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<th>Biomarkers in Parkinson disease: studies on clinical, radiological and biological biomarkers</th>
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<tr>
<td><strong>Author(s)</strong></td>
<td>Crotty, Grace F.</td>
</tr>
<tr>
<td><strong>Publication date</strong></td>
<td>2018</td>
</tr>
<tr>
<td><strong>Type of publication</strong></td>
<td>Doctoral thesis</td>
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Biomarkers in Parkinson Disease: Studies on Clinical, Radiological and Biological Biomarkers.

Dr. Grace F. Crotty MB BCh BAO, MRCPI

Department of Medicine
National University of Ireland, Cork

A thesis submitted for the Doctor of Medicine degree.
Submitted July 2018

Head of Department: Professor Fergus Shanahan

Supervisors: Professor Aideen Sullivan, Dr. Gerard O’Keeffe & Dr. Sean O’Sullivan
I would like to dedicate this thesis to my grandfather, Dr. Tom Crotty, who continued to publish his research with such enthusiasm and motivation until his death at 90 years old. He is such an inspiration to us all and is dearly missed.
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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and resources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

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Acknowledgements

Over the past 4 years, this MD has given me the opportunity to meet many amazing people in the field of Neuroscience and in my local community.

I would especially like to acknowledge Professor Aideen Sullivan, Dr. Gerard O'Keeffe and Dr. Sean O'Sullivan for their support and guidance throughout my MD research.

I would also like to thank my family, especially my parents, my sister Jillian and my husband Eoghan, all of whom helped me make it to the finish line.

I would like to acknowledge my colleagues in the Neurophysiology department of the Cork University Hospital and especially Brendan Coleman who tirelessly helped me study autonomic neuropathy in PD subjects.

I would also like to thank Margaret Cole from UCC who provided expert guidance in the analysis of data in my autonomic neuropathy study. Her patience and enthusiasm were unwavering.

I would like to acknowledge all the patients with PD and their families who took part in my studies. Their interest and support for our research was greatly appreciated.

I would like to thank our collaborators in University Hospital Limerick, Limerick; Santry Orthopaedic Clinic, Dublin; and Queen Square Hospital, University College London, England. Individual contributions are mentioned in each individual chapter.

Finally, I am greatly appreciative of the financial support provided by the UCC Professor Denis O'Sullivan Fellowship and the UCC Translational Research Access Programme (TRAP) grant.
Abstract of thesis

Parkinson disease is the second most common neurodegenerative disorder after Alzheimer disease. It affects 2 to 3 percent of those over 65 years with an age-dependent prevalence. Currently, the diagnosis of PD is hampered by the limited sensitivity and specificity of the available investigations. The diagnosis is usually made based on the clinical presentation which has a number of significant limitations. First of all, the disease has been present for decades before motor symptoms develop. Secondly, using clinical exam alone, the misdiagnosis rate remains high with both over- and under-diagnosis common. It is important to make an expeditious and correct diagnosis of PD, especially in this era of increasing interest in neuroprotective strategies for PD and other neurodegenerative conditions. Delaying the diagnosis until motor symptoms develop is suboptimal as more than 40% of dopaminergic neurons have been destroyed at this stage. We also need to ensure that true cases of PD are being enrolled in PD trials and that these trials are not being confounded by the inclusion of individuals with other causes of parkinsonism. To accomplish these goals, there is a need for PD biomarkers that are both sensitive and specific.

The objective of this thesis was to investigate, using a case-control study design, a number of potential biomarkers for PD. These biomarkers included clinical, biological and radiological markers.

In the first study, we investigated the role of autonomic neuropathy as a clinical biomarker for PD. Using thermal threshold testing, nerve conduction testing and questionnaires, the PD group demonstrated a higher prevalence of autonomic neuropathy. Other outcome measures, including the presence of non-motor symptoms, pain, depressive symptoms and electrophysiological evidence of large fiber neuropathy were also found to be more prevalent in the PD group.

In the second and third studies, we explored the potential role of CSF biological biomarkers in PD. In the second study, we evaluated CSF cytokine levels with the aim of identifying a unique cytokine pattern in the CSF of PD subjects. We failed to detect a cytokine pattern and found no difference in cytokine levels between PD and control groups. However, within a cohort of the PD group, we identified an association between
IL-2 levels and disease severity, with higher concentrations of IL-2 seen in those with more severe disease.

In the third study, we measured GDF5 protein levels in the CSF and found lower concentrations of GDF5 in the PD group compared to controls. GDF5 levels were lower in the female PD subjects compared to males. There was no association between GDF5 concentrations and PD characteristics, age or cognition.

In the final study, we assessed the utility of SPECT imaging of dopamine transporters in the striatal region of the brain (DaTSCAN) as a radiological biomarker for PD in our healthcare system. Following a review of scans over a five-year period, 69% of scans showed evidence of dopaminergic deficit, supporting a diagnosis of PD. Review of request forms for DaTSCAN, demonstrated inappropriate referrals in 13% of cases. Chart review in a subgroup of scans documented a change in patient management in 65% of cases, based on the result of the scan.

In this thesis, we sought to identify potential biomarkers for PD. We found significant differences between the subjects with PD and controls using clinical and biological tests. We also demonstrated findings that support the utility of a radiological biomarker in clinical practice. Our studies showed promising results and require further research. In the future, we envision studies investigating a multimodal biomarker approach in large cohorts of PD subjects.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAN</td>
<td>American Academy of Neurology</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>APD</td>
<td>Atypical parkinsonian disorders</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone morphogenetic protein</td>
</tr>
<tr>
<td>BPI</td>
<td>Brief Pain Inventory</td>
</tr>
<tr>
<td>CBD</td>
<td>Corticobasal degeneration</td>
</tr>
<tr>
<td>DIP</td>
<td>Drug-induced parkinsonism</td>
</tr>
<tr>
<td>DLB</td>
<td>Dementia with Lewy bodies</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ET</td>
<td>Essential tremor</td>
</tr>
<tr>
<td>GBA</td>
<td>Glucocerebrosidase</td>
</tr>
<tr>
<td>GDF5</td>
<td>Growth differentiation factor 5</td>
</tr>
<tr>
<td>GDNF</td>
<td>Glial cell-line derived neurotrophic factor</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association study</td>
</tr>
<tr>
<td>HAAS</td>
<td>Honolulu- Asia Aging study</td>
</tr>
<tr>
<td>H&amp;Y</td>
<td>Hoehn &amp; Yahr scale</td>
</tr>
<tr>
<td>IEFNND</td>
<td>Intraepidermal nerve fiber density</td>
</tr>
<tr>
<td>LEDD</td>
<td>Levodopa-equivalent daily dosage</td>
</tr>
<tr>
<td>LRRK2</td>
<td>Leucine-rich repeat kinase 2</td>
</tr>
<tr>
<td>mDA</td>
<td>midbrain dopaminergic</td>
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<tr>
<td>MDS</td>
<td>Movement Disorder Society</td>
</tr>
<tr>
<td>MIBG</td>
<td>123 I-Meta-IodoBenzylGuanidine</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-mental state examination</td>
</tr>
<tr>
<td>MOCA</td>
<td>Montreal Cognitive Assessment</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSA</td>
<td>Multiple system atrophy</td>
</tr>
<tr>
<td>NCS</td>
<td>Nerve conduction studies</td>
</tr>
<tr>
<td>NiL</td>
<td>Neurofilament light chain</td>
</tr>
<tr>
<td>NMS</td>
<td>Nonmotor symptoms</td>
</tr>
<tr>
<td>NTN</td>
<td>Neurturin</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson disease</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PIGD</td>
<td>Postural instability with gait disturbance</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>PSP</td>
<td>Progressive supranuclear palsy</td>
</tr>
<tr>
<td>PSPN</td>
<td>Persephin</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated on Activation, Normal T cell Expressed and Secreted</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>RBD</td>
<td>REM sleep behavior disorder</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SNCA</td>
<td>Synuclein, alpha gene</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>TCS</td>
<td>Transcranial Ultrasound</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>TTT</td>
<td>Temperature threshold testing</td>
</tr>
<tr>
<td>UKPDSBB</td>
<td>United Kingdom Parkinson Disease Society Brain Bank</td>
</tr>
<tr>
<td>UPDRS</td>
<td>United Parkinson Disease Rating Scale</td>
</tr>
<tr>
<td>VMAT2</td>
<td>Vesicular Monoamine Transporter type 2</td>
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Please note that Chapters 1-4 (pp. 12-82) are unavailable due to a restriction requested by the author.

CORA Cork Open Research Archive [http://cora.ucc.ie](http://cora.ucc.ie)
Chapter 5: DaTSCAN imaging in PD
5.1 Abstract:

Introduction: Dopamine transporter scans are FDA approved as a diagnostic biomarker in the diagnosis of clinically undefined Parkinsonism. Our aim was to assess the indications for imaging usage and its impact on future clinical management in the Irish health service.

Methods and materials: Retrospective review of scans ordered and their corresponding results over a five-year period. A chart review was carried out on a cohort of scans to assess changes in clinical management.

Results: One hundred and eighty scans (69% of total) were reported as showing evidence of dopaminergic deficit. A chart review in 81 patients showed a change in clinical management in 53 patients (65%). Scans were ordered inappropriately in 34 patients (13%).

Conclusions: \textsuperscript{123}I-FP-CIT SPECT scans are being more frequently ordered and if used correctly can alter clinical management. Increased education on indications for use is required to reduce waste of resources and risk to patients.
5.2 Introduction

Parkinson disease (PD) is the second most common neurodegenerative disease and is characterised by the presence of bradykinesia plus one of rigidity, tremor, or postural instability (2). Misdiagnosis rates from 10 to 50% have been found using clinical exam alone, when compared to the gold standard pathological diagnosis (262,356,357). The most common PD mimics include tremor disorders, drug-induced Parkinsonism (DIP), vascular parkinsonism (VP) and Parkinson-plus conditions. The prognosis and management of each disorder differs significantly from PD, and from each other. Therefore, the ability to distinguish between different parkinsonian entities is of clinical importance, allowing for optimal treatment and avoiding unnecessary therapeutic trials or other tests.

*In-vivo* functional imaging of dopamine transporters (DAT) can improve diagnostic accuracy in atypical cases of Parkinsonism. DaTSCAN, which is the trade name for striatal presynaptic dopamine transporter imaging using $^{123}$I-FP-CIT [(123)I-N-omega-fluoropropyl-2beta-carbomethoxy-3beta-nortropane] Single Photon Emission Computed Tomography [SPECT], has been licensed by the European Medicines Agency (EMA), The Society of Nuclear Medicine (SNM), and the US Food and Drug Administration (FDA) for certain indications (Table 1) (111,118). Reductions in $^{123}$I-FP-CIT SPECT striatal uptake is demonstrated to have 95% sensitivity and 95% specificity with a high positive predictive value for identifying parkinsonian syndromes (PS) (111). $^{123}$I-FP-CIT SPECT initial imaging results have been remarkably consistent with the clinical diagnoses made at three years follow-up (117,357). Almost 100% concordance has been found between Neuroradiologists on interpreting this imaging (357,358). The Parkinsonian syndromes (PS) which include PD, Multiple System Atrophy (MSA), Progressive Supranuclear Palsy (PSP), and Corticobasal Degeneration (CBD) show nigrostriatal degeneration on DaTSCAN neuroimaging. Other conditions, such as essential tremor (ET), DIP, vascular parkinsonism (VP), psychogenic parkinsonism, normal aging, normal pressure hydrocephalus, and dystonic tremor may demonstrate features of parkinsonism, but do not have nigrostriatal degeneration on neuroimaging (111). In the appropriate clinical setting (such as where the differential diagnoses being queried include a neurodegenerative Parkinsonism versus another mimic disorder),
123I-FP-CIT SPECT can be a very useful investigation. However, as each scan costs approximately €1,200 per patient, this is a resource that should not be used routinely.

Table 1: Indications for DaTSCAN as per FDA, EMA and SNM guidelines (111,118)

<table>
<thead>
<tr>
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<tr>
<td>4</td>
<td>Differentiate Essential tremor from parkinsonian disorders</td>
</tr>
<tr>
<td>5</td>
<td>Differentiate Dementia with Lewy bodies from Alzheimer’s disease</td>
</tr>
<tr>
<td>6</td>
<td>Distinguish drug-induced parkinsonism from parkinsonian disorder</td>
</tr>
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</table>

Our primary aim was to evaluate the current use of 123I-FP-CIT SPECT in our health service as a diagnostic biomarker for parkinsonian syndromes. We reported on the indications for ordering 123I-FP-CIT SPECT along with assessing trends of referral by different specialties. We investigated for correlations between dopaminergic deficit on imaging and demographics or symptomatology. We identified inappropriate referrals and assessed the impact of these scans on subsequent clinical management.

5.3 Methods and materials
Study design: A retrospective review of 123I-FP-CIT SPECT request forms and their corresponding results over a five-year period from 2008 to 2013 in two tertiary care hospitals, Cork University Hospital [CUH], Cork, Ireland and University Hospital Limerick [UHL], Limerick, Ireland. Patients in two tertiary care hospitals who underwent 123I-FP-CIT SPECT over this five-year period were included in the study. No additional exclusion or inclusion criteria were applied. Scans were carried out as per each institution’s protocol and in accordance with international guidelines. Patients were instructed to discontinue all potential confounding medications prior to scan. All scans were read by experienced radiologists who were aware of clinical history and differential diagnoses as documented on referral. Demographics including gender, age at scan, symptoms, medications, and scan report details including indication for scan, referring specialty, and institution were manually gathered. Inappropriate referrals were defined as referral indications not approved by FDA, SNM, or EMA guidelines and included differentiating PD from other PS, dystonia, vascular parkinsonism, dementia, and unknown along with assessing progression (see Table 1 for recommended indications.
for scan). We reviewed all available handwritten charts in our two hospitals in order to evaluate the utility of scans for future clinical management.

Study Ethics was received from both the Clinical Research Ethics Committee of the Cork Teaching Hospitals and the Research Ethics Committee of UHL prior to the initiation of the study (appendix).

**Statistical analysis:** Data was inputted into SPSS version 20.4.1. Two groups were formed for statistical analysis: those with and without dopaminergic deficits. Descriptive statistics, frequencies along with Pearson’s chi-squared test were used.
5.4 Results

Patient demographics and referral sources
Two hundred and sixty-one patients underwent $^{123}$I-FP-CIT SPECT over a five-year period. One hundred and forty-eight (56.7%) were male and median age was 67 years (Table 2). The number of scans ordered increased every year with the most scans completed in the final full calendar year. Scans were predominantly ordered by neurologists (54.4%), geriatricians (34.5%) and psychiatrists (6.1%). Thirteen scans were referred from other specialties including general medicine (n=6), rheumatology (n=2), respiratory (n=1), nephrology (n=1), gastroenterology (n=1) and emergency department (n=1). Fifty-five percent (55%) of scans were outside referrals, ordered by physicians working outside of our two hospitals.

Table 2: Demographics of DaTSCANs ordered

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>261</td>
</tr>
<tr>
<td>Age at scan, years</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>65.6 (12.22)</td>
</tr>
<tr>
<td>Median (interquartile range)</td>
<td>67.0 (58.5 - 75.5)</td>
</tr>
<tr>
<td>Min, Max</td>
<td>24, 91</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>113 (43.4%)</td>
</tr>
<tr>
<td>Male</td>
<td>148 (56.7%)</td>
</tr>
<tr>
<td>Mean (median) follow-up after scan in years at time of chart review</td>
<td>4.9 (5.0)</td>
</tr>
</tbody>
</table>
Referral reason
The most common reason for ordering a scan was for assessment of a parkinsonian syndrome (PS), accounting for 62.5% of referrals. Other frequent referral reasons were differentiating drug-induced Parkinsonism (DIP) from PS at 17.2% and PS vs. PD at 7.3%. Inappropriate referrals were seen in 13% of cases (Table 3).

Table 3: $^{123}$I-FP-CIT SPECT indications along with results

<table>
<thead>
<tr>
<th>$^{123}$I-FP-CIT SPECT indications</th>
<th>Number (% of all $^{123}$I-FP-CIT SPECT scans)</th>
<th>% of $^{123}$I-FP-CIT SPECT scans showing reduced striatal dopamine transporter signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinsonian syndrome</td>
<td>163 (62.5%)</td>
<td>71.8</td>
</tr>
<tr>
<td>Lewy body dementia</td>
<td>7 (2.7%)</td>
<td>57.1</td>
</tr>
<tr>
<td>Drug-induced vs PS</td>
<td>45 (17.2%)</td>
<td>57.8</td>
</tr>
<tr>
<td>PD vs ET</td>
<td>12 (4.6%)</td>
<td>50</td>
</tr>
<tr>
<td>PD vs dementia</td>
<td>2 (0.8%)</td>
<td>50</td>
</tr>
<tr>
<td>PS vs PD</td>
<td>19 (7.3%)</td>
<td>89.5</td>
</tr>
<tr>
<td>PD progression</td>
<td>2 (0.8%)</td>
<td>100</td>
</tr>
<tr>
<td>PD vs vascular PD</td>
<td>8 (3.1%)</td>
<td>62.5</td>
</tr>
<tr>
<td>PD vs dystonia</td>
<td>2 (0.8%)</td>
<td>50</td>
</tr>
<tr>
<td>Unclear</td>
<td>1 (0.4%)</td>
<td>100</td>
</tr>
</tbody>
</table>

Symptomatology
We were interested in the documentation of parkinsonism, in particular the symptoms needed for The United Kingdom Parkinson’s Disease Society Brain Bank Clinical Diagnostic (UKPDSBB) criteria for diagnosing Parkinson disease (2). One hundred and thirty-three patients (51%) had a tremor, 88 patients (33.7%) had rigidity, 69 patients (26.4%) had bradykinesia and 5 patients (1.9%) had postural instability documented on their request forms for imaging.
123I-FP-CIT SPECT results and subgroup analysis

One hundred and eighty patients (69% of total) had positive scans with dopaminergic deficit qualitatively assessed. Seventy-seven (42.7%) of these scans showed correct laterality between symptom sidedness and dopaminergic deficit on imaging. Thirty-two scans (17.9%) showed bilateral dopaminergic deficits in the presence of unilateral symptoms. Twenty-one scans (11.8%) showed dopaminergic deficit on the incorrect side to unilateral symptoms. In the remaining fifty scans (27.5%), the symptom sidedness was not documented on the referral forms. When comparing the demographics and symptomatology of those with evidence of dopaminergic deficit against those with a normal scan, no statistically significant difference was found regarding age, gender, indication for scan or symptoms (p>0.05).

Change of management

This was assessed by review of handwritten medical notes available in our two hospitals. Eighty-one charts were available for review. Forty-three (53%) of these scans were ordered by neurologists; 25 (30.9%) ordered by geriatricians; 7 (8.6%) ordered by psychiatrists and 6 (7.4%) ordered by general medical physicians. Documentation of further management was noted in 53 of these charts (65.4%). Twenty-five patients (30.9%) had a change of diagnosis from ET or DIP to PS. Seventeen patients (21%) were started on new medications or had an increase in medication doses after confirmation of diagnosis. Eleven patients (13.5%) had either discontinued treatment or didn’t start planned medication. Regarding specialties, changes in management were noted in 65.9% of neurology (27 patients), 94.7% of geriatrics (18 patients), 50% of psychiatry (2 patients) and 100% of general medicine (6 patients) referrals.

5.5 Discussion

Over the five-year period the number of scans ordered almost quadrupled, from 21 scans in the first year to 79 in the final year. We think this reflects the increased awareness of the utility of 123I-FP-CIT SPECT in diagnosing parkinsonian syndromes (PS). Many of our patients (62.5%) were referred for this scan in cases of PS, although it was often unclear from the referral forms what other diagnoses were being considered in addition to PS. The second most common indication was for DIP versus PS (17.2%).
Prior studies report DIP accounting for 24 to 51% of cases of parkinsonism (359). DIP can present similar to PS with rest tremors or asymmetric parkinsonism (14,359). DIP is important to identify as withdrawal of the offending drug can reverse the symptoms of parkinsonism (237). $^{123}I$-FP-CIT SPECT scans are ideal for these patients as neuroleptics predominantly affect the postsynaptic dopamine receptors with only a negligible affinity for the dopamine transporter (DAT) (360,361). In our study, 26 patients (57.8%) with a psychiatric diagnosis or prescribed neuroleptics were found to have evidence of dopaminergic deficit on neuroimaging, supporting a diagnosis of PS rather than DIP. Despite being susceptible for DIP, these patients were also at risk for PS given their older age, with 88.5% of them, greater than 60 years of age. The DaTSCAN results in these cases could be presymptomatic PS with subclinical SN degeneration. Research has suggested that antidopaminergic medications, like neuroleptics can unmask subclinical SN degeneration, resulting in overt parkinsonism (14,359).

Inappropriate referrals for $^{123}I$-FP-CIT SPECT are important to identify and prevent as they are a waste of resources and cause an unnecessary risk to patients without benefit (111,362). We found inappropriate referrals in 34 cases (13% of total) resulting in an estimated cost of €48,000 euros to the health service. Documented reasons for ordering the scan included differentiating PS from PD; PD from dystonia; PD from Dementia with Lewy Bodies (DLB); or assessing progression in PD. Although ongoing research is investigating variant mapping techniques of $^{123}I$-FP-CIT SPECT and other biomarkers for these reasons (111), they are currently not clinical indications for the scan and are not licensed by the EMA, SNM and FDA guidelines (table 1). Positive scans (i.e. evidence of qualitative dopaminergic deficit) were seen in 69% of cases. Although not evaluated in our study, there is an age-related decline of radiotracer uptake in normal patients of 3.3 to 10% per decade (363), making the interpretation of results in an older age group more difficult.

Eighty-one scans (31% of total) were normal with no evidence of dopaminergic deficit. Possible diagnoses for normal scans include ET, dystonia, dementia not related to SN degeneration, vascular parkinsonism, DIP and psychogenic parkinsonism. SWEDDDs (Scans Without Evidence of Dopaminergic Deficit) is a controversial term
used to described subjects with parkinsonism and normal DaTSCANs. It is now commonly associated with dystonia or dystonic tremor but can be associated with a variety of etiologies (364).

As 55% of patients were referred from outside institutions, we were limited in our chart review. However, we were able to assess change of management in patients under the care of neurologists, geriatricians, psychiatrists, and general medical physicians. A change in management after \(^{123}\text{I}-\text{FP-CIT SPECT}\) was clearly documented in 65% of our chart review subgroup. This was consistent with a recent multicenter, open, non-randomized study which showed change in planned management in 72% of their patients after DaTSCAN (358). Another retrospective review, reported a change in management in 63% of cases (361). Interestingly in three of our patients, the scans results were not accepted by the ordering physician suggesting some uncertainty in the scan’s validity.

The overdiagnosis of PD at initial presentation occurs in 10 to 47% of patients in both community and hospital settings (11,365). This misdiagnosis of PD is more likely with non-specialists compared to Movement disorder experts (115). In our study, only three scans were requested by a recently appointed Movement disorder specialist, supporting early referral of patients with parkinsonism to specialists. Prior research had scans ordered solely by neurologists (366). However, patients with parkinsonism can present to any specialty and the feasibility and cost-effectiveness of restricting the ordering of these scans to only neurologists or Movement disorder experts is debatable (367).

Given the retrospective nature of the study, the large percentage of outside referrals to our centers for these scans and the reliance on handwritten scan request forms and chart reviews for data collection there were some limitations to this study. We were only able to assess change of management in one-third of scans due to either unclear documentation or inability to access the handwritten medical records.

Dopamine transporter scan is a diagnostic biomarker which is increasingly being used in the clinical and research setting. In fact, the EMA has recently endorsed its application in PD clinical trials (119). Given its limitations and expense, it is not feasible to use it for the clinical diagnosis in all patients with parkinsonism. However, it is a
useful biomarker in challenging cases, especially early in the disease when signs are minimal; when atypical features are present or when there are other comorbidities. In our study, we showed increased awareness and utility of dopamine transporter scans in diagnosing parkinsonian syndromes in our health service. We found that dopamine transporter imaging can assist with diagnosis and change clinical management, if used for the correct indications. We also identified a small, yet significant number of inappropriate referrals. These referrals will be important to address in the future, in order to reduce the waste of resources and prevent unnecessary radiation exposure to patients. Potential solutions include better education of the medical community or listing strict indications for the scan on the request forms.
Chapter 6: Conclusion
6.1 Summary of results

In this thesis, we completed several case-control studies investigating clinical, radiological and biological biomarkers in Parkinson disease. We identified several differences between the subjects with PD and controls.

In the first study, we detected a higher prevalence of autonomic neuropathy in PD subjects using a novel approach of SCOPA-AUT questionnaire and temperature threshold testing to diagnose autonomic neuropathy. We also found a trend towards more large fiber neuropathy in the PD group than in controls, with increased prevalence of neuropathy in those subjects on a higher levodopa-equivalent daily dosage (LEDD), a finding previously reported in the literature. Other non-motor symptoms, including depression, pain, gastrointestinal disturbances and urinary dysfunction, were more prevalent in PD subjects than in controls. Potentially, this approach of SCOPA-AUT questionnaire and temperature threshold testing could be used in clinical practice to diagnose autonomic or small fiber neuropathy and thus avoid skin biopsy or more labour-intensive autonomic function testing.

In the second study, we failed to identify a distinct cytokine pattern in the PD group. There was no difference in the concentration of each cytokine examined, and no difference in the presence or absence of individual cytokines between the PD and control groups. Interestingly, in the PD group we found a strong correlation between IL-2 levels and disease severity, with higher IL-2 levels associated with more severe disease on the H&Y scale. Our results suggest that CSF cytokine levels are not useful in diagnosing PD. However, the association of IL-2 with disease severity is intriguing and suggests a possible role for anti-inflammatory medications in hastening disease progression.

In our third study, we measured GDF5 protein levels in the CSF of both PD subjects and controls. We found a significantly lower concentration of GDF5 protein in the CSF of the PD group compared to controls. GDF5 levels in PD subjects correlated with gender, with higher levels seen in males. There was no relationship between GDF5
levels and disease duration or disease stage. Our results suggest a potential role for GDF5 protein in neuroprotective strategies, although further studies are needed to replicate this finding.

Finally, we found that DaTSCAN, a proposed radiological biomarker for diagnosing PD can be useful when applied correctly in diagnostically-challenging cases of parkinsonism. In our chart review, we observed a change in clinical management in two-thirds of patients. However, there was also evidence of inappropriate referrals in a small but significant number of cases. Increased education on the use of this biomarker in clinical practice is warranted in order to reduce waste and risk to patients.

### 6.2 Strengths of these studies

There are several strengths in our research studies. First of all, we reviewed a myriad of potential biomarkers in diverse domains including clinical, biological and radiological. We chose these biomarkers as the testing equipment was readily available in our department and therefore could be used for both our research and potential future research or clinical settings. Secondly, we collaborated extensively both inside and outside our institution, which increased the expertise levels used in our study. It also broadened the applicability of our results to the general population. We worked with other members of the CUH Neurology department to identify potential subjects with PD; with the Neurophysiology department in training for our NCS and TTT; and with the Anesthesiologists and Orthopedic teams for identifying potential controls for our CSF studies. We collaborated with Limerick Regional Hospital, Limerick for our DaTSCAN study and with St James Hospital, Dublin, and Queen Square Hospital, University College London, England for our CSF studies. Using the recently created Parkinson’s Disease Research Cluster (PDRC) at University College Cork (UCC), Ireland, we collaborated with other PD researchers in CUH and UCC. We also participated locally in our community with the PD society in Cork city. Thirdly, we applied a novel approach to diagnosing autonomic neuropathy using the validated SCOPA-AUT questionnaire and TTT. Lastly, to the best of our knowledge, we are the first group to study GDF5 protein levels in the CSF of people.
6.3 Limitations of our studies

However, we are also aware of some limitations in our studies. Firstly, our sample size for the clinical and biological biomarkers’ studies was relatively small. At the initiation of this MD, there was no established database of PD patients and no biobank of biological samples in Cork. Therefore, all subjects were freshly recruited from the community or clinics and enrolled into these studies; or attained through new collaborations with other institutions. Due to the small sample sizes, we may have missed clinically significant results or conversely, seen associations that would not be replicated in larger studies. Secondly, although collaboration is important for research and allowed us to develop relationships with other institutions and ultimately increase our sample sizes, it may have introduced confounding variables into our CSF samples. We tried to reduce sample variabilities by ensuring that the collection, processing and storage protocols were similar between institutions. Thirdly, the ELISAs applied in our CSF studies were not specifically validated for CSF. Although cytokines and GDF5 protein were detectable in most samples, they remained at very low levels. This may have been due to their low concentrations in the lumbar CSF, as seen in other studies, or due to poor sensitivity of these ELISAs for CSF, confounding our results. Other limitations are related to those that have been previously recognized in other biomarker studies, including the recruitment of non-PD subjects into the PD group; PD is a heterogeneous disorder and the different phenotypes are not equivalent; and lastly, the assessment of relatively non-specific markers which are also present in control subjects and the elderly.

6.4 Future research directions

Biomarkers and biomarker discovery are areas of increased interest and active research in PD. Identifying a sensitive and specific biomarker is essential for better understanding of the disease pathogenesis; more rapid and correct diagnosis; informing prognosis in PD; monitoring disease progression; and evaluating disease-modifying effects of new therapies in clinical trials.

In this thesis, we evaluated clinical, biological and radiological biomarkers; and identified differences in subjects with PD, in all of these individual domains. However,
there was often overlap in these markers between the two groups. In future research, we expect that all of these avenues will continue to be explored and that a multimodal approach is likely to result in the most sensitive and specific biomarker for PD.

Our clinical biomarker study on autonomic neuropathy in PD identified a higher prevalence of autonomic neuropathy in PD subjects compared to controls. However, we were surprised by the lack of association between SCOPA-AUT and TTT and thus, more research is warranted comparing the predictive value of our approach using TTT and SCOPA-AUT with that of more formalized autonomic function testing, skin biopsy, or possibly, cardiac SPECT imaging. As previously mentioned, using our approach, there was overlap between subjects with PD and controls in symptoms of dysautonomia, suggesting that dysautonomia on its own is too nonspecific as a clinical biomarker. Other clinical biomarkers that are actively being investigated are REM sleep behavior disorder, olfactory loss and technological applications for detecting subclinical motor impairments. Using the MDS prodromal PD criteria, it is now possible to calculate an individual’s pretest probability for developing PD (19).

The study of biological biomarkers in PD is a minefield with an endless list of candidate markers currently being measured. Research on neuroinflammation and neurotrophic factors is critical given the clear laboratory and epidemiological evidence of both neuroinflammation and depletion of neurotrophic support in PD pathogenesis, along with the availability of potential therapies i.e. NSAIDs, immunomodulator drugs and injection of growth factors. Based on our research and others, we think that the study of cytokine levels is too inconsistent to be pursued further, at least as a sole biomarker. Multiple studies have failed to identify a reliable inflammatory marker in the serum or CSF. Other modalities, specifically PET imaging may provide a better marker for inflammation and be more suited for monitoring inflammation in PD.

In regard to neurotrophic factors, our study on CSF GDF5 protein expression is the first to examine its levels in humans and in subjects with PD. The identification of a new neurotrophic factor for further investigation in subjects with PD is intriguing, especially as GDF5 protein has been shown in vitro and in vivo to protect dopaminergic neurons. Our research group continues to investigate GDF5’s role in PD. Our results will need to
be replicated in larger human cohorts. If GDF5 is consistently shown to be lower in subjects with PD, therapeutic trials could be considered in the future.

The study of biological biomarkers requires the availability of large well-characterized cohorts with standardized collection procedures, in order to reduce the inconsistent results currently seen in the literature, presumably secondary to differences in study population and methodology. We also think that unbiased screening of hundreds or thousands of markers at once will be more beneficial than the current “candidate biomarker approach” which targets a single marker due to its known involvement in the pathophysiology of PD. Fortunately, there are now several biobanks and international collaborations, making it possible to carry out this type of research, as detailed in the introductory chapter.

Lastly, the utility of DaTSCAN imaging in PD as a biomarker continues to be explored. As seen in our study, DaTSCANs are increasingly being used in the investigation of parkinsonism. The recent endorsement by the EMA for the use of DaTSCANs in clinical trials in PD is exciting for biomarker research. SURE-PD3, a phase 3 clinical trial in PD has included two DaTSCANs in its protocol (368). The first scan acts as a diagnostic biomarker, confirming dopaminergic deficit and excluding SWEDDs; and the second scan at the end of the trial investigates its use as a prognostic biomarker. Nevertheless, ongoing research on DaTSCAN imaging is warranted. The current qualitative nature of reporting DaTSCANs is too subjective and thus, both objective striatal-binding ratios and machine-algorithms are being studied (369,370). It is also an expensive test with limited specificity, being unable to differentiate idiopathic PD from other parkinsonian syndromes. This limitation is significant as these parkinsonian syndromes have different pathologies and prognosis.

The long-term aim is to have biomarkers that can recognize or corroborate the presence of pre-clinical or clinical disease, assess disease severity, and predict disease prognosis. By identifying these biomarkers, we will then be better equipped in the most important mission which is to discover effective neuroprotective therapies for PD.
Chapter 7: References


Stiasny-Kolster K, Doerr Y, Möller JC, Höffken H, Behr TM, Oertel WH, et al. Combination of “idiopathic” REM sleep behaviour disorder and olfactory dysfunction as possible indicator for alpha-


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247. Isobe C, Abe T, Terayama Y. Levels of reduced and oxidized coenzyme Q-10 and 8-hydroxy-2’deoxyguanosine in the cerebrospinal fluid of patients with living Parkinson’s disease demonstrate that mitochondrial oxidative damage and/or oxidative DNA damage contributes to the neurodegenerative process. Neurosci Lett. 2010 Jan 18;469(1):159–63.


305. Hasegawa Y, Inagaki T, Sawada M, Suzumura A. Impaired cytokine production by peripheral
Mar;101(3):159–64.

Serum and Cerebrospinal Fluid of Patients With Alzheimer’s Disease by Color-Coded Bead Technology. J

307. Blum-Degen D, Müller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and
interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer’s and de novo Parkinson’s disease

308. Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1 beta, IL-2,
IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in

309. Wilms H, Rosenstiel P, Sievers J, Deuschl G, Lucius R. Cerebrospinal fluid from patients with
neurodegenerative and neuroinflammatory diseases: no evidence for rat glial activation in vitro.

inflammatory markers in Parkinson’s disease – Associations with depression, fatigue, and cognitive

concentrations of inflammatory markers in Parkinson’s disease and atypical parkinsonian disorders. Sci

May;17(5):427–42.

mini-mental state examination, montreal cognitive assessment, and dementia rating scale-2 scores in

314. Chen X, Hu Y, Cao Z, Liu Q, Cheng Y. Cerebrospinal Fluid Inflammatory Cytokine Aberrations in
Alzheimer’s Disease, Parkinson’s Disease and Amyotrophic Lateral Sclerosis: A Systematic Review and

(TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci

316. Furukawa Y, Kondo T, Nishi K, Yokochi F, Narabayashi H. Total biopterin levels in the ventricular
CSF of patients with Parkinson’s disease: a comparison between akineto-rigid and tremor types. J Neurol

317. Sawada M, Imamura K, Nagatsu T. Role of cytokines in inflammatory process in Parkinson’s
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Chapter 8: Appendices
8.1 Ethical approval for conduct of ‘Autonomic neuropathy in PD study’ (chapter 2)

9th August 2012

Dr Sean O’Sullivan
Consultant Neurologist
Department of Neurology
National Neuroscience Centre
Cork University Hospital
Wilton
Cork

Re: An assessment of the involvement of pain and autonomic symptoms in patients with parkinsonism.

Dear Dr O’Sullivan

Expedited approval is granted to carry out the above study at:

➤ Cork University Hospital.

The following documents have been approved:

➤ Application Form
➤ Invitation Letter
➤ Consent Form (Remove the word “sample” for title of consent form)
➤ Detailed Protocol
➤ The Brief Pain Inventory
➤ Mini-Mental State Examination
➤ Scopa-aut
➤ Beck Depression Inventory.

The co-investigator involved in this study will be:

➤ Dr Sean O’Dowd

Yours sincerely

Dr Michael Hyland
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals
8.1 Ethical approval for conduct of ‘DaTSCAN imaging in PD’ (chapter 3)

17th December 2012

Dr Sean O’Sullivan
Consultant Neurologist
Cork University Hospital
Wilton
Cork

Re: A clinical audit of DaTSCAN use in the CUH.

Dear Dr O’Sullivan

Expedited approval is granted to carry out the above study in:

- Cork University Hospital.

The following documents have been approved:

- Application Form
- Data Collection Sheet.

We note that the co-investigators involved in this study will be:

- Orsin O’Corragain, Medical Student.

Yours sincerely

Dr Michael Hyland
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the assessment of individual clinical audits. The Committee is fully compliant.
8.1 Ethical approval for conduct of ‘DaTSCAN imaging in PD’ (chapter 3)

28th April, 2014.

Dr. Grace Crotty,
Neurology Movement Disorder Research Fellow,
UCC.

Re: Protocol Title
A Clinical Audit of DaTSCAN Use in the University Hospital Limerick.

Dear Dr. Crotty,

The Research Ethics Committee at the University Hospital Limerick has received a submission for ethical approval for the above study.

The following documents were reviewed and approved by the Research Ethics Committee:

Application to the Research Ethics Committee
Approved

From an insurance perspective, please note that cover does not extend to those parties not employed by the Health Service Executive (HSE), or non-HSE Institutions.

Yours sincerely,

Fionnuala O’Brien,
Clinical Programmes Co-Ordinator,
(For and on behalf of the Research Ethics Committee & the Risk Management Department).
8.1 Ethical approval for conduct of ‘Cytokine and GDF5 levels in PD CSF samples’ (chapter 4 & 5)

11th May 2012

Dr. Sean O’Sullivan
Consultant Neurologist
Cork University Hospital
Wilton
Cork

Re: Potential biomarkers for Parkinson’s Disease: comparison of levels of neurotrophic factors in cerebrospinal fluid and serum of Parkinson’s disease patients with those in healthy controls

Dear Dr. O’Sullivan

Expedited approval is granted to carry out the above study in

- Cork University Hospital
- University College Cork.

The following documents were approved:

- Signed Application Form
- Detailed Protocol
- CV for Chief Investigator
- Consent Form Version 1 dated 15th March 2012
- Participant Invitation Letter
- GP Letter.

We note that the co-investigators involved in this study will be:

- Dr. Aideen Sullivan, Dr. Daniel Castello and Dr. Gerard O’Keeffe.

Yours sincerely,

[Signature]

Dr. Michael Hyland
Chairman
Clinical Research Ethics Committee of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Community (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Good Clinical Practice Guidelines as they relate to Ethics Committees and the conditions and procedures of Good
8.1 Ethical approval for conduct of ‘Cytokine and GDF5 levels in PD CSF samples’ (chapter 4 & 5)

26th November 2013

Dr. Sean O’Sullivan
Consultant Neurologist
Cork University Hospital
Wilton
Cork

Re: Potential biomarkers for Parkinson’s disease: comparison of levels of neurotrophic factors in cerebrospinal fluid and serum of Parkinson’s disease patients with those in healthy controls.

Dear Dr. O’Sullivan

The Chairman approved the following:

- Amendment Application Form
- Increased Study Population
- Addition of Dr. Grace Crotty as a co-investigator in the above study.

Full approval will be granted subject to receipt of the following:

- Revised Consent Form – Put a tick box section on Page 3 so that participants can tick separately whether or not they agree to storage of samples.

Yours sincerely

[Signature]

Professor Michael G. Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals
8.2 Publication and published abstracts:
Crotty GF, O'Corragain OA, Bogue C, Crotty J, O'Sullivan SS. The Utility of Dopamine Transporter Scans for Diagnosing Parkinsonian Disorders. Ir Med J. 2018 May; 111 (5).

Published abstracts:


Chapters 1,3 and 4 are prepared manuscripts for submission, currently undergoing co-authours’ reviews, prior to submission

Chapter 2 submitted to journal, currently awaiting journal review