Biomarkers and Parkinson’s disease

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Summary

Biomarkers are characteristics that can be measured as an indicator of a normal biological process, and they have special relevance in Parkinson’s disease. Parkinson’s disease is a chronic neurodegenerative disorder that is difficult to study, given the site of pathology and because the resultant clinical phenotype fluctuates over time. We currently have no definitive diagnostic test, and thus for the clinician there is hope that biomarkers will help diagnose symptomatic and presymptomatic disease or provide surrogate end-points to demonstrate clinical efficacy of new treatments, such as neuroprotective therapies, and help stratify this heterogeneous disease. No biomarker is likely to fulfil all these functions, so we need to know how each has been validated in order to understand their uses and limitations, and be aware of potential pitfalls. In this review we discuss the current potential biomarkers for Parkinson’s disease, highlight the problems with their use, and conclude with a discussion of future alternatives.

Keywords: biomarkers; Parkinson’s disease; diagnosis

Abbreviations: DAT = dopamine transport; MIBG = metaiodobenzylguanidine; MSA = multiple system atrophy; SPECT = single-photon emission computed tomography


Introduction

Parkinson’s disease is a common chronic neurodegenerative disorder in which there is a loss of dopaminergic nigrostriatal neurons in the substantia nigra pars compacta with evidence of intracytoplasmic inclusions known as Lewy bodies. The classical clinical features are of progressive tremor, rigidity and bradykinesia. However, neuronal loss occurs beyond the dopaminergic system, and consequently patients display autonomic, affective and cognitive deficits. Parkinson’s disease can be difficult to diagnose in its early stages, and may be mimicked by other diseases, such as essential tremor, multiple system atrophy (MSA) and progressive supranuclear palsy (see reviews by Galvin et al., 2001; Burn and Lees, 2002; Poewe and Wenning, 2002).

Optimization of our treatment of Parkinson’s disease requires accurate information both about the ongoing disease process in the brain and its corresponding clinical syndrome. However, in Parkinson’s disease the key pathology is in the brainstem, hidden from direct study during life, and this, coupled to a fluctuating clinical syndrome over time, makes it difficult to monitor in an unbiased and objective manner.

Biomarkers aim to improve our data collection and knowledge about both the clinical and pathological parameters of disease (see text box 1 for a definition; Biomarkers Definitions Working Group, 2001), which is complicated in Parkinson’s disease by a rather poor correlation between the underlying pathology and the subsequent clinical phenotype. This review therefore aims to clarify the understanding of biomarkers and their relevance to Parkinson’s disease with a discussion of current candidates and their potential successors.

Why do we need a marker for Parkinson’s disease?

To improve diagnosis

To many, the most intuitive goal for a biomarker is to help diagnose disease. For Parkinson’s disease this may be divided into two separate aims: differentiation of susceptible individuals from normals before symptoms develop (sensitivity), and identification of true idiopathic Parkinson’s disease from its imitators once symptomatic (specificity).

Even in highly specialized centres the sensitivity of the clinical diagnosis of Parkinson’s disease in symptomatic patients is only about 91% (Hughes et al., 2002), and it is likely to be far less in other settings (Rajput et al., 1991; Hughes et al.,...
extrapyramidal motor symptoms (Fig. 1).

Surrogate marker A biomarker that is intended to substitute for a clinical end-point. A surrogate end-point is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiological, therapeutic, pathophysiological or other scientific evidence.

Surrogate markers are a subset of biomarkers.

Clinical end-point A characteristic or variable that reflects how a patient feels, functions or survives.

Disease-modifying therapy Treatment that affects the underlying pathophysiology of disease rather than purely its symptoms (although there may be symptom improvement as a result of these treatments).

For Parkinson’s disease, neuroprotective and neurorestorative therapies are potential disease-modifying therapies.

1992). Thus it is clear that any way of detecting true idiopathic Parkinson’s disease and distinguishing it from the numerous causes of a similar clinical syndrome would be beneficial, enabling better epidemiology and natural history studies and allowing cheaper, more powerful clinical trials.

For Parkinson’s disease the need for early presymptomatic diagnosis is driven by the evolution of putative neuroprotective agents (Stocchi and Olanow 2003) that would ideally be administered as soon as the characteristic pathology is detected, given that ~50% reduction in dopaminergic nigral cells is required before clinical expression (Fearnley and Lees, 1991). It seems likely that this presymptomatic phase of Parkinson’s disease lasts ~5 years, given recent pathological studies (Fearnley and Lees, 1991) and imaging data (Morrish et al., 1996a; Marek et al., 2001). However, patients may develop subtle clinical correlates corresponding to a prodromal syndrome lasting 4–6 years (Gonera et al., 1997), thus providing an opportunity to make an early diagnosis before the onset of characteristic extrapyramidal motor symptoms (Fig. 1).

To monitor disease progression and demonstrate treatment efficacy

Once a diagnosis of Parkinson’s disease is established, the role of biomarkers changes. One valuable role is in longitudinal clinical treatment trials to provide surrogate end-points which, if adequately validated, can provide a degree of objectivity and potentially enable a reduction in both the duration of a trial and the number of patients required for significance. Examples include the measurement of blood pressure and cholesterol that have been clearly linked to mortality from myocardial infarction and stroke in large trials (Scandinavian Simvastatin Survival Study Group, 1994; Hansson et al., 1998) and are now accepted end-points for drug licensing. Unfortunately, at present no such biomarkers exist for Parkinson’s disease.

In established Parkinson’s disease there are two particular problems for surrogate markers to overcome. Firstly it is difficult to be sure about the magnitude of treatment effect by clinical assessment since symptoms fluctuate over time and the majority of patient assessments are subjective. Attempts to overcome subjectivity have included the use of rating scales, such as the Unified Parkinson’s Disease Rating Scale, which has been shown to have reasonable interobserver variability, although it is biased towards assessment of motor deficits (Martinez-Martín et al., 1994).

The second hurdle is that some putative neuroprotective agents (for example dopamine agonists, discussed below) also have symptomatic effects unrelated to their disease-modifying action. This affects the ability of clinical rating scales to detect the neuroprotective action even if patients are assessed after a 12 h washout period, since dopaminergic stimulation lasts much longer than this (Nutt et al., 2002). Thus, there is a need for a biomarker that can reliably detect retarded pathological progression without relying on symptoms.
What is the marker telling us?
Before we can use biomarkers for Parkinson’s disease, it is essential to determine exactly what they are measuring, and therefore what information they can and cannot provide (Fig. 2). On the one hand, there exists an underlying disease process, which might be considered to be one of a number of events, such as Lewy body formation, neuronal degeneration, dopamine depletion and so on. On the other hand, there are a range of clinical phenotypes caused by this process. The problem in Parkinson’s disease is that there is poor clinicopathological correlation, meaning that you cannot reliably predict clinical phenotype if you know the pathology, and vice versa. So, for example, a patient’s symptoms fluctuate hour by hour, but their pathology presumably does not. In addition, ~10% of people over 60 have incidental Lewy bodies in their brain, yet only a fraction of these ever develop symptoms (Fearnley and Lees, 1991; Ben-Shlomo and Wenning, 1994). Furthermore, as discussed above, our ability to predict pathology (diagnose) from clinical phenotype is poor. The result is that a biomarker targeted to the detection of pathology may well not be able to provide clinical information, and vice versa.

Of course, in reality markers that are designed to provide information about pathology can also provide some information about clinical symptoms by correlation. For example, imaging serotonergic function by \(^{11}\)C-WAY100635 PET not only provides information about the degeneration in median raphe signalling but also correlates with rest tremor (Doder et al., 2003). The important point is to appreciate what the biomarker (in this case PET imaging) was primarily designed to monitor (pathology here) and what information is acquired by correlation (clinical phenotype, in particular rest tremor). In this example the PET scan is not a biomarker for rest tremor, and the observed association might be lost in a different population.

What are the problems in using biomarkers in clinical practice?

Pitfalls when using biomarkers in diagnosis
Biomarkers have a positive predictive value, which provides a measure of the chance that a patient with a positive result has the disease. Naturally, we wish to improve the positive predictive value of a biomarker, but this can only be done by increasing its specificity (an intrinsic property of the test that we can not alter) or by an increase in the prevalence of the disease (perhaps by testing only those at increased risk).

There is a natural tendency for clinicians to combine biomarkers when faced with a symptomatic patient they suspect has a disease in whom test results have so far proved negative. Although tests can be statistically combined, in order for an additional test to improve diagnostic accuracy it should be independent of those already in use, which can be difficult in practice. The use of multiple diagnostic biomarkers will tend to increase the likelihood of type I errors—diagnosing an unaffected person as having the disease (for a discussion see Schulzer, 1994 or Rivner, 1994).

Pitfalls with the use of biomarkers as surrogate end-points
Biomarkers to be used as surrogate end-points need to be rigorously validated, ideally using more than one drug for the same indication in a particular population (Temple, 1999). A variety of statistical methods have been proposed to represent the proportion of treatment effect that is captured by a surrogate marker (Cowles 2002), and there are many reasons for dissociation between a surrogate marker and the clinical end-point it is trying to represent (Prentice, 1989) (Text Box 2).

Although sometimes proposed in place of clinical end-points, biomarkers are more often measured in addition to these end-points since this increases the clinicopathological
Text Box 2 Reasons for a dissociation between a surrogate marker and the end-point it is trying to represent

1. False positives
(a) Treatment affects the biomarker but not the disease. A well-known example occurred in the Cardiac Arrhythmia Suppression Trial (CAST), where flecainide suppressed arrhythmias (the biomarker) after myocardial infarction, but did not reduce mortality (the end-point) (Echt et al., 1991).
(b) Treatment affects a clinically unimportant aspect of pathophysiology that is faithfully represented by the biomarker.

2. False negatives
Treatment does not alter the biomarker even though it does alter a useful aspect of the disease pathophysiology. For example, when testing the therapeutic effect of interferon-γ on recurrent infections in patients with chronic granulomatous disease, the biomarker (superoxide production by phagocytes) did not change even though there was clinical benefit (International Chronic Granulomatous Disease Study Group, 1991).

3.Insensitive
Treatment does alter the biomarker, but many unexpected outcomes of the treatment are not represented.

4. More sensitive
In some situations, such as during the long presymptomatic phase that occurs in Parkinson’s disease, a biomarker sensing pathological change may be more sensitive than the clinical outcome.

Potential biomarkers for Parkinson’s disease
The large number of potential markers for Parkinson’s disease will be reviewed by dividing the potential candidates into three main categories.

1. Imaging as a biomarker

Functional imaging
PET and single-photon emission computed tomography (SPECT) imaging have numerous potential applications, including following the decline in neurotransmitter function, examining metabolic activity (using 18F-fluorodeoxyglucose) and monitoring regional cerebral blood flow. Furthermore, new ligands in development may be able to provide pathological information, such as the microglial response to cell loss (e.g. 11C-PK11195; Cicchetti et al., 2002; Gerhard et al., 2003) or accumulation of proteins such as α-synuclein and β-amyloid (Zhuang et al., 2001). In general, whilst PET scans have greater spatial resolution (especially in 3D mode), SPECT scans are cheaper and are more widely available—an important point if such a marker is to be adopted clinically.

To determine what information is obtained by PET or SPECT imaging is complicated and comes down to validation of a particular ligand in a particular situation. For example, 18F-dopa is taken up by dopaminergic neurons and converted to 18F-dopamine; therefore a scan using this ligand will provide a representation of L-dopa uptake through the blood–brain barrier, aromatic amino acid decarboxylase activity that converts L-dopa to dopamine, and the dopamine storage capacity in synaptic vesicles. Early pathological validation studies have shown that striatal 18F-dopa uptake correlates well with nigral cell count in humans with various diseases (Snow et al., 1993), and that the striatal 18F-dopa influx constant (K_i) correlates with dopamine levels, synthetic enzyme activity and nigral cell counts in monkeys lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Pate et al., 1993).

It is worth reiterating, however, that this validation is specific for a particular set of conditions, and that this relationship may be lost. For example, in early Parkinson’s disease the uptake of 11C-methylphenidate, a marker of the dopamine transporter (DAT), is reduced relatively more than 18F-dopa (Lee et al., 2000), which may represent compensatory down-regulation of DAT to try to maintain dopamine levels in the synapse. Furthermore, dopaminergic medications may directly affect these scans (for example, by possibly causing DAT down-regulation), which would drastically affect their interpretation, especially in the context of neuroprotection (reviewed recently by Brooks et al., 2003).

Presymptomatic detection. One of the goals of functional imaging is the presymptomatic detection of patients and there are now several publications to suggest this may be possible. For example, patients with hemi-Parkinson’s disease showed reduced uptake on the ipsilateral as well as the expected contralateral side (Schwarz et al., 2000), twin studies have shown a dopaminergic deficit in asymptomatic twins of patients with Parkinson’s disease (Burn et al., 1992; Holthoff et al., 1994; Laihinen et al., 2000), and dopaminergic dysfunction has been shown in four people after taking 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine prior to the onset of symptoms (Calne et al., 1985). However, one unresolved issue with these studies is whether subjects with preclinical imaging abnormalities actually go on to develop Parkinson’s disease, although data from one of the twin studies suggests that at least some do (Piccini et al., 1999b).

At present, PET and SPECT images show significant variation between normal subjects, such that a preclinical cell loss of less than 50% will be difficult to detect. There are therefore ongoing efforts to increase the specificity of the test, including detailing the topography of the dopaminergic deficiency that seems to affect the dorsal putamen early in the disease (Sawle et al., 1994; Morrish et al., 1996b). Alternatively, the positive predictive value of the test might be improved by scanning only those at particularly high risk of developing Parkinson’s disease, such as family members (Piccini et al., 1997) or...
Differenating symptomatic Parkinson’s disease from atypical Parkinsonian disorders. A different potential diagnostic use for functional imaging is to help determine which symptomatic patients really have Parkinson’s disease. Assessment of dopaminergic function of the caudate and putamen using 18F-dopa PET showed that in 64% of patients the PET diagnosis agreed with clinical diagnosis of Parkinson’s disease, increasing to 70% for progressive supranuclear palsy, but the agreement was far less for MSA (Burn et al., 1994). 11C-raclopride PET is emerging as a potential alternative, providing an indirect estimate of synaptic dopamine levels from changes in tracer D2 receptor binding potentials (Brooks et al., 1992; Antonini et al., 1997). Rather than studying dopaminergic function, some groups have chosen quantitative 18-fluorodeoxyglucose PET (18FDG PET) to monitor metabolic activity, and the results suggest that some patients with clinically diagnosed striatognial degeneration can be differentiated from those with Parkinson’s disease and normal controls because of their reduced caudate and putamen signal (Eidelberg et al., 1993).

However, one of the problems in interpreting these studies is that they are not particularly representative of real life, where the challenge is to distinguish the different forms of parkinsonism when the diagnosis is in doubt. In most comparative imaging studies the patients are readily distinguishable clinically. Furthermore, this clinical diagnosis becomes the gold standard against which the scan result is compared even though we know that our clinical diagnosis is not totally accurate [although a recent study has suggested a positive predictive value of up to 98.6% for the clinical diagnosis of idiopathic Parkinson’s disease in a specialist movement disorder centre using pathological confirmation (Hughes et al., 2002)].

Nevertheless, with improved scan resolution and the use of newer ligands there are likely to be significant advances in the next few years to enable further clinical use of these promising tools. Thus it may prove useful, for example, to assess striatal D2 dopamine receptors on medium spiny GABAAergic neurons using (125I)-S(-)IBZM (iodobenzamide) since in Parkinson’s disease there is no change in D2 binding, whereas in MSA and progressive supranuclear palsy it seems to be diffusely reduced (discussed by Brucke et al., 2000). Alternatively, it may be that different scanning approaches are adopted, such as the use of 1H magnetic resonance spectroscopy to study cerebral metabolites (O’Neill et al., 2002) or accurate structural MRI (Savoiardo, 2003; Yekhlef et al., 2003). As with functional imaging of the dopaminergic system, the intersubject variation between normal people and those with Parkinson’s disease currently limits the accuracy of these tools.

Monitoring disease progression. In the REAL PET study the 18F-dopa influx constant (Ki) in the putamen was measured in patients randomized to receive either levodopa or ropinirole over 2 years. At baseline 11% of patients with the clinical diagnosis of Parkinson’s disease were felt to have normal images from the caudate and putamen. In the remainder there was a significant difference in the deterioration of putamen 18F-dopa uptake with ropinirole compared with levodopa (~13% versus −20%, P = 0.02). The investigators concluded that ropinirole caused ~30% slowing of the progression of Parkinson’s disease over this period (Whone et al., 2003).

The intrasubject test–retest reproducibility of 18F-dopa PET is relatively good and by using the latest PET machine, co-aligning three dimensional image sets and looking at putamen 18F-dopa influx constants (Ki) the variability can be as low as 2% (Brooks, 2003), suggesting that it is potentially a powerful way of following disease progression in a patient. In fact it was estimated that by using 18F-dopa PET it is possible to detect a 30% protective effect with 80% power in a 2 year study with 60 patients treated with levodopa versus 60 with a neuroprotective agent (Brooks, 2003). However, as discussed above, it is possible that levodopa alters the uptake of 18F-dopa and therefore changes the 18F-dopa influx constant, which would clearly alter the interpretation of the REAL PET results (Fahn, 2002).

In contrast, in the CALM-PD study, 2β-carboxymethoxy-3β(4-iodophenyl)tropane (123I-β-CIT) SPECT was used to study the integrity of the dopaminergic system (reuptake of dopamine into axons via the DAT) in patients treated with pramipexole versus levodopa. Overall the results suggested an ~40% reduction in the rate of loss of striatal 123I-β-CIT uptake with pramipexole compared with levodopa (Parkinson Study Group, 2000, 2002), with some correlation to clinical phenotype in that the percentage reduction in striatal 123I-β-CIT uptake correlated with the change from baseline in the UPDRS at 46 months (r = −0.4, P = 0.001).

The issues encountered in monitoring disease progression with 18F-dopa PET, 123I-β-CIT SPECT and (+)-1C-dihydrotetrabenazine PET [which reflects storage of dopamine in synaptic vesicles via VMAT2 (the vesicular monoamine transporter)] have recently been reviewed elsewhere (Brooks et al., 2003; Morrish, 2003). As well as use in neuroprotective studies, these imaging tools have been used to show that cell transplantation can restore dopaminergic function in line with clinical improvement (Piccini et al., 1999a), providing evidence that clinical benefit relates to the dopaminergic activity within the graft.

Can functional imaging assess the validity of other biomarkers? Finally, great caution needs to be exercised when functional imaging is used as a surrogate marker with which to assess the efficacy of other potential biomarkers. For example, Wolters and colleagues performed a study comparing sense of smell with 123I-β-CIT SPECT in first-degree relatives of patients with Parkinson’s disease (Wolters et al., 2000), since they are known to have an increased risk of developing the disease (Payami et al., 1994; Marder et al., 1996). They hypothesized that the 50 best and worst smellers of a group of 500 would have significantly different striatal binding of 123I-β-CIT. However, even if subjects demonstrating a poor result on a potential new test also have poor SPECT scans it must be shown that this same group does in fact go on to
develop Parkinson’s disease in order to show they could be good biomarkers.

**Transcranial ultrasound**

Using a temporal bone acoustic window, it is possible to use ultrasound to determine the echogenicity of the substantia nigra (Becker and Berg, 2001). In Parkinson’s disease this tends to be increased bilaterally, whereas in extrapyramidal neurological disorders it is often normal, although in ~30% of cases this examination could not distinguish these two groups (Berg et al., 2001b; Walter et al., 2002). As yet the pathophysiological significance of this increase is not clear, but it seems to relate to iron and ferritin levels, and potentially therefore an increase in iron-derived free radicals causing cell damage (Berg et al., 2002).

There is some evidence to suggest that this technique might detect presymptomatic disease. For example, the substantia nigra hyperechogenicity seen in healthy asymptomatic subjects correlated with a reduction in 18F-dopa uptake in the putamen using PET (Berg et al., 2002), and psychiatric patients with high echogenicity developed worse extrapyramidal signs and symptoms on neuroleptics compared with those with lower echogenicity (Berg et al., 1999, 2001a). Furthermore, elderly people without a diagnosis of Parkinson’s disease but with substantia nigra hyperechogenicity showed more frequent and more severe slowing and hypokinesia than age-matched subjects with normal echogenicity, although it is unknown how many of these go on to develop frank Parkinsonism (Berg et al., 2001c).

**Cardiac metaiodobenzylguanidine (MIBG) scintigraphy**

Many patients with Parkinson’s disease complain of autonomic dysfunction, such as bladder irritability, sweating or constipation, prior to developing characteristic extrapyramidal signs. Furthermore, there is post-mortem evidence of Lewy body formation diffusely within the autonomic nervous system, including enteric nerves and cardiac plexus (Qualman et al., 1984; Singaram et al., 1995; Wakabayashi and Takahashi, 1997). In the light of these findings some researchers have chosen to study autonomic function as a potential biomarker in Parkinson’s disease.

MIBG is an analogue of noradrenaline that is transported into sympathetic neurons and can act as a tracer of catecholaminergic neurons if labelled with radiolabelled iodine and detected by scintigraphy. A recent review of the use of such imaging in a total of 246 Parkinson’s disease patients and 45 cases of MSA suggested that the cardiac to mediastinal uptake ratio of MIBG could correctly identify idiopathic Parkinson’s disease with 89.7% sensitivity and 94.6% specificity from the group with MSA (Braune, 2001).

However, caution must be exercised in interpreting these promising results since this biomarker is affected in other diseases that might imitate Parkinson’s disease, such as dementia with Lewy bodies (Yoshita et al., 2001). In addition, we do not know exactly what the reduced MIBG uptake in the heart of patients with Parkinson’s disease means since it does not reflect denervation, and it seems to be reduced even without overt autonomic failure.

### 2. Clinical testing procedures

**Firstly let us consider diagnosis.** Any form of clinical testing from neuropsychology to clinical neurophysiology relies on the phenotypic expression of the disease process, and could therefore not detect the preclinical period of neuronal loss (this would require a marker reflecting pathology). However, these testing procedures might be able to sensitively detect subtle early symptoms and signs in at-risk groups that would otherwise be missed on routine clinical assessment. They may therefore effectively shorten the prediagnostic period and might be useful in precipitating early treatment. For example, in Huntington’s disease there is evidence that poor performance on tests of attentional set shifting and semantic verbal fluency can be found in ‘asymptomatic’ gene-positive individuals (Lawrence et al., 1998), at the time of reduced striatal D1 and D2 receptor binding on 11C-raclopride PET (Andrews et al., 1999).

Secondly, a biomarker that accurately measures clinical phenotype will be of limited use for assessing pathological progression since it would be affected by purely symptomatic effects of disease modifying medication (see above in ‘Monitoring disease progression’ – the dopamine agonist trials). However, clinical markers are essential for monitoring symptom fluctuation in response to treatment, which is the ultimate end-point for patients and clinicians. Furthermore, given that Parkinson’s disease is extremely heterogeneous (Foltynie et al., 2002a), these markers are likely to help stratify the diseased population by patterns of presentation and progression. This might prove helpful in targeting novel treatments, such as cell transplantation, since early data suggest that some subgroups of the Parkinson’s disease population respond better than others (Freed et al., 2001; Olanow et al., 2003).

### Affective and psychological tests

Prior to developing Parkinson’s disease many patients suffer a range of rather non-specific symptoms, such as depression, anxiety and musculoskeletal pain, that might herald subsequent disease development (Gonera et al., 1997). It is possible to use depression rating scales and other tools to monitor some of these symptoms (Montgomery et al., 2000a, b), but the results tend to be rather variable, which is partly because the early symptoms of Parkinson’s disease might be similar to prodromal depressive symptoms and because rating scales such as the Beck Depression Inventory (Beck et al., 1961) are not specifically designed to capture the relevant early depressive symptoms.

An alternative might be to develop a test that concentrates on certain features of the depression that are more specific to Parkinson’s disease patients, such as feelings of self-reproach (Huber et al., 1990) or anxiety-related depression (Hoogendijk...
et al., 1998). Early studies suggested that Parkinson’s disease sufferers showed emotional repression, self-reliance, introversion and punctuality, although this was based on small numbers of patients and anecdotal evidence (for reviews see Todes and Lees, 1985; Menza, 2000). Nevertheless, it may be that a personality questionnaire looking at such traits may go some way to predicting mood disorder and help with early diagnosis in susceptible individuals, although the issue of identifying the latter group remains.

There is evidence that cognitive dysfunction may affect executive processes and precede the motor manifestations of Parkinson’s disease both in humans and a slowly progressing animal model of Parkinson’s disease (Schneider and Pope-Coleman, 1995; Brown et al., 1998). This may remain unrecognized by relatives because external cues in the environment and a familiarity with daily tasks allow the sufferer to function normally. It is possible, however, that complex neuropsychological tests requiring an intact working memory and executive function may pick up these deficits early in susceptible people (Cooper et al., 1991). However, one study using the Tower of London test of problem-solving and executive function did not reliably detect early Parkinson’s disease (Owen et al., 1992).

Beyond a role in diagnosis it seems increasingly likely that cognitive behaviour may provide a powerful way of defining disease heterogeneity and increasing our understanding of the neural correlates of cognitive dysfunction within the disease (Lewis et al., 2003a, b; Fohtynie et al., 2004).

**Tests of motor performance**

Any test of motor performance in Parkinson’s disease can really be viewed as an extension of the clinical examination, which has been refined in the light of post-mortem data to be as diagnostically accurate as possible (Rajput et al., 1991; Hughes et al., 1992b). Performance of complex tasks such as writing, visually guided movement or sequential tasks has been used to examine actions that involve high-level motor control, whereas some researchers have focused on simple parameters, such as the velocity of movement or reaction time.

Using these procedures there is some evidence of a motor abnormality before symptoms are declared by the patient. So, for example, when testing patients with unilateral symptoms of Parkinson’s disease, motor abnormalities have frequently been detected on the ‘normal’ side (Horstink and Morrish, 1999). Visuomotor testing has also revealed impairments on the asymptomatic side of hemiparkinsonian patients in the control of movement direction during tracing tests, and of movement velocity during target tracking (Hocherman and Giladi et al., 1999). However, many tests looking at simple movements are unrewarding in their conclusions if taken in isolation (Kraus et al., 2000; Montgomery et al., 2000a, b) and contingent on mood and motivation.

Overall, with refinement there may be a testing algorithm that might help in the early diagnosis of disease. To be really useful it would need to differentiate atypical cases from true Parkinson’s disease when there is clinical uncertainty, and at present there are no trial data to support this. Therefore at the present time these tests would seem to be more useful in monitoring clinical progression and response to symptomatic treatment rather than diagnosis.

**Clinical neurophysiology**

There are two rather different clinical neurophysiological approaches to Parkinson’s disease. The first of these has been to describe and subdivide the exact motor phenotypes resulting from disease of the basal ganglia as there are neurophysiological counterparts of the key clinical features of the disease. Thus, tremor can be monitored by surface EMG or accelerometer, bradykinesia by reaction times or ballistic movements, and rigidity by surface EMG or long-latency stretch reflexes (Valls-Sole and Valdeoriola, 2002). This descriptive approach allows the objective monitoring of the effects of treatment and any change in clinical signs over time. Furthermore, it provides valuable insight into the pathological cause of these clinical complaints, which should help us understand and subclassify this heterogeneous disease.

Alternatively, we know that pathological changes occur in Parkinson’s disease outside the basal ganglia, which, if they can be detected and accurately monitored, might themselves provide a useful marker. For example, it seems likely that sensory motor integration is affected in Parkinson’s disease, and could be measured using somatosensory evoked potentials (Rossini et al., 1989), or transcranial magnetic stimulation to reveal abnormal higher cortical processing (Lewis and Byblow, 2002). Furthermore, there is some evidence for a reduced amplitude and slope of the cerebral electrical activity that precedes and accompanies voluntary internally paced movements (the Bereitschaftspotential) in Parkinson’s disease compared with controls (Filipovic et al., 2001). The sympathetic skin response and electrocardiogram R–R interval variation have been used to help confirm and monitor clinical dysautonomia (Zakrzewska-Pniewska and Jamrozik, 2003), which may help more in stratifying disease than in diagnosis or monitoring disease progression. Finally, several groups have investigated the use of anal sphincter EMG, which can help distinguish subjects with MSA from normals, but unfortunately it is unable to reliably distinguish MSA from Parkinson’s disease, given the range of neurogenic changes in Parkinson’s disease, especially in advanced disease (Giladi et al., 2000; Libelius and Johansson, 2000; Vodusek, 2001).

**Olfaction**

The loss of smell detection, identification or discrimination often goes unnoticed early in neurodegenerative disease, before the development of extrapyramidal signs. In Parkinson’s disease this may in part reflect neurodegeneration within the olfactory bulb since there is evidence that this might precede both nigral degeneration and symptoms (Braak et al., 2002). Support for the presymptomatic deterioration of olfaction has been provided by Berendese and colleagues, who showed olfactory dysfunction in first-degree relatives of patients with Parkinson’s disease together with reduced...
striatal dopamine transporter binding, as assessed by $^{125}\text{T-}\beta$-CIT in four out of 25 SPECT scans of these hyposmic relatives (Berendse et al., 2001). Two of the relatives with hyposmia and reduced striatal dopamine transporter binding subsequently developed Parkinson’s disease, suggesting that olfaction might be a useful presymptomatic biomarker.

Once symptoms have developed, it has been suggested that olfactory dysfunction might help distinguish patients with idiopathic Parkinson’s disease from healthy subjects (Tissingh et al., 1999). Perhaps more usefully, however, it may be that alteration in the sense of smell can also help distinguish true cases of Parkinson’s disease from their imitators, such as progressive supranuclear palsy (Doty et al., 1993; Hawkes, 2003). In a recent study it was found that patients with idiopathic Parkinson’s disease were either anosmic or hyposmic, whereas all but one of the patients with MSA or progressive supranuclear palsy had only mild to moderate hyposmia (Muller et al., 2002), and patients with corticobasal degeneration or psychogenic movement disorders were found to be normosmic.

**Vision**

There are many ways in which vision might be affected in Parkinson’s disease (Bodis-Wollner and Onofri et al., 1987). For example, it has been suggested that colour vision and contrast sensitivity might be abnormal as a result of a change in intraretinal dopaminergic transmission in amacrine and interplexiform cells, and colour vision has indeed been found to be abnormal in some Parkinson’s disease patients (Buttner et al., 1995) but not all (Vesela et al., 2001). Furthermore, there seem to be differences in contrast sensitivity, visual evoked responses and electroretinograms in Parkinson’s disease patients compared with controls, but the diseased and normal values overlap (Price et al., 1992). Given that the pathological process in Parkinson’s disease affects retinal cells, it may be that tests of retinal function will correlate more closely with pathological changes in the basal ganglia than clinical phenotype.

On the other hand, abnormalities of eye movement might turn out to be more closely related to motor phenotype than pathology since, for example, it seems that visual landmarks improve antisaccade performance (a saccade made in the opposite direction to a stimulus) in Parkinson’s disease more than controls, in a fashion analogous to target-directed pointing (Briand et al., 1999). Several studies have recorded eye movements in Parkinson’s disease compared with controls, and although there does seem to be some difference between Parkinson’s disease patients and controls during voluntary saccade paradigms (Briand et al., 1999) their potential as biomarkers is not fully characterized.

**3. Biochemical and genetic tests**

The ideal biochemical biomarker would need to be easily measured and to reflect accurately the ongoing degenerative process in the basal ganglia. Furthermore, any correlation with clinical phenotype should only arise secondarily to this relationship and the marker should not, for example, be influenced by symptomatic drug therapies.

**Blood tests**

Parkinson’s disease is thought to be due, at least in part, to increased oxidative stress (Jenner, 2003) and considerable effort has been spent in the search for a marker of this process. For example, it has been noted that patients with Parkinson’s disease show a selective reduction in mitochondrial complex I in their substantia nigra (Schapira et al., 1990) and that platelets exhibit a similar deficiency (Parker et al., 1989). Platelets have been used for a long time to model serotonergic and dopaminergic neuron behaviour (Da Prada et al., 1988), and since they are easily collected they make appealing candidates as peripheral biomarkers of oxidative stress, rather than other sources such as skeletal muscle (Blin et al., 1994). However, there may be a degree of tissue specificity, and at least one study has reported a lack of difference in complex I levels in the platelets of patients with Parkinson’s disease versus controls (Mann et al., 1992), which would limit the ability of peripheral assays to detect central changes.

The concentrations of several other potential markers of oxidative stress have been measured in blood, such as malondialdehyde, superoxide radicals (Ilic et al., 1999), the coenzyme Q10 redox ratio (Gotz et al., 2000), 8-hydroxy-2’-deoxyguanosine from oxidized DNA and 8-hydroxyguanosine from RNA oxidation (Kikuchi et al., 2002; Abe et al., 2003). The levels tend to be abnormal in Parkinson’s disease compared with control groups, providing valuable insight into the nature of the oxidative stress, but none is sufficiently robust to be useful as a diagnostic biomarker of the disease process in clinical practice. A similar situation exists if instead the levels of protective enzyme systems are compared, such as glutathione reductase, or copper and zinc superoxide dismutase (Ilic et al., 1999).

An alternative haematogenous biomarker for Parkinson’s disease may arise from studies looking at dopamine metabolism in the periphery. There is some evidence of a reduction in dopaminergic transporter immunoreactivity in lymphocytes of Parkinson’s disease patients (Caronti et al., 2001). In addition there is evidence that platelet monoamine oxidase B activity is increased and plasma β-phenylethylamine is reduced in patients with Parkinson’s disease, and that this may be reversed by selegiline treatment (Bonuccelli et al., 1990; Zhou et al., 2001). Attempts to detect a difference in plasma homovanillic acid in Parkinson’s disease patients versus controls have so far been unrewarding (Jenner et al., 1993).

Finally, some researchers have opted to look instead at α-synuclein in platelets of controls versus patients with Parkinson’s disease since there is evidence for a dose effect of this protein (Singleton et al., 2003) as well phosphorylation in human synucleinopathies (Fujiwara et al., 2002). Unfortunately, although α-synuclein is present in platelets (Hashimoto et al., 1997) early studies do not suggest there is any difference in the amount in controls versus Parkinson’s disease (Li et al., 2002) (A.W. Michell, L.M. Luhesti, M.G. Spillantini, R.A. Barker, unpublished results).
**CSF**

CSF is a less appealing source of biomarkers in comparison with blood because of the difficulty of obtaining samples. None the less, many researchers have investigated its chemical composition in Parkinson’s disease, especially in relation to potential markers of oxidative stress, some of which have also been investigated in blood. For example, the levels of 8-hydroxy-2′-deoxyguanosine and 8-hydroxyguanosine were found on average to be elevated in Parkinson’s disease (Kikuchi et al., 2002; Abe et al., 2003). Similarly, the levels of the reactive oxygen species malondialdehyde have been recorded in the CSF and found to be significantly increased in Parkinson’s disease compared with controls (Ilic et al., 1999).

Numerous other potential CSF biochemical markers have been studied. These have revealed that in Parkinson’s disease compared with control subjects there are reduced levels of CSF β-phenylethylamine that correlated negatively with Hoehn and Yahr clinical stage (Zhou et al., 1997). Furthermore, ventricular CSF levels of orexin were lower in Parkinson’s disease patients than controls, especially in those with advanced disease (Drouot et al., 2003). On the other hand, there seem to be similar amounts of α-synuclein (Borghì et al., 2000) and insulin (Jimenez-Jimenez et al., 2000), and only slightly differing levels of homovanillic acid, 5-hydroxy-indoleacetic acid and acetylcholinesterase (Hartikainen et al., 1992; Jenner, 1993; Loeffler et al., 1995).

Of course the above studies throw some light on disease pathogenesis and were not carried out purely to detect potential biomarkers, and as diagnostic tests they would provide at best poor sensitivity and specificity, like the blood tests described earlier. This may be in part because of treatment or compensatory mechanisms that maintain the levels of dopamine and its metabolites by increased production from the surviving cells (LeWitt et al., 1992; Zigmond et al., 2002).

**Genetic testing**

For the majority of patients there is no clear link between genotype and the development of Parkinson’s disease, although genetic susceptibility is suggested by several lines of evidence, such as the familial clustering of Parkinson’s disease (Sveinbjörnsdóttir et al., 2000; Foltynie et al., 2002b) and the increased incidence of Parkinson’s disease in the identical twin of some patients, especially the young (Burn et al., 1992; Tanner et al., 1999). Of course there are extremely rare and well publicized autosomal dominant cases of Parkinson’s disease (Polymeropoulos et al., 1997; Kruger et al., 1998), as well as autosomal recessive transmission (Kitada et al., 1998). For these cases alone, genetics is uniquely useful to help diagnosis and to help subdivide this heterogeneous disease, since patients with the autosomal dominant or recessive types of disease tend to have rather different clinical courses.

In sporadic Parkinson’s disease the use of genetic markers is not so clear-cut (Foltynie et al., 2002b; Dekker et al., 2003). There have been a number of candidate gene studies using techniques such as familial linkage analysis, direct DNA sequencing and allelic association studies in an attempt to associate specific genes, such as tyrosine hydroxylase and many others, with Parkinson’s disease (Warner and Schapira, 2003). There has been little success for sporadic disease, but one hope is that if an association is found it might help stratify a diseased population; however, it would not be powerful enough to make predictions for a single patient, given the number of unknown interacting genetic and environmental factors, differing gene penetrance and so on.

**Future directions**

Advances in our understanding of genetics may well eventually provide useful biomarkers for Parkinson’s disease. For example, single-nucleotide polymorphisms and haplotype maps could be used to investigate subtle genetic differences in drug receptors in the light of differing clinical responses to a new drug. This could help determine the molecular basis for clinical variation, and once validated the single-nucleotide polymorphism or haplotype map might serve as a predictive biomarker of clinical response. Alternatively, RNA profiling can provide a map of the genes that are actively being transcribed that, once correlated to drug efficacy, could help explain the biochemical basis of that efficacy. In patients it may eventually be possible to use this technique on leucocytes in a blood sample to gain insight into why a patient is responding well or adversely to a new drug. Given the complex interaction between genes and their environment, these techniques are unlikely to help with diagnosis or individual risk prediction in Parkinson’s disease.

An alternative systems biology approach is to look further down the line and assess the pattern of peptide fragments (proteomics) or metabolites (metabonomics) from a peripheral tissue sample. Using techniques such as nuclear magnetic resonance spectroscopy, we can test for a large variety of compounds in different tissues and subsequently compare the patterns achieved (which represent a metabolic profile) in the diseased and control state to provide an insight into the metabolic defect in disease. Recently, such a technique has been shown to be powerful enough to help diagnose coronary heart disease (Brindle et al., 2002). For Parkinson’s disease this technique might help presymptomatic diagnosis, as well as improve our understanding of the metabolic defect in disease, drug toxicology and the reason that some patients respond better to treatment than others.

**Conclusions**

There is clearly a need for biomarkers that accurately reflect pathological change, given the advent of putative neuroprotective agents. There is already evidence that this is possible to some extent using PET or SPECT imaging of the basal ganglia, but an appealing alternative would be to gain insight into the pathological changes from a continuously variable biochemical marker that can be assayed economically and easily.
Characteristics of the ideal biomarker that is designed to reflect a change in pathological or clinical trait X

- Close (first-degree) association with X without relying on intermediate variables, thereby minimizing the risk of dissociation. It must sensitively reflect even small changes in X.
- Treatment has no direct effect on the biomarker; it only changes with a true change in X.
- The biomarker changes linearly (either negatively or positively) in response to a change in X.
- Measurements are reproducible at a different time or in a different centre.
- The biomarker should ideally capture all changes in X so that no information is lost.
- The optimal clinical biomarker should be cheap, non-invasive and quick to measure by untrained staff.
- Appropriately thorough validation of the above (depends on the use of the biomarker and implications of error).

Alternatively, a new motor, neurophysiological or neuro-psychological biomarker may provide some form of objective measure of clinical phenotype. As well as potentially providing another objective end-point for treatment, this type of clinical biomarker will help stratify this heterogeneous disease.

The inaccuracy of our clinical diagnosis of idiopathic Parkinson’s disease is well recognized, and hence there is a demand for a diagnostic biomarker. Markers reflecting pathology may allow the earliest presymptomatic diagnosis, whereas either clinical or pathological markers may allow the differentiation of true idiopathic Parkinson’s disease from its many imitators once symptoms are overt.

Overall, it is clear that no biomarker will be able to do it all in Parkinson’s disease (Text Box 3). Given the heterogeneity of disease, it is likely that a biomarker will only prove useful in certain situations, whilst the strength of the clinical examination is its breadth and ability to detect non-dopaminergic symptoms. In the search for improved diagnostic fidelity it may be that a stepwise approach is required despite the potential pitfalls in combining diagnostic tests. For example, clinical assessment might be supplemented by a specific neuropsychological questionnaire or physiological test, with subsequent confirmation by imaging or a biochemical marker. Finally, any biomarker used in clinical trials as a surrogate end-point requires extensive evaluation over years in different populations of Parkinson’s disease patients to ensure its validity. The key in each situation is to appreciate the limitations of the biomarker and what it actually measures.

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References


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