REVIEW

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Neurochemical Approaches in the Laboratory Diagnosis of Parkinson and Parkinson Dementia Syndromes: A Review

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The diagnosis of Parkinson disease (PD) is rendered on the basis of clinical parameters, whereby laboratory chemical tests or morphological imaging is only called upon to exclude other neurodegenerative diseases. The differentiation between PD and other diseases of the basal ganglia, especially the postsynaptic Parkinson syndromes multisystem atrophy (MSA) and progressive supranuclear palsy (PSP), is of decisive importance, on the one hand, for the response to an appropriate therapy, and on the other hand, for the respective prognosis of the disease. However, particularly at the onset of symptoms, it is difficult to precisely distinguish these diseases from each other, presenting with an akinetic-rigid syndrome. It is not yet possible to conduct a neurochemical differentiation of Parkinson syndromes. Therefore, a reliable biomarker is still to be found that might predict the development of Parkinson dementia. Since this situation is currently the subject of various different studies, the following synopsis is intended to provide a brief summary of the investigations addressing the field of the early neurochemical differential diagnosis of Parkinson syndromes and the early diagnosis of Parkinson dementia, from direct α-synuclein detection to proteomic approaches. In addition, an overview of the tested biomarkers will be given with regard to their possible introduction as a screening method.

Review Criteria

- The information used in this review was obtained using the online database PubMed until September 2008. Here, a selection of interesting papers concerning neurochemical approaches in akinetic-rigid syndromes and their differential diagnoses was performed. The different studies were analyzed by comparing the diseases included, the demographic data and the sample pretreatment and storage, the analytical methods used, the establishment of the respective limit values, and the statistical evaluation.
- Until today, Parkinson disease (PD) and Parkinson dementia and the respective differential diagnoses are still clinically based diagnoses. As patients with PD are at high risk of developing dementia, markers are needed for early diagnosis. This will particularly apply once neuroprotective therapies become available. Although there are lots of promising studies investigating potential biomarkers, it is not currently feasible to introduce any of these proteins into the clinical workflow because of a high overlap of values, marginal reproducibility, or even contradictory results.

Introduction

Parkinson disease (PD), also called idiopathic Parkinson syndrome, with an incidence of about 85%, is more common than the familial, autosomal hereditary form, at up to 15% [1]. Three mutations in human α-synuclein are known at present (A30P, E46K, and A53T) that play an important role in the rare hereditary form of PD [2]. An increasing prevalence can be detected for PD in advanced age, 1% among 60-year-olds and 3% in the 80-year-old age group [3]. The characteristic symptoms are linked to the demise of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Eosinophilic, cytoplasmatic

bodies incorporated in the SNpc, the so-called Lewy bodies (LB) [4], can also be detected, as are also observed in Lewy body dementia (DLB) [5]. It has been shown that these incorporated bodies contain α-synuclein, a presynaptic filament protein that is expressed in high concentrations in the terminal ends of neurons [6].

Regarding the formation of α-synuclein-containing inclusion bodies and their importance in neuropathological alterations, Braak et al. were able to indicate a topographical extent of these lesions with an initial onset in the dorsal motor nucleus of the glossopharyngeal and vagal nerves and anterior olfactory nucleus in the brain stem proceeding with an ascending course to cortical structures, beginning with the anteromedial temporal mesocortex [7]. From there, the neocortex succumbs, commencing with high-order sensory association and prefrontal areas. Related to disease ongoing, first-order sensory association/premotor areas and primary sensory/motor fields then follow suit. His group discusses the option of an uninterrupted series of susceptible neurons that extend from the enteric to the central nervous system being involved in the pathology of PD, and the existence of such an unbroken neuronal chain lends support to the hypothesis that a putative environmental pathogen capable of passing the gastric epithelial lining might induce α-synuclein misfolding and aggregation in specific cell types of the submucosal plexus and reach the brain via a consecutive series of projection neurons. In the brain, the process apparently begins in the brainstem (dorsal motor nucleus of the vagal nerve) and advances through susceptible regions of the basal mid- and forebrain until it reaches the cerebral cortex [8].

It is known that up to 40–50% of patients with PD already show cognitive deficits in the sense of a minimal cognitive impairment [9] in early stages of the disease, compared with an age-matched control (CON) group. Several hypotheses have been put forward to explain this. On the one hand, it is possible that the reduced cognitive performance is dependent on L-dopa, but does not respond to exogenously administered L-dopa, on the other hand, nondopaminergic systems that are responsive to cholinergic, noradrenergic, and serotonergic neurotransmitters also come into question as the cause [10]. The development of dementia in patients suffering from PD is not uncommon. Up to 30% of the patients develop PD with dementia (PDD) over the course [11]. These patients have a roughly six times higher risk than an age-matched, healthy CON group [12]. Several risk factors for the development of dementia could be detected in patients with PD. These include the age at onset of the disease, the duration of the disease and atypical symptoms such as an akinetic-rigid syndrome with preferential symmetric development, impaired balance, depression as well as autonomic disorders, and a poor response to the administration of L-dopa [13]. Overall, as in DLB, a fluctuation of the symptoms in patients with PDD is typical. Above all, subcortical lesions are considered to be the pathophysiological cause, although a precise connection between dopaminergic, serotonergic, and cholinergic deficits and the development of dementia has not been established to date. It is assumed that a participation of all three neurotransmitter systems influences the reduced cognitive capacity [10,12,13]. Up to today, there is still no diagnostic marker available for early diagnosis of this dementia syndrome. For optimal therapy of dementia, especially in early stages—in analogy to the mild cognitive impairment concept in Alzheimer disease—it would be beneficial to establish a reliable marker. However, this marker is needed for early therapeutic intervention, with the goal of not only treating the extrapyramidal symptoms but also stabilizing the cognitive abilities of patients. In the subsequent summary of studies, we will mention and discuss the potential approaches to dealing with this clinical problem.

In addition to PD, progressive supranuclear palsy (PSP), a disease belonging to the tauopathies, and multisystem atrophy (MSA), which is attributed to the synucleopathies, are subsumed under the diseases with an akinetic-rigid syndrome [14]. An important differential diagnosis for PD is still PSP, which is not only characterized by a degeneration of the SNpc and reticularis but also shows a loss of neuronal structures in the caudatum, putamen as well as the cerebral frontal and limbic cortex [15]. Although it is possible to distinguish these two neurodegenerative diseases from each other clinically, neuropsychologically, and by morphological imaging, this in some cases is impossible, especially in early detection of these diseases [16].

MSA, a neurodegenerative disease affecting the central-motoric, cortico-cerebellar, pontin-medullary, and preganglionic autonomic parts of the nervous system, also represents a differential diagnosis for PD that can be difficult under certain circumstances. A reliable differentiation can only be achieved by conducting postmortem studies at present. For this reason, attempts to differentiate these diseases is a common subject of studies at the moment [12], and the most promising of them are listed in the following synopsis.

The following parts are subsumed to main categories that mirror cardinal similarities like proteins involved in neurodegeneration/neuroprotection, peptides related to nonmotor features of the diseases, and trophic factors that play a role in metabolic pathways.

Tables at the end of each part give an overview of detailed study data as well as results that are not mentioned in the text. At the end, a summary table containing all

main information about cutoff values, sensitivities as well as specificities is designed to provide an overview of the numerous facts mentioned in this synopsis (Table 17). Finally, a summary paragraph with a brief insight into our estimation of promising leads is given.

Evaluation of neurodegenerative processes

α-Synuclein: A Specific Diagnostic Option?

α-synuclein is an abundant brain protein that is present in high concentrations at presynaptic terminals and is found in both soluble and membrane-associated fractions of the brain. Under physiological conditions, α-synuclein is believed to be involved in the development of synapse plasticity, neuronal differentiation, and regulation of dopamine synthesis. It could be shown that this protein is able to provide a certain protection against oxidative stress on overexpression [17]. Neurodegenerative diseases of the central nervous system display a common feature in their pathogenesis: a misfolding and a progressive polymerization of soluble proteins. There seems to be a tendency for soluble, neuronal proteins to assume a different spatial conformation, either as a type of ageing process or caused by genetic mutation. This can subsequently be accompanied either by a dysfunction or by cell death of neuronal structures [18]. Like amyloid-beta (Aβ) in Alzheimer dementia (AD), the conversion of soluble α-synuclein into an aggregated, insoluble form plays a key role in the pathogenesis here. As we investigated aggregation procedures, the *in vitro* studies showed the existence of not only soluble monomers but also partially folded intermediates that lead to the formation of the amyloidogenic nucleus and fibrils [19]. These oligomeric intermediates may emerge in a transient or stabilized form. The fact that the transient oligomers disappear at the same rate that fibrils appear suggests that the fibrils may be assembled directly from them via a longitudinal association of the oligomers [20]. The stable structured oligomers are said to have significant secondary and tertiary structure and are substantially more compact than monomeric α-synuclein. It is not clear whether they would necessarily be neurotoxic [21].

Cystein-string protein (CSP) is another abundant synaptic vesicle protein and is said to function as a co-chaperone, which is essential for neuronal survival. In CSP knockout mice that were sacrificed at the age of 4 months, Chandra et al. demonstrated that a simultaneous transgenic expression of α-synuclein in these mice prevents the lethal effect of CSP knockout. [2]. It may thus play a protective role in injuries to terminal nerve endings. Not only *in vitro* but also *in vivo* studies examined the potential neuroprotective role of α-synuclein. Here, the data provide an indication of the importance of co-chaperone molecules like CSP in this process. It is known that the primary structure of CSP contains a DNA J-domain typical for heat shock protein (Hsp)-40-type co-chaperones [22] and CSP is able to activate the Adenosintriphosphatase (ATPase) activity of Hsc-70 [23]. The transgenic expression of α-synuclein in the knockout study of Chandra et al. not only showed an abolishment of the lethal phenotype created by deletion of CSP but also an acceleration of the lethality of CSP gene deficiency in case of endogenous α-synuclein knockout. These observations indicate an *in vivo* activity of α-synuclein in abrogating the lethal effects of CSP-deletion, and this may be an important information about the physiological role of α-synuclein in protecting synapses against injury, on the one hand and, it demonstrates the complexity of this homeostasis and the involvement of other proteins that interact with, support, or inhibit α-synuclein proteins on the other hand.

It has been shown that α-synuclein can be detected both in plasma and in cerebrospinal fluid (CSF). Several studies have therefore investigated α-synuclein as a potential marker for the differentiation of PD from other neurodegenerative diseases [17]. Some of these approaches appear very promising, although the results were not confirmed by all of the studies.

Already 10 years ago, Jakowec et al. examined full-length α-synuclein concentrations in CSF as well as in brain samples using Western blot with commercially available antibodies. The full-length α-synuclein represented by the 19-kDa band could only be detected by an N-terminal binding antibody in brain tissues but not in CSF samples. Here, an additional band of α-synuclein of 42 kDa was detected using a C-terminal binding antibody, but this band turned out to be not specific for α-synuclein (Table 1) [24]. A further investigation on protein concentrations of α-synuclein in CSF samples utilizing immunoprecipitation as well as immunoblotting detected the 19-kDa band in CSF samples using a C-terminal-recognizing antibody. The amount of the CSF 19-kDa protein did not significantly vary in PD and normal cases, so that α-synuclein did not appear to represent a peripheral marker of PD pathology. This specific anti-α-synuclein antibody revealed an additional 14-kDa band that was not distinct in PD patients in comparison to CON [25]. Inquiries have also been conducted on the protein concentration of α-synuclein in plasma of patients with neurodegenerative diseases in ELISA approaches. El-Agnaf et al. were able to detect significantly elevated concentrations in patients with PD [26]. Therefore, his group developed an ELISA that uses a nondenaturating approach designed to recognize oligomeric species of

Table 1 Studies investigating relative α-synuclein levels or absolute α-synuclein concentrations in CSF samples, brain tissue homogenate, and plasma samples

	[24]	[25]	[26]	[27]	[28]	[29]
Diagnosis	PD/CON	PD/CON	PD/CON	PD/MSA/CON	PD/CON	PD/DLB/CJD/CON
n	8/4	12/10	34/27	105/38/51	33/38	8/38/8/13
Age (mean)	n.m.	n.m.	69/69.5	65/60/63	63/47	76/71/71/64
Method	Western blot 25 μg/lane	Western blot 50 μg/lane	ELISA	ELISA	ELISA	ELISA
Concentration	-	-	Mean $\pm$ SE: 0.353 $\pm$ 0.687 0.014 $\pm$ 0.281	79.9 $\pm$ 4.0 pg/mL 78.1 $\pm$ 3.5 pg/mL 76.1 $\pm$ 3.9 pg/mL	18.16 μg/mL -	3.0 $\pm$ 1.3 pg/μL 3.8 $\pm$ 3.3 pg/μL 300 $\pm$ 248 pg/μL 6.0 $\pm$ 5.7 pg/μL
Material	CSF, brain	CSF	CSF, plasma	Plasma	CSF	CSF
α-synuclein species	42 kDa	19 kDa, 14 kDa	aa 121–125 Supposed oligomers	aa 117–131 Supposed oligomers	aa 121–125 Supposed oligomers	Total a-synuclein
Result	No difference	No difference	Plasma: PD $\uparrow$	PD/MSA $\uparrow$	PD $\downarrow$	PD/DLB $\downarrow$ /CJD $\uparrow$

Analyzed subject groups are Parkinson disease (PD), Parkinson disease dementia (PDD), multisystem atrophy (MSA), dementia with Lewy bodies (DLB), Creutzfeldt–Jakob disease (CJD), and controls (CON), respectively. Results are given as mean $\pm$ SD. *n* = number of subjects; n.m. = not mentioned; aa = amino acid

human α-synuclein. Here, they detected elevated concentrations of these supposed oligomers in plasma samples of PD patients in comparison to control patients. However, a prominent overlap was observed. Similar results were obtained for CSF collected post mortem, indicating an importance of different soluble molecular forms of α-synuclein in the pathology of PD. Lee et al. could additionally demonstrate identical results in plasma for patients with MSA using ELISA [27]. In CSF samples of living patients, Tokuda et al. found reduced values of α-synuclein in PD as well as in elderly individuals, but without concluding that they had detected oligomers [28].

Recently, again, the group of El-Agnaf presented a new ELISA to measure total α-synuclein levels in unconcentrated CSF. Here, α-synuclein levels were just lower in patients with the synucleopathies. Surprisingly, the difference between the groups becomes more apparent if α-synuclein levels are divided by the total protein concentration [29].

These antithetic findings raise the question of causative reasons for these data. Western blotting seems to be the improper method for investigations in α-synuclein concentrations, eventually depending on the condition of different antibodies or maybe insufficient protein concentration. It is also possible that the limited numbers of patients or center effects or even preanalytic handling may cause a statistically relevant bias. The application of ELISA pointed to a more promising field, especially in plasma, but it remains for further investigations to confirm or disprove these results.

Regarding α-synuclein functions, it is important to differentiate between the various conformational stages of this protein and account for its disposition of structural transformation. Concerning the monomer and transient intermediate forms, the question arises whether these transient oligomers directly transform into fibrils or dissociate into monomeric species, which then add to the growing fibrils. Furthermore, it is known that α-synuclein occurs not only in a cytosolic fraction but is found additionally as membrane bound. For comparison of *in vivo* and *in vitro* studies, the cellular localization of this protein is crucial because existing investigations detected that the presence of membranes can accelerate or inhibit fibrillation [30]. This maybe reflects the varying results based on different conditions used in the experiments.

For all that, in the light of these somewhat contradictory results, it remains open whether neurochemically or laboratory chemically based differential diagnosis of PD by means of the presynaptic filament protein α-synuclein can be established. It would be a very interesting issue to examine the role of α-synuclein and go further into the question of its possible neuroprotective or neurodegenerative properties.

Hypocretin/Orexin System: Common Pathophysiology for PD and Narcolepsy?

Beside neuropsychiatric symptoms, patients with PDD are also found to suffer from sleep disorders in the sense

Table 2 Studies comparing hypocretin levels in CSF or brain samples of patients with akinetic-rigid syndromes, Parkinson disease (PD), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), and controls (CON)

	[34]	[35]	[37]	[38]
Diagnosis	PD/ CON	PD/CON	PD/PSP/DLB	PD/CON
n	62/64	11/5	62/16/13	10/20
Age (mean)	61/61	79/77	70/72/76	69/69
Method	RIA	Immunohistochemistry	RIA	RIA
Concentration	306.0 ± 42.0	-	302 ± 38	307 ± 235
	317.4 ± 8.4		258 ± 37	407 ± 86
			297 ± 38	
Material	CSF	Brain	CSF, brain	CSF
Result	PD ↓	PD ↓	PD ↓	No differences

Hypocretin concentrations determined by radioimmunoassay (RIA) are given in pg/mL (mean ± SD).

of sleep attacks in up to 20% of cases, fragmented nocturnal sleep with strong tiredness during the day, and motoric behavioral disorders in the REM sleep phases, which can often already be detected years before the occurrence of other symptoms. The combination of these symptoms may possibly indicate an etiological connection with a narcoleptic syndrome. The cause of this disease is held to be a loss of hypocretin (orexin)-producing neurons, which is reflected by a reduced concentration in the CSF that may be below the detection limit. It is known that these hypocretin-producing neurons are localized in the lateral part of the hypothalamus, have an excitatory effect on autonomic, metabolic, and endocrine systems, and are involved in the regulation of arousal reactions [31]. In PD, it was possible to detect not only degenerative processes of dopaminergic neurons in the substantia nigra (SN) but also pathological changes in the hypothalamus [32]. In addition, the Parkinson-typical LBs were found to be present in the hypothalamus [33]. This raises the question of an analogical pathomechanism in PD because of the given neurodegeneration or the development of a second-line-like impairment. If this theory were true, hypocretin measurement in PD patients with sleep disorders would be advantageous for adequate therapy.

Under the assumption of hypocretin system impairment in patients with PD or associated syndromes, numerous studies were conducted on this subject, but unfortunately they do not present a common consensus (Table 2).

Using radioimmunoassays (RIA), Mingnot et al., Yasui et al., as well as Baumann et al. investigated CSF samples of Parkinson syndromes. Decreased concentrations of hypocretin were detected by Mignot et al. [34]. Thannickal et al. examined hypocretin-producing cells of the hypothalamus of patients with PD and control persons for the presence of hypocretin 1. The research group determined an increasing loss of hypocretin-expressing cells with an increasing progression of the disease—23% in stage I of PD, and 62% in stage V, according to Hoehn and Yahr [35,36]. The authors concluded that PD is additionally characterized by a strong loss of hypocretin-containing cells, and this means that degenerative processes not only of the SNpc but also of the hypothalamus may have an influence on the clinical picture of patients in PD. In a further study on this subject, three different measurements of hypocretin concentrations were conducted: first of all, the protein concentration in ventricularly localized CSF obtained post mortem. Second, the content of hypocretin was detected in peptide extracts of the cerebral cortex. Third, the number of hypocretin-producing neurons was determined directly in the lateral hypothalamus in patients with PD. Using RIA, a reduction was found in patients with PD compared with healthy CON both in the number of hypocretin neurons and in the hypocretin concentration of the ventricularly obtained CSF as well as in the protein concentration in the prefrontal cortex [37]. However, another study failed to confirm this correlation. Apart from performing polysomnography, Baumann et al. tested the concentration of hypocretin in CSF samples. Here, no deviating protein values were found in CSF of patients with PD compared with the CON group [38].

As a result of the conflicting data position, it is currently not possible to postulate common pathophysiological mechanisms for PD and narcolepsy or consider hypocretin as a suitable biomarker in CSF for screening methods. In spite of these data, it would be important to continue with further approaches to hypocretin investigation and reconsider the reason for these conflicting data. One cannot rule out that application of different antibodies—against hypocretin 1 and 2 or orexin 1 and 2—may have an influence on the available data. Furthermore, the intraday and circadian release of these peptides

as well as the effect of food intake or fasting (catabolic metabolism) [39] have to be taken into consideration for prospective examinations.

Tissue Transglutaminase (tTG): Connection to α-Synuclein?

As apoptosis of neuronal cells is considered to be an important process in the progressive loss of dopaminergic, nigrostriatal neurons in PD, and the activation of tTG can sometimes be detected as an event in the course of apoptosis, this enzyme was investigated in more detail as a possible biomarker of PD. In cultured cells, tTG may exert both pro- and antiapoptotic effects, depending upon the type of cell, the kind of death stimuli, the intracellular localization of the enzyme, and the type of its activities switched on. The actual data support the notion that transamidation by tTG can both facilitate and inhibit apoptosis, while the GTP-bound form of the enzyme generally protects cells against death [40]. tTG is a 76-kDa protein that is not able to pass the blood–brain barrier. However, it can be detected as a normal component of neuronal structures. Physiologically, the activation of tTG during embryogenesis is involved in the development and differentiation of the nervous system. In cells that are subject to apoptosis, in late stages of the ongoing apoptosis cascade, an activation can be detected that is connected with an extensive polymerization and stabilization of intracellular proteins, before they are taken up by means of phagocytosis [41]. Nonetheless, there are data suggesting that tTG may play a role in neurodegenerative processes by stabilizing toxic oligomers of the disease-relevant proteins, but further studies are necessary to validate this hypothesis.

At present, there are only two investigations of tTG in PD versus control persons using ELISA and Western blotting (Table 3). Both were able to differentiate PD samples from nondemented CON—one of the studies in CSF [42], the other in brain samples [43]. Adringa et al. were able to draw a connection between expression of tTG and crosslinking of α-synuclein in a postmortem study of PD and control persons. Using immunohistochemistry, immunoprecipitation, and Western blot, they detected a relation of crosslinked α-synuclein, formed at the expense of the total α-synuclein monomer, to disease progression [43].

Table 3 Tissue transglutaminase expression levels in CSF and brain samples of patients suffering from Parkinson disease (PD) in comparison to controls (CON)

	[42]	[43]
Diagnosis	PD/ CON	PD/CON
n	54/34	6/4
Age (mean)	73/66	74/77
Method	ELISA	Western blot 10 μg/lane
Concentration	70.3 ± 75.0 7.6 ± 10.5	-
Material	CSF	Brain
Result	PD ↑	PD ↑

Concentrations are given in pg/mL (mean ± SD).

Several other approaches display a correlation between the tTG-induced formation of insoluble protein aggregates and the development of senile plaques [44,45]. Segers-Nolten et al. were able to demonstrate that tTG concentrations in nanomolar ranges were sufficient for complete inhibition of fibrillization by effective α-synuclein crosslinking, resulting predominantly in intramolecularly crosslinked monomers accompanied by an oligomeric fraction [46]. Possibly, tTG crosslinking may impose structural constraints on α-synuclein, preventing the assembly of structured oligomeres required for disruption of membranes and progression into fibrils, and a hindrance of progression into pathogenic species may be assumed [47].

Networking of α-synuclein monomers by tTG with a possible impairment of their physiological function will present an important issue to be engaged in the future. For a possible establishment of tTG with regard to its diagnostic importance, however, further studies will have to be conducted on reproducibility and thus on the validity of the present data situation.

Tau Proteins and Aβ Peptides: Biomarkers Established in the Diagnosis of AD

A different study design was used to investigate the concentration of the proteins total tau (t-tau), phosphorylated tau (p-tau), and Aβ 1–42 in CSF samples from patients with AD and patients with other neurodegenerative diseases, including PD. T-tau is a protein that binds to microtubuli of the axon and leads there to a regulation of stability. As t-tau is secreted into the CSF, it can provide information about the death of neurons. P-tau, which can be detected in the hyperphosphorylated state, particularly in AD, loses its connecting function and, via instability of the axon, can lead to reduced transport capacity. The peptide Aβ 1–42 is a cleavage product of amyloid precursor protein and is detected in low concentrations, especially in AD [48]. In contrast to AD, however, no elevated/decreased values could be found for the proteins t-tau, p-tau, or Aβ 1–42 in the CSF of PD patients (Table 4) [49,50]. The question remains how the PDD can be differentiated from PD or other neurodegenerative diseases, especially other types of dementia, particularly in

Table 4 Studies analyzing tau and amyloid β concentrations in CSF or plasma of patients with akinetic-rigid syndromes

	[51]	[52]	[53]	[54]	[55]	[56]	[57]	
Diagnosis	PD/ CON	PD/CON	PD/MSA/PSP/CON	PD/PDD/CON	PD/PSP/MSA/DLB/CBD/CON	PDD/DLB/CON	PD/CON	
n	23/32	15/17	48/36/15/32	23/74/41	11/20/18/20/12/19	21/21/23	20/15	
Age (mean)	68/70	70/72	62/63/68/65	72/72/70	70/69/62/73/65/67	69/71/72	72/62	
Method	ELISA tau	ELISA tau	ELISA Aβ 1–42	ELISA: tau/ Aβ 1–42	Western blot, Aβ 1–42	Western blot, Aβ 1–42, Aβ 1–40*	ELISA: tau, Aβ 1–42	
					25 μg/lane	25 μL/lane		
Concentration	313 ± 125	7.45 ± 1.59	812 ± 147	Tau: 216 ± 170/214 ± 149/148 ± 119	-	-	tau:	Aβ:
	610 ± 167	8.33 ± 2.83	552 ± 192	Aβ: 559 ± 154/466 ± 198/641 ± 199			0.25 ± 0.19	0.53 ± 0.22
			729 ± 148				0.20 ± 0.13	0.81 ± 0.32
			800 ± 228					
Material	CSF	CSF	CSF	CSF	CSF	CSF	CSF	
Result	No differences	No differences	No differences	PDD: tau ↑/Aβ 1–42 ↓	PSP/CBD: Aβ 1–42 ↓	PDD: Aβ 1–40* ↓, DLB: Aβ 1–40* ↑	PD: Aβ 1–42 ↓	

Concentrations determined in the respective patient samples are given in pg/mL (mean ± SD) with the exception of reference [52] where concentration is indicated in pM and of reference [56] where concentration is indicated in ng/mL.

PD = Parkinson disease; PDD = Parkinson disease dementia; MSA = multisystem atrophy; PSP = progressive supranuclear palsy; DLB = Lewy body dementia; CBD = corticobasal degeneration; CON = control.

early, subclinical stages, in order to ensure that the patients receive an appropriate therapy. On this subject, investigations are available on biomarkers in CSF as well as in blood samples.

In 2000, first investigations of CSF samples of patients with PD versus CON using standardized ELISA for measurement were performed. Here, no significant differences between PD and the CON groups could be found [51]. Comparable results were achieved by Sjögren et al. 1 year later [52] as well as by Holmberg et al. [53], who examined a more comprehensive group including PD, CON, and the postsynaptic Parkinson syndromes PSP and MSA. We carried out two investigations on this problem—in 2006, we found comparable values in PDD to those that are established in AD (decrease of Aβ 1–42 and elevation in tau protein) [54]. Similar findings with lower p-tau levels were described recently in PDD [50]. A year later, in addition to PD and CON, we examined a broader collective including PSP, MSA, DLB, and corticobasal degeneration (CBD) in CSF samples using Western blot [55]. Here, we again found decreased values of Aβ 1–42 in patients suffering from PSP and CBD.

In early clinical stages of PD, dementia is often quite difficult regarding differentiation of PDD from DLB, especially when the onset of the cognitive impairments cannot be precisely established. A laboratory marker would be helpful here. In such an investigation, CSF samples of patients with PDD were compared with DLB and nondemented CON. Aβ SDS-PAGE/Western blot was used for the investigations. In this connection, the authors detected a new isoform of the Aβ family, the isoform Aβ 1–40*. This peptide, which is considered to be an oxidized α-helical isoform of Aβ 1–40, was able to distinguish DLB from PDD [56]. However, the conclusions of this study have been a matter of controversial debate, as the concentrations of oxidized Aβ 1–40* may be dependent on the storage of the samples, and thus, potentially elevated levels may be detected, depending on the length of sample storage (personal observation).

By means of ELISA, the same study group tested tau and Aβ 1–42 concentrations not only in CSF but also in plasma samples of patients with PD, PDD, and CON. Concerning plasma, no difference was found between the groups [57].

In our understanding, it is currently impossible to distinguish the degenerative disorders mentioned above by means of tau/Aβ measurement. These biochemical markers were able to indicate neurodegenerative procedures, but they are not capable of giving support in their differential diagnoses. Furthermore, the use of different patient subgroups and the examination of various Aβ isoforms hamper the comparability of the relevant studies. Additionally, the application of different ELISA kits makes

it difficult to compare the specific results among each other.

Reelin and Its Role in Neurodegeneration via the Apolipoprotein System

Reelin, an extracellular protein with a mass of 420 kDa, which binds to the transmembranous receptors abetalipoprotein receptor-2 and VLDL receptor, was investigated in a unique study. During embryogenesis, reelin is involved in regulation of neuronal migration in the central nervous system. In adult tissues, it is believed to have an influence on neuronal plasticity, synaptogenesis, and cognitive performances in the sense of memory capacity [58]. It is assumed that it plays a role in degenerative processes by binding to the apolipoprotein E (ApoE) receptor. It was shown that an ApoE 4/4 polymorphism is a possible risk factor for the development of AD. In addition, the loss of reelin is apparently linked to an increased phosphorylation of tau [59]. In the case of AD, it was shown that tau hyperphosphorylation leads to the formation of intracellular fibrillary tangles and neuronal degeneration. In this respect, analogies between AD and PD pathology might be taken into account, and the detection of reelin as a potential biomarker for early occurrence of cognitive impairment is an interesting feature. For investigation of reelin concentration—by means of a Western blot in samples of CSF and plasma—Botella-Lopez et al. enrolled patients with PD, PSP as well as CON in their study. All of the groups with Parkinson syndromes examined showed a marked expression of three reelin bands (420 kDa, 310 kDa, and 180 kDa) in comparison to the control persons (Table 5) [60]. Reelin may be regarded as a possible marker for neurodegenerative processes, but—according to this unique study—it is not able to differentiate between those diseases either in CSF or in plasma.

Furthermore, Chin et al. were able to underline the suggestion that alterations in reelin signaling may contribute to neuronal dysfunction associated with AD [61]. In human postmortem brain tissues, they found reductions of reelin-expressing pyramidal neurons in the entorhinal cortex of AD samples. This could be confirmed by Saez-Valero et al. who found an elevated 180-kDa band of reelin in CSF samples of AD and frontotemporal lobar degeneration (FTLD) persons compared with control persons using Western blot [62]. Displaying a promising field for further examinations, we unfortunately found these to be the only studies engaging in reelin expression, and additional investigations will be necessary to confirm or disprove the absorbing role of reelin in Parkinson syndromes.

Table 5 Reelin expression in Parkinson disease (PD) and progressive supranuclear palsy (PSP) compared with controls (CON)

	[60]
Diagnosis	PD/ PSP/CON
n	6/6/11
Age (mean)	70
Method	Western blot 20 μg/lane
Material	CSF, plasma
Result	No differences

ST13 and HSP70: Changes in the Neuroprotective Status of Cells

ST13 is a protein that is considered to be a cofactor of heat shock protein 70 (HSP 70) and is able to stabilize its function as a chaperone molecule. HSPs provide a line of defense against misfolded, aggregation-prone proteins and are among the most potent suppressors of neurodegeneration. HSP 70 is involved in the folding of α-synuclein and can reduce toxic effects of various influencing parameters on this protein in cells [63].

Scherzer et al. used whole blood from patients who were still in an early stage of PD and compared them, by means of the results obtained by microarray, with age-matched healthy CON (Table 6). A lower concentration of ST13 mRNA was found in the PD patients [63]. Regarding ST13 as a chaperone of HSP 70, a reduced anti-aggregation effect of the latter protein was assumed, but it remains doubtful whether blood samples are in a position to reveal intracerebral pathogenic events. Apart from this, Kawamoto et al. demonstrated elevated concentrations of HSP 70 in brain samples of patients with MSA in comparison to CON [64].

However, as comparative control studies with a similar design are lacking on this subject, it is not yet possible to draw definitive conclusions about the reliability of these data with regard to a precise diagnosis, but HSPs are a promising and interesting field that is worth following up, especially with regard to anti-α-synuclein aggregation effects.

Table 6 Studies on the chaperones heat shock protein HSP 70 and ST13 in Parkinson disease (PD) and multisystem atrophy (MSA) compared with controls (CON)

	[63]	[64]
Diagnosis	PD/CON	MSA/CON
n	50/55	15/7
Age (mean)	69/64	67/68
Method	Microarray/real time PCR	Western blot 10 μg/lane
Chaperone	ST13	HSP 70
Material	Plasma	Brain
Result	PD $\downarrow$	MSA $\uparrow$

Table 7 Analyses of neurofilament levels in the pathogenesis of Parkinson disease

	[65]	[66]	[67]
Diagnosis	PD/MSA	PD/MSA/PSP/CBD/CON	MSA/ILOCA
n	31/19	22/21/21/6/45	27/18
Age (mean)	53/53	63/62/70/63/64	61/61
Method	ELISA	ELISA	ELISA
Concentration	NfL: 6.7 ± 31.0/33.4 ± 18.0 NfHp35: 56 ± 31/191 ± 18	n.m.	NfL: 42 ± 25/11.7 ± 7.0 NfHp35: 234 ± 144/115 ± 49
Material	CSF	CSF	CSF
Result	MSA: NfL; NfHp35 ↑	MSA/PSP: NfH^{SM135} ↑	MSA: NfL, NfHp35 ↑

Concentrations are given as mean ± SD in ng/L.
PD = Parkinson disease; MSA = multisystem atrophy; PSP = progressive supranuclear palsy; CBD = corticobasal degeneration; CON = control; n.m. = not mentioned; ILOCA = idiopathic late-onset cerebellar ataxia; NfH = neurofilament heavy chain; NfL = neurofilament light chain.

Neurofilaments: Structural Proteins in the Pathogenesis of Neurodegeneration

In an investigation of CSF samples, the protein neurofilament heavy chain (NfH) was detected as a possible marker for the differential diagnosis of Parkinson syndromes. The protein NfH is an important diagnostic parameter for axonal cell damage as phosphorylated neurofilaments are components of the cytoskeleton and are mainly expressed in axonal compartments of neurons there. If the neuronal axons are damaged, these proteins are released and can subsequently be detected in CSF.

Nevertheless, Abdo et al. detected increased levels of NfH^{SM135} as well as neurofilament light chain (NfL) for MSA in CSF investigated in an ELISA study (Table 7) [65]. Interested in NfH^{SM135} concentrations of CSF samples as well, Brettschneider et al. analyzed a comprehensive cohort by means of commercially available ELISA (patients with PD and other Parkinson syndromes [MSA, PSP, CBD] in comparison to CON samples were included in the study). An elevated protein concentration was found in the atypical Parkinson syndromes MSA and PSP compared with PD and the CON groups [66]. Because it is also difficult to distinguish patients with MSA-C from idiopathic late-onset cerebellar ataxia (ILOCA), Abdo et al. analyzed different markers in CSF of such patients. They detected elevated concentrations of NfL as well as NfHp35 in each of the MSA-C patients compared with ILOCA [67].

Almost all of these studies were performed without application of controls, but they still indicate a common tendency of neurofilaments to play a part in neurodegeneration. Representing a marker for axonal damage and possibly for impairments of the cytoskeleton, neurofilaments are putative indicators for neuropathological transformations, and it is important that further investigations go into more detail.

Serpins: A Model for Conformational Diseases

Several biological processes require a balance between proteases that initiate proteolytic pathways essential to life and the inhibitors that limit excessive protease activity. There are many different families of protease inhibitors, but of these, just one exceptional family of serine protease inhibitors, the serpins, appears to control key intracellular and extracellular pathways [68]. The role of serpins in these critical functions has been very important, because they differ from all other families of protease inhibitors in having a complex mechanism of action that involves a drastic change in their shape. There are inherent disadvantages of such conformational mechanisms, because they are associated with a vulnerability to misfolding [69]. Like aggregation of α-synuclein, there are indications that depositions of serpins are able to cause neurodegenerative diseases accompanied by dementia [70,71].

The first investigations of α-1 antichymotrypsin (ACT) in CSF as well as in serum samples of patients with PD detected no differences in comparison to the CON group using RIA (Table 8) [72]. By means of ELISA, Pirtilla et al. were able to reproduce these data [73]. Unlike these results, examinations of DLB patients in comparison to control persons found elevated levels of ACT in the patient group using rocket electrophoresis [74].The fact that serpins are able to cause aggregations such as those detected for α-synuclein may be of great pathological importance for neurodegenerative processes. Regarding the inconsistent study results mentioned above, one has to take into consideration that only patients with PD and DLB were included in the studies. Furthermore, it would be interesting to investigate not only α-1 ACT but also other members of the serpin family such as neuroserpins that are considered to play a crucial role in neuroplasticity [75].

Table 8 Serpin concentrations in CSF and serum of Parkinson disease patients (PD), dementia with Lewy body patients (DLB), and controls (CON)

	[72]	[73]	[74]
Diagnosis	PD/CON	PD/CON	DLB/CON
n	18/89	20/42	38/37
Age (mean)	66/57	64/61	75/72
Method	RIA	ELISA	Rocket electrophoresis
Concentration	1.32 ± 0.42	3.14 ± 1.17	10.2 ± 13.7
	1.35 ± 0.33	2.52 ± 0.76	7.6 ± 12.3
Material	CSF, serum	CSF, serum	CSF, serum
Result	No differences	No differences	DLB ↑

Concentrations are given as mean ± SD in mg/L for serum values.

Catecholamine and Indolamine Metabolites

Neurotransmitter Metabolites: A Representative Family of Adrenergic, Serotonergic, and Dopaminergic Functions

Neurofilaments and metabolites of different neurotransmitters such as the adrenergic metabolite methoxy-hydroxy-phenyl-ethylene glycol (MHPG) crop up regularly as an important matter of investigation within the context of studies designed to differentiate neurodegenerative diseases. MHPG is a metabolite of norepinephrine degradation, especially in the brain, where it is the principal norepinephrine metabolite and indicates recent sympathetic nervous system activity. Representing the dopaminergic pathway, homovanillic acid (HVA), as a degradation product of dopamine, is of laboratory interest. 5-hydroxyindoleacetic acid (5-HIAA) is the main metabolite of serotonin and typifies this metabolite pathway.

In a study investigating in this field, the concentrations of the neurotransmitter metabolites 5-HIAA and MHPG were compared in CSF of patients with MSA and PD (Table 9). The analysis showed that the mean concentrations of 5-HIAA and MHPG were significantly reduced in the patient group with MSA compared with the PD patient group. The authors established a connection between the decreased concentrations of 5-HIAA and HMPG and that of MSA, in combination with autonomic symptoms. On the basis of this, they conclude that neuronal damage appears to be more severe in patients with MSA compared with PD patients. In summary, it is implied that determination of the variables MHPG and 5-HIAA may possibly contribute to a differentiation between the diseases MSA and PD, and this specifically at a time when the clinical diagnosis cannot yet be clearly rendered [76]. The same group replicated their results using ELISA for CSF samples of PD and MSA patients 3 years later. Here, they were able to reproduce the decrease of MHPG in MSA patterns [65]. Another study using HLPC instead of ELISA demonstrated reduced values not only for HVA but also for MHPG in PD [77]. The findings may suggest a correlation between dementia, on the one hand, and mesocorticolimbic and mesostriatocortic dysfunction with dopaminergic and noradrenergic deficiencies, on the other hand, in PD patients.

Investigating the monoamine metabolite HVA, the controlled clinical trial Deprenyl (selegiline) and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP), which examined the effects of selegiline and tocopherol in 800 subjects with early, untreated PD, measured the CSF HVA concentration at baseline and again 4 weeks after the study endpoint [78]. The hypothesis of this study is that if selegiline offers neuroprotection in PD patients, the HVA levels should not decrease over time as much as in those receiving placebo. The important treatment arms concerning HVA are selegiline-placebo versus active selegiline hydrochloride

Table 9 Neurotransmitter metabolites in the differentiation of Parkinson disease (PD), multisystem atrophy (MSA), and control (CON)

	[65]	[76]	[77]	[78]
Diagnosis	PD/MSA	PD/MSA/CON	PD/CON	PD/CON
n	31/19	35/29/62	22/16	256/544
Age (mean)	53/53	53/61/52	67/62	55
Method	ELISA	ELISA	HPLC	ELISA
Concentration	46 ± 29 nM	45 ± 29 nM	24.95 ± 16.71 ng/mL	9.2 ± 12.7 ng/mL
	32 ± 18 nM	31 ± 9 nM	43.54 ± 21.39 ng/mL	3.2 ± 14.4 ng/mL
		46 ± 29 nM		
Material	CSF	CSF	CSF	CSF
Result	MSA: MHPG ↓	MSA: 5-HIAA, MHPG ↓	PD: HVA/MHPG ↓	PD: HVA ↑

HVA = homovanillic acid; MHPG = methoxy-hydroxy-phenyl-ethylene glycol; HPLC = high-performance liquid chromatography.

(10 mg/day) ($n = 256$). Evaluation of the data demonstrated that decline in CSF HVA concentration was significantly greater in subjects assigned to receive selegiline (9.2 ± 12.7 ng/mL) than in subjects not receiving selegiline (3.2 ± 14.4 ng/mL), indicating persistent monoamine oxidase (MAO) inhibition by selegiline. The conclusions drawn from this study were an indication of the long duration of MAO inhibition by selegiline, on the one hand, and its limited suitability as a marker of severity or progression in PD, on the other hand.

It is rather important that the stability of metabolites is contingent on the pH value of the respective tissue, and discrepancies between physiological data lead to a rapid autooxidation—with possibly inaccurate measurements. Even if immediate freezing is performed, it is not possible to estimate the influence of circulation on metabolite degradation in CSF—incidentally, a problem that applies to all CSF proteins.

To recapitulate, it seems that the identification of neurotransmitter metabolites is an interesting but nonspecific field for the differential diagnosis of PD. The failure of these proteins to be introduced as potential biomarkers traces back to the fact that at least two, or better three, metabolites were necessary to distinguish extrapyramidal syndromes—for clinical practice, that is unrealistic at present.

Evaluation of Trophic Factors

Heart Fatty Acid-Binding Protein (H-FABP): Effects of Lipid Peroxidation

H-FABP was originally discussed in IPG-two-dimensional differential in gel electrophoresis (2D DIGE) analysis as a potential marker for Creutzfeldt–Jakob disease (CJD). However, in an independent evaluation, we were able to show markedly elevated values for this protein in the serum of patients with DLB. In a further study, we observed that determination of the H-FABP concentration by means of an ELISA analysis of serum could differentiate DLB from PD, with a sensitivity of 84% and a specificity of 82% (Table 10). In addition, PD can be distinguished from PDD with a sensitivity of 69% and a specificity of 80%. By determining the serum concentration of H-FABP, PDD can also be differentiated from healthy subjects. In this case, the sensitivity was 92%, with a specificity of 64%. By determining the quotient of serum H-FABP/CSF tau protein, PDD could be differentiated from AD with a sensitivity of 88% and a specificity of 74%. However, PDD could not be differentiated from DLB within the context of this study [79]. Pathophysiologically, it is interesting that H-FABP has a high homology with α-synuclein. As a hypothesis, one might postulate that serum H-FABP is the peripheral analog to central α-synuclein, but scores of biochemical as well as pathophysiological studies will be necessary to investigate this issue in detail.

Table 10 H-FABP concentrations in Parkinson disease, either with or without dementia (PD, PDD) and in dementia with Lewy bodies (DLB) compared with controls (CON)

	[80]
Diagnosis	PD/PDD/DLB/CON
n	45/25/33/51
Age (mean)	69/74/70/70
Method	ELISA
Concentration	2.71 ± 2.41
	6.7 ± 9.27
	10.19 ± 15.52
	3.73 ± 6.82
Material	CSF, serum
Result	PDD, DLB ↑

Concentrations are indicated as mean ± SD in pg/mL.

An analysis of the concentrations of H-FABP in serum and CSF as a possible marker for the differentiation of neurodegenerative diseases was performed in a further study. The analysis of the data revealed significantly elevated values for H-FABP in the CSF, whereby PDD could be differentiated from the CON group. In addition, serum concentrations of H-FABP enabled a significant differentiation between the individual Parkinson syndromes (comparison of PDD and CON, DLB and PDD, as well as PDD and PD) at different cutoff values, depending on the clinical differential diagnosis. Here, the sensitivity was 80%, with a specificity of 76% [80]. Wada-Isoe et al. investigated serum H-FABP levels of patients with PD, DLB, and AD as well as the heart-to-mediastinum (H/M) ratio by means of iodine-123 metaiodobenzylguanidine (^{123}I-MIBG) cardiac scintigraphy. They found significantly higher levels of serum H-FABP in DLB as well as PD patients than in AD patients. The H/M ratios of the DLB and PD patients were significantly lower than those of AD patients. Unfortunately, the examination of serum H-FABP levels did not allow discrimination between DLB and PD patients [81].

Cardiac sympathetic nerve dysfunction may possibly be associated with the elevation of serum H-FABP in DLB and PD patients, and it remains for further studies to determine the clinical availability of H-FABP for the differential diagnosis of PD. At present, it cannot be ruled out that measurement of H-FABP may serve not only as a potential diagnostic but also as a prognostic marker with regard to the increased lethality provoked by autonomic dysfunctions.

Growth Hormones: Involved in Somatotropic Functions

Considering that growth hormones (GH) might play a role in the development of neurodegenerative diseases and associated repair mechanisms, various investigations were conducted on their release and on a possible differentiation of PD from MSA.

The activation of the hypothalamic α2-adrenoceptor and the muscarinic cholinergic receptor induces release of GHs via growth hormone-releasing hormone (GHRH) and inhibition of somatostatin. Both clonidine (an α2-adrenoceptor agonist) and the amino acid arginine lead to an activation of the cholinergic system and thus to the release of GH. It is known that, similar to the tuberoinfandibular, dopaminergic signal pathway, there is an intrahypothalamic cholinergic signal transduction pathway that can probably be held responsible for the effect of cholinergic medications on somatotropic functions. It is established that a defect of this intrahypothalamic, cholinergic system can occur in MSA, as biochemical analyses of the hypothalamus of MSA patients have shown a reduction of ACE activity. In this context, a reduced vasopressin release in response to cholinergic medication has been described in patients with MSA [82]. In several studies, a clonidine test (clonidine growth hormone test [CGHT]) was performed in patients with PD and MSA as well as control persons in order to detect a possible difference in release of GH and thus to enable a differentiation of these two diseases.

On this subject, a research group investigated patients with PD, MSA, and CON (Table 11). None of the subjects suffering from disease had been treated with medication for their condition previously. Blood was taken 15 minutes before the administration of 2 μL/kg body weight of clonidine and after clonidine administration every 15 minutes for the duration of the subsequent study. An increase in GH was found in patients with PD and in the CON group, whereas patients with MSA did not show any change in GH concentration [83]. In another study with the same design, patients with MSA and PD were investigated. Half of the PD patients had no previous medication, the other half was examined during the intake of L-dopa or dopamine agonists. No differences could be found between the three groups investigated [84]. By contrast, Tranchant et al. analyzed PD patients under a long-term treatment with L-dopa or dopamine agonists in comparison to MSA patients and they detected elevated concentrations of GH in PD [85]. These results were verified by Strijks et al. [86], Lee et al. [87] as well as Schaefer et al. [88] who performed their investigations under equivalent study conditions.

Table 11 Growth hormone concentrations in serum samples of Parkinson disease (PD), multisystem atrophy (MSA), progressive supranuclear palsy (PSP), and controls (CON)

	[82]	[83]	[84]	[85]	[86]	[87]	[88]	[89]
Diagnosis	PD/MSA/CON/ILOCA	PD/MSA/CON	PD/MSA	PD/MSA	PD/MSA	PD/MSA	PD/CON	PD/MSA/PSP/CON
n	7/6/8/4	13/31/27	9/17	19/7	21/11	21/45	10/10	26/26/23/80
Age (mean)	n.m.	74/74/-	62/65	68/70	55/59	63/58	55/55	65/66/63/65
Method	RIA	RIA	RIA	RIA	RIA	RIA	RIA	RIA
Concentration	20.3 ± 7.0 ng/mL	11.9 ± 2.4 mU/L	3.2 ± 2.7 mU/L	2.44 ± 0.88 mU/L	27.1 ± 38.0 mU/L	4.19 ± 0.92 ng/mL	502.4 ± 202.6 ng/mL	8.74 ± 0.98 ng/mL
	24.1 ± 6.9 ng/mL	3.4 ± 1.2 mU/L	2.8 ± 2.5 mU/L	0.47 ± 0.16 mU/L	8.6 ± 20.2 mU/L	0.83 ± 0.61 ng/mL	312.0 ± 98.5 ng/mL	1.46 ± 0.29 ng/mL
	21.1 ± 3.5 ng/mL	18.6 ± 3.6 mU/L						6.64 ± 0.82 ng/mL
	24.8 ± 6.0 ng/mL							8.59 ± 0.44 ng/mL
Material	Serum	Serum	Serum	Serum	Serum	Serum	Plasma	Serum
Result	PD/MSA ↑	PD, CON ↑	No differences	PD ↑	PD ↑	PD ↑	PD ↑	PD/PSP/CON ↑

Concentrations are given as mean ± SD.
RIA = radioimmunoassay; ILOCA = idiopathic late-onset cerebellar ataxia.

Apart from clonidine tests, a further trial is available with the aid of the amino acid arginine, which can also lead to an increased release of GHs. In a study regarding the influence of arginine on concentrations of GH, the GH response to three different stimuli, clonidine, arginine, and GHRH + arginine was investigated in patients suffering from MSA and PD and healthy subjects. In all three groups, the medication being taken was discontinued 4 weeks before the start of the test. The analysis showed no response to the administration of GHRH + arginine (no increase of GH, nor of IGF-I nor of IFG-binding protein-3). After administration of clonidine, elevated values of GH were found in the CON group and PD patients as well as in subjects with MSA [82] and PSP [89]. The intake of arginine brought out an increase in GH concentration comparable to clonidine. In an additional study with PD and the postsynaptic Parkinson syndromes MSA and PSP, GH concentrations were specified in comparison to non-demented CON. The values were increased in PD and PSP, replicating the results for MSA.

Again in the investigation of GHs, the data obtained demonstrated a common tendency in almost all of the studies. The variations of these data may be explained by the time point of blood sampling—there is a circadian release of GH, with a maximum in the late evening and night, and a minimum in the morning hours. Furthermore, the studies did not mention the state of disease according to Hoehn and Yahr—which is in our understanding an important point besides the intake of medication. These investigations only demonstrated elevated levels of GH at the end of the performed time span (average of 30 or 45 minutes after clonidine/arginine intake)—making an introduction of this test procedure into clinical flow quite impossible, due to excessive costs.

Oxidative Stress and Generation of Reactive Oxygen Species (ROS)

Tetrahydrobiopterin (BM4): Neurodegeneration as a Result of Oxidative Stress

Under the assumption that neurodegenerative diseases might have an endogenous toxic cause, an approach was taken to investigate the biosynthesis of nitrogen monoxide (NO), a free radical with a half-life in the range of seconds. This unstable molecule is relatively rapidly metabolized by tissue oxygen to the stable compounds nitrate and nitrite. The biosynthesis of NO is dependent on the amino acids L-arginine, the substrate of NO synthase, and L-glutamate, which can stimulate NO synthesis via activation of the NMDA receptor. L-citrulline is formed by this catalyzation. It is also known that formation of NO is coupled with the presence of tetrahydrobiopterin (BM4), a coenzyme of NO synthase. In earlier investigations, a reduction of BM4 was detected in cerebral tissue of patients with AD and PD [90]. This would be accompanied by a reduced production of NO, which might result in a deterioration of neuronal functions, whereas an elevated concentration of NO can exert toxic effects on neuronal structures. However, it could also be shown that, under suboptimal concentrations of BM4 or L-arginine, NO synthase can catalyze the so-called reactive oxygen species (ROS), which can have an even more toxic effect than NO itself [91].

Therefore, a study was conducted to detect the concentration of these three amino acids in CSF of patients with PD, PDD, and MSA and control persons by means of high-performance liquid chromatography (HPLC) (Table 12). Elevated protein-amino acid concentrations of L-citrulline were detected in patients suffering from MSA compared with the CON group [92]. Examining the same question, Molina et al. found significantly reduced values of L-citrulline and L-arginine in plasma of PD patients [93]. A third study group examined CSF as well as serum in PD patients versus control persons and found reduced concentrations of L-glutamate in CSF of PD patients [94].

These results may highlight impaired signaling in NO synthesis—on the one hand, one might assume lower concentrations of NO (L-arginine and L-glutamate reduction) in neurodegeneration, on the other hand, altered homeostasis may cause augmentation of ROS with consecutive destruction of physiological structures. Apart from these controversial study results, this promising approach should be followed up in detail in further investigations in order to draw more precise conclusions from the data obtained to date with regard to their diagnostic value. Oxidative stress, accumulation of ROS, and mitochondrial functioning, on the one hand, and (neuro-) degeneration or aging, on the other hand, are interesting fields that would be worth pursuing.

Osteopontin: Another Molecule Inducing ROS?

Another molecule that plays a role in the pathogenesis of NO production is osteopontin (OPN), a glycosylated phosphoprotein. It is supposedly involved in processes that result in oxidative stress and apoptosis and in reactions that cause damage to mitochondrial structures. A regulation of different cytokines that play a role both in chemotaxis and in NO synthesis could also be demonstrated. The glycoprotein OPN may thus be involved in the pathogenesis of Parkinson syndrome and was investigated in the following study as a possible biomarker in CSF in terms of its diagnostic potential [95].

To this end, both serum and CSF from patients with PD receiving Parkinson-specific medication with L-dopa

Table 12 Amino acids relevant for synthesis of NO in the differentiation of Parkinson disease with or without dementia (PD, PDD), multisystem atrophy (MSA), and controls (CON)

	[92]	[93]	[94]
Diagnosis	PD/PDD/MSA/CON	PD/CON	PD/CON
n	89/19/15/21	31/45	10/10
Age (mean)	66/75/66/65	62/58	65/57
Method	HPLC	HPLC	RIA
Concentration	2.6 ± 0.8 μM	1.5 ± 1.0 ng/mL	16.7 ± 8.5 μM
(mean $\pm$ SD)	3.0 ± 1.3 μM	16.4 ± 4.3 ng/mL	124.6 ± 39.7 μM
	2.9 ± 0.9 μM		
	2.2 ± 0.6 μM		
Material	CSF	CSF, plasma	CSF, serum
Result	MSA: L-citrulline ↑	PD plasma: L-citrulline, L-arginine ↓	PD CSF: L-glutamate ↓

HPLC = high-performance liquid chromatography; RIA = radioimmunoassay.

or dopamine agonists and a CON group were investigated by means of ELISA (Table 13). In the analysis, a significantly elevated protein concentration of OPN was found in the serum of patients with PD compared with the CON group. A significant elevation in OPN concentration was also demonstrated in the CSF. Positive correlations could be detected between the severity of disease, on the one hand, measured by stages according to Hoehn and Yahr, and the OPN concentration in CSF, on the other hand. In addition, high values for OPN in CSF were linked to the occurrence of a demential syndrome in patients with Parkinson syndrome. The authors were also able to show that a specific drug therapy with L-dopa or dopamine agonists has positive effects on the protein amount of OPN (a Parkinson-specific therapy was correlated with low values) [96]. Within the context of this study, the localization of OPN gene expression was demonstrated in human brain samples from patients with PD, and in comparison with these, in tissues from healthy CON. In PD subjects, gene expression of OPN was found preferentially in the SN and in almost all somata of nerve cells, especially in the neuromelanin-containing zone, whereas almost all LBs had positive staining for OPN [96]. Iczkiewicz et al. were able to support these data by lesioning the SN using 6-hydroxydopamine and mechanical vehicle-inducing lesioning. They detected an increase in OPN expression and discussed this in the context of nigral cell survival regulation [97].

Table 13 Osteopontin concentrations in CSF and serum of Parkinson disease (PD) compared with controls (CON)

	[96]
Diagnosis	PD/CON
n	30/30
Age (mean)	70/70
Method	ELISA
Concentration	718.3 ± 770.7
	468.2 ± 282.9
Material	CSF, serum
Result	PD ↑

Concentrations are given as mean $\pm$ SD in ng/mL.

OPN is a multifunctional molecule, highly expressed also in chronic inflammatory and autoimmune diseases, and it is specifically localized in and around inflammatory cells. OPN is furthermore a secreted adhesive molecule and is thought to aid in recruitment of monocyte and macrophages and regulate cytokine production in macrophages, dendritic cells, and T cells. OPN has been classified as a T-helper 1 cytokine and thus is believed to exacerbate inflammation in several chronic inflammatory diseases [98]. It is possible that OPN plays the role of an important regulator of inflammatory events in neurodegenerative diseases as well, and its manipulation may provide a means of achieving neuroprotection in PD—a circumstance that could be examined in further studies, especially in terms of a potential therapeutic agent.

Metals: Believed to Be (Co)Factors of Aggregation

It is known that the risk of developing a Parkinson syndrome can be increased in predisposed individuals through synergistic effects of a wide variety of metals. A number of metals will be presented as examples to show the influence on diverse different tissues and signal pathways. One of the most important representatives is iron, which is able to form free radicals and initiate redox reactions. In addition, iron is able to accumulate in a wide

variety of different brain tissues, especially in the SNpc, in order to interact with neuromelanin to form complexes that are possibly in a position to cause cell death of neurons by inducing oxidative stress [99,100]. Highest iron concentrations in the brain are found in the globus pallidus, followed by the red nucleus,SN, putamen, and caudate nucleus, the areas most vulnerable to neurodegenerative disorders associated with parkinsonian syndromes. Under physiological conditions, iron is, by its capacity to transport electrons and generate hydroxyl radicals, vitally involved in many processes such as neuronal development, gene expression, enzyme function, synthesis of vital molecules (heme, iron-sulphur clusters, and dopamine), as well as the respiratory chain. On the other hand, there is evidence that iron may play a primary role in neurodegenerative processes in PD. This hypothesis is traced back to the finding that long-term occupational exposures to different combinations of metals, including iron, have been associated with PD. Furthermore, several studies have suggested that iron interacts with α-synuclein, enhancing the conversion of unfolded or α-helical α-synuclein to β-pleated sheet conformation, the primary form in LBs.

In the case of MSA, increased concentrations of iron have been described primarily not only in the putamen but also in the SN and caudate nucleus in postmortem and magnetic resonance imaging (MRI) studies. PSP is distinguished by an increase in iron detected in the SN and, to a lesser extent, in the putamen and later in the disease. Marked iron accumulation of the SN has also been described in patients diagnosed with CBD (reviewed in Berg et al. [101]).

Manganese is also able to form ROS and toxically acting catecholamines, an important basis for neurodegenerative processes [102]. The metal copper equally plays a role in metabolism of the cell insofar as it acts as a cofactor of several enzymes and proteins with a detoxifying function, for example, SOD and coeruloplasmin. However, it can also cause cell death of neurons under conditions of oxidative stress and mitochondrial impairments and, among other things, may play a role in the pathogenesis of PD [103]. As regards Ca^{2+} and Mg^{2+}, it has been shown that intracellular free Ca^{2+} is present in elevated concentrations in neurons subject to neurodegenerative processes, and that this can be correlated with an induction of cell death [104].

Although aluminium can only be detected in small concentrations in brain tissues under physiological conditions, elevated concentrations of this metal have been found in neurofibrillary tangles of PD patients. This metal is not a promotor of free radicals *de facto*, but, in combination with aluminium salts given as bivalent iron, it is able to generate an iron-induced peroxidation of lipids [105]. At the same time, silicon can apparently interact with aluminium to reduce its bioavailability and thus have a protective effect. On the other hand, silicon can combine with aluminium to form an aluminium–silicate complex that accumulates in neurofibrillary tangles [106].

Data are available ascribing iron metabolism an important role in the pathogenesis of Parkinson syndromes. An elevated concentration of total iron has been detected in the SN of PD patients [107]. As iron is able to generate highly toxic hydroxyl radicals via an oxidation of iron II to iron III and as it also leads to cleavage of H_2O_2 via Fenton's reaction, a closely regulated iron metabolism is necessary under physiological conditions. The increased iron deposition detected in PD thus supports the hypothesis of oxidative stress during pathogenesis and implicates an underlying impaired iron metabolism [108].

Coeruloplasmin, a 132-kDa glycoprotein, not only oxidizes multiple substrate molecules but also plays an important role in iron metabolism via its ferroxidase function. This enzyme catalyzes oxidation of iron II to iron III, but no ROS are produced in contrast to iron. Through loss of function, mutations in the coeruloplasmin gene seen in hereditary acoeruloplasminaemia and in double knockout animal experiments on mice, an elevated iron-induced peroxidation of fatty acids and a dysfunction of mitochondrial processes in the basal ganglia could be found [109]. Further evidence of coeruloplasmin relevance in the neurodegenerative processes of Parkinson syndromes was provided by immunohistochemical studies, which were able to show a colocalization of this protein with LBs. Hochstrasser et al. investigated the coeruloplasmin-coding gene in patients with Parkinson syndrome compared to corresponding control subjects and succeeded in identifying three mutations: I63T, associated with PD, R793H, correlating with a hyperechogenicity of the SN, and D544E, to which both applied [110]. This was the basis for further investigations of PD patients who carry these mutations with regard to a possibly impaired iron metabolism. Protein concentrations of coeruloplasmin, iron, ferritin, and transferrin were measured and, in addition, the activity degree of ferroxidase was detected in serum of patients with diagnosed PD in whom the abovementioned mutations had been demonstrated. A functional relevance of the coeruloplasmin mutations I63T and D544E was found. In patient samples with an I63T mutation, a 50% reduced protein concentration of coeruloplasmin and a 70% reduced activity of ferroxidase were observed. In addition, reduction of iron concentrations by half and reduced transferrin saturations were detected. The D544E polymorphism also showed significantly reduced values of serum coeruloplasmin and ferroxidase activity. From these results, the authors concluded that a changed

Table 14 Metal concentrations in CSF and blood samples in Parkinson disease patients (PD) and controls (CON)

	[112]	[113]	[114]	[115]
Diagnosis	PD/CON	PD/CON	PD/CON	PD/CON
n	26/13	71/44	36/21	37/37
Age (mean)	65/64	65/52	71/62	66/62
Method	Atomic emission spectrometry	Photometer	Atomic emission spectrometry	Atomic emission spectrometry
Concentration	Iron: 401.6 ± 92.1/324 ± 33.8 μg/L Zinc: 4.7 ± 892/4.0 ± 652 μg/L Copper: 994 ± 171/1.2 ± 326 μg/L	1.122 ± 432 ng/mL 1.596 ± 442 ng/mL	96 ± 11 μg/L 161 ± 31 μg/L	0.10 ± 0.06 mg/L 0.17 ± 0.14 mg/L
Material	Serum, plasma	Serum, plasma	CSF, serum	CSF
Result	PD: plasma iron/zinc ↑ PD: serum copper ↓	PD: iron ↓	PD: CSF zinc ↓	PD: zinc ↓

Concentrations are given as mean ± SD.

activity of coeruloplasmin might represent a possible vulnerability factor for iron-induced oxidative stress on neurons of the SN [111]. Using serum as well as plasma samples, Forte et al. found inconsistent results concerning iron levels (Table 14) [112,113], indicating that measurement of this metal may not be helpful in the diagnosis of PD.

Examinations of zinc concentrations in CSF of PD patients revealed lower values in comparison to control persons [114,115]. Under the assumption that metal ions, demonstrated in the SNpc of PD patients, might also play a role in the pathogenesis of α-synuclein formation, Ubersky et al. investigated various valent metal ions by means of biophysical methods with regard to possible effects on fibril formation and thus on conformational changes of α-synuclein. The thioflavin T assay was used to detect kinetics of fibril development in the presence of a wide variety of different mono-, di-, and trivalent metal ions. Using respective concentrations of 2 mM $AlCl_3$, $FeCl_3$, $CoCl_3$, and $CuCl_2$, it was not only shown that fibril formation is induced by metal ions but also shown that this induction is accelerated 50-fold. The divalent representatives zinc, magnesium, and calcium, in contrast, had no influence on α-synuclein fibril formation. $AlCl_3$ showed by far the strongest induction of fibril development accompanied by a change in protein conformation of α-synuclein at much lower $AlCl_3$ values [116]. It is known that metal-induced generation of oxidative stress can result in damage to molecules and that this can be correlated with the initiation of various events like mitochondrial dysfunction, excitotoxicity, and an elevation of intracellular calcium with the subsequent death of neurons. In addition, it could be shown in PD that oxidative stress manifests itself with an elevated metal accumulation accompanied by the absence of an appropriate amount of antioxidants [117]. In the oxidation of proteins by metal ions, di-thyrosine is catalyzed with a specific, detectable fluorescence emission spectrum. In their investigations, Ubersky et al. failed to detect a fluorescence signal for di-thyrosine, so one might assume that the fibril formation of α-synuclein does not take place via a metal-induced oxidation of this protein. The authors therefore put forward the theory that a direct interaction of this presynaptic protein with metal ions and, here in particular with $AlCl_3$, plays an important role in the pathogenesis of α-synuclein in Parkinson syndrome, and that the known structural changes of α-synuclein are possibly connected to resulting aggregations.

The role of α-synuclein in this context remains to be clarified: does the fibril formation represent an attempt to retard neurodegenerative processes in terms of neuroprotection or is it the chief cause of pathology? Inducing environmental stress, metals are an ideal instrument for further investigations in this ambiguous field.

Complex I: Disturbances in the Respiratory Chain: Causes or Effects of Neurodegeneration?

Concerning oxidation in the respiratory chain and aerobic oxygen production, metals likewise play an important role: notably in complex I iron as the prosthetic group for NADH dehydrogenase—as well as complex II iron as the prosthetic group for succinate dehydrogenase. A connection is often made between the presence of oxidative stress and accompanying impairments of the respiratory chain, whereby it has not yet been definitely clarified which of these two parameters is the cause and which is the effect. Assuming that PD is a multifactorial disease with a combination of genetic, individual, and environmental aspects as causative parameters for individual

Table 15 Analyses of the expression levels of respiratory chain complex I (NADH: ubiquinone-oxyreductase) in total brain tissue, frontal cortex, and substantia nigra pars compacta (SNpc) from patients with Parkinson disease (PD) and controls (CON)

	[118]	[119]	[120]
Diagnosis	PD/CON	PD/CON	PD/CON
n	10/12	11/11	5/4
Age (mean)	68/67	72/73	79/71
Method	Western blot 10 mg/lane	ELISA 100 μg/well Western blot 50 μg/lane	ELISA, spectrophotometer
Material	Brain, SNpc	Brain, SNpc	Brain, frontal cortex
Result	PD ↓	PD ↓	PD ↓

etiopathogenesis, investigations on stress defenses are an auxiliary field in which this theory can be examined.

Keeney et al. investigated the activity degree of complex I from patients with PD and age- and sex-matched control subjects (Table 15). For this purpose, mitochondrial tissue was isolated from brain samples obtained post mortem and immunoblots were performed [118]. The intensities of the porin band (mainly occurring in the outer mitochondrial membrane) as well as N-cadherin (preferentially detected in the plasma membrane) were then compared. In doing so, no difference between the two groups investigated could be established. In order to measure the catalytic activity of complex I (NADH: ubiquinone oxyreductase), NADH consumption was detected in the presence of coenzyme Q10. Here, after normalization to intensity of the porin band in Western blot, a significant reduction in complex I activity was seen in samples of patients with PD compared with healthy subjects. In order to further verify the data obtained to date on the reduced activity of complex I, the electron flow in this complex was investigated. The underlying principle here is an interaction of NADH-originating electrons of complex I with oxygen and the resulting formation of superoxides, which can be measured by means of fluorescence assays after conversion into hydrogen peroxide by the enzyme SOD-2. The samples of PD patients showed a significant reduction of NADH-mediated electron flow in contrast to the CON group. In an analogous study design, Devi et al. were able to confirm the reduction of complex I activity in brain samples of SNpc for PD patients not only by Western blot but also by means of ELISA [119]. Nevertheless, it appears not to be a specific effect of the basal ganglia, as Parker et al. found equivalent results in brain samples of frontal cortex, suggesting that impairments of the respiratory chain may be a global phenomenon in PD [120].

Further data concerning complex I in the pathogenesis of neurodegenerative diseases is given in excellent reviews on this topic [121–123].

Proteomics

Proteomic Approaches: A Promising Field for the Future

The classical workflow in proteome analysis involves the isolation of the proteome or a subproteome from an organism, the separation of proteins by means of electrophoretic or chromatographic methods, and the identification and quantification of these proteins. Further steps involve the characterization of proteins, the determination of their activity or the function of the proteins as well as the elucidation of protein–protein and protein–ligand interactions. In the postgenome era, proteomics provides a powerful approach for analysis of normal and transformed cell functions, for identification of disease-specific targets, and for uncovering novel endpoints for the evaluation of chemoprevention agents. However, expression-level analysis may not reflect the functional state of proteins and is biased toward long-living abundant proteins. This may be one explanation of why it is so difficult to find a generally valid diagnostic marker for differentiation of neurodegenerative diseases.

The choice and selection of the particular proteomic approach depend on the physiological model or disease process being studied. Understanding the experimental system will greatly improve the chances of success on proceeding to the next phase of a proteomic project or at least determine whether proteomics is a suitable experimental design for the goal. Concerning sample attributions, determining protein concentration, dynamic range, and degree of solubility may be a way of establishing changes. The next issue that has to be taken into account is whether direct quantification of changes is required versus analyzing the changes qualitatively. These are just a few aspects of filtering out the most promising proteomic approach, and it makes no claim to be complete.

In our understanding, proteomics is the most promising up-and-coming domain for filtering out interesting

Table 16 Proteomic approaches in the differential diagnosis of Parkinson disease (PD) compared with samples of dementia with Lewy bodies (DLB) patients and controls (CON)

	[5]	[124]	[125]	[126]
Diagnosis	PD/CON	PD/CON	PD/DLB/CON	PD/CON
n	4/4	5/5	10/5/10	5/5
Age (mean)	75/74	n.m.	63/69/65	84/77
Method	2D DIGE, MALDI MS	ICAT/LTQ MS, Western blot	iTRAQ, MudPIT, MS/MS	2 D DIGE, MALDI MS
Protein amount	100 μg sample	10μg/lane	-	100 μg sample
Material	Brain, SNpc	Brain, SNpc	CSF	Brain, SNpc
Result	PD ↑	PD ↓	PD/DLB	PD ↑
Regulated proteins	Periredoxin II, mitochondrial complex III, ATP synthase D, complexin I, profilin, L-type calcium channel, fatty acid-binding protein	Mortalin	↓: ceruloplasmin, VitD binding protein, Apo H, Apo C1 ↑: chromogranin B, β-fibrinogen, haptoglobin, T-cadherin	Annexin V, ferritin H, glutathion S transferase Mu3, glutathion S transferase P1, glutathion S transferase omega 1, glial maturation factor beta, brain-derived neurotrophic factor, glial fibrillary acidic protein, galectin 1, cellular retinoid-binding protein 1, beta tubulin cofactor A, S-adenosyl homocystein hydrolase

2D DIGE = 2-dimensional differential gel electrophoresis; MS = mass spectrometry; MALDI MS = matrix-assisted laser disorption and ionization mass spectrometry; LTQ-MS = linear ion trap quadropole mass spectrometry; ICAT = isotope-coded affinity tagging; iTRAQ = isobaric tagging for relative and absolute quantification; MudPIT = multidimensional protein identification technology; SNpc = substantia nigra pars compacta, n.m. = not mentioned.

proteins that have subsequently to be validated with other—more established—techniques.

In a proteomic analysis of human brain tissue SN, investigated post mortem in PD patients and an age- and sex-matched reference group, two-dimensional gel electrophoresis and subsequent mass spectroscopy were conducted to detect specific proteins. Significant elevations, in particular of presynaptic proteins, were seen in the group of PD patients compared with the CON group (Table 16). These proteins included periredoxin II, mitochondrial complex III, ATP-synthetase D, complexin I, profilin, L-type calcium channel D, and H-FABP [5]. The authors consider these elevated protein concentrations to be a reaction of afferent fibres to nigral dopaminergic neurons, as a reactive release in response to excessive cell death in the SN during the pathogenesis of PD. Two theories have been put forward as a possible cause–effect relationship: on the one hand, a relative elevation in presynaptic proteins may lead to an increased dopamine release through neurons of the SN, on the other hand, this afferent hyperactivity may also be induced by glutamatergic fibres of subthalamic structures, a view that can be reconciled with the hypothesis of cell death in the SN as a consequence of exotoxic agents [5].

There are also proteomic approaches investigating α-synuclein-mediated neurotoxicity. Gillardon et al. conducted their experiments on this subject by means of an α-synuclein (A30P) transgenic mouse model for PD, using 2D-DIGE. The focus was mainly directed toward changes in presynaptic proteins of transgenic mice showing early symptoms. For this purpose, microdissected brain tissue and, in particular, the synaptosomal fraction of transgenically altered animals were prepared and compared with unaltered mice. Six differentially expressed proteins could be identified: the diseased animals showed low protein concentrations of the enzymes methylglutaconyl-CoA hydratase and ATP-alpha chain synthase as well as an increased concentration of the LIM and SH3 domain protein, sorting nexin-12, as well as serotransferrin. As a significantly reduced concentration of two mitochondrial proteins was detected in those diseased animals—ATP synthase, which is involved in oxidative phosphorylation of mitochondria, and methylglutaconyl-CoA hydratase, an enzyme playing an important role in energy metabolism of the mitochondria—further experiments were conducted in order to investigate a possible impairment of mitochondrial functions. For this purpose, oxygen consumption

of mitochondria-enriched, fractionated tissue of mouse brainstem was investigated by means of an Oxygraph-2k system (Oroboros, Instruments, Innsbruck, Austria), whereby this research group failed to detect any dysfunction of mitochondrial respiration [124].

Thus, in this mouse model, it does not appear to demonstrate an elevated concentration of neuroprotective proteins, such as HSPs, or enzymes of glycolysis as a reactive response to an increased detectable amount of α-synuclein, although the abovementioned mitochondrial enzymes were detected in a significantly reduced concentration. Another promising method of proteomic approaches also used by this research group is expression analysis of microRNA (miRNA). These are noncoding transcripts of 19–24 nucleotides that are produced by the RNAase Dicer. It is known that, during embryonic development of the nervous system, gene expression of various different miRNAs that are involved in processes of cell specification and sprouting of axonal structures takes place [125].

There are also data available implying a possible connection between generally reduced gene expression of miRNA, on the one hand, and neurodegenerative processes, on the other hand [126]. In our study, lower signal intensities of miR-10a, miR-10b, miR-212, miR-495, and miR-132 were detected in brainstem of α-synuclein (A30P) transgenic mice compared with a wild-type CON group. In summary, the authors concluded that determination of miRNA may be a possible, but currently not yet unified, method for rendering the diagnosis of PD [124].

Investigations on a possible influence of oxidative stress on the pathogenesis of Parkinson syndrome are a common subject of not only current protein biochemical approaches but also proteomic approaches. On the basis of investigations in which oxidative stress was generated under laboratory conditions in the form of neurotoxins such as rotenone [4] or methyl-phenyl-tetrahydropyridine (MPTP) [127], inhibition of complex I of the respiratory chain was shown, followed by spontaneous cell death. For their experiments, Jinghua et al. selected a proteomic method that is known under the name shotgun proteomics multidimensional protein identification technology (MudPIT), with which a quantitative analysis of corresponding proteins can be performed. For this purpose, consecutive experiments are performed by means of multidimensional LC and mass spectrometry (MS) in order to separate and fragment the peptides obtained for protein identification. To this end, an investigation of patients with Parkinson syndrome and healthy control subjects was conducted. After isolation of the mitochondria-rich fraction from tissues of the SNpc, 119 regulated proteins could be detected in comparison to the CON group. Of these, a detailed investigation of mortalin (mthsp70/GRP75), a protein influencing regulative processes of the respiratory chain, was conducted. Mortalin was found to be present in reduced concentrations in mitochondrial fractions of dopaminergic cells. In subsequent cell culture experiments with overexpression or inhibition of mortalin, it could be shown that this protein has an influence on the survival rate of cells in low concentrations, whereby a connection could be found between mortalin and rotenone. Rotenone, which is not concentrated in dopaminergic neurons in contrast to MPTP and paraquat, but still results in apoptosis of neuronal cells, leads to an inhibition of the respiratory chain. This gives rise to the assumption that dopaminergic cells of the SNpc react extremely sensitively to dysregulations of the respiratory chain. In addition, it is known that a rotenone-mediated toxicity can result in development of LB-like, intracytoplasmatic incorporated bodies, consisting of α-synuclein and ubiquitin [128].

With the data they collected, Jin et al. provided support for the theory that mortalin may have a rotenone-mediated toxic influence on mitochondrial and proteasomal functions, above all in the presence of oxidative stress [129]. The hypothesis has been put forward that mortalin has an antiapoptotic effect on cells. Studies on this have shown that overexpression of this protein resulted in malignant transformation of the cells investigated (NIH 3T3) [130]. Apart from this, a prolonged survival rate of normal fibroblasts was detected under overexpression of mortalin [131]. In addition, elevated protein concentrations of mortalin were found in tumors of the central nervous system [132]. After reduction of mortalin concentrations generated by antisense gene expression, tumor growth in immortalized cells was arrested [133]. It is probable that well-balanced concentrations of mortalin are necessary for physiological homoeostasis, and that the smallest discrepancies in this system may have serious consequences for both individual cells and tissue assembly. The use of mortalin may provide a reliable method for differential diagnosis of extrapyramidal syndromes and has to be verified in future studies.

There are further ongoing studies using proteomic approaches to detect a possible biomarker differentiating the group of parkinsonian syndromes. One of these study designs employed an unbiased quantitative proteomic approach called isobaric tagging for relative and absolute protein quantification (iTRAQ) to label prefractionated human CSF followed by MudPIT prior to identification and quantification of CSF proteins with tandem MS. This multiplex format allowed the authors to compare simultaneously the proteome of CSF in patients suffering from AD, patients with PD and DLB and healthy, age-matched CON [134]. In total, 136, 72, and 101 of the 1500

Table 17 Tabular listing of possible biomarkers for the diagnosis and differential diagnosis of Parkinson syndromes including Parkinson disease (PD), Parkinson disease dementia (PDD), multisystem atrophy (MSA), Lewy body dementia (DLB), and progressive supranuclear palsy (PSP)

	PD	PDD	MSA	DLB	PSP	Control	Cutoff	CSF	Brain	Serum	Sensitivity	Specificity	Reference
5-HIAA	n		↓				62 nM	∗			71%	90%	[76]
Aβ 40∗	n	n	n	↑	n	n	0.848 ng/mL	∗			81%	71%	[56]
Aβ 42	n	n	↓		n	n	41.4 pg/mL	∗			87%	87%	[56]
$\alpha-$synuclein	↑					n	79.9 pg/mL	∗		∗	53%	85%	[26]
GH	↑		n			↑	#	∗			80%	75%	[89]
H-FABP	n	↑		↑		n	2.8 pg/mL	∗		∗	84%	82%	[80]
Hypocretin	↓				↓	n	110 pg/ml	∗	∗		#	#	[37]
L-citrullin	n	n	↑			n	2.9 μM/L	∗			#	#	[92]
MBP	n		↑				0.7 μg/L	∗			72%	86%	[76]
MHPG	n		↓				42.5 nM	∗			86%	75%	[67]
NFHp35			↑			n	129.5 ng/L	∗			87%	83%	[67]
NfH^{SM135}	n		↑		↑	n	1.4 ng/mL	∗			76%	94%	[66]
NFL			↑			n	24.4 ng/L	∗			79%	94%	[67]
NSE	n		↑				8.4 μg/L	∗			47%	91%	[76]
Osteopontin	↑					n	3.6 ng/mL	∗	∗	∗	#	#	[95]
p-tau	n	n	↑		n	n	128 ng/L	∗			95%	77%	[49]
Reelin	↑	n		↑	↑	n	#	∗		∗	#	#	[60]
Serpin				n		n	#	∗		∗	95%	78%	[74]
t-tau	n	n	n	n	n	n	#	∗			86%	91%	[49]
tTG	↑					n	0.5 pg/mL	∗			#	#	[42]

5-HIAA = 5-hydroxy-indole acetic acid; Aβ = amyloid β; MBP = myelin basic protein; MHPG = methoxy-hydroxy-phenylethylene glycol; NfH = neurofilament heavy chain; NfL = neurofilament light chain; NSE = neuron-specific enolase; p-tau/t-tau = phospho-tau/total-tau; tTG = tissue transglutaminase; n = normal levels; ∗ = investigated material; # = no data available; ↓ = reduced values; ↑ = elevated values.

identified proteins displayed quantitative changes unique to AD, PD, and DLB, respectively, so that in further experiments, eight unique proteins were confirmed closely by Western blot analysis. The proteins ApoH, as a complex participating in agglutination, ApoC1, inhibiting a cholesteryl ester transfer protein, resulting in increased atherosclerosis, ceruloplasmin, a transport protein for copper in human plasma, chromogranin B, ubiquitously found in cores of amine and peptide hormones and neurotransmitter dense-core secretory vesicles, β-fibrinogen, known for its role in coagulation and inflammation, haptoglobin, a binding and transport protein for hemoglobin, T-cadherin, involved in cell–cell contacts and reorganization of the dynamic cytoskeleton accompanied by phenotype changes, and VitD BP, that is, besides its role in binding and transport of vitamin D molecules, able to interact with the actin filaments and takes part in processes concerning chemotaxis—were part of these eight more closely investigated molecules. Several panels of unique markers were capable of distinguishing AD, PD, and DLB patients from each other as well as from CON. The authors suggested potentially good combinations of markers: β-fibrinogen plus VitD BP for patients with AD, chromogranin B plus ceruloplasmin for patients with PD, and ApoC1 plus chromogranin B for patients with DLB. These combinations were able to differentiate the examined neurodegenerative diseases and might be potential biomarkers for differential diagnosis [134]. Further investigations with regard to reproduction of the available data have to be carried out.

Werner et al. examined brain samples of SN in patients with PD and a CON group using 2D DIGE and matrix assisted laser disorption and ionization (MALDI)-ToF for identification of probable proteins. They found lots of differentially regulated proteins (Table 16) elevated in PD and gathered from these results a heterogeneous etiopathogenesis of the diseases [135].

Finally, proteome analyses are a very promising field for selection and overview of potential proteins. Nevertheless, it is currently necessary to confirm the obtained results using well-established protein biochemical procedures. Furthermore, it is difficult, given the massive amount of data obtained using proteome analyses, to segregate those candidates that have the greatest promise for future investigations.

Conclusion

In conclusion, it can be stated that a variety of different, very promising studies are available on possible diagnostic differentiation of Parkinson syndromes and associated diseases (Table 17). They all have in common the attempt to detect a single or a few specific biomarkers with the help of which a universal screening method can be

established with simple laboratory chemical and, here in particular, protein biochemical and proteomic methods. This ideal case would enable the early detection and differentiation of the diseases mentioned. However, up to now, it has not been possible to separate a single marker that additionally has sufficient sensitivity and specificity in the respective analytical method and with which identical, reproducible results can be achieved in subsequently conducted CON. In addition, the use of nonuniform parameters, such as number of patients investigated, sample pretreatment, sample storage, analytical methods used, establishment of the respective limit values, and statistical evaluation in studies investigating the same biomarker, hampers direct comparison of the results obtained. Apart from this, there are individual, very promising studies on a particular marker, without control studies conceived with the same design on the same subject, with the help of which the reproducibility of the data obtained could be verified. In this respect, further neurochemical studies with CSF and blood samples as well as morphological imaging and neuropsychological investigations also performed by other specialized disciplines will undoubtedly follow in the future in order to detect possible parameters that should be tested in subsequent studies for their suitability as established diagnostic markers.

In our understanding, none of the proteins specified above is really applicable as a potential marker for diagnosis or differential diagnosis of PD. This overview suggests the existence of many proteins involved in neurodegenerative processes that seem to mirror neuropathological changes, on the one hand, but lack specificity, on the other hand.

In our view, the specific biomarker for a certain neurological disease is yet to be identified—or better, several biomarkers. We think that a combination of multiple or at least two proteins will be necessary to differentiate PDs as well as dementing syndromes from each other, as demonstrated for AD (tau/Aβ) and CJD (tau, protein S-100B, and protein 14–3-3). Furthermore, we assume that the diagnostic question has to be very precise (e.g., PD or PDD, MSA versus PD). In these situations, existing biomarkers may provide additional information by adjusting proper cutoff values.

It is our hope that—using clinical proteomic tools—further candidates can be found to improve the early and differential diagnosis of Parkinson syndromes and PDD.

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Conflict of Interest

Concept/design: Sarah Jesse, Petra Steinacker, Bastian Hengerer, and Markus Otto; critical revision of article: Markus Otto, Petra Steinacker, Stefan Lehnert, Bastian Hengerer, Frank Gillardon, and Sarah Jesse; approval of article: Markus Otto, Petra Steinacker, Stefan Lehnert, Bastian Hengerer, Frank Gillardon, and Sarah Jesse; and data collection: Sarah Jesse and Markus Otto. The authors have no conflict of interest.

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