD, Taylor KS, Counsell CE. Mortality in Parkinson's disease: a systematic review and meta-analysis. Mov Disord 2014;29:1615-22.

[2] Posada IJ, Benito-León J, Louis ED, Trincado R, Villarejo A, Medrano MJ, et al. Mortality from Parkinson's disease: a population-based prospective study (NEDICES). Mov Disord 2011;26:2522-9.

[3] Levy G, Tang MX, Louis ED, Côté LJ, Alfaro B, Mejia H, et al. The association of incident dementia with mortality in PD. Neurology 2002;59:1708-13.

[4] Hely MA, Morris JG, Traficante R, Reid WG, O'Sullivan DJ, Williamson PM. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 1999;67:300-7.

[5] de Lau LM, Verbaan D, Marinus J, van Hilten JJ. Survival in Parkinson's disease. Relation with motor and non-motor features. Parkinsonism Relat Disord 2014;20:613-6.

[6] Olanow CW. Levodopa: effect on cell death and the natural history of Parkinson's disease. Mov Disord 2015;30:37-44.

[7] Lo RY, Tanner CM, Albers KB, Leimpeter AD, Fross RD, Bernstein AL, et al. Clinical features in early Parkinson disease and survival. Arch Neurol 2009;66:1353-8.

[8] Willis AW, Schootman M, Kung N, Evanoff BA, Perlmutter JS, Racette BA. Predictors of survival in patients with Parkinson disease. Arch Neurol 2012;69:601-7.

[9] Ba F, Martin WR. Dopamine transporter imaging as a diagnostic tool for parkinsonism and related disorders in clinical practice. Parkinsonism Relat Disord 2015;21:87-94.

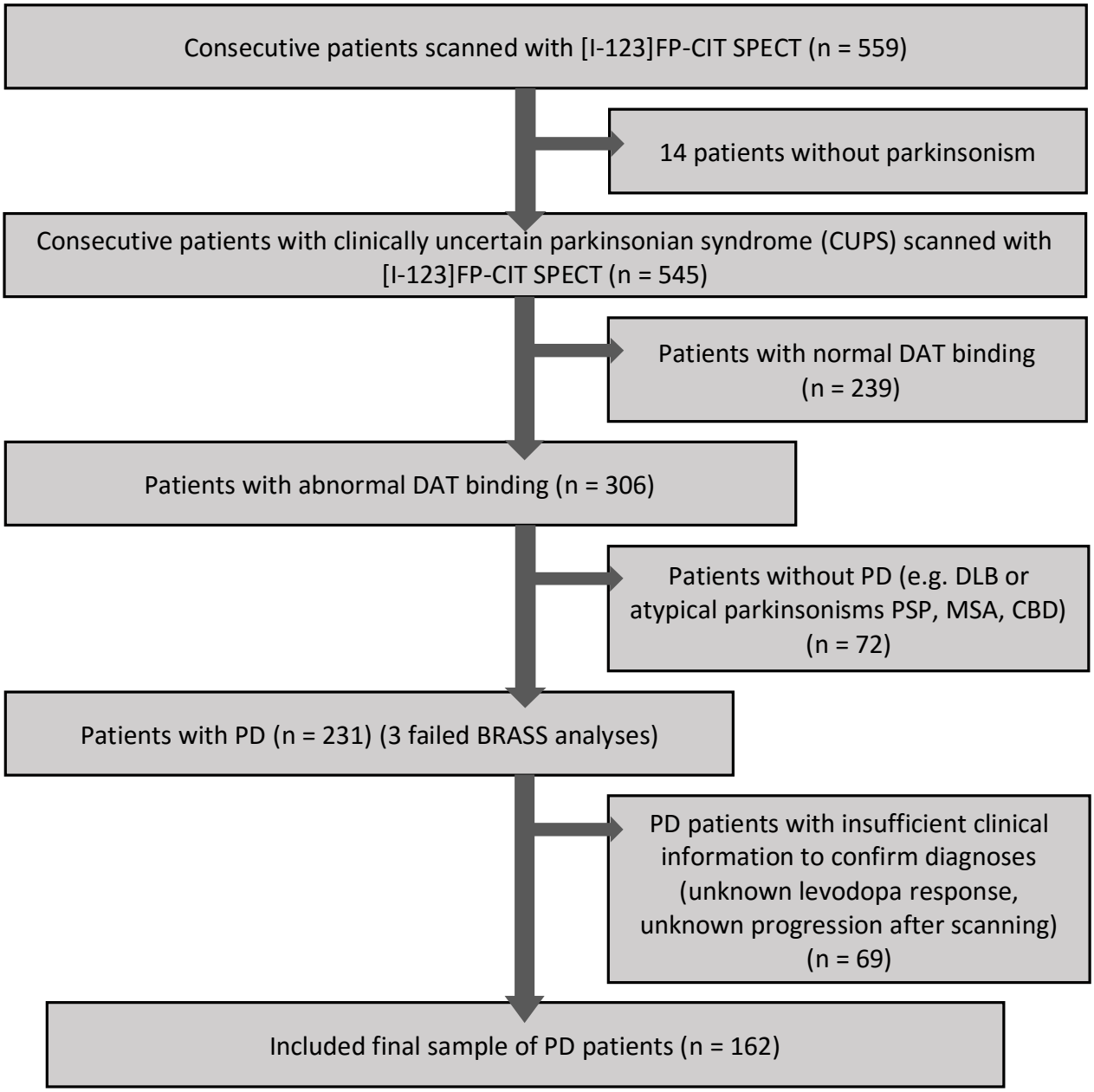
[10] Colloby SJ, McParland S, O'Brien JT, Attems J. Neuropathological correlates of dopaminergic imaging in Alzheimer's disease and Lewy body dementias. Brain 2012;135:2798-808.

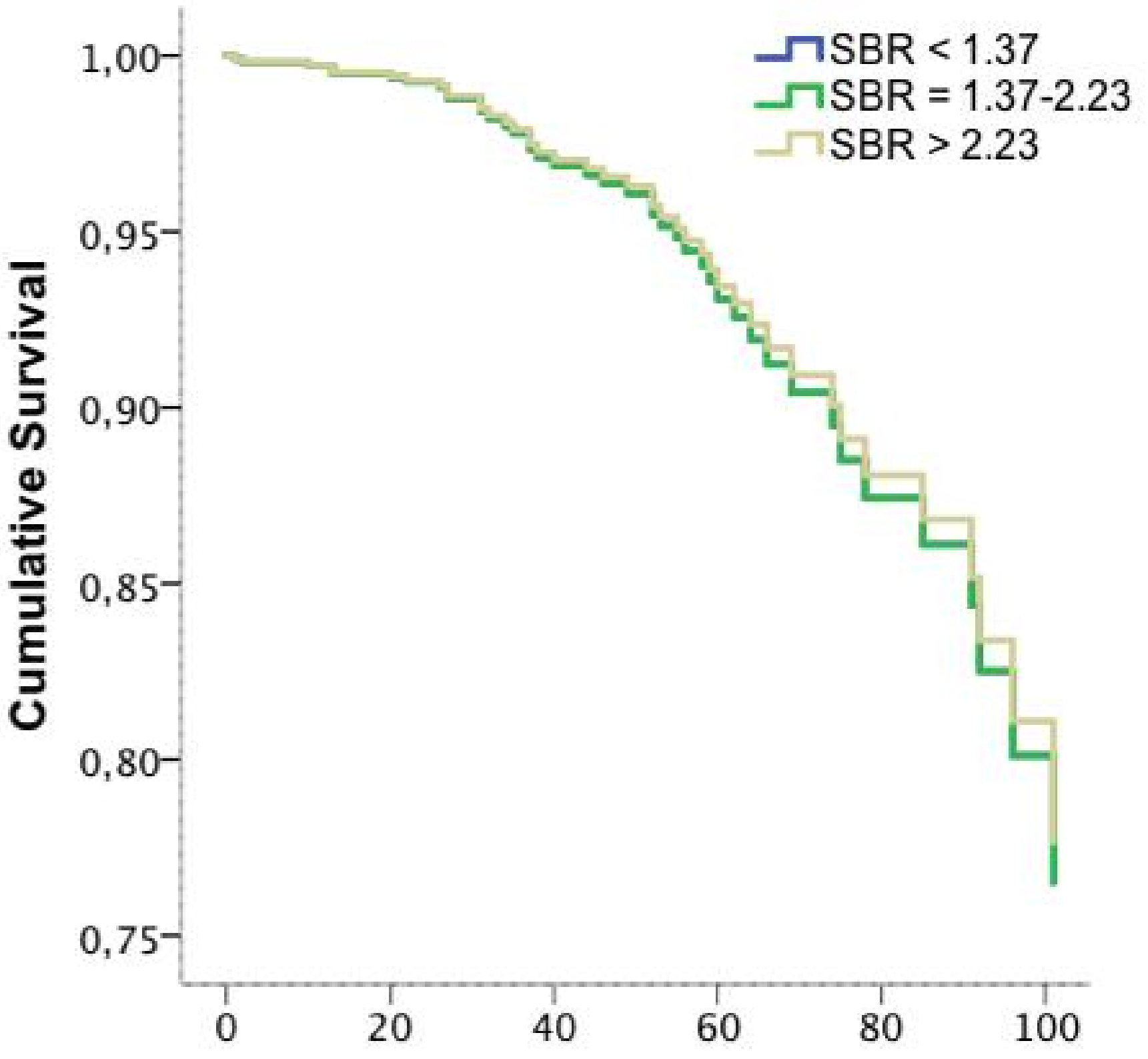
[11] Kraemmer J, Kovacs GG, Perju-Dumbrava L, Pirker S, Traub-Weidinger T, Pirker W. Correlation of striatal dopamine transporter imaging with post mortem substantia nigra cell counts. Mov Disord 2014;29:1767-73.

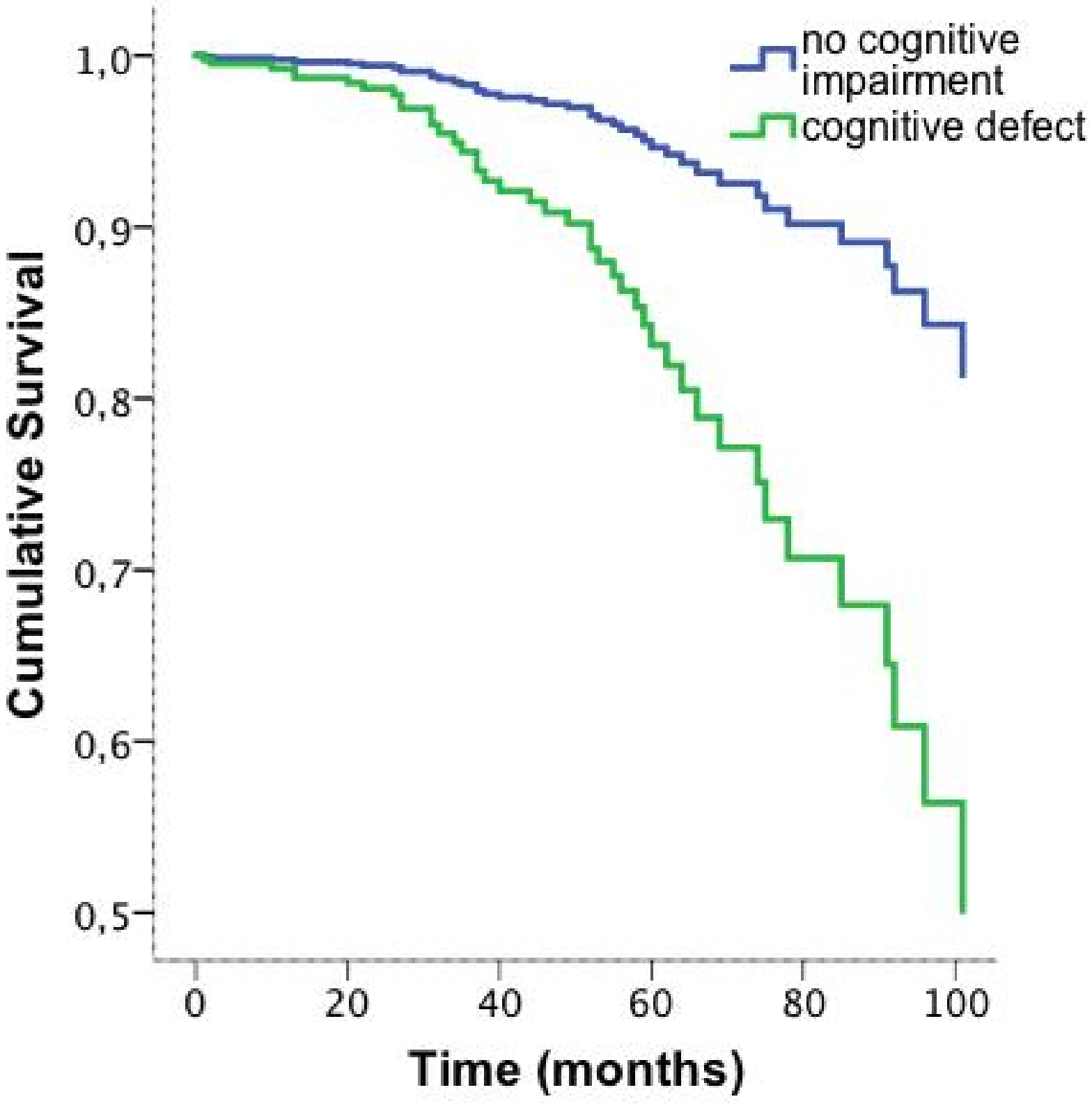
[12] Saari L, Kivinen K, Gardberg M, Joutsa J, Noponen T, Kaasinen V. Dopamine transporter imaging does not predict the number of nigral neurons in Parkinson disease. Neurology 2017;88:1461-7.

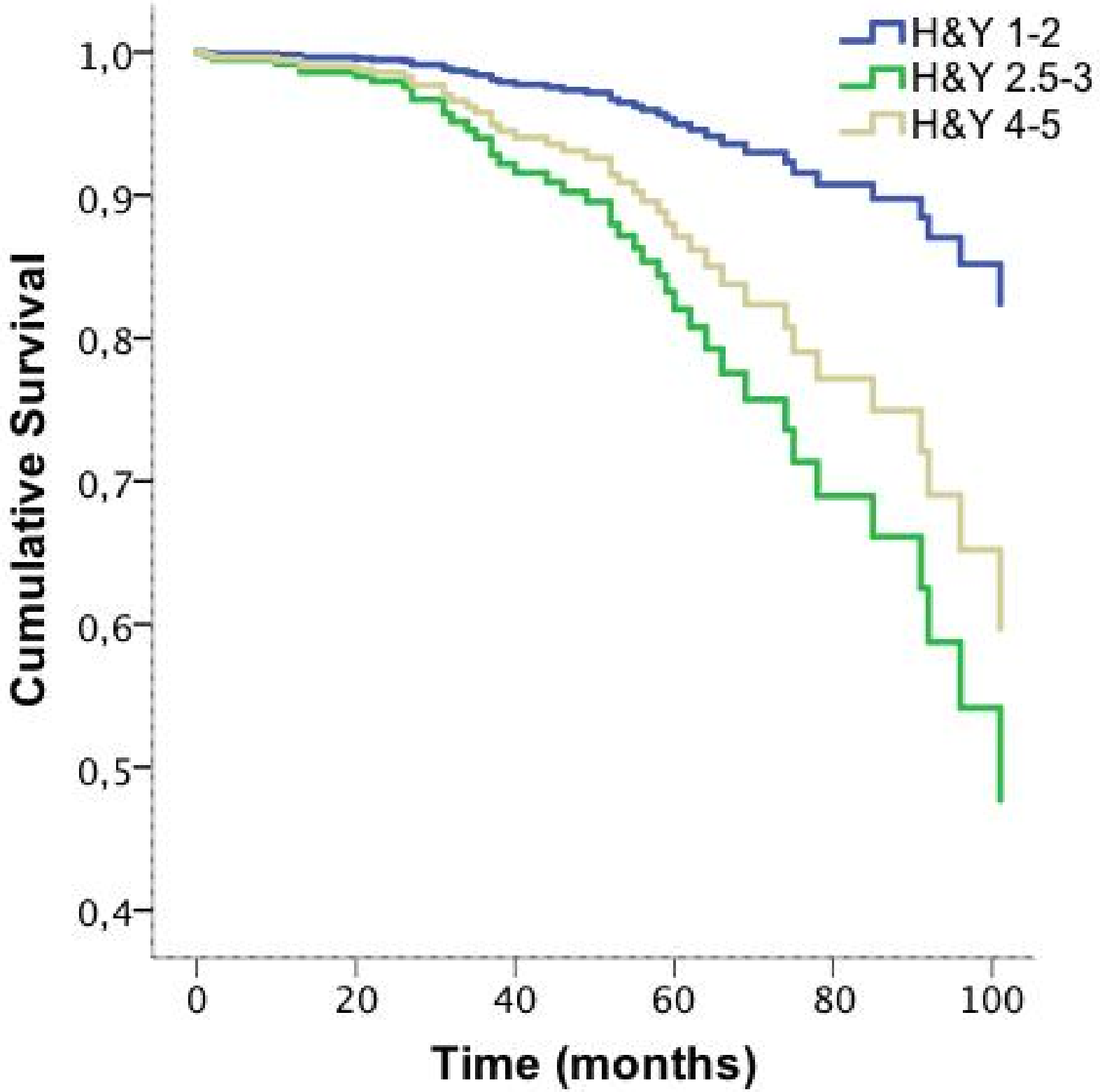
[13] Ravina B, Marek K, Eberly S, Oakes D, Kurlan R, Ascherio A, et al. Dopamine transporter imaging is associated with long-term outcomes in Parkinson's disease. Mov Disord 2012;27:1392-7.
[14] Staffen W, Mair A, Unterrainer J, Trinka E, Ladurner G. Measuring the progression of idiopathic Parkinson's disease with [123I] beta-CIT SPECT. J Neural Transm (Vienna) 2000;107:543-52.
[15] Djaldetti R, Treves TA, Ziv I, Melamed E, Lampl Y, Lorberboym M. Use of a single [123I]-FP-CIT SPECT to predict the severity of clinical symptoms of Parkinson disease. Neurol Sci 2009;30:301-5.
[16] Kaasinen V, Kinos M, Joutsa J, Seppänen M, Noponen T. Differences in striatal dopamine transporter density between tremor dominant and non-tremor Parkinson's disease. Eur J Nucl Med Mol Imaging 2014;41:1931-7.
[17] Ziebell M, Andersen BB, Pinborg LH, Knudsen GM, Stokholm J, Thomsen G, et al. Striatal dopamine transporter binding does not correlate with clinical severity in dementia with Lewy bodies. J Nucl Med 2013;54:1072-6.
[18] Järvelä JT, Rinne JO, Eskola O, Kaasinen V. Mortality in Parkinson's disease is not associated with the severity of early dopaminergic defect. Parkinsonism Relat Disord 2014;20:894-7.
[19] Lee CS, Samii A, Sossi V, Ruth TJ, Schulzer M, Holden JE, et al. In vivo positron emission tomographic evidence for compensatory changes in presynaptic dopaminergic nerve terminals in Parkinson's disease. Ann Neurol 2000;47:493-503.
[20] Ribeiro MJ, Vidailhet M, Loc'h C, Dupel C, Nguyen JP, Ponchant M, et al. Dopaminergic function and dopamine transporter binding assessed with positron emission tomography in Parkinson disease. Arch Neurol 2002;59:580-6.
[21] Eshuis SA, Jager PL, Maguire RP, Jonkman S, Dierckx RA, Leenders KL. Direct comparison of FP-CIT SPECT and F-DOPA PET in patients with Parkinson's disease and healthy controls. Eur J Nucl Med Mol Imaging 2009;36:454-62.
[22] Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010;25:2649-53.
[23] Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, et al. Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord 2004;19:1020-8.
[24] Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage 2007;38:95-113.

[25] Joutsa J, Johansson J, Kaasinen V. Is Occipital Cortex a Valid Reference Region in 123I-FP-CIT SPECT Imaging? Clin Nucl Med 2015;40:615-6.
[26] Kozina EA, Kim AR, Kurina AY, Ugrumov MV. Cooperative synthesis of dopamine by non-dopaminergic neurons as a compensatory mechanism in the striatum of mice with MPTP-induced Parkinsonism. Neurobiol Dis 2016;98:108-21.
[27] Brooks DJ, Tambasco N. Imaging synucleinopathies. Mov Disord 2016;31:814-29.
[28] Marek KL, Seibyl JP, Zoghbi SS, Zea-Ponce Y, Baldwin RM, Fussell B, et al. [123I] beta-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. Neurology 1996;46:231-7.
[29] Irwin DJ, Grossman M, Weintraub D, Hurtig HI, Duda JE, Xie SX, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. Lancet Neurol 2017;16:55-65.
[30] Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. Lancet Neurol 2017;16:66-75.


Consecutive patients scanned with [I-123]FP-CIT SPECT (n = 559)
14 patients without parkinsonism
Consecutive patients with clinically uncertain parkinsonian syndrome (CUPS) scanned with [I-123]FP-CIT SPECT (n = 545)
Patients with normal DAT binding (n = 239)
Patients with abnormal DAT binding (n = 306)
Patients without PD (e.g. DLB or atypical parkinsonisms PSP, MSA, CBD) (n = 72)
Patients with PD (n = 231) (3 failed BRASS analyses)
PD patients with insufficient clinical information to confirm diagnoses (unknown levodopa response, unknown progression after scanning) (n = 69)
Included final sample of PD patients (n = 162)


SBR < 1.37
SBR = 1.37-2.23
SBR > 2.23
Cumulative Survival
1,00
0,95
0,90
0,85
0,80
0,75
0
20
40
60
80
100


no cognitive impairment
cognitive defect
Cumulative Survival
1,0
0,9
0,8
0,7
0,6
0,5
0
20
40
60
80
100
Time (months)


H&Y 1-2
H&Y 2.5-3
H&Y 4-5
Cumulative Survival
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0
20
40
60
80
100
Time (months)

Elsevier Editorial System(tm) for
Parkinsonism & Related Disorders
Manuscript Draft

Manuscript Number: PARKRELDIS-D-17-00176

Title: Survival in Parkinson's disease in relation to striatal dopamine transporter binding

Article Type: Full Length Article

Keywords: Parkinson's disease; SPECT; dopamine; survival; mortality

Corresponding Author: Ms. Elina Mäkinen, Bachelor of Medicine, PhD Student

Corresponding Author's Institution: University of Turku and Turku University Hospital, Turku, Finland

First Author: Elina Mäkinen, Bachelor of Medicine, PhD Student

Order of Authors: Elina Mäkinen, Bachelor of Medicine, PhD Student; Juho Joutsa, PhD; Tero Vahlberg, MSc; Valtteri Kaasinen, PhD

Abstract: Objective

To investigate whether dopamine transporter (DAT) binding, as measured with single photon emission computed tomography (SPECT), can be used to predict mortality in patients with Parkinson's disease (PD).

Methods

A total of 162 patients with PD and abnormal [I-123]FP-CIT SPECT were clinically followed for a median of 5.8 years. A multivariate Cox regression model was used to investigate survival with the independent predictors of age, gender, severity of motor impairment, levodopa-equivalent daily dose of medication, presence of cognitive defects, and putaminal specific binding ratio (SBR) of [I-123]FP-CIT. In addition, associations between striatal and extrastriatal SBRs and survival were investigated using voxel-based analyses.

Results

The Kaplan-Meier estimate for mortality was 36%. Higher age ($P < 0.001$), presence of cognitive defects ($P = 0.001$), and more severe motor symptom severity ($P = 0.002$) were significantly associated with increased mortality. No associations were found between putaminal DAT binding and survival ($P = 0.99$). There were no significant differences in SBRs in any striatal or extrastriatal region between survivors and non-survivors, and no associations were found between SBRs and scan-to-death intervals among non-survivors.

Conclusions

Unlike the severity of motor and cognitive symptoms, the level of striatal dopaminergic defect in DAT SPECT does not predict mortality in PD. Although presynaptic dopaminergic functional imaging may have value

as a diagnostic tool, the clinical symptom-based characteristics are superior for predicting lifespan.

Author Declaration

Parkinsonism & Related Disorders is committed to proper scientific conduct and the protection of animal and human research subjects. Submission of this manuscript implies compliance with the following ethical requirements. Please affirm that you are representing all of the authors in stating compliance with these policies by checking the box at the end of this section.

1. Studies with human subjects must have been conducted in accordance with the Declaration of Helsinki. All persons must have provided informed consent prior to being included in the study.

2. Studies with animal subjects must have been conducted in accordance with the Guide for the Care and Use of Laboratory Subjects as adopted by the US National Institutes of Health and/or according to the requirements of all applicable local, national and international standards.

3. Protocols with animal or human subjects must have been approved by the relevant local committee(s) charged with ensuring subject protection. Studies that entail pain or distress will be assessed in terms of the balance between the distress inflicted and the likelihood of benefit.

4. The authors declare that the manuscript is original, that it is not being considered for publication elsewhere, and that it will not be submitted elsewhere while still under consideration for Parkinsonism & Related Disorders or after it has been accepted by Parkinsonism & Related Disorders.

5. All authors have seen and approved the manuscript in the form submitted to the journal. The authors declare that they have conformed to the highest standards of ethical conduct in the submission of accurate data and that they acknowledge the work of others when applicable.

6. All sources of financial support for the work have been declared in the Acknowledgements section of the manuscript. Any additional conflicts of interest must also be declared. Please include declarations of any consultancy or research funding received from relevant companies from three years prior to performance of the research until the time of manuscript submission. If the research is supported by internal funds, that should be stated as well.

To indicate compliance with the preceding declaration and that you have obtained agreement from all of the authors of this paper to declare their compliance as well, please place an x here: X___

In cases of uncertainty please contact an editor for advice.

*Cover Letter

R.F. Pfeiffer and Z.W. Wszolek,

Editors-in-Chief

Parkinsonism & Related Disorders

26th February 2017

Dear Prof. Pfeiffer and Prof. Wszolek,

We would like to submit our manuscript "*Survival in Parkinson's disease in relation to striatal dopamine transporter binding*" for publication as a *Full-length article* in *Parkinsonism & Related disorders.*

Our study shows that striatal dopamine transporter (DAT) binding, as measured with [I-123]FP-CIT SPECT, cannot be used as a predictor of mortality in Parkinson's disease as there is no relationship between the level of striatal DAT binding and survival. We feel that our study is important as it uncovers complex mortality mechanisms in Parkinson's disease that are probably more related to proteinopathic than dopaminergic events.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. No related papers from the same study have been published or submitted. E. Mäkinen was in charge of writing the first draft of the manuscript, took part in the organization and execution of the study as well as in the design, execution and review of the statistical analyses. V. Kaasinen carried out the conception of the study, took part in the organization and the execution of the study as well as in the design, execution, review and critique of the statistical analyses. J. Joutsa and T. Vahlberg took part in the execution, review and critique of the statistical analyses. All of the authors took part in the review and critique of the manuscripts, have accepted the final manuscript and agree with submission and publication in *Parkinsonism & Related Disorders*. There are no ghost writers. The authors declare no conflicts of interest relevant to this work. This study was supported in part by the Finnish Parkinson Foundation, Turku University

Foundation and Turku University Hospital (ERVA-funds). The study was approved by the ethical committee of the local hospital district and was conducted according to the principles of Helsinki.

Please address all correspondence to:

Dr. Elina Mäkinen

Division of Clinical Neurosciences

University of Turku and Turku University Hospital

POB 52, FIN-20521

Turku, Finland

Tel. +358-2-3130000

Email: elmama@utu.fi

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Elina Mäkinen

Highlights

- The level of dopamine transporter binding has no effect on survival in Parkinson's disease.
- Motor and cognitive symptoms at the time of DAT imaging are associated with future survival in PD.
- Mortality in PD may be more related to proteinopathic than dopaminergic events.

Survival in Parkinson's disease in relation to striatal dopamine transporter binding

Elina Mäkinen [a], Juho Joutsa [a, b c, d], Tero Vahlberg [e], Valtteri Kaasinen [a, d]

[a] Division of Clinical Neurosciences, University of Turku and Turku University Hospital, Turku, Finland
[b] Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[c] Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA
[d] Turku PET Centre, University of Turku, Turku, Finland
[e] Department of Biostatistics, University of Turku and Turku University Hospital, Turku, Finland

Corresponding author:
Dr. Elina Mäkinen
Division of Clinical Neurosciences,
University of Turku and Turku University Hospital
POB 52, FIN-20521
Turku, Finland
Tel. +358-2-3130000
Email: elmama@utu.fi

Key words: Parkinson's disease; SPECT; dopamine; survival; mortality

Abstract

Objective

To investigate whether dopamine transporter (DAT) binding, as measured with single photon emission computed tomography (SPECT), can be used to predict mortality in patients with Parkinson's disease (PD).

Methods

A total of 162 patients with PD and abnormal [I-123]FP-CIT SPECT were clinically followed for a median of 5.8 years. A multivariate Cox regression model was used to investigate survival with the independent predictors of age, gender, severity of motor impairment, levodopa-equivalent daily dose of medication, presence of cognitive defects, and putaminal specific binding ratio (SBR) of [I-123]FP-CIT. In addition, associations between striatal and extrastriatal SBRs and survival were investigated using voxel-based analyses.

Results

The Kaplan-Meier estimate for mortality was 36%. Higher age ($P < 0.001$), presence of cognitive defects ($P = 0.001$), and more severe motor symptom severity ($P = 0.002$) were significantly associated with increased mortality. No associations were found between putaminal DAT binding and survival ($P = 0.99$). There were no significant differences in SBRs in any striatal or extrastriatal region between survivors and non-survivors, and no associations were found between SBRs and scan-to-death intervals among non-survivors.

Conclusions

Unlike the severity of motor and cognitive symptoms, the level of striatal dopaminergic defect in DAT SPECT does not predict mortality in PD. Although presynaptic dopaminergic functional imaging may have value as a diagnostic tool, the clinical symptom-based characteristics are superior for predicting lifespan.

Introduction

Parkinson's disease (PD) is associated with increased mortality as the mortality hazard ratio in PD patients is generally between 1.2 and 2.4 compared to in individuals without PD [1]. There is evidence of factors that are associated with the increased mortality in PD, such as cognitive impairment and an older age at the disease onset [1-3] with mixed results concerning the influence of gender on mortality [1, 4, 5]. A recent meta-analysis found no evidence to suggest that the use of levodopa reduces mortality [6]. From a clinical perspective, increased mortality in PD appears to be connected to the phenotype of postural instability and gait disorder (PIGD), symmetricity of motor signs and the general severity of the motor symptoms [5, 7]. Although demographic and clinical factors impact survival in PD [8], identification of the primary neurobiological survival-related mechanisms is important for the development of treatments that aim to increase lifespan in patients with PD.

Brain dopamine transporter (DAT) imaging is mainly used during the early diagnostic phase of clinically uncertain parkinsonism, with [I-123]FP-CIT SPECT being the most widely used method [9]. DAT imaging has been shown to separate PD patients from healthy controls with high precision [9], but the exact mechanism and relevance of the striatal DAT binding defect is unclear. For instance, although correlations have been reported in mixed patient populations [10, 11], striatal DAT does not seem to correlate with the loss of dopamine-producing tyrosine hydroxylase (TH)-positive neurons in the substantia nigra pars compacta of PD patients, at least after some years of disease progression [12]. In addition, even though lower DAT binding at the early stages of PD has been reported to be associated with a higher UPDRS motor score later on [13], and the Hoehn & Yahr stage appears to be associated with DAT binding [14], dopaminergic imaging parameters do not strongly correlate with all clinical symptom-based ratings such as tremor [15, 16]. A recent study on patients with dementia with Lewy bodies (DLB) even suggested that DAT binding may show no correlation with the clinical disease severity of DLB as neither the H&Y stage nor the MMSE is correlated with DAT [17]. The mixed results suggest that correlations between DAT binding and clinical symptoms are generally modest and could even be absent in Lewy body spectrum diseases.

Our earlier results in PD patients scanned with 6-[18F]fluoro-L-DOPA (FDOPA) PET suggested that striatal [18-F]FDOPA uptake does not predict mortality [18]. The results of the 88 unmedicated PD patients scanned in the early stages of the disease showed that, while age and motor severity (UPDRS) were associated with survival, FDOPA uptake was not. It is important to acknowledge that marked compensatory upregulation of FDOPA uptake has been described in the early stages of PD [19-21], which could worsen the predictive value of FDOPA-PET. On the other hand, DAT reuptake does not seem to be associated with upregulation but rather with downregulation in PD, which could make DAT SPECT more sensitive for finding clinical correlates compared to FDOPA [19]. While FDOPA uptake reflects central aromatic L-amino acid decarboxylase (AADC) enzyme activity and dopamine storage in nerve terminals of the nigrostriatal dopaminergic pathway, [I-123]FP-CIT is a tracer that binds to DAT proteins in dopaminergic axons involved in the reuptake of synaptic dopamine [21]. In light of the questions of the cellular mechanisms related to increased mortality in PD, as well as the different functions of FDOPA and [I-123]FP-CIT in the measurement of the presynaptic dopaminergic system, and the possibility of up- and downregulation affecting these functions, we sought to investigate whether the level of DAT binding in [I-123]FP-CIT SPECT can predict survival in Parkinson's disease in a large clinical sample of patients.

Patients and Methods

Study population

The original study sample included 559 patients scanned with [I-123]FP-CIT SPECT in the years 2007-2012 at Turku University Hospital, Finland due to clinically uncertain parkinsonism [16]. One-hundred and sixty-two patients with clinically confirmed diagnoses of idiopathic PD at the end of the follow-up, abnormal DAT SPECT scans, and sufficient clinical information were selected as the final study sample. These patients were followed until death or the end of the follow-up, and the survivors were followed for a minimum of 3.8 years. The date of exit for the follow-up was set at October 4th 2016, whereas the entry date of the survival analysis was the date of imaging. Thus, the follow-up interval for the survival analysis ranged from 1 to 115 months (0.1 to 9.6 years), and the median follow-up interval was 69 months (5.8 years).

Clinical and demographic details at the time of imaging were collected, including gender, age at scan, anti-parkinsonian medication comprising levodopa daily dose and levodopa-equivalent daily dose (LEDD) [22], presence of cognitive impairment or dementia, and modified Hoehn & Yahr (H&Y) stage [23]. Out of the 162 patients, 96 (59.3%) were male and 66 (40.7%) female, and the mean (SD) age at the scan was 66.4 (10.4) years. Fifty-seven patients (35.2%) were treated with antiparkinsonian medications at the time of imaging with a median LEDD of 200 mg. Thirty-two patients (19.8%) were receiving levodopa, with a median daily dose of 300 mg, whereas 26 patients (16.0%) were receiving other antiparkinsonian drug(s), e.g., selegiline, ropinirole and pramipexole, and nine patients (5.6%) were receiving both. Thirty-three patients (20.4%) had a cognitive defect at the time of imaging on the basis of MMSE ($\leq$ 24) and/or clinical medical records of cognitive impairment. The clinical characteristics of the study sample are shown in Table 1. The study was approved by the ethical committee of the local hospital district and was conducted according to the principles of the Declaration of Helsinki.

Image analysis

The scans were analysed using an automated semi-quantitative method (BRASS, version 3.6, Hermes Medical Solutions, Stockholm, Sweden) as previously described [16]. The specific binding ratio (SBR = [region-occipital]/occipital) of tracer binding in the posterior putamen of the most

severely affected hemisphere was used as the marker of the level of dopaminergic defect. The median, bottom and upper SBR quartiles were calculated, and according to the quartiles, the level of putaminal dopaminergic defect was categorized under three categories. Category 1 included patients in the bottom quartile of SBR with the most severe dopaminergic defect (SBR < 1.37), category 2 included patients with an average DAT binding (SBR = 1.37-2.23), and category 3 included patients in the top quartile of SBR with a less severe dopaminergic defect (SBR > 2.23).

Survival analysis

Initially, Kaplan-Meier analyses were performed to investigate the effect of the following factors on survival: gender, modified H&Y stage, use of antiparkinsonian medication other than levodopa, levodopa daily dose in three categories (1. no levodopa, 2. levodopa dose < 300 mg, 3. levodopa dose $\geq$ 300 mg), LEDD in three categories (1. no medication, 2. LEDD < 200 mg, 3. LEDD $\geq$ 200 mg) and presence of cognitive defects at the time of imaging (Table 1). The modified H&Y scale was combined into the following three clinical categories: category 1 (H&Y 1, 1.5 and 2) including patients without impairment of balance, category 2 (H&Y 2.5 and 3) including patients with bilateral symptoms and (slight) postural impairment, and category 3 (H&Y 4 and 5) including patients with severe motor disability and postural impairment. First, univariate Cox regression was used to investigate the effect of factors on survival. Finally, factors significantly associated with survival in the univariate model as well as on the basis of clinical interest were included in multivariate Cox proportional hazard model. The multivariate model was also separately used to investigate the effect of these factors on survival in less advanced patients (H&Y stages 1, 1.5 and 2, n = 120). Results were expressed using hazard ratios (HR) with 95% confidence intervals (CI). IBM SPSS Statistics Version 24 (IBM Corp., New York, USA) was used in the survival analyses.

The association between survival and striatal and extrastriatal SBRs were also investigated voxel-by-voxel over the entire brain using Statistical Parametric Mapping software (SPM8; running in MatlabR2015a (Mathworks Inc., Natick, MA, USA). Out of the 162 patients, 14 were excluded from the SPM analysis because of an inappropriate field of view (i.e., midbrain cut out from the image), leaving 148 patients for the final sample. The images were warped to the Montreal Neurological Institute (MNI) space using an in-house [I-123]FP-CIT SPECT template as described earlier [24, 25]. The reference region uptake was extracted from the occipital cortex and SBR images were created by dividing the original image voxel values by the reference region average uptake. Finally, the SBR

images were smoothed using an 8 mm isotropic Gaussian kernel to improve the signal-to-noise ratio. The SBR images were then analysed using a general linear model (GLM). Subjects who had died by the 5-year follow-up were compared to subjects who were alive at 5 years after the scan date. In addition, an association between SBRs and scan-to-death intervals was analysed in non-surviving patients similarly using a GLM. Both analyses were conducted with and without covariates (age, H&Y category and cognitive defect) in the model. An analysis mask was used which limited the search volume to regions of the cerebral cortex, midbrain and pons where the tracer uptake exceeded the uptake in the reference region, as described earlier [25]. Family-wise error (FWE) correction was applied at the voxel- or cluster-level, and *P* values less than 0.05 were considered significant.

Results

The Kaplan-Meier estimate for mortality was 36%. The main demographics of the study population are shown in Table 1.

The use of antiparkinsonian drug(s) other than levodopa showed no significant association with survival in the univariate Cox regression analysis ($P = 0.11$), and this factor was left out of the multivariate Cox regression analysis. In the other univariate analyses, age at scan ($P < 0.001$), LEDD ($P = 0.01$), levodopa daily dose ($P < 0.001$), presence of cognitive defects ($P < 0.001$), modified H&Y combined into three categories ($P < 0.001$), and the categorical putaminal SBR of DAT binding ($P = 0.03$) were associated with survival, except gender ($P = 0.17$). LEDD and levodopa daily dose were highly correlated, and LEDD was chosen for the multivariate Cox regression model. The results of the multivariate Cox regression analysis are shown in Table 2, and the graphical survival functions are shown in Figure 1. No significant associations were found between the level of putaminal DAT binding and survival (Table 2, Figure 1a). Gender and LEDD categories were not associated with survival (Table 2). The mortality risk increased with age, and was clearly increased in patients with cognitive defects at the time of imaging (Table 2, Figure 1b). H&Y stage was associated with survival (Table 2, Figure 1c) as the mortality risk was increased in H&Y category 2 (stages 2.5 and 3) and category 3 (H&Y 4 and 5) compared to patients in H&Y category 1 (H&Y 1, 2.5 and 2) (Table 2, Figure 1c). Also in the separate analysis of 120 less advanced patients (H&Y 1, 1.5 and 2), no associations were found between the level of putaminal DAT binding and survival ($P = 0.76$).

In the SPM analysis, there were no significant differences between any striatal or extrastriatal regional SBR between survivors and non-survivors at the 5-year time point. In addition, no statistically significant association was found between regional SBR and the scan-to-death interval in patients who died during the follow-up. The significance of the results did not change when adjusting for age, H&Y category and cognitive defect.

Discussion

The results of this study show no relationship between striatal or extrastriatal DAT binding and survival in PD. However, a higher mortality risk was associated with older age, cognitive defects and a greater motor symptom severity at the time of imaging, and these effects, supported by other studies [1-3, 5, 7], serve as positive controls verifying that the sample was quantitatively and qualitatively sufficient to demonstrate possible associations with DAT. Nevertheless, the independent significance of DAT in predicting mortality was clearly not significant ($P = 0.99$), which suggests that even a major increase in sample size would not have changed the essential result. It would therefore appear that, unlike age and clinical symptoms, striatal dopaminergic defects do not predict mortality in PD patients.

The results of this study are in line with our earlier findings of survival in a smaller sample of PD patients scanned with FDOPA-PET [18]. Thus, the different presynaptic dopaminergic target molecular mechanisms of [I-123]FP-CIT and FDOPA demonstrated no differences in the outcome. Although there may be a correlation between the clinical severity and dopaminergic tracer binding in the early stages of the disease in PD [13], these factors may separate when the disease progresses. The clinical symptom severity is tightly connected with clinical disability and death, whereas brain dopamine function may be influenced by numerous coexisting pathological and compensatory mechanisms complicating the interpretation. The exact mechanism of the decrease in striatal DAT function in PD detected with DAT SPECT remains unclear as it may reflect either lower endogenous dopamine levels, dysfunctional DAT expression or the degeneration of dopaminergic nigrostriatal nerve endings [19, 21]. There is recent evidence to suggest that DAT binding does not correlate with post mortem substantia nigra neuron counts [12], although it remains possible that the DAT tracer uptake reflects neuronal function or activity and not the number of neurons. The loss of dopamine neurons in the substantia nigra may lead to an increased synthesis and release of dopamine and increased dopaminergic activation in the surviving dopaminergic nerve endings, and the dopamine reuptake by DAT may correspondingly be downregulated in the striatum [19]. In addition, a recent study on mice models suggested that besides dopaminergic neurons, there are also striatal non-dopaminergic monoenzymatic neurons containing TH and AADC, the dopamine synthesis enzymes, and the production of dopamine in

these neurons increases as the dopamine deficiency rises in the degraded parkinsonian striatum [26]. Finally, although striatal DAT function loss is approximately 50% in the contralateral posterior putamen at the onset of clinical parkinsonism [27, 28], this varies between patients with equal mild hemi-parkinsonian symptoms [17, 28]. Likely, this variable individual dopaminergic threshold obscures later correlations with clinical end-points such as disability and death.

Mortality in PD is not necessarily directly associated with the underlying loss in the number of dopamine-producing neurons, but proteinopathic mechanisms may be more relevant. PD and other Lewy body diseases are associated with intraneuronal inclusions of alpha-synuclein protein [17, 29], and the rate in the accumulation of toxic alpha-synuclein aggregates could be a more important neuropathological event in predicting mortality. Indeed, recent neuropathological findings suggest that alpha-synuclein pathology, as well as the burden of neurofibrillary tangles and amyloid plaque pathology are the strongest pathological predictors of survival in synucleinopathies [29]. In PD associated with mixed Alzheimer's disease neuropathology, the increasing level of cerebral tau neurofibrillary tangles may shorten the lifespan [29]. There is also evidence that the deposition of nigral neurofibrillary tangles may induce parkinsonian symptoms [17]. The proteinopathic cascade to cortical regions could forecast disability and death more accurately than binding characteristics of striatal dopaminergic tracers.

In our study, cognitive impairment was related to a decreased survival as the mortality risk was over three-fold higher in patients with cognitive disturbances compared to those without cognitive impairment. Indeed, the presence of cognitive defects have previously been shown to be the greatest risk factors of higher mortality in PD [1, 2]. Although the cognitive decline may be hard to predict, it seems that even a mild cognitive dysfunction, with an incidence of up to 25% in de novo PD patients, is associated with a high risk of progression to dementia [29, 30]. The connection between cognition and mortality in PD suggests that cortical biomarkers, such as alpha-synuclein– and tau–sensitive PET-ligands, may be more accurate in predicting advancing disability than basal ganglia–specific dopamine tracers.

There are limitations in this study including the retrospective study design and the lack of neuropathological confirmation of PD diagnoses. However, the clinical PD diagnoses were confirmed both by abnormal DAT SPECT scans and clinically relevant follow-up periods. It should be noted that the mortality risk was higher in patients with H&Y stages 2.5 and 3 than in patients

with H&Y stages 4 and 5 (n = 5) probably due to the low number of patients with the highest severity stages. However, the differences between these categories was not significant, and the differences between severe and mild (H&Y 1, 1.5 and 2) patients were clear. Importantly, the correlation between DAT binding and survival remained non-significant when advanced patients (H&Y > 2) were excluded from the analysis, indicating that the results were not affected by the higher mortality probability of patients with the highest motor disability. Survival was not related to either dopaminergic medication or gender. The findings on the medication effect are generally in accordance with earlier studies [6], although there are discrepant results concerning the effect of gender on mortality in PD [1, 4, 5].

To conclude, we have shown that DAT SPECT imaging cannot be used as a clinical predictor of mortality in Parkinson's disease as the level of striatal dopaminergic defect in DAT SPECT does not influence long-term survival unlike older age, higher clinical motor symptom severity and presence of cognitive defects. Notably, even large differences in DAT SPECT findings (mild to severe abnormality in DAT binding) do not seem to be associated with future lifespan in PD patients. We must underline, however, that the results of the present study do not imply that the binding characteristics of dopaminergic radiotracers are irrelevant in diagnostics or clinical monitoring but, rather, that there may be compensatory changes and confounding factors in the nigrostriatal dopamine transmission in patients with PD, which lessen the value of functional dopaminergic imaging in predicting survival. The proteinopathic cascade to cortical regions might forecast disability and death more accurately than the nigrostriatal dopaminergic degeneration as measured with functional imaging.

Conflicts of interest

None.

Sources of Funding

This study was supported in part by the Finnish Parkinson Foundation, Turku University Foundation and Turku University Hospital (ERVA-funds).

Author contributions

E.M. was in charge of writing the first draft of the manuscript, took part in the organization and execution of the study as well as in the design, execution and review of the statistical analyses. V.K. carried out the conception of the study, took part in the organization and the execution of the study as well as in the design, execution, review and critique of the statistical analyses. J.J. and T.V. took part in the execution, review and critique of the statistical analyses. All of the authors took part in the review and critique of the manuscript and have accepted the final manuscript.

References

[1] Macleod AD, Taylor KS, Counsell CE. Mortality in Parkinson's disease: a systematic review and meta-analysis. Mov Disord 2014;29:1615-22.

[2] Posada IJ, Benito-León J, Louis ED, Trincado R, Villarejo A, Medrano MJ, et al. Mortality from Parkinson's disease: a population-based prospective study (NEDICES). Mov Disord 2011;26:2522-9.

[3] Levy G, Tang MX, Louis ED, Côté LJ, Alfaro B, Mejia H, et al. The association of incident dementia with mortality in PD. Neurology 2002;59:1708-13.

[4] Hely MA, Morris JG, Traficante R, Reid WG, O'Sullivan DJ, Williamson PM. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 1999;67:300-7.

[5] de Lau LM, Verbaan D, Marinus J, van Hilten JJ. Survival in Parkinson's disease. Relation with motor and non-motor features. Parkinsonism Relat Disord 2014;20:613-6.

[6] Olanow CW. Levodopa: effect on cell death and the natural history of Parkinson's disease. Mov Disord 2015;30:37-44.

[7] Lo RY, Tanner CM, Albers KB, Leimpeter AD, Fross RD, Bernstein AL, et al. Clinical features in early Parkinson disease and survival. Arch Neurol 2009;66:1353-8.

[8] Willis AW, Schootman M, Kung N, Evanoff BA, Perlmutter JS, Racette BA. Predictors of survival in patients with Parkinson disease. Arch Neurol 2012;69:601-7.

[9] Ba F, Martin WR. Dopamine transporter imaging as a diagnostic tool for parkinsonism and related disorders in clinical practice. Parkinsonism Relat Disord 2015;21:87-94.

[10] Colloby SJ, McParland S, O'Brien JT, Attems J. Neuropathological correlates of dopaminergic imaging in Alzheimer's disease and Lewy body dementias. Brain 2012;135:2798-808.

[11] Kraemmer J, Kovacs GG, Perju-Dumbrava L, Pirker S, Traub-Weidinger T, Pirker W. Correlation of striatal dopamine transporter imaging with post mortem substantia nigra cell counts. Mov Disord 2014;29:1767-73.

[12] Saari L, Kivinen K, Gardberg M, Joutsa J, Noponen T, Kaasinen V. Dopamine transporter imaging does not predict the number of nigral neurons in Parkinson disease. Neurology 2016;in press.

[13] Ravina B, Marek K, Eberly S, Oakes D, Kurlan R, Ascherio A, et al. Dopamine transporter imaging is associated with long-term outcomes in Parkinson's disease. Mov Disord 2012;27:1392-7.

[14] Staffen W, Mair A, Unterrainer J, Trinka E, Ladurner G. Measuring the progression of idiopathic Parkinson's disease with [123I] beta-CIT SPECT. J Neural Transm (Vienna) 2000;107:543-52.

[15] Djaldetti R, Treves TA, Ziv I, Melamed E, Lampl Y, Lorberboym M. Use of a single [123I]-FP-CIT SPECT to predict the severity of clinical symptoms of Parkinson disease. Neurol Sci 2009;30:301-5.

[16] Kaasinen V, Kinos M, Joutsa J, Seppänen M, Noponen T. Differences in striatal dopamine transporter density between tremor dominant and non-tremor Parkinson's disease. Eur J Nucl Med Mol Imaging 2014;41:1931-7.

[17] Ziebell M, Andersen BB, Pinborg LH, Knudsen GM, Stokholm J, Thomsen G, et al. Striatal dopamine transporter binding does not correlate with clinical severity in dementia with Lewy bodies. J Nucl Med 2013;54:1072-6.
[18] Järvelä JT, Rinne JO, Eskola O, Kaasinen V. Mortality in Parkinson's disease is not associated with the severity of early dopaminergic defect. Parkinsonism Relat Disord 2014;20:894-7.
[19] Lee CS, Samii A, Sossi V, Ruth TJ, Schulzer M, Holden JE, et al. In vivo positron emission tomographic evidence for compensatory changes in presynaptic dopaminergic nerve terminals in Parkinson's disease. Ann Neurol 2000;47:493-503.
[20] Ribeiro MJ, Vidailhet M, Loc'h C, Dupel C, Nguyen JP, Ponchant M, et al. Dopaminergic function and dopamine transporter binding assessed with positron emission tomography in Parkinson disease. Arch Neurol 2002;59:580-6.
[21] Eshuis SA, Jager PL, Maguire RP, Jonkman S, Dierckx RA, Leenders KL. Direct comparison of FP-CIT SPECT and F-DOPA PET in patients with Parkinson's disease and healthy controls. Eur J Nucl Med Mol Imaging 2009;36:454-62.
[22] Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010;25:2649-53.
[23] Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, et al. Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord 2004;19:1020-8.
[24] Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage 2007;38:95-113.
[25] Joutsa J, Johansson J, Kaasinen V. Is Occipital Cortex a Valid Reference Region in 123I-FP-CIT SPECT Imaging? Clin Nucl Med 2015;40:615-6.
[26] Kozina EA, Kim AR, Kurina AY, Ugrumov MV. Cooperative synthesis of dopamine by non-dopaminergic neurons as a compensatory mechanism in the striatum of mice with MPTP-induced Parkinsonism. Neurobiol Dis 2016;98:108-21.
[27] Brooks DJ, Tambasco N. Imaging synucleinopathies. Mov Disord 2016;31:814-29.
[28] Marek KL, Seibyl JP, Zoghbi SS, Zea-Ponce Y, Baldwin RM, Fussell B, et al. [123I] beta-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. Neurology 1996;46:231-7.
[29] Irwin DJ, Grossman M, Weintraub D, Hurtig HI, Duda JE, Xie SX, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. Lancet Neurol 2017;16:55-65.
[30] Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. Lancet Neurol 2017;16:66-75.

Table 1. The clinical characteristics of the study population at the time of the follow-up onset (the time of imaging).

	All	Survivors	Non-Survivors
N (%)	162	120 (74.1)	42 (25.9)
Age at scan, mean (SD), years	66.4 (10.4)	63.1 (9.3)	75.9 (7.1)
Gender (male/female)	96/66	68/52	28/14
Cognitive defect, n (%)	33 (20.4)	19 (15.8)	14 (33.3)
H&Y, median (range)	1.5 (4.0)	1.5 (3.0)	2.8 (4.0)
1, 1.5 and 2, n (%)	120 (74.1)	104 (86.7)	16 (38.1)
2.5 and 3, n (%)	25 (15.4)	11 (9.2)	14 (33.3)
4 and 5, n (%)	17 (10.5)	5 (4.2)	12 (28.6)
Levodopa, median (range), mg	300 (750)	300 (700)	300 (700)
No levodopa, n (%)	130 (80.2)	105 (87.5)	25 (59.5)
Levodopa daily dose < 300 mg, n (%)	10 (6.2)	4 (3.3)	6 (14.3)
Levodopa daily dose ≥ 300 mg, n (%)	22 (13.6)	11 (9.2)	11 (26.2)
LEDD, median (range), mg	200 (893)	142 (893)	300 (723)
No antiparkinsonian medication, n (%)	105 (64.8)	82 (68.3)	23 (54.8)
LEDD < 200 mg, n (%)	27 (16.7)	21 (17.5)	6 (14.3)
LEDD ≥ 300 mg, n (%)	30 (18.5)	16 (11.3)	13 (31.0)
Putamen SBR, median (range)	1.76 (2.83)	1.80 (2.79)	1.67 (2.13)
SBR < 1.37, n (%)	39 (24.1)	25 (20.8)	14 (33.3)
SBR = 1.37-2.23, n (%)	82 (50.6)	61 (50.8)	21 (50.0)
SBR >2.23, n (%)	41 (25.3)	34 (28.3)	7 (16.7)

SD = Standard deviation
n = number of patients
H&Y = Hoehn & Yahr stage. Category 1: modified H&Y stages 1, 1.5 and 2; category 2: modified H&Y stages 2.5 and 3; and category 3: modified H&Y stages 4 and 5.
LEDD = Levodopa equivalent daily dose.
SBR = Specific binding ratio of tracer [I-123]FP-CIT in the posterior putamen of the most severely affected hemisphere in the automated analysis (BRASS). Category 1: SBR < 1.37 (bottom quartile), category 2: SBR = 1.37-2.23, category 3: SBR > 2.23 (upper quartile).

Table 2. The association of the factors on survival in Parkinson's disease (n=162).

	Adjusted HR (95% CI)	*P*-value
Age at scan (years)	1.14* (1.09-1.20)	< 0.001
Male gender	1.84 (0.90-3.84)	0.10
Cognitive defect	3.35 (1.67-6.72)	0.001
Hoehn & Yahr		0.002
H&Y category 2 vs. 1	3.83 (1.75-8.36)	0.001
H&Y category 3 vs. 1	2.67 (1.11-6.39)	0.03
LEDD		0.10
LEDD <200 vs. no medication	0.60 (0.22-1.64)	0.32
LEDD ≥200 vs. no medication	1.79 (0.83-3.89)	0.14
Putamen SBR		0.99
Putamen SBR category 2 vs. 1	1.00 (0.49-2.05)	1.00
Putamen SBR category 3 vs. 1	0.95 (0.35-2.58)	0.91

HR = Hazard ratio for mortality after adjustment for other factors in the model
CI = Confidence interval
*HR for one-year increase in age
H&Y category 1: modified H&Y stages 1, 1.5 and 2; category 2: modified H&Y stages 2.5 and 3; category 3: modified H&Y stages 4 and 5.
LEDD = Levodopa equivalent daily dose.
SBR = Specific binding ratio of tracer [I-123]FP-CIT in the posterior putamen of the most severely affected hemisphere in the automated analysis (BRASS). Category 1: SBR < 1.37, category 2: SBR = 1.37-2.23, category 3: SBR > 2.23.

Fig.1A

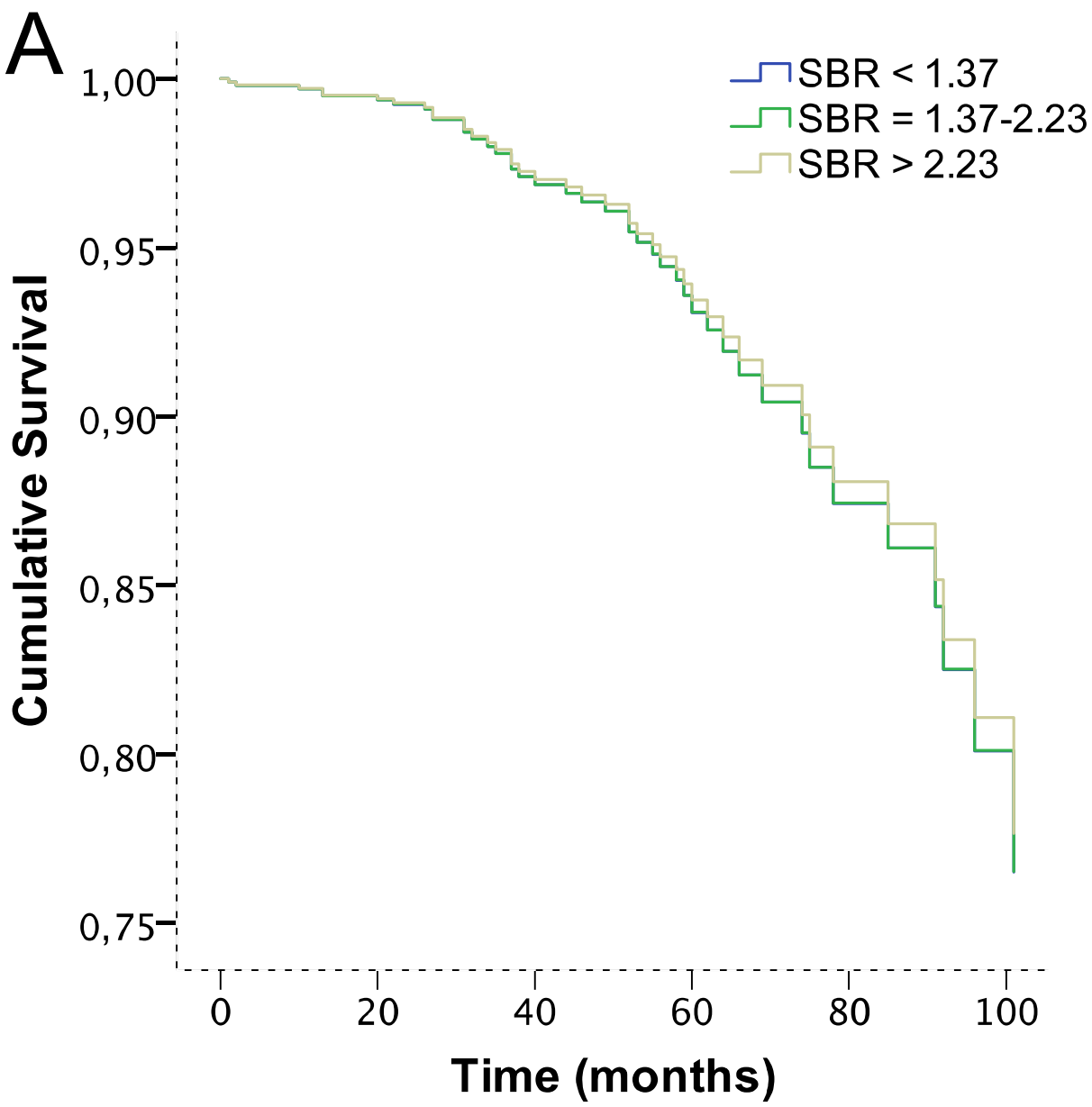

A
1,00
0,95
0,90
0,85
0,80
0,75
Cumulative Survival
SBR < 1.37
SBR = 1.37-2.23
SBR > 2.23
0
20
40
60
80
100
Time (months)

Fig.1B

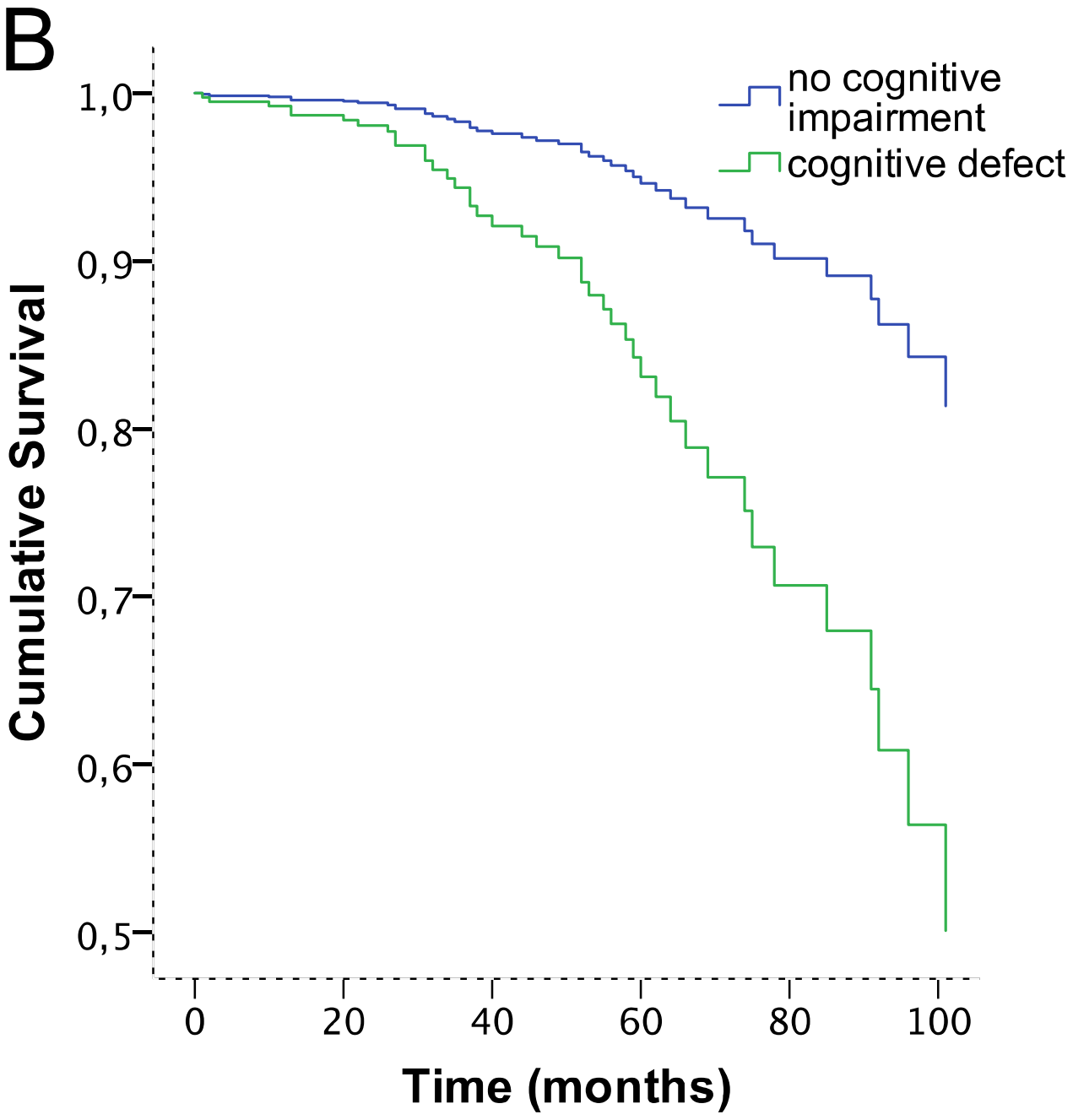

B
1,0
0,9
0,8
0,7
0,6
0,5
Cumulative Survival
no cognitive impairment
cognitive defect
0
20
40
60
80
100
Time (months)

Fig.1C

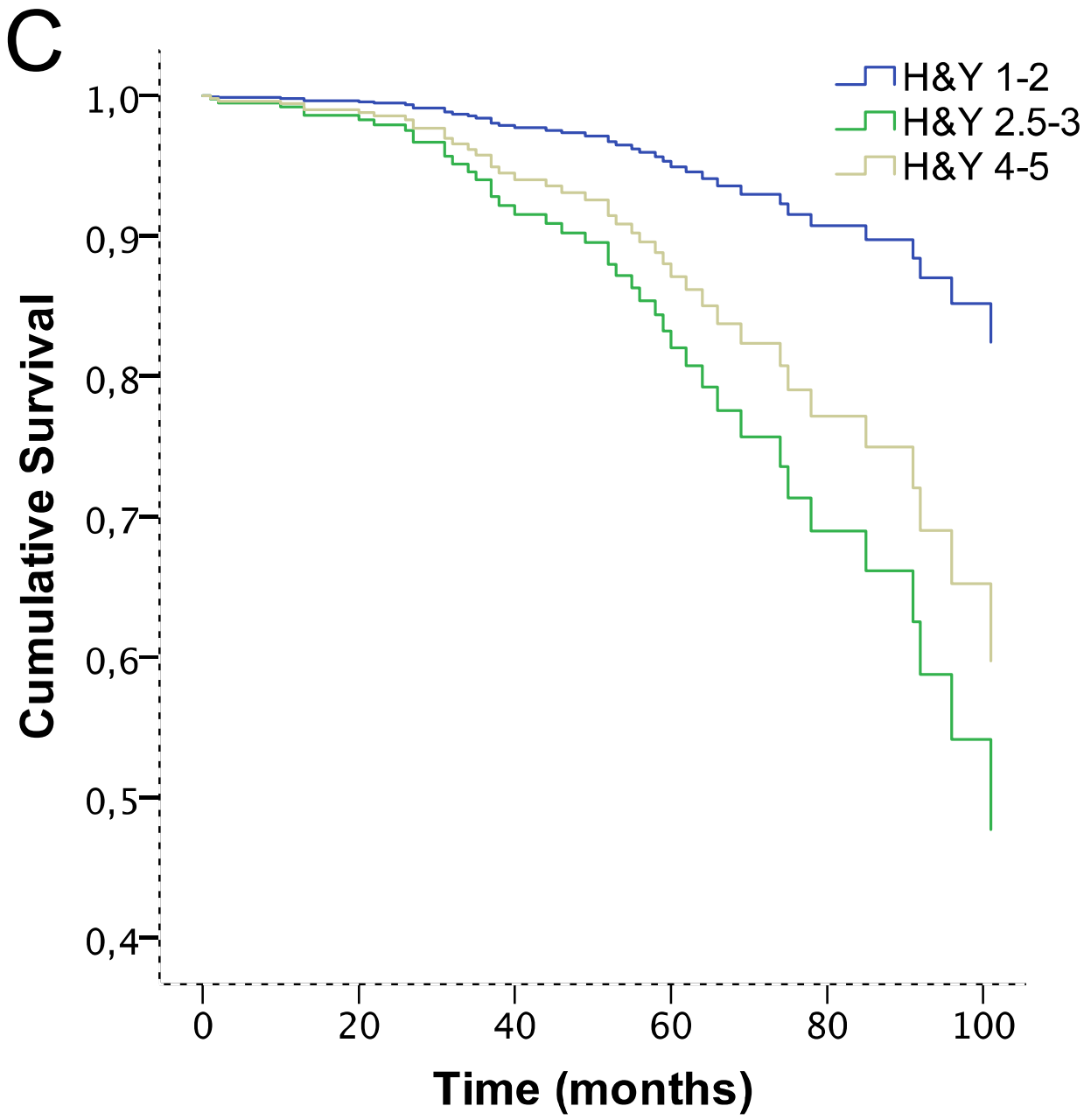

C
H&Y 1-2
H&Y 2.5-3
H&Y 4-5
Cumulative Survival
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0
20
40
60
80
100
Time (months)

Figure 1. Survival function graphs showing the independent impact of three factors on survival in patients with PD, after adjusting each factor for other variables in the multivariate Cox regression model. Cumulative survival is plotted against the clinical follow-up time. A = Specific binding ratio (SBR) of tracer [I-123]FP-CIT in the posterior putamen of the most severely affected hemisphere in the automated analysis (BRASS); B = Patients with and without cognitive impairment at the time of imaging; C = Hoehn & Yahr stage combined into three clinical categories at the time of imaging.