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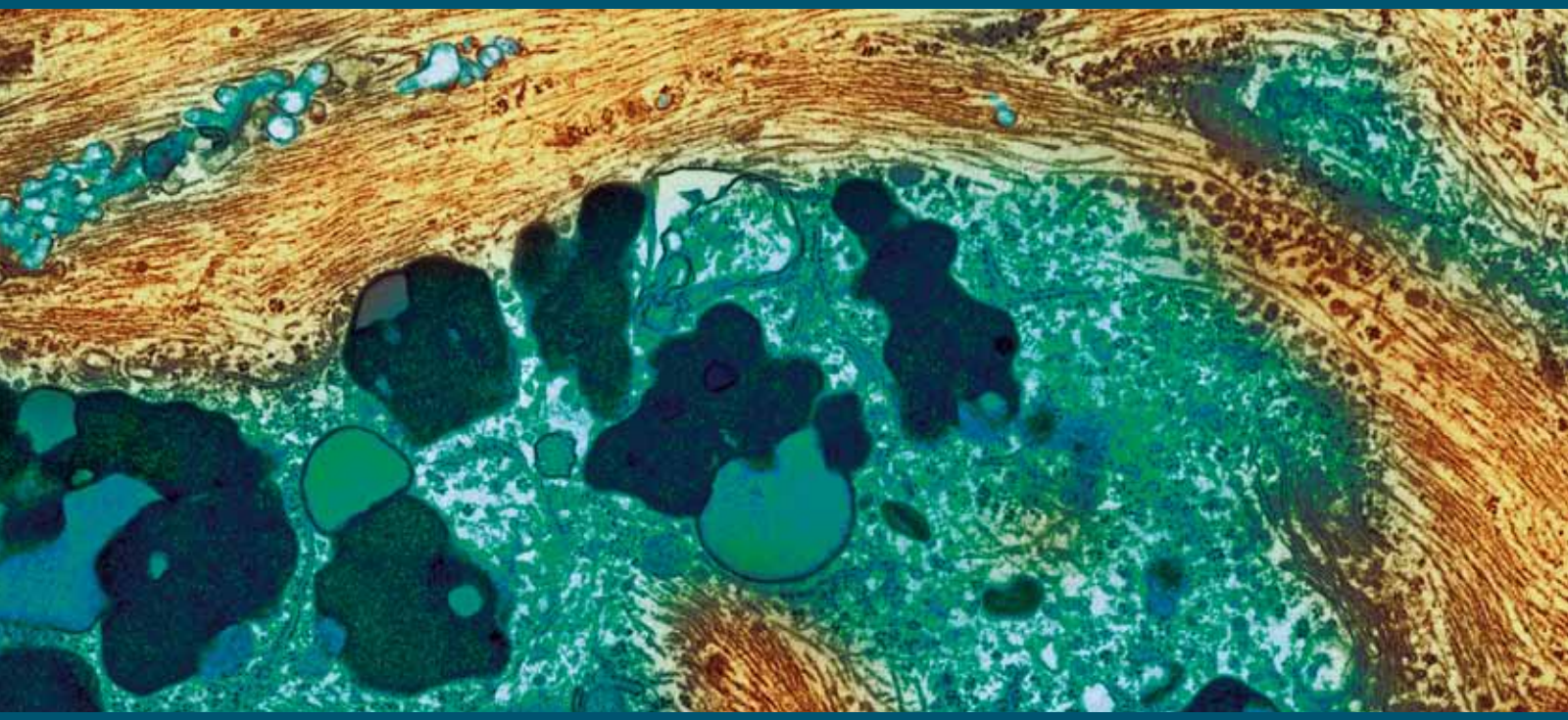
Biomarkers in Alzheimer's Disease and Lewy Body Disorders with Dementia

Guest Editors: Thomas Leyhe, Taher Darreh-Shori, Christoph Laske, Michelle M. Mielke, and Walter Maetzler



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Editorial Board

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Editorial

Biomarkers in Alzheimer's Disease and Lewy Body Disorders with Dementia

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The present special issue provides a synthesis of current materials and techniques used to not only explore, but also to validate and evaluate novel markers, potentially new biomarkers, in the field of dementias associated with Alzheimer's and Lewy body pathology. This topic is of particular interest as only a very minor proportion of potential markers detected in preclinical studies will ultimately be included in larger clinical studies or for routine clinical use.

The review article by Wang et al. focuses on an exciting and novel topic in the field, the potential of the quantification of small brain protein degradation fragments in blood, which are generated specifically by brain-derived proteases, to diagnose and follow neurodegenerative processes. The authors thoroughly review the currently available literature. They then speculate on the utility of this strategy as a method of choice for the development of novel biomarkers for disease progression, stage of disease, and treatment efficacy in large cohorts of demented patients as well as individuals at risk for neurodegenerative dementias. A specific focus is on the potential of tau-, APP-, and alpha-synuclein-associated fragments.

The review of Deleidi and Maetzler argues that disturbances in protein clearance mechanisms in the brain substantially contribute to neurodegenerative dementias associated with Abeta and Lewy body pathology. In fact, much evidence has been accumulated pointing to defective protein clearance mechanisms involved in the initiation and progress of sporadic Alzheimer's disease. The authors provide, for the first time, an extensive overview of the literature dealing with protein clearance aspects in the field of Lewy body-associated dementias, with a particular focus on intraneuronal and extraneuronal clearance mechanisms of Abeta and alpha-synuclein.

Alpha-synuclein, and the detection of its monomeric and oligomeric species in cerebrospinal fluid and blood of patients with dementia with Lewy bodies, is also the central focus of the review provided by Kasuga et al. The authors conclude that the determination of altered alpha-synuclein species such as truncated, phosphorylated, and oligomeric forms in body fluids may have a far higher potential to differentiate between healthy and disease state compared to the measurement of unaltered alpha-synuclein levels.

In addition to the review papers, there are several research papers included in this special issue highlighting new research in the field. Baldin et al. assess the associations between neuropsychological outcomes and pro- and anti-inflammatory serum markers in a large cohort of patients with Alzheimer's disease and controls. Their results suggest that individuals with cognitive impairment possess different

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proinflammatory and anti-inflammatory "strategies" compared to those without. The authors argue that combinations of inflammation and neuropsychological measures, as presented in their study, may improve diagnostic quality. As this is a baseline assessment of a longitudinal study, we expect that the initial results concerning the predictive diagnostic value of this approach—which are even more interesting—will soon be available. This article fits well in a rapidly growing number of important articles reporting about the usefulness of *combinations* of outcome measures to define state, trait, and risk of chronic (neurodegenerative) diseases.

Schreitmüller et al. assess vascular markers by Alzheimer's disease severity. Their research is based on the idea that in Alzheimer's disease a continuous vascular activation, induced by hypoperfusion and factors and processes associated with angiogenesis, occurs. In their study, the authors investigated serum levels of the proangiogenic factor Angiopoietin-1 in patients with Alzheimer's disease, mild cognitive impairment, and controls. They found higher levels in patients with Alzheimer's disease and concluded that Angiopoietin-1 is a potential candidate for a respective biomarker panel.

Duchesne et al. provide another (if not the most important) piece in the biomarkers' development puzzle. They nicely illustrate the importance of implementing adequate verification, validation, and evaluation steps before a promising marker can be integrated into large scale studies or even routine clinical assessment. More specifically, they report both the within-session scan/repeat and across-session scan/rescan reliability (a component of validity) of a self-developed assessment tool that detects medial temporal lobe atrophy via MRI images. In their article, they end up with a power calculation, that is, an estimation of minimum precision threshold that must be added to the effect size, to obtain true cohort sizes for clinical trials.

Lastly, Liepelt-Scarfone et al. report on the diagnostic utility of neuropsychological test results obtained from a large cohort of nondemented and demented patients with Parkinson's disease. In this paper, the authors introduce factor and cluster analysis strategies that enable a differentiation between different subtypes of Parkinson's disease patients, a detection of similarities between test results, and a reduction of a large number of markers on an explorative, hypothesis-free basis. These are particularly important approaches to increase the quality of clinical data that are included in biomarker studies, and to improve our understanding about pheno- and endophenotypes in respective disorders.

It must be emphasized that the evaluation of any therapy is heavily depending on the existence of sufficient trait and state markers which are currently, in particular in Lewy body disorders, not available. With this respect, we feel that this special issue offers both review articles and original research which provide a comprehensive overview of promising (combinations of) biomarkers as well as state-of-the-art techniques to evaluate them. We hope this issue will stimulate the continuing efforts towards developing promising and reliable markers for these particularly burdensome disorders.

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Review Article

Protein Clearance Mechanisms of Alpha-Synuclein and Amyloid-Beta in Lewy Body Disorders

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Protein clearance is critical for the maintenance of the integrity of neuronal cells, and there is accumulating evidence that in most—if not all—neurodegenerative disorders, impaired protein clearance fundamentally contributes to functional and structural alterations eventually leading to clinical symptoms. Dysfunction of protein clearance leads to intra- and extraneuronal accumulation of misfolded proteins and aggregates. The pathological hallmark of Lewy body disorders (LBDs) is the abnormal accumulation of misfolded proteins such as alpha-synuclein (Asyn) and amyloid-beta (Abeta) in a specific subset of neurons, which in turn has been related to deficits in protein clearance. In this paper we will highlight common intraneuronal (including autophagy and unfolded protein stress response) and extraneuronal (including interaction of neurons with astrocytes and microglia, phagocytic clearance, autoimmunity, cerebrospinal fluid transport, and transport across the blood-brain barrier) protein clearance mechanisms, which may be altered across the spectrum of LBDs. A better understanding of the pathways underlying protein clearance—in particular of Asyn and Abeta—in LBDs may result in the identification of novel biomarkers for disease onset and progression and of new therapeutic targets.

1. Introduction

Lewy body disorders (LBDs) is an umbrella term that includes diseases with alpha-synuclein (Asyn) aggregates as fibrils in Lewy bodies (LBs) and Lewy neurites. Several lines of evidence support a pathogenic role of misfolded Asyn in LBDs [1–3]. Parkinson's disease (PD) without dementia (PDND) is the most common subtype of LBDs, followed by PD with dementia (PDD) and dementia with Lewy bodies (DLBs) [4, 5]. Like in PD, the core feature of PDD is a diagnosis of PD according to the UK Brain Bank Society criteria [6] but also includes cognitive symptoms severe enough to fulfil dementia criteria at least one year after PD diagnosis with insidious onset, slow progression, and impairment in more than one cognitive domain [7]. DLB is the second most prevalent neurodegenerative dementia after Alzheimer's disease (AD). Clinical diagnosis is based on the presence of a dementia syndrome, accompanied by at least two out of the three following symptoms: fluctuating cognition with pronounced variations in attention and alertness, visual hallucinations, and Parkinsonism [8]. In contrast to PDD, the onset of dementia in DLB is before or within one year of any Parkinsonism.

About one-fourth of LBDs patients show cortical amyloid-beta (Abeta) deposition, with the highest proportion in DLB subjects, followed by PDD subjects [9–14]. According to clinical, neuroimaging, and neuropathological data, co-occurrence of Asyn and Abeta is regularly associated with dementia in LBDs [13–15].

Based on the central role that Asyn and Abeta play in the pathogenesis of LBDs and the increasing body of literature pointing to defective clearance of misfolded proteins as a key mechanism to the pathogenesis of LBDs, this paper aims at providing a condensed review of this latter topic. Although not exhaustive, it may provide a basic understanding of such mechanisms eventually contributing to the development of

novel disease biomarkers (which are currently not available [16, 17]) and neuromodulatory treatment strategies for these still incurable and chronic diseases.

2. General Mechanisms of Protein Clearance

The main *(intraneuronal)* pathways for the degradation and recycling of proteins are the ubiquitin/proteasome system (UPS) and the autophagy-lysosomal pathway (macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA)).

The UPS degrades short-lived nuclear and cytosolic proteins or misfolded proteins in the endoplasmic reticulum [18]. It plays a key role in signal transduction, cell cycle progression, apoptosis, and cellular differentiation and has been implicated in several human diseases, including neurodegenerative diseases, cancer, inflammation, and autoimmunity [18–20].

Autophagy is a process involving the degradation of components inside lysosomes [21, 22]. It has a variety of physiological and pathophysiological roles in protein and organelle clearance, development, defence against microorganisms, cell death, and antigen presentation [23]. In macroautophagy, organelles and macromolecular components are first surrounded by a double membrane, defined as the autophagosome or autophagic vacuole (AV), which then fuses with lysosomes to form autolysosomes. In microautophagy, the transfer of cytosolic components the lysosomal compartment happens by direct invagination of the lysosomal membrane without prior sequestration into the autophagosome. Finally, in CMA, individual proteins are targeted to lysosomes by the binding of a hsc70-containing chaperone/cochaperone complex.

All these pathways are observable in neurons, and both impairment and excessive activation of these pathways are linked to neurodegenerative processes [24].

(Extraneuronal) clearance pathways include interaction of neurons with astro- and microglia and with infiltrating macrophages. Microglial cells are considered the professional phagocytes in the brain. However, other populations of cells may also act as phagocytes, including astrocytes, neural stem cells, and neurons [25–28]. There is no clear evidence for a defective protein clearance by the brain innate immune system as a primary pathogenetic event in neurodegenerative diseases. However, phagocytosis of misfolded proteins by astro- and microglia triggers the release of proinflammatory cytokines and chemokines and reactive oxygen/nitrogen species, which may, under pathological conditions, further promote neuronal dysfunction and degeneration [29, 30].

The adaptive immune system is also involved in the clearance of misfolded proteins in the brain. Naturally occurring autoantibodies are detectable in body fluids of healthy controls and in patients with neurodegenerative disorders [31–33]. Finally, protein transport from the parenchyma to the cerebrospinal fluid (CSF) and across the blood-brain barrier are relevant clearance mechanisms and have been shown to be affected in LBDs.

Intra- and extraneuronal clearance mechanisms closely interact: as an example, protein aggregates can stimulate the cell surface innate immune receptors, initiating intracellular signaling cascades that, in turn, stimulate phagocytosis. An overview of how these mechanisms may interact is schematically illustrated in Figure 1.

In the following, all these mechanisms are discussed in more detail with special emphasis on Asyn and Abeta clearance.

3. Asyn Clearance

3.1. Intraneuronal Mechanisms. Abnormal deposition of Asyn occurs early in the disease process of LBDs and may follow an ascending pattern in most of the cases, starting from lower brainstem areas and then affecting limbic and cortical areas [34]. Deposition may start even earlier in the autonomic peripheral nervous system [35, 36]. The mechanisms responsible for Asyn degradation have been controversial, but it appears that, under normal conditions, Asyn is degraded by both the UPS and the autophagy-lysosomal pathway [37–39], whereas the autophagy-lysosomal pathway mediates clearance of accumulated and aggregated Asyn [38]. In agreement with this observation, activation of autophagy leads to increased wild-type Asyn clearance and neuroprotection [37, 40]. Normal Asyn binds to the CMA-specific receptor LAMP-2A on the lysosomal membrane and is subsequently degraded by CMA. However, mutant forms of Asyn (A53T and A30P) and Asyn modified by dopamine tightly bind to the CMA receptors on the lysosomal membrane and inhibit both their own degradation and that of other CMA substrates [38, 41]. The dysfunction of CMA triggers neuronal dysfunction and increases vulnerability to stress. Interestingly, both mutant and wild-type Asyn can decrease proteasomal activity and increase vulnerability to neurodegeneration, leading to a vicious cycle where an increased amount of intraneuronal Asyn can block its clearance by itself [38, 42–46].

Several studies provide evidence for impaired autophagy-mediated clearance mechanisms in PD. The main proteins involved in CMA (Lamp2a and Hsc70) are decreased in the SN and amygdala from PD patients [46]. Microtubule-associated protein 1A/1B-light chain 3 (LC3, a marker for autophagic vacuoles) colocalizes with Asyn in most LBs and Lewy neurites [46].

Genetic mutations leading to Parkinsonism support the hypothesis that defective clearance mechanisms are centrally involved also in idiopathic forms of LBDs. Interaction between PINK1 and Parkin can modulate mitophagy [47, 48]. PINK1 itself directly activates autophagy and interacts with autophagic proteins such as Beclin1 [49]. A reduced clearance of mitochondria was also demonstrated in cells lacking DJ-1 which is another protein associated with recessive forms of Parkinsonism [50]. Mutations in the *LRRK2* gene, the most common form of late onset autosomal dominant Parkinsonism, may also cause neuronal cell death via impairment of protein degradation pathways as they influence the autophagy-lysosomal pathway,

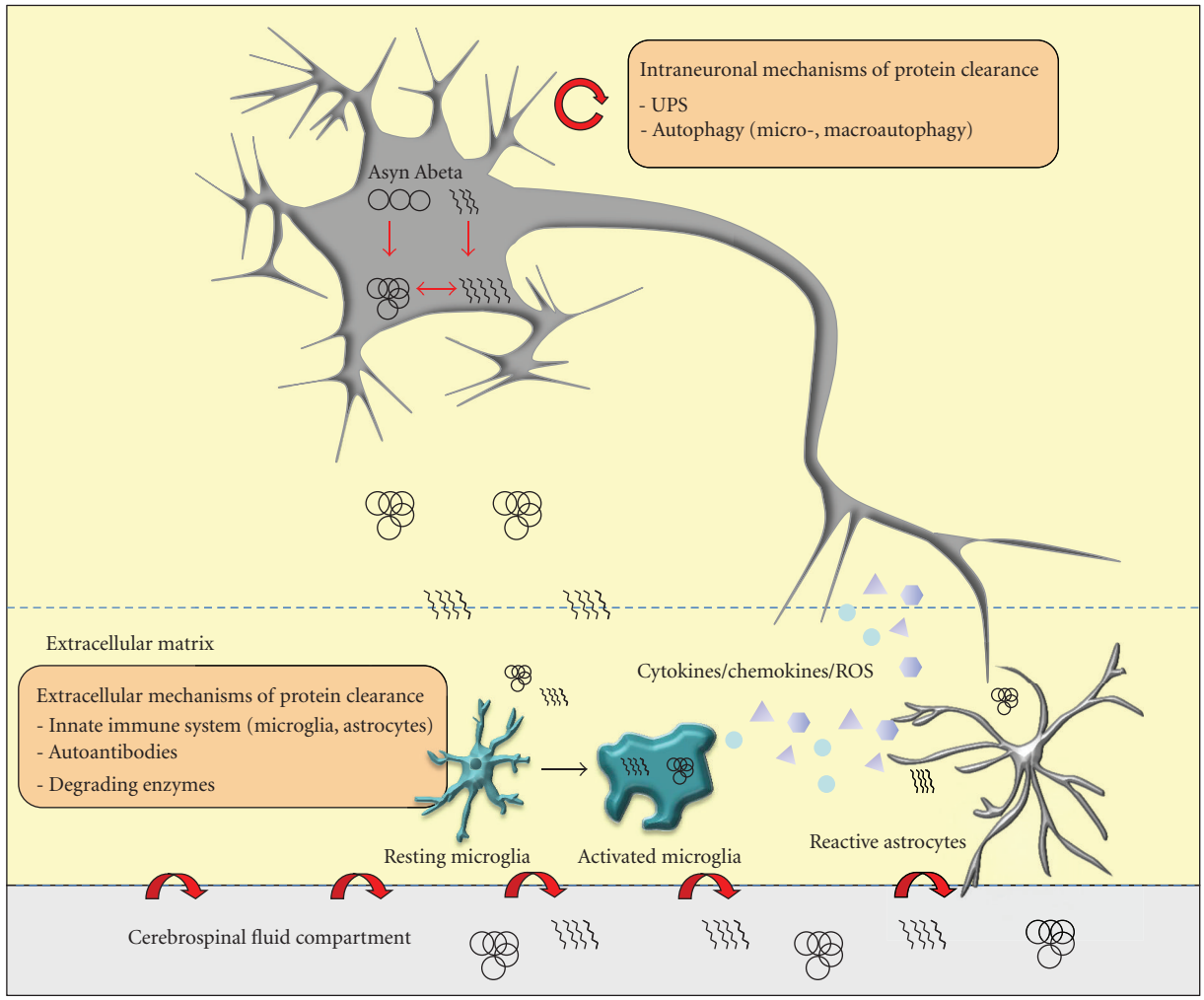

Figure 1: Intra- and extraneuronal mechanisms of protein clearance. The main intracellular pathways for the degradation and recycling of proteins are the ubiquitin/proteasome system (UPS) and the autophagy-lysosomal pathway (microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA)). Extracellular clearance pathways include interaction of neurons with astro- and microglia, and with infiltrating macrophages, autoantibodies, and protein transport from the parenchyma to the cerebrospinal fluid and across the blood-brain barrier. The engulfment of misfolded proteins by astro- and microglia triggers the release of proinflammatory cytokines and chemokines as well as reactive oxygen/nitrogen species, which may, under pathological conditions, further promote neuronal dysfunction and degeneration.

leading to Asyn accumulation and aggregation [51, 52]. Heterozygous mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (GBA) convey an approximately 5-fold risk for PD. These mutations are associated with lysosomal dysfunction and influence binding of Asyn to its specific receptor at the lysosome membrane: a recent study demonstrated that the accumulation of the GBA substrate glucosylceramide stabilizes Asyn soluble oligomers which, in turn, can inhibit normal GBA lysosomal activity. This creates a positive feedback loop that could directly contribute to the neurodegenerative process [53]. The heterozygous *GBA* mutation also seems to directly influence fatty acid metabolism: PD patients with these mutations have lower CSF levels of palmitoleic, oleic, linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic acids compared with both idiopathic PD patients and controls [54].

3.2. Extraneuronal Mechanisms (Mainly) . Although Asyn is a cytosolic protein, a low quantity of the protein is secreted via vesicle exocytosis and is then present in biological fluids including plasma and CSF [55–57]. The mechanisms responsible for the extraneuronal clearance of Asyn are not entirely clear; however, there is increasing evidence that adjacent cells such as astrocytes and microglia as well as the adaptive immune system and local protein transport mechanisms are crucially involved.

Loss of midbrain dopaminergic neurons and striatal degeneration can be preceded by neuroinflammation marked by activated microglia and an increase in proinflammatory cytokines and chemokines [29, 30, 58–62]. Astrocytes are also involved in the initiation and progression of the disease [63]. A recent study showed that astrocytic Asyn initiates noncell autonomous killing of neurons [64].

Indeed, extraneuronal forms of Asyn can activate glial cells and trigger inflammation and subsequent release of proinflammatory molecules, a common pathological hallmark of LBDs [60, 65]. Several studies have reported Asyn-containing inclusions in astroglia of PD and DLB patients [66], and phagocytic microglial cells are very efficient scavengers of extraneuronal Asyn aggregates [63, 67].

Results of recent GWAS studies argue for the relevance of the immune system, in the pathophysiology of LBDs, as variations in the *human leukocyte antigen (HLA)* region were associated with occurrence of LBDs [68, 69]. This may also be mediated through microglia as these cells are capable of presenting antigens to lymphocytes [70] via the HLA domain. Activation of HLA-positive microglia is observable in affected brain regions of PD patients [71]. Asyn autoantibodies are more prevalent in sera of PD patients than in controls [31]. These results were basically confirmed by a recent study where serum Asyn autoantibody levels were higher in demented LBDs patients than in controls [72].

An overload of local Asyn can also occur due to defective transport mechanisms of Asyn from the neuron to the CSF. Most studies investigating CSF total Asyn levels in PD showed that these levels are reduced in the CSF of PD patients compared to controls, whereas oligomeric Asyn levels seem to be higher in PD compared to control CSF [57, 73–77]. A recent study showed that CSF levels of phosphorylated Asyn correlate weakly with PD severity and, if corrected for total Asyn, contributed to the differential diagnosis between PD, multiple system atrophy (MSA), and progressive supranuclear palsy (PSP) [78]. However, it is neither clear to date how Asyn is transported from the parenchyma to the CSF, nor why total Asyn is reduced and pathological forms of Asyn are elevated in the CSF of PD patients.

4. Abeta Clearance: Lessons from Alzheimer's Disease Research

Comparable to LBDs pathophysiology, in AD, only some rare genetic mutations lead to increased APP expression or to changes in Abeta stability or aggregation [79]. The common late onset sporadic AD seems to be far better explained by impaired Abeta clearance mechanisms [80]. Thus, assuming that similar mechanisms may occur in (late onset) AD and LBDs and that Abeta pathology is a pathophysiologically relevant feature of LBDs, a discussion of (ineffective) Abeta clearance mechanisms as they occur in AD may substantially contribute to our understanding of how Abeta may (or may not) be cleared in LBDs.

4.1. Intraneuronal Mechanisms. There is convincing evidence from electron microscopy studies [81] that the autophagy-lysosomal pathway is involved in the pathogenesis of AD. AVs which are the major reservoir of intraneuronal Abeta are abundant in affected neurons, especially in those with neurofibrillary tangles [82]. These studies argue for an impaired maturation of autophagolysosomes and impaired intraneuronal retrograde transport in AD. Defective clearance of Abeta-generating AVs may result in Abeta accumulation [83, 84]. Several further findings corroborate the hypothesis that impaired autophagy plays a key role in the pathogenesis of neuronal degeneration in AD. Beclin1 is decreased in AD brains, and decreased neuronal autophagy and subsequent lysosomal dysfunction and neurodegeneration are observed in mice carrying a heterozygous deletion of Beclin1 [85]. Presenilin 1 (PS1) is essential for maturation of the lysosomal proton pump and affects autophagocytosis and protein turnover [86].

4.2. Extraneuronal Clearance Mechanisms (Mainly). Abeta concentration is tightly regulated by amyloid-degrading proteolytic enzymes and perivascular drainage [87–89]. Neprilysin (NEP) is an Abeta-degrading protein found at presynaptic terminals and in body fluids [90, 91]. It is a preferentially membrane-bound, presynaptically located protein with an extracellular catalytic site which can degrade Abeta [90, 91]. A soluble form of NEP is detectable in body fluids such as blood and CSF, emanating from a slow release from the membranes [92]. Most interestingly, reduced CSF NEP activity levels have been shown to occur in early AD [93, 94].

Another protein involved in defective clearance mechanisms in AD is cystatin C. Neurons, among other cells, are able to produce and secrete this protein [95]. Fourfold higher levels of cystatin C in the CSF than in blood [96] indicate a relevant role of the protein in CNS pathways. Cystatin C binds monomeric Abeta and carries soluble Abeta [97, 98]. There is evidence that AD patients have reduced CSF cystatin C levels [99, 100]. This makes it intriguing to hypothesize that a deficient Abeta-binding capacity, as induced by a lack of (functional) cystatin C, may contribute to the amyloidogenic process in AD [99]. Indeed, increased expression of this protein has been shown to reduce parenchymal Abeta load in mouse models of AD [101, 102]. Of note, the *BB* genotype of the cystatin C-encoding gene—which leads to reduced cystatin C secretion from the neuron to the extracellular space [103, 104]—conveys susceptibility to AD [105].

Inflammatory reactions, characterized by activated microglia and astrocytes surrounding amyloid deposits, are intimately associated with the onset and progress of AD [106–108]. Reactive astrocytes with Abeta-positive granules are found in close proximity to amyloid plaques. Human astrocytes express scavenger receptors and several Abeta-degrading enzymes such as NEP, insulin-degrading enzyme (IDE), endothelin-converting enzyme (ECE), angiotensin-converting enzyme (ACE), plasminogen activators, and the matrix metalloproteinases-9 and -2 (MMP-9, MMP-2) [109–112]. These findings suggest an important role of astrocytes in Abeta clearance.

Clinicopathological studies suggest that microglial activation is an early event in AD pathology [113, 114]. Activated microglia surround amyloid fibril deposits, and postmortem studies have shown significant amounts of Abeta in microglial cells of AD patients treated with immunization therapy [115, 116]. Microglia express toll-like receptors (TLRs), a family of highly conserved molecules that

recognize pathogen-associated molecular patterns, including both exogenous and endogenous ligands [117]. TLR2 and TLR4 have been associated with the removal of Abeta, indicating that the innate immune system plays a key role in preventing the brain from Abeta deposits [118–120].

In addition, the adaptive immune system is obviously involved in CNS parenchyma clearance mechanisms. Naturally occurring antibodies directed against Abeta have been detected in the CSF and plasma of patients with AD and healthy control subjects. Some studies have shown reduced CSF levels of anti-Abeta antibodies in patients with AD compared with healthy control subjects [32, 121] and in individuals at increased risk for AD [122]. Another study reported that a subset of conformation-specific, cross-reactive antibodies that may protect against amyloidogenic toxic peptides are reduced in AD patients [123]. As a consequence of these findings, a number of phase II and III clinical trials are currently under way to test the effect of such autoantibodies in AD patients [124]. First results are promising: a recent study using carbon 11-labeled Pittsburgh Compound B ([11C]PiB) positron emission tomography (PET) has shown that passive immunization can reduce the level of brain amyloid *in vivo* after 18 months of antibody treatment [125].

5. Abeta Clearance in Lewy Body Disorders

5.1. Intraneuronal Mechanisms. To the best of our knowledge there is no study available that investigated intraneuronal mechanisms of Abeta clearance in LBDs. There is however indirect evidence that deficits of (intraneuronal) defence mechanisms against Abeta toxicity may exist, at least in demented LBDs patients. We recently showed that CSF levels of uric acid, an antioxidant detectable in neurons and associated with PD progression, were significantly lower in demented than in nondemented LBDs patients. In addition, these levels correlated positively with CSF Abeta42 levels, with highest correlation values in controls and lowest in demented LBDs patients [126]. In the light of the recent finding that CSF Abeta levels increase within hours after trauma and thus reflect a sufficient and fast response to neuronal stress [127], a weak correlation of CSF Abeta42 with uric acid may indicate deficits in this repair mechanism.

5.2. Extraneuronal Mechanisms (Mainly). Lowered CSF NEP activity levels have been found in demented LBDs patients, compared to nondemented LBDs patients and controls [128]. In addition, CSF NEP activity levels correlated positively with CSF Abeta42. These data argue for a role of NEP in the pathophysiology of cognitive decline in LBDs.

Also cystatin C seems to be relevantly involved in Abeta-associated cognitive decline in LBDs. Our group investigated CSF and serum levels in LBDs patients [129] and found lower CSF cystatin C levels in demented LBDs patients compared to PDND and controls. In additions, these levels correlated positively with age at onset of dementia but not with parameters associated with Parkinsonism. Notably, the correlation between CSF cystatin C and CSF Abeta42 levels was highly significant in nondemented individuals, but not significant in demented patients. This indicates that cystatin C-related Abeta transport from the neuron to the CSF is impaired in demented LBDs patients. This hypothesis is corroborated by genetic results [129]. The risk genotype of the *CST3* gene, *BB*, was detectable only in demented LBDs patients and was associated with low CSF cystatin C levels.

The role of the innate immune system in Abeta clearance in LBDs is not well understood although neuroinflammatory reactions are a common finding in LBDs and are considered to play a key role in the neurodegenerative process [63]. There is a tight association of microglia with degenerating LB-containing neurons [130]. Activated microglia is associated with Asyn-positive oligodendrocytes in MSA patients and in an animal model of this disease [131, 132]. Astrocytic abnormalities also occur [133, 134].

The adaptive immune system is also involved in Abeta-associated mechanisms in LBDs. Autoantibodies against Abeta were elevated in serum and CSF of demented LBDs patients, compared to controls and were even higher than in other forms of dementia such vascular dementia [72]. Still, many questions remain about the contribution of the immune system, such as microglia, macrophages, and T cells but also other immune cells, to clearance of misfolded proteins in the CNS.

6. Conclusion

Although many questions remain open, recent literature suggests that impairment of protein clearance is one of the key factors mediating the degeneration of vulnerable neuronal populations in LBDs. Both intra- and extraneuronal clearance mechanisms are impaired in LBDs. An improved understanding of such pathways can provide the basis for new developments in the biomarker era and, ultimately, contribute to the development of neuromodulatory or even causal treatment strategies.

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Research Article

Cognitive Profiles in Parkinson's Disease and Their Relation to Dementia: A Data-Driven Approach

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Parkinson's disease is characterized by a substantial cognitive heterogeneity, which is apparent in different profiles and levels of severity. To date, a distinct clinical profile for patients with a potential risk of developing dementia still has to be identified. We introduce a data-driven approach to detect different cognitive profiles and stages. Comprehensive neuropsychological data sets from a cohort of 121 Parkinson's disease patients with and without dementia were explored by a factor analysis to characterize different cognitive domains. Based on the factor scores that represent individual performance in each domain, hierarchical cluster analyses determined whether subgroups of Parkinson's disease patients show varying cognitive profiles. A six-factor solution accounting for 65.2% of total variance fitted best to our data and revealed high internal consistencies (Cronbach's alpha coefficients > 0.6). The cluster analyses suggested two independent patient clusters with different cognitive profiles. They differed only in severity of cognitive impairment and self-reported limitation of activities of daily living function but not in motor performance, disease duration, or dopaminergic medication. Based on a data-driven approach, divers cognitive profiles were identified, which separated early and more advanced stages of cognitive impairment in Parkinson's disease without dementia. Importantly, these profiles were independent of motor progression.

1. Introduction

Beyond the characteristic motor signs, a number of non-motor symptoms including cognitive aspects are gaining increasing attention in Parkinson's disease (PD). Recent work revealed a substantial heterogeneity of cognitive impairment, which is apparent in both different profiles and different levels of severity ranging from slight and early cognitive changes up to the diagnosis of PD dementia (PDD) [1]. With the demand for an early, individualized, and better therapeutic treatment, the focus is now to identify the patients with a potentially higher risk of dementia [2]. Already a few cognitive alterations may enhance the risk of PDD. However, it needs to be noted that exactly the same cognitive alterations can also be found in PD patients who did not develop dementia [3, 4]. On subtest level, some studies suggest that early executive dysfunction is predictive of the conversion to dementia [2, 3], while others argue for a crucial role of impaired visuospatial and language abilities [4]. Thus, a distinct clinical profile for patients with a potential risk of developing PDD still has to be identified [1].

Most authors define the stage of mild cognitive impairment in PD (PD-MCI) [5] and the involvement of different cognitive domains by a predominately theoretical [6, 7] rather than a data-driven, quantitative approach. Some data-driven studies on different subtypes of PD found a poor test performance in varying neuropsychological tasks, suggesting that these help to diagnose PDD [8, 9]. Recently, a cluster analysis on a small cohort of PD patients without dementia revealed differences in severity of cognitive deterioration

but not in cognitive phenotypes [10]. We here propose an approach to identify cognitive profiles based on performance differences within quantitatively determined domains using standardized factor scores. These standard values can be used to compare the mean performance of each single PD patient investigated here to the mean performance of the present, total PD cohort. Our study thus was designed by (i) a data-driven identification of different cognitive domains in a large cohort of both PD patients with and without dementia and (ii) a data-based subdivision of PD patients according to exactly these standardized domain scores to characterize subgroups with divers cognitive profiles and potentially different levels of cognitive impairment.

2. Materials and Methods

2.1. Patients. We investigated 121 patients with idiopathic PD according to the UK Brain Bank criteria [11] admitted to the outpatient clinic of the Department of Neurodegenerative Diseases University of Tuebingen and the Gertrudis Clinic Leun-Biskirchen Germany. All patients received their usual, optimized medication and were able to complete all cognitive tasks (Tables 1 and 2 provide all relevant details).

Inclusion criteria were: age $\geq$ 50 years, onset of dementia >2 years from PD diagnosis, adequate or corrected hearing/visual abilities, and German as first language. Exclusion criteria were: other neurological diseases affecting the central nervous system, prior surgery for PD, medication interfering with cognition (i.e., hypnotics or tranquilizers), or a minimental state examination [12] score < 18 (testing not feasible). Patients with identified gene mutations and those reporting more than 2 first or second degree relatives with a definitive diagnosis of PD [7] were excluded to avoid monogenetic subgroups in which cognition can be specifically altered [13]. Most patients identified their spouse as caregiver (72.7%); the others indicated an adult child (12.4%), other family members (8.3%), or nonfamily members (6.6%). The study was approved by the local ethical committee. All patients and caregivers gave written informed consent.

2.2. Neuropsychological Assessment. All examinations and expert evaluations were carried out within a week. Each patient underwent a comprehensive test battery according to the recommendations of the Movement Disorder Society (MDS) Task Force [7] comprising the following tests (see also supplemented Table 1 in Supplementary material available online in doi:10.1155/2012/910757): the Tower of London (TL-D, conceptualization) [14], the Trail Making Test parts A and B (TMT-A, TMT-B; psychomotor speed, set shifting) [15], the digit span (DS forward and backward, working memory capacity), and the figure test (FT, nonverbal memory, set maintenance) from the Nuernberger Alters Inventory a battery to assess mild to advanced cognitive impairments (NAI) [16], as well as 8 subtests, that is, word-list memory (WL), including the number of false positive words (WL-I), word-list recall (WL delay), word-list recognition (WL-R, all verbal memory), the Boston naming test (BNT, language), verbal fluency (VF, animal naming, executive function), as well as the copy task (praxis) and its delayed recall (praxis delay, both visuospatial abilities) from the German version of the Consortium to Establish a Registry on Alzheimer's Disease (CERAD) [15]. Further, we applied the logical memory tasks (LogI and LogII) of the Wechsler Memory Scale-Revised (WMS-R, verbal memory) [17], the object decision part (OD) of the Visual Object and Space Perception battery (VOSP, visuospatial abilities) [18], the Berlin Apraxia Test (BAXT, ideomotor apraxia) [19], and two computerized reaction time tasks (Alertness, Go-Nogo; Test of Attentional Performance, TAP) [20], the first providing a specific measure of the ability to respond to a critical stimulus following an auditory cue (phasic alertness). Analyses are based on standard norms (percentile rank scores) of healthy German control subjects as published in the manuals. Data are corrected either for age (NAI, WMS-R, VOSP) or for age and education (CERAD, TAP, TMT, TL-D).

Diagnostic criteria for dementia followed the recommendation of the MDS task force for probable PDD [7]. In detail, our criteria for PDD were (i) scores 1.5 SD (PR < 7) in at least one test below published group norms of healthy control subjects in at least 2 of the following cognitive domains: attention (as measured by Alertness, Go-Nogo), executive function (DS, TL-D, TMT-A, TMT-B, VF), visuospatial function (praxis, praxis delay, OD), memory (LogI, LogII, WL, WL-I, WL delay, WL-R, FT), or language ability (BNT), (ii) cognitive decline with insidious onset and slow progression reported by either the patients or their proxies, and (iii) impairment of nonmotor activities of daily living (ADL) as verified by a structured patient and/or caregiver interview on the perception of cognitively influenced ADL function in the domestic environment.

2.3. Motor Performance, Behavioral Disturbances, and ADL. Clinical assessment included the Hoehn and Yahr stage, the unified Parkinson disease rating scale part III (UPDRS-III) [21] for motor function and the neuropsychiatric inventory (NPI) for behavioral disturbances (e.g., hallucinations) [22]. The Parkinson's Disease Questionnaire (PDQ-39) [23] and the Beck's Depression Inventory (BDI) [24] provided self-rating scales of health related quality of life and mood. Further, we calculated age-corrected standard scores of the patients' ADL function using (i) a self-rating questionnaire (Nuernberger-Alters-Alltagsaktivitaeten-Skala, NAA) and (ii) its corresponding scale for proxies (Nuernberger-Alters-Beobachtungsskala, NAB) [16].

Full drug history includes the total daily dose of levodopa only and the total daily dose of all dopaminomimetics, which was calculated as levodopa equivalent daily dose (LEDD) according to published conversion rates (see legends of Tables 1 and 2, and [25–27]).

2.4. Data Analyses

2.4.1. Identification of Cognitive Domains: Exploratory Factor Analysis (EFA). First, we performed an EFA on all neuropsychological data to identify cognitive domains in the

Table 1: Demographic, clinical, and neuropsychological characteristics of the two PD groups (PDD patients included) as identified by the first hierarchical cluster analysis.

	Total group of PD and PDD	Cluster-I PD only*	Cluster-II PD and PDD*	*P* value
Number, (%)	121 (100.0)	50 (41.3)	71 (58.7)	
Male gender, *n* (%)	81 (66.9)	33 (66.0)	48 (67.6)	0.85
Age at evaluation, years	68.7 ± 6.9	66.1 ± 6.7	70.6 ± 6.4	<0.001
Neurological assessment				
Disease duration, years	6.6 ± 5.1	5.8 ± 4.7	7.1 ± 5.2	0.17
UPDRS-III motor score (on state)	28.3 ± 11.5	25.3 ± 11.7	30.5 ± 11.0	0.01
Hoehn and Yahr stage, *n* (%)				
1	12 (9.9)	6 (12.0)	6 (8.5)	0.06
1.5	5 (4.1)	3 (6.0)	2 (2.8)	
2	49 (40.5)	24 (48.0)	25 (35.2)	
2.5	32 (26.5)	14 (28.0)	18 (25.4)	
3	16 (13.2)	3 (6.0)	13 (18.3)	
4	7 (5.8)	0 (0)	7 (9.9)	
Medication, daily dose				
Levodopa dose (mg)	351.4 ± 304.7	330.3 ± 343.6	366.3 ± 275.6	0.55
Levodopa equivalent dose (mg)	573.8 ± 417.2	585.0 ± 470.2	566.0 ± 378.8	0.35
Antidepressants, *n* (%)	28 (23.1)	9 (18.0)	19 (26.8)	0.12
Neuroleptics, *n* (%)	14 (11.6)	1 (2.0)	13 (18.3)	0.11
PD patients with dementia, PDD	24 (19.8)	0 (0)	24 (33.8)	<0.001
MMSE (raw score)	26.6 ± 2.6	28.1 ± 1.5	25.5 ± 2.6	<0.001
Beck's Depression inventory	8.7 ± 5.7	7.1 ± 4.7	9.9 ± 6.0	0.009
Neuropsychiatric inventory	4.7 ± 7.3	3.5 ± 5.5	5.5 ± 8.2	0.22
Parkinson's disease Questionnaire-PDQ-39	5.4 ± 4.2	3.4 ± 3.1	6.8 ± 4.3	0.001
NAI: NAA-ADL inventory, patients' self-assessment	48.8 ± 32.4	67.2 ± 22.0	35.9 ± 32.4	<0.001
NAI: NAB-ADL inventory, caregivers' assessment	50.3 ± 31.0	67.1 ± 25.1	38.4 ± 29.4	<0.001
Factor scores	Standardized values of the total PD cohort (PD norms)	Mean group performance in relation to the standardized values, that is, below (−) versus above (+) the average of the total PD cohort		
Factor 1, frontal lobe function	0 ± 1	0.41 ± 0.71	−0.29 ± 1.07	0.005
Factor 2, word-list memory and recall	0 ± 1	0.56 ± 0.90	−0.39 ± 0.87	<0.001
Factor 3, attention	0 ± 1	−0.58 ± 0.87	0.37 ± 0.91	<0.001
Factor 4, logical memory	0 ± 1	−0.68 ± 1.06	0.48 ± 0.60	<0.001
Factor 5, praxis and visual perception	0 ± 1	−0.92 ± 0.90	0.56 ± 0.76	<0.001
Factor 6, fluency and naming ability	0 ± 1	0.62 ± 0.84	−0.44 ± 0.86	<0.001
Neuropsychological assessment	Mean group performance in relation to the standardized values provided by the test manuals, that is, below (−) and above (+) the average of healthy control subjects			
Factor 1:				
Trail Making Test, part B	49.8 ± 39.3	75.2 ± 29.8	32.0 ± 35.3	<0.001
Tower of London	39.0 ± 26.7	48.5 ± 24.0	32.3 ± 26.6	0.024
NAI: digit span	56.3 ± 31.4	70.3 ± 28.7	46.5 ± 29.6	<0.001
NAI: figure test	52.1 ± 27.2	62.6 ± 20.1	44.7 ± 29.1	0.006
Berlin Apraxia Test (raw score)	35.7 ± 5.5	38.7 ± 3.2	33.7 ± 5.9	<0.001

Table 1: Continued.

Neuropsychological assessment	Mean group performance in relation to the standardized values provided by the test manuals, that is, below (−) and above (+) the average of healthy control subjects			
Factor 2:				
CERAD: word-list memory	29.9 ± 27.3	48.5 ± 16.8	16.8 ± 20.3	<0.001
CERAD: word-list recall	36.9 ± 30.0	54.8 ± 29.1	24.2 ± 23.7	<0.001
CERAD: word-list recognition	40.7 ± 34.5	57.2 ± 30.3	20.0 ± 32.7	<0.001
CERAD: word-list intrusion	42.3 ± 33.7	53.7 ± 28.0	34.2 ± 34.0	0.005
Factor 3:				
TAP: phasic alertness	55.4 ± 29.2	44.2 ± 25.7	63.3 ± 29.1	0.001
TAP: Go-Nogo, median RT	40.5 ± 33.5	60.6 ± 29.1	26.4 ± 29.0	<0.001
Factor 4:				
WMS-R: logical memory I	24.4 ± 26.2	41.9 ± 26.9	12.0 ± 17.0	<0.001
WMS-R: logical memory II	25.9 ± 26.2	45.0 ± 26.1	12.5 ± 16.0	<0.001
Factor 5:				
CERAD: praxis	40.2 ± 35.7	63.6 ± 29.9	23.7 ± 29.8	<0.001
CERAD: praxis delay	35.6 ± 35.8	59.0 ± 34.8	19.2 ± 26.2	<0.001
VOSP: object decision	42.4 ± 30.1	56.4 ± 29.6	32.5 ± 26.5	0.001
Factor 6:				
CERAD: verbal fluency	30.0 ± 28.1	52.8 ± 27.7	24.2 ± 21.8	<0.001
CERAD: Boston naming test	46.9 ± 33.2	63.1 ± 28.1	35.4 ± 31.9	<0.001
Trail Making Test, part A	45.8 ± 35.4	70.2 ± 28.0	28.6 ± 29.6	<0.001

Data are given as mean ± SD; lower standard (that is, percentile rank) scores in neuropsychological tests indicate poorer performance except for the MMSE; UPDRS-III: Unified Parkinson's Disease Rating Scale part III; *P* values are corrected for age and UPDRS-III motor score; %: Percentage; PD: Parkinson's disease; PDD: Parkinson's disease with dementia; LEDD: levodopa equivalence daily dose according to the following conversion rates: 100 mg Levodopa equalling 125 mg Levodopa sustained release, 1 mg Pergolide, 1 mg Pramipexol, 5 mg Ropinirole, 5 mg Rotigotin, 10 mg Bromocriptine, 10 mg Apomorphine, 1/5 Entacapone, 1.5 mg Cabergoline. Additionally, 5% was added to the total Levodopa dose for every 5 mg of Selegiline or 1 mg of Rasagiline, up to a maximum of 10%; MMSE: Minimental State Examination; NAI: Nuernberger Alters Inventar; RT: reaction time; *Grouping of patients with PDD following the first hierarchical cluster analysis.

PD group. The factor matrix was optimized with oblique rotation, because factors were expected to be correlated [28]. Variables with a factor loading > 0.5 or < -0.5 were considered as core variables for a given factor [29]. The Kaiser's criterion (eigenvalue > 1) and the corresponding scree plot results were used to determine the number of factors to be retained. Internal consistency was verified by Cronbach's alpha (α) coefficient, which was required to be higher than $\alpha > 0.6$ for each factor to indicate a sufficient internal consistency structure [6].

2.4.2. Identification of Characteristic Profiles in the PD Cohort: Hierarchical Cluster Analysis. Based on the factor loadings from the EFA, individual factor scores were calculated by the Anderson-Rubin algorithm, which produces factor scores that are uncorrelated and standardized with a mean of zero and a standard deviation of 1. A patient with a factor score of 0 (zero) thus shows average performance compared to the total PD cohort; a positive score indicates performance above, a negative score below the average of the whole patient group investigated here.

With these individual factor scores, we performed two separate hierarchical cluster analyses to identify patient subgroups with different cognitive profiles (Ward's-method). The first analysis was conducted on the total group of PD patients ($n = 121$), the second, for validation purposes, on all PD patients except those with dementia ($n = 97$). As PDD patients can be expected to suffer from more severe cognitive, motor, and behavioral impairment, we evaluated this specific influence on our study results by excluding them from the second HCA. Student's *t*- (age, disease duration, UPDRS-III motor score) or χ^2-tests (gender, Hoehn & Yahr stage) were used for between-group comparisons. Analyses of covariance and Mantel-Haenszel statistics accounted for differences in demographics and disease severity. Because of the number of comparisons, the significance levels were set at $P = 0.01$ to optimize the trade-off between false positive protection (type 1) and sensitivity/power (type 2 error). All analyses were conducted using SPSS 17.0 (SPSS Inc, Chicago, III, USA).

3. Results

3.1. Patient Characteristics. Of the 121 patients, seventeen (14.0%) received L-dopa, 25 (20.7%) dopamine agonists, and one (0.8%) patient amantadine only. Both L-dopa and dopamine agonists were given to 78 (64.5%) patients, of whom 27 additionally received amantadine. Twenty-four patients (19.8%) of the total cohort had PDD; six of them were treated with cholinesterase inhibitors (see Table 1 for further details).

Table 2: Demographic, clinical, and neuropsychological characteristics of the two PD groups (PDD patients excluded) as identified by the second hierarchical cluster analysis.

	PDD only	Cluster-I PD only	Cluster-II PD only	*P* value
Number, (%)	24 (19.8)	43 (35.6)	54 (44.6)	
Male gender, *n* (%)	18 (75.0)	28 (65.1)	35 (64.8)	0.97
Age at evaluation, years	74.2 ± 5.9	65.7 ± 6.0	68.7 ± 6.5	0.02
Neurological assessment				
Disease duration, years	9.5 ± 5.6	5.6 ± 4.3	6.1 ± 4.9	0.66
UPDRS-III motor score (on state)	37.5 ± 11.3	25.3 ± 11.5	26.7 ± 9.6	0.52
Hoehn and Yahr stage, *n* (%)				
1	0 (0)	6 (14.0)	6 (11.1)	
1.5	0 (0)	3 (7.0)	2 (3.7)	
2	7 (29.2)	21 (48.8)	21 (38.9)	0.60
2.5	3 (12.5)	11 (25.6)	18 (33.3)	
3	8 (33.3)	2 (4.7)	6 (11.1)	
4	6 (25.0)	0 (0)	1 (1.9)	
Medication, daily dose				
Levodopa dose (mg)	457.2 ± 256.0	323.5 ± 352.6	326.6 ± 277.2	0.57
Levodopa equivalent dose (mg)	665.7 ± 407.8	554.7 ± 435.7	548.2 ± 408.3	0.82
Antidepressants, *n* (%)	7 (29.2)	8 (18.6)	13 (24.1)	0.14
Neuroleptics, *n* (%)	7 (29.2)	1 (2.3)	6 (11.1)	0.67
MMSE (raw score)	23.0 ± 2.7	28.1 ± 1.5	26.9 ± 1.5	0.003
Beck's Depression Inventory	11.6 ± 6.2	7.1 ± 4.8	8.8 ± 5.8	0.06
Neuropsychiatric inventory	9.5 ± 10.3	3.3 ± 5.1	3.3 ± 5.1	0.73
Parkinson's disease questionnaire-PDQ-39	10.4 ± 4.2	3.5 ± 3.1	4.9 ± 3.2	0.03
NAI: NAA-ADL inventory, patients' self-assessment	8.8 ± 16.9	67.6 ± 22.3	51.7 ± 28.9	0.002
NAI: NAB-ADL inventory, caregivers' assessment	11.5 ± 10.3	66.8 ± 24.6	54.4 ± 27.1	0.03
Factor scores	Mean group performance in relation to the standardized values (0 ± 1), that is, below (−) and above (+) the average of the total PD cohort			
Factor 1, frontal lobe function	−1.11 ± 0.86	0.32 ± 0.71	0.23 ± 0.92	0.64
Factor 2, word-list memory and recall	−0.76 ± 0.90	0.73 ± 0.84	−0.25 ± 0.77	<0.001
Factor 3, attention	0.61 ± 0.98	−0.56 ± 0.92	0.17 ± 0.85	<0.001
Factor 4, logical memory	0.51 ± 0.67	−0.73 ± 1.13	0.35 ± 0.62	<0.001
Factor 5, praxis and visual perception	0.92 ± 0.70	−0.67 ± 0.90	0.14 ± 0.79	<0.001
Factor 6, fluency and naming ability	−0.86 ± 0.81	0.69 ± 0.81	−0.17 ± 0.84	<0.001
Neuropsychological assessment	Mean group performance in relation to the standardized values provided by the test manuals, that is, below (−) and above (+) the average of healthy control subjects			
Factor 1:				
Trail Making Test, part B	5.3 ± 15.1	75.9 ± 30.2	48.9 ± 35.0	0.001
Tower of London	14.8 ± 22.0	47.7 ± 25.1	42.9 ± 23.8	0.46
NAI: digit span	39.0 ± 32.2	66.9 ± 29.5	55.6 ± 29.4	0.04
NAI: figure test	26.2 ± 29.4	62.4 ± 21.3	55.3 ± 23.0	0.12
Berlin Apraxia Test (raw score)	29.3 ± 6.6	38.6 ± 3.3	36.4 ± 3.9	0.005
Factor 2:				
CERAD: word-list memory	12.7 ± 21.7	51.2 ± 25.6	21.9 ± 19.4	<0.001
CERAD: word-list recall	18.0 ± 24.5	60.2 ± 27.4	26.7 ± 21.8	<0.001

Table 2: Continued.

Neuropsychological assessment	Mean group performance in relation to the standardized values provided by the test manuals, that is, below (−) and above (+) the average of healthy control subjects			
CERAD: word-list recognition	15.5 ± 24.0	62.8 ± 27.8	34.2 ± 33.3	<0.001
CERAD: word-list intrusion	20.8 ± 31.9	56.9 ± 29.0	40.1 ± 32.8	0.008
Factor 3:				
TAP: phasic alertness	68.7 ± 32.9	43.6 ± 27.1	59.0 ± 25.9	0.007
TAP: Go-Nogo, median RT	16.5 ± 28.1	60.0 ± 30.1	37.3 ± 30.4	<0.001
Factor 4:				
WMS-R: logical memory I	8.5 ± 16.5	43.2 ± 27.9	16.4 ± 18.8	<0.001
WMS-R: logical memory II	7.5 ± 12.2	47.3 ± 27.2	17.1 ± 17.2	<0.001
Factor 5:				
CERAD: praxis	13.1 ± 27.4	63.6 ± 31.3	33.7 ± 31.1	<0.001
CERAD: praxis delay	10.3 ± 21.5	58.9 ± 35.2	28.3 ± 30.5	<0.001
VOSP: object decision	19.9 ± 19.6	56.6 ± 29.2	41.1 ± 28.6	0.04
Factor 6:				
CERAD: verbal fluency	17.6 ± 20.8	55.4 ± 27.3	28.7 ± 22.3	<0.001
CERAD: Boston naming test	21.3 ± 28.8	64.4 ± 28.3	44.3 ± 30.8	0.001
Trail Making Test, part A	9.0 ± 16.8	71.8 ± 27.3	41.4 ± 30.6	<0.001

Data are given as mean ± SD; lower standard (i.e. percentile rank) scores in neuropsychological tests indicate poorer performance except for the MMSE; UPDRS-III: Unified Parkinson's Disease Rating Scale part III; P values are corrected for age; %: Percentage; PD: Parkinson's disease; PDD: Parkinson's disease with dementia; LEDD: Levodopa equivalence daily dose according to the following conversion rates: 100 mg Levodopa equalling 125 mg Levodopa sustained release, 1 mg Pergolide, 1 mg Pramipexol, 5 mg Ropinirole, 5 mg Rotigotin, 10 mg Bromocriptine, 10 mg Apomorphine, 1/5 Entacapone, 1.5 mg Cabergoline. Additionally, 5% was added to the total levodopa dose for every 5 mg of Selegiline or 1 mg of Rasagiline, up to a maximum of 10%; MMSE: Minimental State Examination; NAI: Nuernberger Alters Inventar; RT: reaction time.

3.2. Cognitive Domains. Table 3 shows the result of the EFA and the internal consistency analysis. The EFA was verified by the Bartlett's test of sphericity ($\chi^2 = 872.7$, $df = 171$, $P < 0.001$) and the Kaiser-Meyer-Olkin measures of sampling adequacy (MSA = 0.82). A six-factor solution accounting for 65.2% of total variance explained by the factors fits best to our data (see supplemented Table 1 for information on the rejected five-factor solution). The internal consistency structure of each factor was found to be at least moderately high ($0.67 \leq \alpha \leq 0.86$). Factor 1 consisted of five neuropsychological tasks explaining 33.5% of total variance ($\alpha = 0.67$). Like the TL-D [14], each test was mainly related to aspects of frontal lobe function. Factor 2 comprised four tasks on word-list memory and recall ($\alpha = 0.78$, 8.2% of variance explained). Both Factor 3 (6.8% of variance explained, $\alpha = -0.85$) and Factor 4 (5.9%, $\alpha = 0.86$) consisted of two neuropsychological tasks on attention and episodic memory (5.7% of variance explained), respectively. Factor 5 consisted of tasks on praxis and visual perception ($\alpha = 0.70$, 5.7% of variance explained). Factor 6 comprised three tests that are mainly used for assessing fluency and naming ability (5.2% of variance explained, $\alpha = 0.70$).

3.3. Cognitive Profiles in PD. Table 1 refers to the hierarchical cluster analysis on the total group of PD patients ($n = 121$, incl. 24 PDD), Table 2 to the subsequent hierarchical cluster analysis on all PD patients except those with dementia ($n = 97$). Both analyses revealed two different, independent clusters regarding the degree of cognitive impairment within the domains defined by the EFA (see Figure 1). Our first hierarchical cluster analysis assigned all 24 patients with PDD to Cluster-II, that is, to the group with poorer neuropsychological test performances (see Table 1, PD and PDD). In contrast, all Cluster-I patients showed less cognitive impairment and, crucially had no dementia ("PD only"). The second hierarchical cluster analysis, carried out for validation purposes, replicated the grouping in 92.8% of all PD patients without dementia ($n = 97$, Table 2). Most important, no patient initially assigned to the more severely impaired Cluster-II, was regrouped in Cluster-I.

Our approach of identifying characteristic profiles within the PD cohort (i.e., an identification of those patients with poorer individual performances in the specific tests of the corresponding EFA domain compared to all other PD patients investigated here) revealed a clear-cut division of the six cognitive domains into two subgroups (all P values < 0.005). PD patients with lower factor scores of Factor 3 (attention $P < 0.001$), Factor 4 (logical memory $P < 0.001$), and Factor 5 (praxis and visual perception, $P < 0.001$) were grouped in Cluster-I. In contrast, Cluster-II patients showed lower factor scores in neuropsychological tasks assigned to Factor 1 (frontal lobe function, $P < 0.005$), Factor 2 (word-list memory and recall, $P < 0.001$), and Factor 6 (fluency and naming ability, $P < 0.001$). The analysis without PDD patients revealed comparable results except for frontal lobe functions (Figure 1).

3.4. Subgroup Comparison of Clinical Parameters in Patients with "PD Only". To identify a cognitive profile in patients who had not developed dementia at the time of examination,

Table 3: Results of the exploratory factor analysis and the consistency analysis on the neuropsychological test results of all 121 patients indicating a six-factor model of cognition in PD.

Factor interpretation	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
	frontal lobe function	word-list memory and recall	attention	logical memory	praxis and visual perception	fluency and naming ability
Tower of London	0.62					
Trail Making Test, part B	0.64					
NAI: digit span	0.65					
NAI: figure test	0.69					
Berlin Apraxia Test (raw score)	0.66					
CERAD: word-list memory		0.77				
CERAD: word-list recall		0.84				
CERAD: word-list recognition		0.71				
CERAD: word-list intrusion		0.77				
TAP: phasic alertness			−0.80			
TAP: Go-Nogo, median RT			0.70			
WMS-R: logical memory I				0.88		
WMS-R: logical memory II				0.87		
CERAD: praxis					0.83	
CERAD: praxis delay					0.83	
VOSP: object decision					0.62	
CERAD: verbal fluency						0.84
CERAD: Boston naming test						0.77
Trail Making Test, part A						0.66
Variance explained (%)	33.51	8.15	6.78	5.90	5.70	5.19
Cronbach's alpha coefficient	0.67	0.78	−0.85	0.86	0.70	0.70

Analyses are based on standard norms (i.e percentile rank scores, PR: indicating the patient's relative position in the norm group with a range between 0 and 100) of healthy German control subjects as published in the manuals; data are corrected either for age (NAI, WMS-R, VOSP) or for age and education (CERAD, TAP, TMT, TL-D). Only for the BAXT raw data were used; CERAD: Consortium to Establish a Registry For Alzheimer's Disease, German version; WMS-R: Wechsler Memory Scale-Revised; NAI: Nuernberger Alters Inventory; VOSP: Visual Object and Space Perception battery; TAP: Test of Attentional Performance; RT: Reaction Time.

we compared the clinical parameters of the two Clusters without PDD (Table 2). Cluster-II tended to be older ($P < 0.02$). As age is a risk factor for PDD [30], all P values were corrected for it.

No differences were found for motor disability, disease duration, and psychiatric symptoms. Compared to published group norms that are standardized on healthy control subjects (not to the present factor scores), Cluster-II patients showed overall lower performances in most neuropsychological tests. However, comparison on subgroup level without PDD failed significance, for example, for most executive tasks (see Table 2), suggesting that particularly PDD patients had led to significant differences in the first hierarchical cluster analysis because of marked difficulties in this domain.

Regarding the impact of ADL dysfunction on PD, it is notable that members of Cluster-II (without PDD) rated themselves as more impaired ($P = 0.002$). These self-impressions tended to be confirmed by their caregivers as well as by their reduced quality of life reports (PDQ-39, $P = 0.03$).

4. Discussion

We introduce a data-driven approach to identify different profiles and stages of cognitive impairment in PD. First, we determined cognitive domains based on a comprehensive neuropsychological test battery, and second we identified subgroups that differ with respect to their standardized individual performance in these domains. Whereas the first part has already been addressed to some extent [8–10, 31], the second part provides a first attempt to identify PD patients with a potentially higher PDD risk using PD related rather than healthy control norms. This method allows the differentiation within the group of those PD patients, who show a severe impairment in almost all cognitive tasks and who thus might have a potential risk of developing dementia. While the standard procedure (i.e., using healthy control standard norms) turned out insufficient to differentiate within this overall severely impaired patient group, our approach of using factor scores revealed varying cognitive profiles that differ

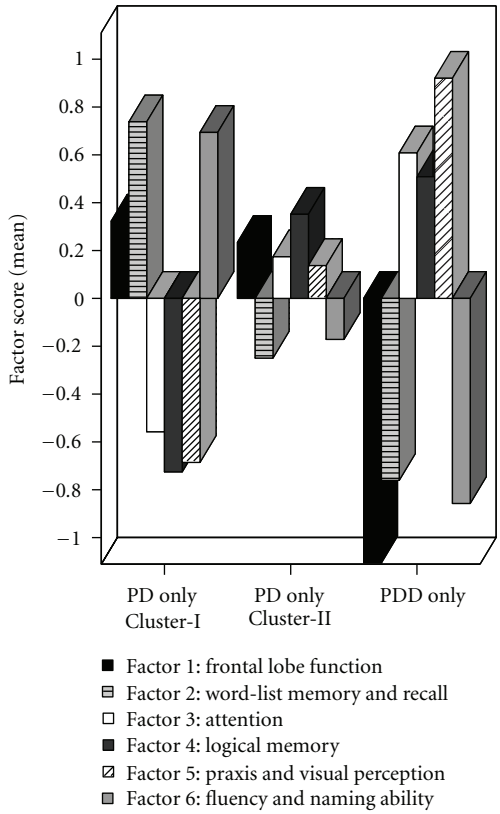

Figure 1: Mean group performance (mean factor scores) of PD patients without dementia clustered into two distinct groups (PD only, $n = 43$, Cluster-I versus PD only, $n = 54$, Cluster-II) as well as of the group of PD patients with dementia (PDD only, $n = 24$).

with respect to both, severity and most affected cognitive functions.

4.1. Factor Analysis. Focusing on both, internal consistency structure and adequate factor correlations, we found that a six-factor solution fitted best to our data. Our results are in accordance with the theoretical assumption of the MDS Task Force [7] and recent work on the factorial structure of cognition in PD [8, 9] that differentiate between executive, long term memory, and retrieval ability as well as language and visual function. In contrast to others [8], we identified six instead of three domains. The rotation algorithm, the greater sample size, and our larger number of neuropsychological tasks may account for this difference. It is well known that the PD phenotype can vary largely. Thus, our more homogenous cohort without potential genetic variants of PD or other confounding factors could also have influenced the results.

In line with previous observations [8], verbal fluency performance was more closely related to psychomotor speed and language tasks than to other frontal lobe assessments (please compare Factor 1). Thus, it may be concluded that the verbal fluency task addresses a different cognitive aspect than the other frontal lobe instruments used here. This interpretation is further supported by the finding that semantic fluency impairment reflects structural grey matter changes in regions that are known to be involved in language networks [32]. Moreover, this task has been found to correlate with disease severity and motor assessment, which may explain an association to psychomotor speed performance in patients with PD [33, 34].

Another interesting finding is that the BAXT, an inventory for the assessment of ideomotor apraxia, was closely related to other frontal lobe tests in our PD cohort. Actually, patients with frontal lobe dysfunction may also show signs of ideomotor apraxia [35]. PD patients are known to suffer from an action-sequence planning deficit [36] that can at least partly explain the clinical signs of apraxia in PD. Recently, a strong association of finger dexterity with praxis function but not with the Parkinson's symptoms has been described [37]. This finding indicates that impaired finger dexterity in PD probably has an apraxic component, which is clinically more apparent in later disease stages. It thus seems that ideomotor deficits may rather contribute to an incorrect selection of action sequences than to a dysfunction in action semantics as suggested for patients with parietal lobe involvement. Indeed, symptoms of apraxia have been reported repeatedly in PD, although they are not as frequent and evident as in other neurodegenerative disorders [38, 39]. It was not the scope of this study to clarify which mechanism causes impairment in different cognitive domains or even in ideomotor apraxia. Nevertheless, our results suggest that apraxia could be a variant of the dysexecutive syndrome in PD. It might be interesting to address this hypothesis in future research.

The two logical memory subtests of the WMS-R did not reveal high loadings on Factor 2 (list learning and recall). In contrast to the CERAD memory tests, the verbal recall of the logical memory tasks may be more demanding with respect to working memory or metamemory, because it requires memory self-monitoring [40]. Thus, one may argue that the corresponding test performance is more dependent on frontal lobe functions [41]. Additionally, impaired logical memory abilities are known to be related to a decline in dopaminergic activity in the basal ganglia in both, healthy persons and PD patients [42–45]. This finding also supports the assumption that logical memory assessments address aspects of frontal lobe and working memory function and additionally may mirror dopaminergic dysfunction.

4.2. Cluster Analysis

4.2.1. Cognitive Profile of Clusters. Based on the individual factor scores, both analyses revealed two independent groups with a subdivided, domain structure regarding the most affected cognitive functions. Crucially, the groups clustered even more closely without PDD patients (see Figure 1), arguing for a validation of the present grouping by our second analysis.

Cluster-II patients without dementia reported more ADL dysfunctions beyond their objectively more advanced cognitive decline. Interestingly, the CamPaIGN study showed that the PDD diagnosis at followup was linked to poorer semantic fluency at baseline and reduced visuoconstruction

[4]. Others found that cognitive progression is strongly associated with memory and visuoconstructive skills [46, 47]. Likewise, all these functions were still more impaired in our overall more affected Cluster-II, even without PDD.

Currently, we can only speculate that Cluster-I and Cluster-II patients suffer from different pathologies. Dopaminergic loss modulates cognition (e.g., attention and psychomotor speed) especially in the early stages [48]. Interestingly, Cluster-I patients showed reduced attention as indicated by the corresponding factor scores. In contrast, advanced PD affects a broad range of cognitive abilities [49] as confirmed by the present Cluster-II. Since this cannot be fully attributed to dopaminergic loss [50], the extent of Lewy body pathology [51], an imbalance of other neurotransmitter systems, a primarily cholinergic deficit [52, 53], or Alzheimer's histopathology [54] should be considered.

4.2.2. Implications for the Characterization of Cognitive Impairment in PD. At first sight there seems to be a contradiction between the cognitive profiles revealed by the two different analyses, that is, by either standard or factor scores. The comparison to the commonly used standard scores showed, as would be expected, that the Cluster-II patients are more impaired in almost all neuropsychological tests. The factor score analysis, however, identified a different cognitive profile in this formerly homogenous patient group (see Figure 1). Crucially, one needs to keep in mind that both, the standardized factor score and the standard score refer to the patients' individual test performance. The major difference is that the results are compared to different standardized group norms. The factor score represents the performance of one individual person in relation to the average performance of the total PD cohort. In contrast, the standard score specifies the individual test performance by normative data from healthy controls comparable with respect to age and education. Most important for the present study is that only the combination of these two sources revealed that patients of Cluster-I are predominately affected in attention performance, visual spatial abilities, and logical memory but not in the other cognitive domains. In contrast, patients of Cluster-II suffer additionally to the impairment of those of Cluster-I from a more extensive impairment in memory, frontal lobe function, fluency, and naming ability. Our alternative approach of analyzing the data driven standardized factor scores (instead of standardized percentile rank scores) offers the opportunity for a more precise differentiation within the PD group. Actually, the presence of two cognitive profiles within the group of PD patients without dementia could only be detected by the use of factor scores and not by the commonly applied standard scores.

At present, the PD-MCI concept [7, 55] is defined theoretically by the severity of dysfunction in one or more cognitive domains. However, its predictive value has not yet been proven [56]. One main difficulty is the heterogeneity of affected cognitive domains and severity of cognitive dysfunction in PD which is supported by many previous [1] as well as our present data. To date, it remains open which of the various cut-off values are most predictive of PDD and which neuropsychological tasks might be the most promising to identify PD patients at risk for dementia according to the MCI concept.

Following our preliminary results of a clinical sample, we argue that a global neuropsychological (domain) score based on standardized assessments and compared to population based PD norms (e.g., factor scores) may help reflect the level of cognitive impairment and its progression more appropriately than various or even single cut-off scores from healthy control subjects. Such PD norms may offer the possibility to specify for each single PD patient whether the deficits occur to a greater or lesser extent compared to other PD patients, resulting in a more sensitive characterization of both kind and severity of cognitive impairment. Such PD norms should be derived from representative PD samples that undergo a well-defined, standardized neuropsychological test battery that is widely used and accepted, for example, following MDS Task Force recommendations [7].

5. Limitations

It needs to be considered that the generalizability of our results is limited by the small sample size. Still, although the cohort is not population based, our PDD patients present the well-known phenotype, that is, they were older and had a longer disease duration (please see [2, 57]).

Further, we are aware of the methodological limitations of explorative factor analyses. Nevertheless, our data driven approach provides a useful alternative to generate even more specific hypotheses on the resulting factor structure, which have to be verified by future research using, for example, confirmatory models. Such studies may offer a promising perspective to evaluate PD patients' cognitive progression or conversion to dementia over time.

6. Conclusions

Our data-driven approach suggests at least two different subtypes of cognitive impairment in PD, which are rather independent of motor function, disease duration, and PD medication but do have an impact on activities of daily living. Moreover, our data driven approach confirms the cognitive domains suggested by the consensus guidelines.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Clinical Study

Elevated Angiopoietin-1 Serum Levels in Patients with Alzheimer's Disease

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Background. Alzheimer's disease (AD) is the most common cause of dementia in the elderly. AD is characterized by the accumulation of amyloid plaques and neurofibrillary tangles and by massive neuronal loss in the brain. There is epidemiologic and pathologic evidence that AD is associated with vascular risk factors and vascular diseases, contributing to cerebral hypoperfusion with consecutive stimulation of angiogenesis and upregulation of proangiogenic factors such as Angiopoietin-1 (Ang-1). *Methods*. In the present study, we measured Ang-1 serum levels in 42 patients with AD, 20 patients with mild cognitive impairment (MCI), and in 40 healthy elderly controls by ELISA. *Results*. We found significantly increased Ang-1 serum levels in patients with AD compared to control subjects ($P = 0.003$). There was no significant difference between MCI patients and healthy controls ($P = 0.553$) or between AD and MCI patients ($P = 0.054$). The degree of cognitive impairment as measured by the mini-mental status examination (MMSE) score was significantly correlated with the Ang-1 serum levels in all patients and healthy controls. *Conclusions*. We found significantly increased Ang-1 serum levels in AD patients. We could also show an association between Ang-1 serum levels and the cognitive status in all patients and healthy controls. Thus, serum Ang-1 could be a potential candidate for a biomarker panel for AD diagnosis.

1. Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease and the most common cause of dementia in the elderly [1]. Epidemiological studies have shown that risk factors for vascular diseases, including hypertension, diabetes, hypercholesterolaemia, hyperhomocysteinemia, and the apolipoprotein-4 genotype, are also important risk factors for AD, which indicate that their pathogenic mechanisms are connected [2]. AD patients also have more severe atherosclerosis in large cerebral arteries at the base of the brain (circle of Willis) than age-matched controls without AD [3]. Consecutively, cerebral blood supply is reduced in AD patients by atherosclerosis-induced vascular narrowing [4]. Reduced cerebral blood supply leads to cerebral hypoperfusion which is besides chronic inflammation one of the major clinical features in AD and could also play a critical role in its pathogenesis [5]. Cerebral hypoperfusion and consecutive hypoxia stimulate vascular activation and angiogenesis [6–8] and lead to the increase of adhesion molecules, cytokines and chemokines, such as Angiopoietin-1 (Ang-1) and vascular endothelial growth factor (VEGF). In addition, hypoxia may facilitate the pathogenesis of AD through a large number of cellular events leading to degenerative changes such as increasing amyloid beta (Aβ) generation, stimulating the hyperphosphorylation of tau, and impairing blood-brain barrier function [9–14].

Several growth factors or their lack have been implicated in the pathogenesis of AD. Angiopoietins are a family of growth factors specific for the vascular endothelium

[15]. The specificity of the angiopoietins for the vascular endothelium results from the restricted distribution of the angiopoietin receptors, Tie1, and Tie2, to these cells. The four known angiopoietins all bind to Tie2, but it is still unclear as to whether they utilize the closely related receptor Tie1 [16]. Ang-1 is a 70 kDa glycoprotein which contains 498 amino acids, including an N-terminal secretory signal sequence. Two regions within the coding sequence display homology to myosin and the C-terminus of fibrinogen, respectively [15]. In the adult, Ang-1 is expressed at a low level in a wide range of tissues, acting as a maturation and stabilizing signal for mature vasculature [17]. Besides, Ang-1 seems to be important for the development of the vasculature [18]. To the best of our knowledge, there are no data in the literature describing a statistically significant difference between Ang-1 serum levels of AD patients, MCI patients, and healthy elderly controls.

The current study aimed to examine Ang-1 serum levels in AD patients, MCI patients, and healthy elderly controls and to examine the association with the degree of cognitive impairment as measured by the mini-mental state examination (MMSE).

2. Materials and Methods

2.1. Subjects. A total of 42 patients with AD, 20 patients with MCI and 40 healthy elderly controls, were included in the study. Baseline characteristics and demographic parameters are displayed in Table 1. AD and MCI patients were outpatients from our Memory Clinic of the Department of Psychiatry and Psychotherapy at the University Hospital of Tuebingen . Patients with AD fulfilled the criteria of ICD-10, DSM-IV, and the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) for probable AD [19]. Patients with MCI fulfilled the criteria of Petersen et al. [20]. The clinical severity of cognitive impairment was assessed by the MMSE [21]. The ethics committee of the University of Tuebingen approved the study and written informed consent that was obtained from each participant.

2.2. Blood Sampling. Peripheral venous blood was sampled into serum tubes between 08:00 and 09:00 hours (fasting state) in order to take in account a possible circadian rhythm. Tubes were immediately immersed in melting ice. To minimize the source of platelets, serum was centrifuged within 30 min after sampling and stored at −20°C until further analysis.

2.3. Measurement of Ang-1 Serum Concentration. Serum levels of Ang-1 were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's (R & D Systems, Wiesbaden, Germany) instructions.

2.4. Data Analysis. All statistical analyses were carried out using the statistical analysis software package SPSS 19 (SPSS, Munich, Germany). For comparisons of Ang-1 serum levels between subject groups (patients with AD, MCI, and healthy controls), we calculated a univariate ANOVA. The data are presented as mean ± S.D. Significance for the results was set at $P < 0.05$. We conducted a bivariate correlation analysis (Pearson correlation) between age, MMSE scores, and Ang-1 serum levels. The two-tailed t-test was used to assess differences between two groups in case of normal distribution. The Mann-Whitney U-test was used to assess differences between two groups in case of nonnormal distribution. The chi-square test was used to assess differences in gender between two groups.

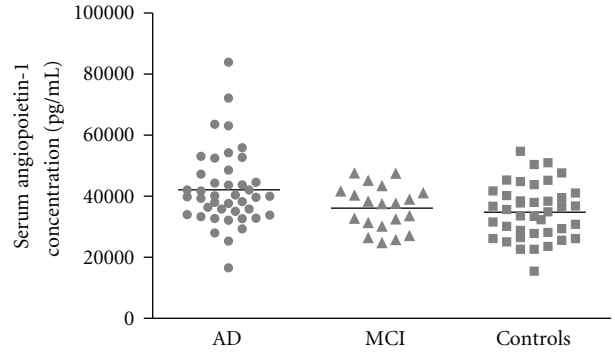

Figure 1: Angiopoietin-1 (Ang-1) serum levels (ng/mL) in Alzheimer's disease (AD) patients, mild cognitive impairment (MCI) patients, and healthy elderly controls. AD patients showed significantly higher Ang-1 serum levels compared with healthy controls ($P = 0.003$). There was no significant difference between MCI patients and healthy controls ($P = 0.553$) nor between AD and MCI patients ($P = 0.054$).

3. Results

Accounting for the age difference between controls and AD patients, we included age as a covariate for the ANOVA. We found a difference in Ang-1 serum levels between the three different groups ($P = 0.003$). Pairwise comparisons with an independent t-test revealed a significant difference between AD patients and controls (AD versus healthy controls [mean ± SD] 42.1 ± 12.6 versus 34.8 ± 8.9 ng/mL; Figure 1). There was no significant difference between MCI patients and healthy controls ($t(58)$-0.596; $P = 0.553$) nor between AD and MCI patients ($t(60) = 1.969$; $P = 0.054$).

There is a significant positive correlation between the MMSE score (as a measure for cognitive status) and Ang-1 serum levels seen in all patients and healthy controls ($n = 102$) ($r = 0.341$; $P < 0.001$; Figure 2). There was no significant correlation between Ang-1 serum levels and MMSE score in AD patients ($r = 0.23$; $P = 0.15$).

4. Discussion

The major findings of the present study are as follows. (1) Ang-1 serum levels are significantly higher in AD patients compared to healthy controls. (2) Taking into account the confounding effect of altered Ang-1 serum levels and MMSE

Table 1: Patients' demographic and clinical details.

Characteristics	All ($n = 102$)	AD ($n = 42$)	MCI ($n = 20$)	Control ($n = 40$)	P value (AD versus controls)	P value (AD versus MCI)
Age (years)	69.9 ± 9.4	73.0 ± 8.0	67 ± 10.2	65.8 ± 8.8	0.001°	0.040
Sex (no. [%])					0.334*	0.597
Females	61 (59.8)	24 (57.1)	10 (50)	27 (67.5)		
Males	41 (40.2)	18 (42.9)	10 (50)	13 (32.5)		
Mini-mental state examination score (MMSE)	24.7 ± 5.6	19.2 ± 4.5	27.3 ± 1.9	29.2 ± 0.7	<0.001	<0.001

°Mann-Whitney U-test, *Chi-square test.

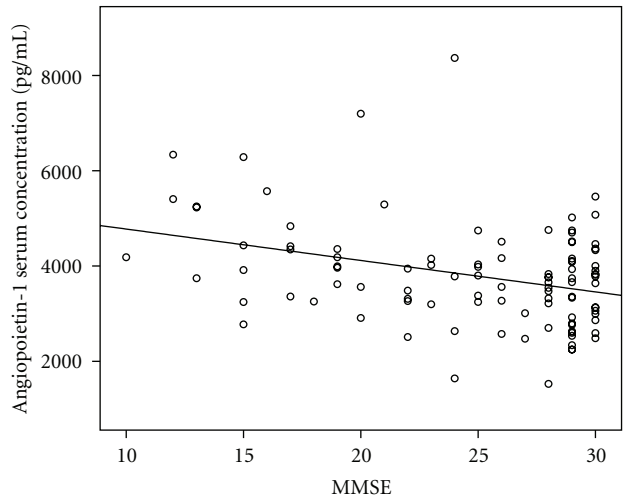

Figure 2: In all patients and healthy controls ($n = 102$) there is a significant correlation between the MMSE score (as a measure for cognitive status) and Ang-1 serum levels ($r = 0.341$; $P < 0.001$).

scores in AD patients, Ang-1 serum levels are significantly inversely correlated with MMSE as a measure for cognitive status in the whole study population. This indicates that a lower degree of cognitive functioning is associated with higher Ang1-serum levels.

Hypoperfusion of the brain caused by atherosclerotic changes of the vessels is assumed to play an important role in the pathogenesis of AD [5]. This hypoperfusion leads to hypoxia-induced angiogenesis via upregulation of hypoxia-inducible genes of Ang-1 and VEGF [8]. Together with VEGF, Ang-1 is capable of augmenting angiogenesis [22]. Coexpression of Angiopoietin-1 and VEGF prevents leakiness associated with VEGF alone [23]. Ang-1 acts as an antipermeability factor which is one of the most important biological roles of the angiopoietins. Vascular permeability is a fundamental component of the inflammatory response and therefore, Ang-1 also has an important anti-inflammatory role [24]. The elevated Ang-1 serum levels in patients with AD could be interpreted as a result of this hypoxia-induced angiogenesis. This is consistent with the findings that cerebral ischemia resulted in the induction of both Ang-1 and Ang-2 genes [25]. In the ischemic brain, expression of Ang-1 and VEGF is temporally and spatially correlated with neovascularization [26]. Another group was also able to show that transgenic overexpression of Ang-1 in the skin of mice produces larger, more numerous, and more highly branched vessels [27].

Angiogenesis is a complex process and consists of several discrete steps beginning with endothelial activation. Under normal conditions, endothelial activation is reversible and self-limiting. In AD, there is a continuous vascular activation induced by hypoperfusion, and factors and processes associated with angiogenesis can be found in the brain [28]. However, there is no evidence for increased vascularity in AD. On the contrary, there are several studies showing decreased microvascular density in the AD brain [29, 30]. One possible explanation could be that in response to a persistent stimulus such as cerebral hypoperfusion brain endothelial cells become activated and acquire an "activated angiogenic phenotype" [31]. No new vessels are formed which is the reason why there is no feedback signal to shut off vascular activation. The endothelial cells become irreversibly activated, and the products of the dysfunctional endothelium could injure or kill neurons [31].

Besides angiogenesis, inflammation plays another important role in the pathogenesis of AD [1]. Nevertheless, it is still not fully clear how and when inflammation arises in the course of AD, and the link between vascular inflammation, neuronal dysfunction, and death has not been clearly defined [32]. At the molecular level, inflammatory mediators are most highly expressed around Aβ deposits and neurofibrillary tangles in the brain from AD patients. There is evidence for inflammatory toxicity in the AD brain. For example, complement fixation and lysis of neurites can be demonstrated ultrastructurally [32, 33]. Several studies strongly suggested that conventional anti-inflammatory drugs may delay the onset or slow the progression of AD [32]. Under inflammatory conditions, there is also a pathological increase in vascular leakage, mediated, for example, by VEGF [34, 35]. In this context, Ang-1 seems to counteract VEGF-induced inflammation and vascular leakage in endothelial cells while having an additive effect on vessel formation [23]. Thus, the increase of Ang-1 serum levels in AD patients as demonstrated in the present study could be interpreted as an attempt of the human organism to encounter vascular inflammation and leakage seen in AD.

In conclusion, we found significantly increased Ang-1 serum levels in AD patients. We could also show an

association between Ang-1 serum levels and the cognitive status in all patients and healthy controls. Thus, serum Ang-1 could be a potential candidate for a biomarker panel for AD diagnosis.

Authors' Contribution

B. Schreitmüller conceived the study, drafted the paper, and took lead on design and coordination of the paper. N. Köhler, T. Leyhe and C. Laske provided considerable aid in conceiving this study, drafting the paper, and providing edits. B. Schreitmüller carried out all statistical analyses. E. Stransky performed ELISA measurements and provided edits to the paper.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Review Article

α-Synuclein as CSF and Blood Biomarker of Dementia with Lewy Bodies

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Dementia with Lewy bodies (DLB) is a common subtype of dementia in the elderly. DLB is neuropathologically characterized by the presence of Lewy bodies and Lewy neurites, both of which are composed of α-synuclein. Although α-synuclein was initially considered to be an exclusively intracellular protein, it has been found to be secreted into biological fluids. α-Synuclein in biological fluids such as cerebrospinal fluid (CSF) and blood has been discussed as a potential biomarker of DLB and α-synuclein-related disorders, because α-synuclein is characteristically accumulated in the brain of patients with these disorders. The α-synuclein level in CSF has been examined by several investigators, and the majority of studies have shown a reduction in CSF α-synuclein level in DLB and α-synuclein-related disorders. Discrepant findings of studies of plasma α-synuclein level in patients with DLB have been reported. Because the level of α-synuclein stored in red blood cells is considerably high, blood contamination and haemolysis during sample collection and processing should be considered as a confounding factor for quantification of α-synuclein. Here, the recent progress in the studies of α-synuclein as a biomarker of DLB and their potential clinical applications are reviewed.

1. Introduction

Dementia with Lewy bodies (DLB) is a common subtype of dementia and is reported to be the second most common neurodegenerative dementia after Alzheimer's disease (AD) in the elderly in several studies [1–3]. DLB is a progressive cognitive disorder characterized by fluctuating cognitive impairment, visual hallucination, and parkinsonism [4]. Diagnosis of DLB in patients with such characteristic clinical features would not be difficult by taking medical history and careful neurological examinations. However, it could be laborious to make an accurate diagnosis of DLB when patients have a substantial degree of concomitant AD pathology, which affects the clinical symptoms with lower rates of visual hallucinations and parkinsonism [5, 6]. Accurate clinical diagnosis of DLB is important because patients may benefit from cholinesterase inhibitors, which improve cognitive function and neuropsychiatric symptoms of DLB [7]. Furthermore, it should be noted that DLB patients are particularly sensitive to neuroleptic drugs [4, 8]. Recent intensive research has given hope for disease-modifying therapeutics for DLB to become a reality. The evaluation of such therapies largely depends on reliable diagnostic and prognostic biomarkers for early detection and monitoring of the stage of DLB. Candidates of such biomarkers are diverse; clinical biomarkers detected on the basis of olfactory function [9], myocardial ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy [10], neuroimaging biomarkers detected by magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) [11], positron emission tomography (PET), and biochemical biomarkers. In this paper, fluid biomarkers, particularly α-synuclein in cerebrospinal fluid (CSF) and blood in patients with DLB and α-synuclein-related disorders were extensively reviewed.

2. Lewy Bodies and α-Synuclein

A century ago, in 1912, Lewy originally described neuronal inclusions in the brain of patients with Parkinson's disease (PD). Seven years later, a Russian neuropathologist,

Tretiakoff, named the inclusions as "corps de Lewy (Lewy bodies)" [12]. Since the discovery, Lewy bodies have been considered as intracytoplasmic, spherical, and eosinophilic neuronal inclusions in the substantia nigra of PD patients. In 1976, Kosaka et al. reported the detection of Lewy bodies in the cerebral cortex of an elderly patient with dementia [13]. After his detailed description of cortical Lewy bodies [14], many similar cases were subsequently reported. Because the characteristic pathological features of DLB and PD are Lewy bodies and Lewy neurites, these disorders are considered to belong to a continuum disorder, and the generic term Lewy body disease (LBD) was proposed [4, 15].

In 1997, Spillantini et al. found that α-synuclein is the main component of Lewy bodies in the brains of PD and DLB patients [16]. Only two months before this report, a mutation was identified in *SNCA*, which encodes α-synuclein in families with autosomal dominant PD [17]. A different *SNCA* mutation was subsequently reported in a family with DLB [18]. In addition, *SNCA* multiplication by duplication/triplication has been identified in a large family with phenotypes ranging from DLB to PD [19]. These neuropathological and genetic findings suggest that α-synuclein is essentially implicated in the pathogenesis of LBD including DLB and PD. Recent studies have suggested that the Lewy body pathology propagates throughout the brain via neuron-to-neuron transmission of α-synuclein aggregates [20]. It has been demonstrated that glial cytoplasmic inclusions, which are unique pathological inclusions found in brains of patients with multiple system atrophy (MSA), are also composed of α-synuclein [21], suggesting an unexpected link between MSA and LBD. These disorders with pathological accumulation of α-synuclein in brains are termed as α-synucleinopathies [22].

α-Synuclein is a 140-residue ubiquitous protein and is highly expressed in neuronal presynaptic terminals under physiological conditions. Although its physiological functions remain unclear, α-synuclein is implicated in synaptic vesicle trafficking particularly in the regulation of synaptic vesicle release [23] and stabilization of SNARE complexes [24]. Deposited α-synuclein in the brain with synucleinopathies is aberrantly phosphorylated at Ser129 [25]. It has been demonstrated that the C-terminal truncated form of α-synuclein promotes the aggregation of α-synuclein [26]. Because α-synuclein has no signal sequence, it was initially considered to be an exclusively intracellular protein. However, it has been shown that α-synuclein is secreted into biological fluids [27, 28].

3. α-Synuclein in CSF

A year after Spillantini et al. reported that α-synuclein is the main component of Lewy bodies, Jakowec et al. examined whether α-synuclein is detectable in CSF [29]. They examined the expression of α-synuclein in CSF from PD patients and control subjects by Western blot analysis using an anti-α-synuclein specific antibody, but failed to detect α-synuclein in CSF [29]. Borghi et al. detected α-synuclein by a method combining immunoprecipitation with immunoblot analysis using two different anti-α-synuclein antibodies [30]. In their study, however, the intensity of the bands immunoreactive to the anti-α-synuclein antibodies showed no significant difference between patients with PD and control subjects [30]. El-Agnaf et al. also confirmed that α-synuclein is detectable in CSF by a similar methodology [27].

Subsequently, Tokuda et al. demonstrated using a new system for enzyme-linked immunosorbent assay (ELISA) that PD patients showed significantly lower α-synuclein levels in CSF than the control groups [31]. Since then, several studies to determine the total α-synuclein level in CSF have been carried out by ELISA or bead-based flow cytometric assay using various combinations of anti-α-synuclein antibodies (Tables 1 and 2). A significantly low level of α-synuclein in CSF from patients with LBD (PD and/or DLB) has been shown by independent studies [28, 31–38]. Our study revealed that total CSF α-synuclein level shows a significant positive correlation with the CSF Aβ42 level in DLB patients [32]. It has been suggested that a low CSF Aβ42 level may be related to amyloid-related pathology in the brains of DLB patients [39]. In addition, experimental studies have suggested that the Aβ42 species strongly enhances the accumulation of α-synuclein [40]. In the CSF from LBD patients, the level of Aβ42 shows a positive correlation with the activity of neprilysin, an enzyme that degrades Aβ [41]. Taken together, the reduction in α-synuclein and Aβ42 levels in CSF suggests the extent of Lewy body pathology and the co-occurrence of amyloid pathology, respectively, in the brain of DLB patients. Interestingly, patients with *SNCA* duplication who showed abundant Lewy body pathology in the brain [42] also show low levels of CSF α-synuclein [32]. Because the predominant source of α-synuclein in CSF is considered to be the central nervous system [43], the decrease in the level of CSF α-synuclein in DLB may reflect a dysfunction in the metabolism or clearance of α-synuclein in the brain, similarly to AD patients with Aβ accumulation in the brain showing a decrease in the level of Aβ42 in CSF.

In contrast, comparable levels of total α-synuclein in CSF between patients with LBD and control subjects have also been reported [45–50]. This discrepancy is probably not due to the misdiagnosis of LBD, because different studies using CSF samples derived from autopsy-confirmed patients showed significantly decreased or comparable α-synuclein levels in LBD patients [34, 50]. In addition to methodological differences in the quantification of α-synuclein, blood contamination of CSF during lumbar puncture should be taken into account when considering the discrepant results. The level of α-synuclein in blood, particularly that stored in red blood cells, is much higher than that in CSF [33, 53]. Haemolysis in the course of sample collection and processing should be considered as a confounding factor for quantification of α-synuclein level in CSF and blood. Other factors such as level fluctuations over time and drug treatment may have less effect on the level of α-synuclein in CSF. Although the level of Aβ in CSF fluctuates over time [54], the level of α-synuclein in CSF does not significantly change [52]. It is also reported that drugs such as L-dopa and dopamine agonists do not affect the level of α-synuclein in CSF [34, 35].

Table 1: Studies on quantification of α-synuclein level in CSF of patients with DLB and other synucleinopathies.

Level of total α-synuclein	Study	Blood contamination was considered*	Controls		Synucleinopathies				Results
			Healthy controls	Neurological controls	AD	Lewy body diseases			
						DLB	PD	MSA	
↓	Tokuda et al. [31]	No	9	29	—	—	33	—	PD patients showed significantly lower α-syn level than the controls ($P < 0.0001$). The level of α-syn decreased significantly with age ($P = 0.0076$) and correlated to inversely assigned Hoehn and Yahr stage ($P < 0.0001$).
↓	Mollenhauer et al. [28]	No	—	13	13	38	8	—	The level of α-syn in DLB and PD patients were lower than AD patients and controls ($P = 0.025$).
↓	Kasuga et al. [32]	No	—	21	31	34	—	—	The level of α-syn in DLB patients was significantly lower than those in patients with AD ($P < 0.05$) and other dementias ($P < 0.01$). In DLB patients, reduced α-syn level correlated with the lower level of CSF Aβ42 ($P = 0.01$). Patients with *SNCA* duplication showed a decrease of CSF α-syn.
↓	Tokuda et al. [44]	No	16	12	—	—	32	—	The level of total α-syn was lower in PD patients than in age-matched controls. The level of α-syn oligomers was significantly higher in PD patients than in age-matched controls.
↓	Mollenhauer et al. [34]	Yes	— —	76 20	62 3	55 66	51 273	29 15	Upper and lower rows indicate training and validation cohorts, respectively. The level of α-syn was significantly lower in DLB, PD, and MSA patients than in other neurological diseases.
↓	Hong et al. [33]	Yes	92	—	38	—	86	20	The level of α-syn was decreased in PD and MSA patients.
↓	Parnetti et al. [37]	Yes	—	32	48	32	38	—	The level of α-syn was lower in patients with neurodegenerative diseases than in cognitively normal subjects, but the level of α-syn alone did not distinguish synucleinopathies from tauopathies. An inverse correlation between α-syn and total tau levels was observed ($P < 0.01$).
↓	Tateno et al. [36]	No	—	11	9	6	11	11	The levels of α-syn of DLB, PD, and MSA were lower than AD.
↓	Wennstrom et al. [38]	No	24	—	26	18	—	—	The level of α-syn in female DLB patients was lower than AD ($P = 0.041$) patients and controls ($P = 0.028$).

Table 1: Continued.

Level of total α-synuclein	Study	Blood contamination was considered*	Controls		AD	Synucleinopathies			Results
			Healthy controls	Neurological controls		Lewy body diseases			
						DLB	PD	MSA	
	Öhrfelt et al. [45]	Yes	55	—	66	15	15	—	PD, DLB patients and controls showed comparable levels of α-syn. AD patients showed significantly lower level of α-syn than the controls ($P < 0.001$). AD patients with MMSE scores below 20 had significantly lower level of α-syn than AD patients with MMSE scores of 20 or higher ($P = 0.02$).
	Noguchi-Shinohara et al. [46]	No	—	—	21	16	—	—	The level of α-syn did not differ between DLB and AD patients. In DLB patients, the duration of illness was associated with lower level of α-syn ($P < 0.05$).
	Spies et al. [47]	No	57	—	131	40	—	—	The level of α-syn was comparable between DLB, AD, and controls. The level of α-syn decreased with age ($P = 0.001$).
⟶	Reesink et al. [48]	Yes	34	—	63	35	18	—	The level of α-syn was not different among PD, DLB, AD, and controls. In DLB patients, lower α-syn was related to lower MMSE scores ($P < 0.05$) and worse category fluency ($P < 0.05$).
	Aerts et al. [49]	Yes	57	—	—	3	58	47	The level of α-syn was comparable among PD, MSA, DLB patients and controls. In PD group, the level of α-syn was negatively correlated with age at time of lumber puncture ($P < 0.006$).
	Foulds et al. [50]	Yes	20	—	—	16	38	8	The level of total α-syn was not different between PD, DLB, MSA and control groups. Oligomeric phosphorylated α-syn was significantly high in patients with MSA ($P < 0.001$).
	Park et al. [51]	No	18	11	—	—	23	—	The level of total α-syn in PD patients was comparable to that of control groups. The level of α-syn oligomer in PD patients was significantly higher than controls ($P = 0.005$).

Arrows indicate decreased (↓) and comparable (→) levels α-synuclein. Sample numbers are shown in each category. *Erythrocyte counts or haemoglobin levels were considered as a confounding factor. AD: Alzheimer's disease; DLB: dementia with Lewy bodies; PD: Parkinson's disease; MSA: multiple system atrophy; α-syn: α-synuclein; MMSE: minimental state examination.

Table 2: Summary of antibodies used to quantify α-synuclein in biofluids.

Study	Target molecule	Anti-α-synuclein antibodies	
		Capture antibody	Detecting antibody
Tokuda et al. [31]	Total α-synuclein	211 (m)	FL-140 (p)
Mollenhouer et al. [28]	Total α-synuclein	mSA-1 (p)	Syn-1 (m)
Öhrfelt et al. [45]	Total α-synuclein	Syn1b (m)	Syn3b (m), Syn5d (m)
Noguchi-Shinohara et al. [46]	Total α-synuclein	211 (m)	FL-140 (p)
Spies et al. [52]	Total α-synuclein	211 (m)	FL-140 (p)
Kasuga et al. [32]	Total α-synuclein	Syn-1 (m)	FL-140 (p)
Reesink et al. [48]	Total α-synuclein	211 (m)	FL-140 (p)
Tokuda et al. [44]	Total α-synuclein	211 (m)	FL-140 (p)
	Oligomeric α-synuclein	211 (m)	Biotinylated 211 (m)
Aerts et al. [49]	Total α-synuclein	211 (m)	FL-140 (p)
Mollenhouer et al. [34]	Total α-synuclein	mSA-1 (p)	Syn-1 (m)
Hong et al. [33]	Total α-synuclein	211 (m), LB509 (m), rabbit anti-α-synuclein (p)	Biotinylated goat anti-human α-synuclein (p)
Parnetti et al. [37]	Total α-synuclein	211 (m)	FL-140 (p)
Foulds et al. [50]	Total α-synuclein	211 (m)	FL-140 (p)
	Oligomeric α-synuclein	211 (m)	Biotinylated 211 (m)
	Phosphorylated α-synuclein	N-19 (p)	pS129 (m)
	Oligomeric phosphorylated α-synuclein	pS129 (m)	Biotinylated pS129 (m)
Tateno et al. [36]	Total α-synuclein	Not described	Not described
Wennstrom et al. [38]	Total α-synuclein	Commercial kit (Invitrogen)	
Park et al. [51]	Total α-synuclein	211 (m)	FL-140 (p)
	Oligomeric α-synuclein	211 (m)	Biotinylated 211 (m)

m: monoclonal antibody; p: polyclonal antibody.

Several groups have recently conducted studies to detect the oligomeric forms of α-synuclein in CSF, because the oligomer species of α-synuclein are considered to be toxic and could enhance pathological accumulation of α-synuclein and disease propagation [55]. Tokuda et al. demonstrated that the levels of α-synuclein oligomers in CSF are significantly higher in patients with PD than in patients with progressive supranuclear palsy (PSP) or AD [44]. In their study, the level of total α-synuclein in the CSF from PD patients tends to decrease [44]. Increased level of α-synuclein oligomers in CSF from PD patients was also shown by other investigator [51]. Foulds et al. showed that the level of oligomers composed of phosphorylated α-synuclein is higher in the postmortem CSF from MSA patients than in that from with PD, DLB, or PSP patients [50]. Sierks et al. also showed a significant increase in the level of α-synuclein oligomers in postmortem CSF from patients with PD by electrochemical impedance spectroscopy [56]. These findings suggest a possibility that α-synuclein oligomer species are detectable in CSF and that their levels may increase in some patients with synucleinopathies. A potential concern is that the oligomeric forms of α-synuclein detected in their studies using different methods may be heterogeneous in size and toxicity; hence, further validation is still needed.

Correlation analysis of clinical parameters, such as minimental state examination (MMSE) and Hoehn Yahr scale scores with the total α-synuclein level in CSF, has shown inconsistent results. Tokuda et al. showed an inverse correlation between the total α-synuclein level in CSF and disease severity determined by the Hoehn Yahr scale [31]. A low α-synuclein level was reported to correlate with a low MMSE score of DLB patients [48]. These findings suggest that α-synuclein level in CSF may reflect the severity of pathological changes occurring in patients with LBD. This notion is supported by the findings of a study that the disease duration in patients with DLB is closely associated with a low α-synuclein level in CSF [46]. In contrast to these findings, other studies revealed no significant association of the α-synuclein level in CSF with MMSE score, gender, age at examination, or disease duration in DLB or AD patients [32]. Shi et al. examined whether CSF α-synuclein level correlates with dopaminergic dysfunction determined by PET in asymptomatic carriers with leucine-rich repeat kinase 2 (LRRK2) gene mutation [64]. They detected no significant

Table 3: Studies of quantification of α-synuclein level in blood of patients with DLB and other synucleinopathies.

		Methods	Samples*	Results**
↑	El-Agnaf et al. [57]	ELISA	Plasma Cont (27), PD/DLB (34)	α-Synuclein oligomers were elevated in patients with PD/DLB compared to controls.
	Lee et al. [58]	ELISA	Plasma Cont (51), PD (105), MSA (38)	The α-synuclein level was increased in patients with PD (79.9 pg/mL) and in those with MSA (78.1 pg/mL) compared with controls (76.1 pg/mL). The α-synuclein level was significantly higher in patients with PD than in those with MSA.
	Duran et al. [59]	ELISA	Plasma Cont (60), PD (95)	The α-synuclein level was elevated in patients with PD compared to healthy controls. Antiparkinsonian treatment does not change plasma α-synuclein level.
	Foulds et al. [60]	ELISA	Plasma (not described)	The level of phosphorylated α-synuclein was higher in patients with PD than healthy controls. None of the levels of total α-synuclein, oligomeric α-synuclein, or oligomeric phospohorylated α-synuclein was different between PD patients and controls.
→	Shi et al. [61]	Bead-based flow cytometric assay	Plasma Cont (95), AD (33), PD (117)	No significant difference was found among patients with PD (36.8 ng/mL), AD (32.4 ng/mL), and those with healthy controls (39.5 ng/mL).
	Park et al. [51]	ELISA	Plasma Cont (29), PD(23)	There was no difference in oligomeric and total α-synuclein in plasma between PD patients and controls.
↓	Li et al. [62]	IP-Western blot	Plasma Cont (11), PD (27)	The α-synuclein level was significantly lower in patients with PD than in those with age-matched healthy controls. Early-onset PD patients had lower α-synuclein levels than late-onset PD patients.
	Laske et al. [63]	ELISA	Serum Cont (40), AD (80), DLB (40)	The α-synuclein level was significantly lower in patients with DLB (4.7 ng/mL) than in those with AD (7.0 ng/mL) and healthy controls (8.1 ng/mL).

Arrows indicate increased (↑), comparable (→), and decreased (↓) levels of α-synuclein. *Sample numbers are shown in parenthesis. **Values are indicated as mean or median. AD: Alzheimer's disease; Cont: controls; DLB: dementia with Lewy bodies; IP: immunoprecipitation; MSA: multiple system atrophy; PD: Parkinson's disease.

correlations, indicating that CSF total α-synuclein level may not be a sensitive biomarker of the preclinical phase of PD.

4. α-Synuclein in Blood

Several studies on the quantification of α-synuclein in blood have been carried out because drawing blood is much less invasive than lumbar puncture to obtain CSF from patients (Table 3). El-Agnaf et al. detected α-synuclein in plasma of patients with LBD by immunoprecipitation using an anti-α-synuclein antibody [27]. Subsequently, they found higher levels of α-synuclein oligomers in plasma from PD patients than in that from control subjects by ELISA [57]. A similar increase in α-synuclein level was observed in plasma from patients with PD and MSA [58, 59]. Lee et al. found that plasma α-synuclein level is higher in patients with PD than in those with MSA [58]. Duran et al. demonstrated that drugs such as L-dopa, dopamine agonists, and MAO/COMT inhibitors do not affect the plasma α-synuclein level in patients with PD [59]. The phosphorylated α-synuclein level in plasma quantified by ELISA as well as Western blot analysis is higher in patients with PD than in control subjects [60]. In their study, the level of α-synuclein remained stable within the same individuals at least over 3 months.

By contrast, Li et al. found a significantly decreased α-synuclein level in plasma from patients with PD by Western blot analysis, which detected only full-length monomeric α-synuclein [62]. Laske et al. also reported a similar decrease in serum α-synuclein level in DLB patients compared with AD patients and control subjects [63]. Comparable levels of plasma α-synuclein were found among patients with PD, AD, and control subjects in other studies [61].

5. Conclusions

Results of measurements of α-synuclein level in CSF and blood have been variable; hence, it is difficult to unequivocally conclude whether α-synuclein is a promising fluid biomarker of DLB and other α-synucleinopathies. More discriminating results for DLB patients could be obtained

by examining specific α-synuclein species such as truncated, phosphorylated, and oligomeric species on the basis of their analogy to Aβ42 and phosphorylated tau species whose changes in levels are found to be reliable CSF biomarkers of AD. In addition, a multicenter study is required to validate the usefulness of α-synuclein as a biomarker by standardized methods of quantifying α-synuclein. Continuous efforts will be required to establish useful fluid biomarkers for early diagnosis of DLB and evaluation of disease-modifying therapeutics for DLB.

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Research Article

Test-Retest Reliability of a New Medial Temporal Atrophy Morphological Metric

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Clinicians and researchers alike are in need of quantitative and robust measurement tools to assess medial temporal lobe atrophy (MTA) due to Alzheimer's disease (AD). We recently proposed a morphological metric, extracted from T1-weighted magnetic resonance images (MRI), to track and estimate MTA in cohorts of controls, AD, and mild cognitive impairment subjects, at high-risk of progression to dementia. In this paper, we investigated its reliability through analysis of within-session scan/repeat images and scan/rescans from large multicenter studies. In total, we used MRI data from 1051 subjects recruited at over 60 centers. We processed the data identically and calculated our metric for each individual, based on the concept of distance in a high-dimensional space of intensity and shape characteristics. Over 759 subjects, the scan/repeat change in the mean was 1.97% (SD: 21.2%). Over three subjects, the scan/rescan change in the mean was 0.89% (SD: 22.1%). At this level, the minimum trial size required to detect this difference is 68 individuals for both samples. Our scan/repeat and scan/rescan results demonstrate that our MTA assessment metric shows high reliability, a necessary component of validity.

1. Introduction

Early detection of Alzheimer's dementia (AD), critical for treatment success, is a high-priority research area. The development of disease-modifying treatment strategies requires objective characterization techniques and quantitative biomarkers able to identify AD with higher accuracy and at a much earlier stage than clinically based assessment [1]. Given that structural magnetic resonance imaging (MRI) (e.g., T1 weighted) on 1 to 3 Tesla clinical scanners allows the *in vivo* assessment of changes such as medial temporal lobe atrophy (MTA) due to AD, it has been proposed to fulfill the role of quantitative biomarkers in recent reports [2, 3].

We have developed a sophisticated automated image processing method for the purpose of evaluating MTA in the context of AD. We recently proposed a single, high-dimensional morphological metric called the disease evaluation factor (DEF) extracted from T1-weighted MRI and able to track and estimate disease state [4]. In our previous report we provided estimates of this metric's efficiency at the discrimination of cognitively normal, control subjects (CTRL) from probable AD patients, as well as the prediction of conversion in mild cognitive impairment (MCI) subjects to probable AD.

Thorough technique verification, validation, and evaluation are necessary, however, in order for imaging biomarkers such as the DEF to be used in clinical trials enrichment, and more importantly, as a diagnostic aid to community physicians. As an essential component of the verification process, comprehensive metrological investigation of MRI-based metrics must include reliability testing.

Reliability is an important component of the precision of a measurement and relates to the consistency of measurements taken by a single person or instrument on the same item and under the same conditions. A less-than-perfect test-retest reliability causes test-retest variability, reducing confidence in the result and decreasing the test's statistical power. Reliability testing is particularly important for MRI-based metrics, which, while acquired with similar protocols, will show dissimilar intensity contrasts for the same tissue

types [5]. These systematic and random variations are machine dependent and can be corrected for the most part via image denoising [6], bias field inhomogeneity estimation [7], and intensity standardization [8].

In this paper we investigated the reliability of our DEF metric through analysis of cross-sectional (i.e., one timepoint) scan/repeat scan and scan/rescan images from two multicentric studies. First, we took advantage of the fact that subjects in the Alzheimer's Disease Neuroimaging Initiative (ADNI) study received two within-session T1-weighted scans at their baseline visit to test for scan/repeat scan analysis. Further, we employed data on three participants in the Pilot European ADNI that had been scanned at seven different sites in a short timeframe to test for Scan/Rescan reliability. We report minimum clinical trial sample size increases at various different levels based on the calculated detection threshold.

Reliability analysis is an important, necessary, and often overlooked step between bench and bedside in the research and clinical contexts.

2. Materials and Methods

2.1. Ethics. Institutional review boards of all participating institutions approved the procedures for this study. Written informed consent was obtained from all participants or surrogates. More information about ADNI[1] and Pilot European ADNI investigators are provided in the Acknowledgments.

2.2. Subjects. In this study we used data from three different studies, totaling 1051 subjects from over 60 centers.

(i) The first was the *Mapping group*, consisting of 145 young control subjects from the International Consortium for Brain Mapping database [9].

(ii) The second was the *Classification group*, which consisted in 70 probable AD and 69 CTRL subjects from the LENITEM database [10]. We required those first two groups to build our high-dimensional metric;

(iii) The third was the *Scan/Repeat Test Group*, which consisted in 1518 baseline MRIs (scan + same-session repeat scans) from 759 CTRL, MCI, and probable AD subjects participating in ADNI, acquired on more than 50 different 1.5T scanners using a similar 3D T1-weighted MP-RAGE protocol [11]. Inclusion criteria to the ADNI study were as follows.

(a) CTRL are MMSE scores [12] between 24–30 (inclusive), a CDR [13] of 0, nondepressed, non-MCI, and nondemented. The age range of normal subjects was roughly matched to that of MCI and mild AD subjects.

(b) MCI subjects are MMSE scores between 24–30 (inclusive), a memory complaint, objective memory loss measured by education adjusted scores on Wechsler Memory Scale Logical Memory II [14], a CDR of 0.5, absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living, and an absence of dementia.

(c) Mild AD is MMSE scores between 20–26 (inclusive), CDR of 0.5 or 1.0, and meets NINCDS/ADRDA criteria for probable AD [15].

From the complete ADNI dataset of 822 subjects at baseline, we selected individuals for the *Scan/Repeat Test Group* that had both valid entry images and processed images that passed *automated* quality control [16].

(iv) Finally, the fourth was the *Scan/Rescan Test Group*, which was obtained with permission from the multicentric Pilot European ADNI project [17]. It included data from three healthy volunteers acting as human quality control phantoms for the study.

2.3. MRI Acquisitions. Subjects in the *Mapping group* were scanned in Montreal, QC, Canada on a Philips Healthcare Gyroscan 1.5T scanner (Best, The Netherlands) using a T1-weighted fast gradient echo sequence (sagittal acquisition, TR = 18 ms, TE = 10 ms, $1 \times 1 \times 1\,\text{mm}^3$ voxels, flip angle 30°).

Subjects in the *Classification group* were scanned in Brescia, Italy on a single Philips Healthcare Gyroscan 1.0T scanner (Best, The Netherlands) using a T1-weighted fast field echo sequence (sagittal acquisition, TR = 25 ms, TE = 6.9 ms, $1 \times 1 \times 1,3\,\text{mm}^3$ voxels).

Subjects in the *Scan/Repeat Test Group* were scanned on over 50 different 1.5T scanners (GE Medical Systems; Siemens Healthcare; Philips Healthcare) using a 3D T1-weighted MP-RAGE protocol or its equivalent [11]. In this protocol, within the same scan session, there were two 3D T1-weighted images acquired, allowing us to test reliability on this scan/repeat pair. The subject was not taken out of the scanner between acquisitions.

Subjects in the *Scan/Rescan Test Group* were scanned within the span of few weeks at seven different European centers (Sites 1 to 7), using the ADNI study 3D T1-weighted MP-RAGE protocol [11]. Six centers collected scan/rescan sessions, where the subject was taken out of the scanner between acquisitions. This allows us to estimate scan/rescan reliability on 18 comparison pairs.

2.4. Initial Image Processing. We processed all MRI volumes identically using the MINC image processing toolbox (http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage) and local software as follows: (a) noise removal [6]; (b) raw scanner intensity inhomogeneity correction [7]; (c) global registration (12 degrees of freedom) [18] to the reference image space defined by the BrainWeb T1-weighted image [19] (1-mm resolution, 0% noise, 0% nonuniformity), maximizing the mutual information between the two volumes [20]; (d) resampling to a 1-mm^3 isotropic grid; (e) linear clamping to (0–100) intensity range; (f) intensity standardization [8]; (g) nonlinear registration of individual standardized subject images to the BrainWeb reference;

(h) computation of determinants of the Jacobian of the deformation field [21].

2.5. High-Dimensional Metric. We generated a low-dimensional feature space with the *Mapping group* using Principal Components Analysis of (a) T1w MRI intensity z-score maps, as a proxy of tissue composition and (2) determinant maps, as a proxy of tissue atrophy. After computing components, data from the *Mapping group* were no longer used in the study.

We then projected intensity and determinant data from the *Classification group* into the space defined by the principal components and used a system of supervised linear classifiers with forward stepwise regression (*p-to-enter* 0.05) to identify a restricted set of eigenvectors $\{\lambda_f\}$ forming a hyperplane that best separated the two classes under study (CTRL versus probable AD). After computing the classification function, data from the *Classification group* were no longer used in the study.

Finally, we projected *Test Group* data in the $\{\lambda_f\}$ eigenvector space. The morphological DEF metric is based on the concept of distance within the space defined by eigenvectors $\{\lambda_f\}$ [4]. Specifically, in this embodiment it consists in the calculated Mahalanobis distance (1) for each subject's image between the position p of a subject's image in the *Mapping group* feature space, along the restricted set of principal components, and the centroids of coordinates formed by the CTRL subjects of the *Classification group*.

The Mahalanobis distance between p and a group G is given by

$$\text{mahal}(p, G) = \sqrt{(p - \mu_G) S_G^{-1} (p - \mu_G)}, \quad (1)$$

where μ_G and S_G are respectively the mean and covariance matrix of group G.

2.6. Experimental Design. We first tested reliability in the ADNI *Scan/Repeat Test Group*, that is, between within-session scan/repeat scan pairs, at a single study timepoint (namely, baseline scans). Secondly, we tested reliability in the Pilot European ADNI *Scan/Rescan Test Group*, that is, within-scanner scan/rescan pairs. For each reliability estimate, we calculated the change in the mean, standard deviation, and Pearson retest correlation. Finally, we estimated the impact of the reliability thresholds on the minimum trial size required to discriminate probable AD versus CTRL subjects, using conservative power assumptions, for cross-sectional evaluations.

3. Results

3.1. Scan/Repeat Scan Reliability. Over the 759 subjects of the ADNI dataset, the scan/repeat change in the mean was 1.97% (95% CI: 0.46%–3.48%), with standard deviation 21.2% (*cf.* Figure 1), and Pearson retest correlation $r = 0.9381$.

We ensured there were no statistical differences in reliability between scan/repeat scans in either CTRL or probable AD groups using the diagnostic provided by ADNI (*cf.* Figure 2).

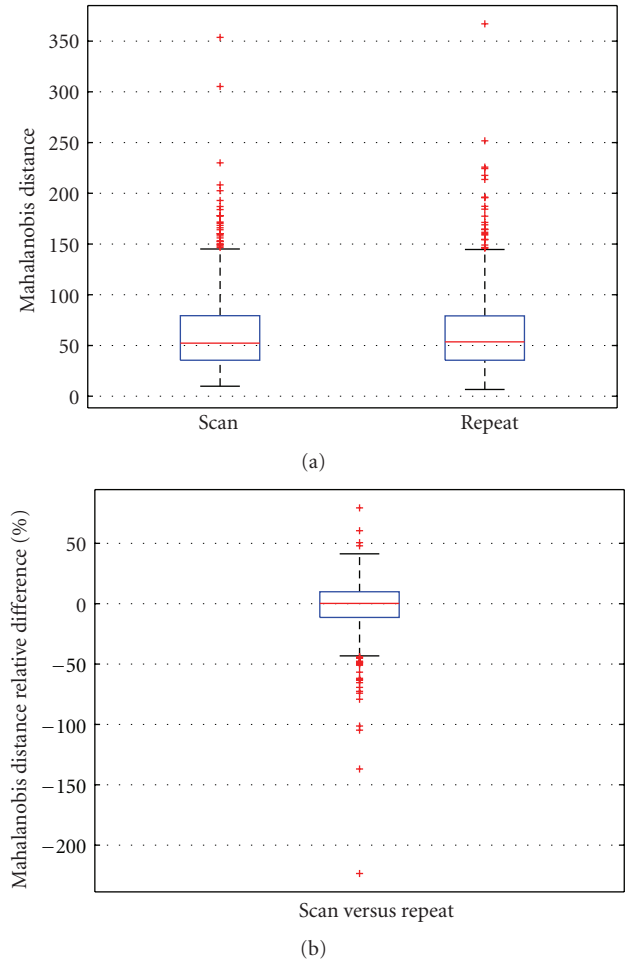

Figure 1: Absolute distances (a) and relative difference in % (b) for the DEF factor between within-session scan and repeat T1-weighted MR scans for 759 baseline ADNI subjects. The change in mean was 1.97%, with 95% confidence intervals 0.46%–3.48%.

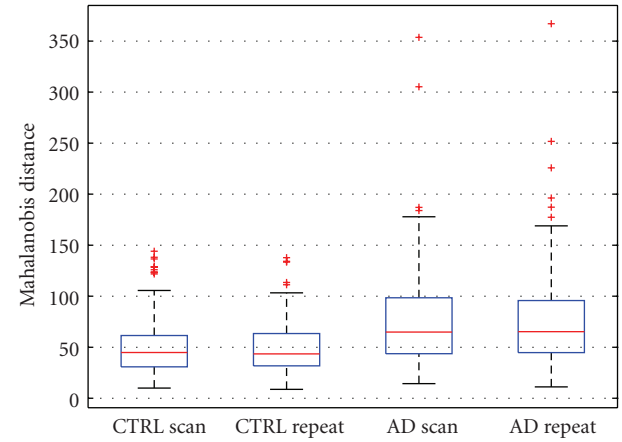

Figure 2: Absolute Mahalanobis distances (DEF factor) between scan/repeat scans for ADNI 203 CTRL subjects (left) and 169 probable AD subjects (right). While the between-group difference was significant, there were no statistical differences in reliability within each diagnostic group.

As reported previously [4], the difference in DEF averages between probable AD and CTRL was 55%. At this level, the minimum trial size required to detect this difference is 62 individuals for both samples ($\alpha = 0.05$; $\beta = 0.50$) (*cf.* Figure 3). Due to the 1.97% minimum precision threshold of the technique, to reach identical power the trial size must increase to 68 individuals.

To evaluate whether the scan/repeat scan distance was smaller than the distance to any one image's nearest neighbor (scan or repeat), we proceeded by calculating all pairwise distances between subjects in the scan/repeat dataset. The comparison shows that the nearest neighbor in nearly all cases was the scan/repeat pair, as opposed to one of the possible neighbor (*cf.* Figure 4).

3.2. Scan/Rescan Reliability. Over the three subjects of the Pilot European ADNI dataset, the scan/rescan change in the mean was 0.89% (95% CI: −14.34%, +12.56%) (*cf.* Figure 5), standard deviation 22.1%, and retest Pearson correlation $r = 0.8609$. Based on similar assumptions, the 0.89% precision threshold of the technique implies an increase in trial size from 62 to 64 individuals.

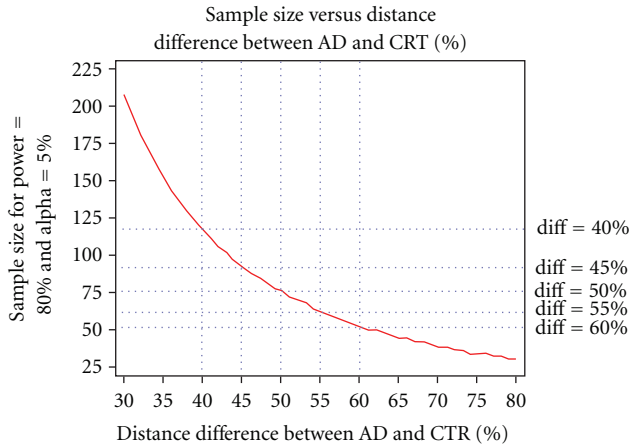

Figure 3: Sample sizes necessary to detect a given DEF difference (in %) between groups at 80% power and alpha level of 0.05.

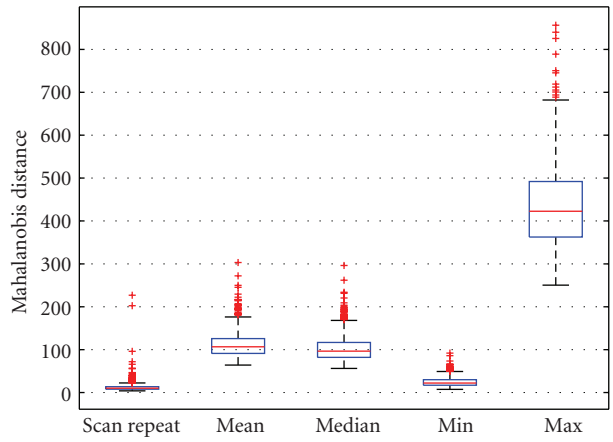

Figure 4: Comparison of scan/repeat scan distance versus all pairwise distances in the *Scan/Repeat Test Group* of 759 ADNI subjects shows that the closest image in the high-dimensional feature space remains its own repeat.

4. Discussion

Imaging biomarkers such as DEF should be thoroughly verified, validated, and evaluated (following ISO9000:2008) before they can be used to enrich populations in clinical trials and aid community physicians to diagnose prodromal AD clinically. *Verification* consists in assessing that the system is built according to its specifications (i.e., assessing that the system is built correctly) and that test data is accurate. *Validation* consists in assessing that the system actually fulfills the purpose for which it was intended (i.e., assessing that the correct system was built). *Evaluation* consists in assessing that the system is accepted by the end-users and performs well for a specific purpose (i.e., assessing that the system is valuable). These are important, necessary, and often overlooked steps between bench and bedside in the research and clinical contexts.

In this study, we proposed a reliability analysis of our high-dimensional morphological metric in a large-scale multicenter setting. Reliability is a *necessary, but not sufficient, component of validity*. Our scan/repeat and scan/rescan results demonstrate that DEF is a reliable metric for medial temporal lobe atrophy estimations.

We further estimated minimum precision threshold that must be added to the effect size to obtain true cohort sizes in the case of clinical trials. While this resulted in increased number of subjects, this increase is somewhat negligible, especially when comparing trial sizes using DEF to those obtained with other metrics, for example, ADAS-Cog [22] or MMSE [12], as mentioned in Schuff et al. [23].

While large datasets represent one of the strengths of the current study, it is not without its limitations. First is the lack of systematic pathological evaluation in both the *Classification group* and the ADNI data. The former implies that the classification function is not optimal for the task of discriminating CTRL from AD; the latter relates to the stability of the DEF. Further, while the mean and confidence intervals are relatively tight, standard deviations tend to be elevated. While it makes the DEF metric suitable for group studies, more work would be required for individual predictions. However, by design, we refrained from using techniques (e.g., within-subject registration, within-subject intensity normalization) that are specifically aimed at removing random and/or systematic errors in individual subject scanning that are not relevant to the pathology. For example, it is expected that within-subject registration would increase spatial concordance, and hence positional variability in the projected intensity and deformation spaces. Such techniques should be considered when continuing our investigations regarding the longitudinal reliability and overall validity of the DEF.

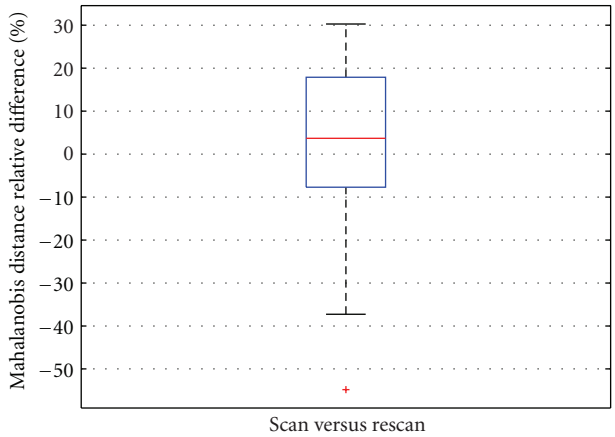

Figure 5: Relative difference in % for the DEF factor between different scan/rescan image pairs for Pilot European ADNI subjects (3 subjects at 6 sites; 18 scan/rescan pairs). The change in mean was 0.89%, with 95% confidence intervals (−14.34%, +12.56%).

Abbreviations

AD: Alzheimer's disease
ADNI: Alzheimer's disease neuroimaging initiative
CTRL: Control subjects
DEF: disease evaluation factor
MCI: mild cognitive impairment
MRI: magnetic resonance imaging
MTA: medial temporal lobe atrophy.

Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Data used in preparation of this paper were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this paper. A complete listing of ADNI investigators can be found at http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

Authors' Contribution

All the authors were guarantors of integrity of the entire study. They conducted the study concepts and design. S. Duchesne did the Literature research. Clinical studies and data acquisition included ADNI, Pilot European ADNI, and ICBM. Methods, analysis and interpretation were conducted by S. Duchesne, F. Valdivia, and N. Robitaille. Statistical analysis was conducted by S. Duchesne, A. Mouiha, and N. Robitaille. The first author helped in the preparation of the paper. All the authors were involved in the revision and review of the paper, paper definition of intellectual content, editing, and final version approval.

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Endnotes

1. Data used in the preparation of this paper for the *Scan/Repeat Test Group* were obtained from the Alzheimer's Disease Neuroimaging Initiative database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations, as a $60 million, 5-year public private partnership. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is

intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California San Francisco CA, USA. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the USA and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. For up-to-date information, see. http://www.adni-info.org/.

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Research Article

The Relation between Inflammation and Neuropsychological Test Performance

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Background. Considerable research documents an association between pro- and anti-inflammatory markers and Alzheimer's disease (AD), yet the differential relation between these markers and neuropsychological functioning in AD and nondemented controls has received less attention. The current study sought to evaluate the relationship between peripheral markers of inflammation (both pro- and anti-inflammatory) and neuropsychological functioning through the Texas Alzheimer's Research and Care Consortium (TARCC) cohort. *Methods*. There were 320 participants (Probable AD $n = 124$, Controls $n = 196$) in the TARCC Longitudinal Research Cohort available for analysis. Regression analyses were utilized to examine the relation between proinflammatory and anti-inflammatory markers and neuropsychological functioning. Follow-up analyses were conducted separately by case versus control status. *Results*. Proinflammatory and anti-inflammatory markers were found to be associated with neuropsychological testing. Third tertile proinflammatory markers were negatively associated with measures of attention and language, and anti-inflammatory markers were positively associated with measures of immediate verbal memory and delayed verbal and visual memory. *Conclusions*. These findings support the link between peripheral inflammatory markers and neuropsychological functioning and suggest the utility of examining profiles of inflammatory markers in the future.

1. Introduction

Inflammation has been hypothesized to modulate a number of pathogenic processes that are associated with Alzheimer's disease (AD), including the amyloid cascade [1–3], although the underlying etiology and full nature of the relationship remains unclear. Both proinflammatory and anti-inflammatory proteins have been shown to be stimulated by injury, β-amyloid toxicity, and ischemia [4], and inflammation is increased in both hypertension and atherosclerosis [5]. Furthermore, peripheral inflammation has been related to poorer cognitive performance [6–9], as well as cognitive decline [10], although these findings have not always been consistently supported [11].

Several large population-based studies have examined serum markers of inflammation and their relation to cognitive functioning. The Honolulu Asia Aging Study (HAAS) [12] found that raised levels of C-reactive protein (CRP; an inflammatory marker) in mid-life was associated with a significantly increased risk for vascular dementia (VaD) and AD, with or without the presence of cardiovascular disease (CVD). The Rotterdam Study reported a link between CRP risk for AD and VaD. Additionally, it has been proposed that individuals with cognitive impairment may have a different balance of proinflammatory and anti-inflammatory cytokines compared to those with normal aging [13, 14].

While considerable research has documented that chronic inflammation and high levels of proinflammatory

cytokines are fundamental components of AD, the relationship between these markers and decline in specific neuropsychological performance has received less attention. Research in this area has tended to use brief measures of global cognitive functioning (e.g., mini-mental state examination (MMSE), Short Portable Mental Questionnaire (SPMQ) making it difficult to gain insight into focal cognitive deficits that may be associated with elevated cytokine levels [15].

The current project sought to examine the link between a number of pro- and anti-inflammatory markers and neuropsychological functioning among a sample of patients diagnosed with AD and cognitively normal controls from the Texas Alzheimer's Research and Care Consortium (TARCC) research. It was hypothesized that there would be a significant relationship between serum levels of inflammatory cytokines (a net increase in the proinflammatory profile) and performance on tests of memory and verbal fluency compared to other neuropsychological domains.

2. Materials and Methods

Participants. Participants included 320 individuals (124 diagnosed with Probable AD and 196 normal controls) enrolled the TARCC Longitudinal Research Cohort. The methodology of the TARCC project has been described in detail elsewhere [16]. Briefly, each participant undergoes an annual evaluation that includes a medical examination, interview, neuropsychological testing, and blood draw. All patients met consensus-based diagnosis for probable AD based on NINCDS-ADRDA criteria [17] and controls performed within normal limits on psychometric assessment and were assigned a Clinical Dementia Rating scale (CDR) global score of 0.0. AD patients were included if their CDR global scores were .5 or 1.0; 46 had CDR global scores of 0.5 and 78 had scores of 1.0. The TARCC project received institutional review board approval and all participants and/or caregivers provided written informed consent.

3. Measures

The TARCC neuropsychology core battery consists of neuropsychological instruments administered as part of the established Alzheimer's disease clinical/research platforms at each participating institution and included digit span (WAIS-R, WAIS-III, WMS-R), Trail Making Test, WMS Logical Memory (WMS-R and WMS-III), Boston Naming Test (30- and 60-item versions), verbal fluency (FAS), Clock Drawing Test, the American National Adult Reading Test (AMNART), the Geriatric Depression Scale (GDS-30), mini-mental state examination (MMSE) [18], and the Clinical Dementia Rating scale (CDR) [19]. In order to equate scores from digit span and story memory scales, all raw scores were converted to scale scores based on previously published normative data [20]. For the Boston Naming Test, the current group recently conducted an independent study that demonstrated the psychometric properties of an estimated 60-item BNT score that can be calculated from 30-item versions [21]. Adjusted scale scores were utilized as dependent variables in analyses.

Table 1: Coefficient of variation by marker.

Marker	Combined	AD	Control
TNFalpha	58.18	54.55	59.68
A1A	49.24	44.44	53.13
IL8	11.38	10.29	12.21
CRP	190.02	317.43	139.39
IL-1ra	15.04	15.54	14.15
IL10	31.55	27.96	32.98

A1A: alpha-1 antitrypsin; CRP: C-reactive protein.

4. Assays

Nonfasting samples were collected in serum-separating tubes during clinical evaluations, allowed to clot at room temperature for thirty minutes, centrifuged, aliquoted, and stored at −80°C in plastic vials. Serum samples were sent frozen to Rules-Based Medicine (http://www.rulesbasedmedicine.com/, Austin, TX, USA) where they were thawed for assay without additional freeze-thaw cycles. Rules-Based Medicine conducted multiplexed immunoassay via their human multianalyte profile (human MAP). Multiple proteins, including proinflammatory markers (TNFalpha, Alpha1Antitrypsin, IL-8, and C-reactive protein) and anti-inflammatory markers (IL-1ra, IL-10), were quantified though multiplex fluorescent immunoassay utilizing colored microspheres with protein-specific antibodies. The coefficient of variation (CV), a normalized measure of dispersion of a probability distribution (CV= standard deviation/mean), for each marker (run only once) are shown in Table 1. The proinflammatory and anti-inflammatory markers were chosen due to past research showing their reliability in use in the study of inflammatory processes [22–26].

Additional information regarding assay performance can be found online. Assays conducted by this company utilizing this platform, including TARCC data, have been published elsewhere [27–29]. For additional information, please refer to (http://www.rulesbasedmedicine.com/).

5. Analyses

Statistical analyses were conducted using SPSS version 18.0 (SPSS, Chicago, IL). Comparisons between NC and AD were conducted using t-tests for continuous measures and χ^2 for categorical measures. The relation between inflammatory makers and cognitive test performance was examined via a series of regression models for all participants (AD and NC) with proinflammatory and anti-inflammatory proteins entered in separate blocks as predictor variables and neuropsychological test scores entered as outcome variables. Levels of inflammatory proteins were categorized into tertiles based on the distribution of the variable (i.e., 1st, 2nd, and 3rd group). The number of markers (pro- and anti-inflammatory separately) in the third tertile for each marker was summed for each participant and this summed score was entered as a predictor variable into a linear regression model with neuropsychological test scores as outcome variables (i.e., if the participant's value for IL-8 and IL-10 were in

Table 2: Demographics by diagnosis.

	AD	Control	*P* value
Number of subjects	124	196	—
Sex			
Female	77 (62.1%)	134 (68.4%)	0.25
Age (years)			
Mean (SD)	76.72 (7.80)	70.47 (8.81)	<0.01
Range	57–91	55–90	
Education (years)			
Mean (SD)	14.35 (3.30)	15.52 (2.73)	<0.01
Range	5–22	10–25	
ApoE			
One or more E4	71 (61.7%)	50 (25.8%)	<0.01

the third tertile, the participant had a "2" entered as the predictor variable into the regression model).

Follow-up analyses were then conducted examining the relationship between proinflammatory and anti-inflammatory markers and neuropsychological functioning on case status (NC versus AD). All biomarker results were transformed using log base 10 in order to evenly distribute the scores of the markers. Assumptions for all statistics tests were checked for violations and statistical significance was declared for *P* value <0.01.

6. Results

Average age and education of the control group was 70.5 ± 8.8 and 15.5 ± 2.7, respectively, while average age and education of the AD group was 76.7 ± 7.8 and 14.4 ± 3.3, respectively. The sample was over 95% Caucasian, and there were more females than males, though gender distribution was not significantly different between groups. As expected, AD patients obtained significantly lower MMSE scores (mean = 22.3 ± 4.1, median = 23.0) than controls (mean = 29.4 ± 0.9, median = 30.0) as well as higher CDR sum of boxes scores (AD mean = 5.2 ± 2.3, median = 5; controls mean = 0.0 ± 0.04, median = 0.0). Demographic characteristics of the study population are shown in Table 2.

7. Individual Markers and Neuropsychological Functioning

7.1. Proinflammatory Markers. Higher IL-8 levels were associated with significantly poorer scores in global cognition (MMSE $\beta = -0.185$, $P = 0.002$), as well as immediate visual memory (WMS-III Visual Reproduction I $\beta = -0.157$, $P = 0.009$), and verbal fluency (COWAT $\beta = -0.172$, $P = 0.004$). Higher IL-8 levels were also associated with significantly greater disease severity (CDR Sum of Boxes; $\beta = 0.205$, $P < 0.001$).

Higher CRP levels were associated with significantly greater disease severity (CDR Sum of Boxes; $\beta = -0.201$, $P = 0.001$). However, elevated CRP levels were also related to significantly better global cognition (MMSE; $\beta = 0.533$, $P = 0.006$), verbal (WMS-III Logical Memory I; $\beta = 0.202$, $P = 0.001$; WMS-III Logical Memory II; $\beta = 0.712$, $P = 0.001$), and delayed visual memory (WMS-III Visual Reproduction II; $\beta = 0.710$, $P < 0.001$).

Higher TNF-alpha levels were associated with better delayed verbal (WMS-III Logical Memory II; $\beta = 0.178$, $P = 0.007$) and immediate visual memory (WMS-III Visual Reproduction I; $\beta = 0.196$, $P = 0.002$). Neuropsychological tests by diagnosis are shown in Table 3.

7.2. Anti-Inflammatory Markers. Higher IL-1ra levels were associated with disease severity (CDR Sum of boxes; $\beta = -0.161$, $P = 0.004$), better scores in verbal memory (WMS-III Logical Memory I; $\beta = 0.168$, $P = 0.005$; WMS-III Logical Memory II; $\beta = 0.164$, $P = 0.006$), and visual memory (WMS-III Visual Reproduction I; $\beta = 0.180$, $P = 0.002$; WMS-III Visual Reproduction II; $\beta = 0.180$, $P = 0.002$).

8. Summed Number of Markers by Tertiles and Separated by Diagnostic Category

The sum of the number of proinflammatory markers in the 3rd tertile was found to be associated with significantly better information processing speed (Trails A; $\beta = 0.177$, $P = 0.004$). When looking at AD cases only, the sum of the proinflammatory markers was associated with significantly better immediate verbal memory (WMS-III Logical Memory I; $\beta = 0.163$, $P = 0.004$), but poorer fluency (COWAT; $\beta = -0.173$, $P = 0.010$). In the NC group, the sum of the proinflammatory markers was associated with significantly better processing speed (Trails A; $\beta = 0.165$, $P = 0.010$). See Table 4 for biomarkers by diagnosis.

9. Discussion

The current study demonstrates that the association between inflammatory markers is quite complex. In our sample, proinflammatory markers were found to be both positively associated with immediate and delayed verbal and visual memory, and disease severity and global cognition and negatively associated with measures of immediate visual memory,

Table 3: Neuropsychological testing by diagnosis.

	AD	Control	*P* value
Number of subjects	124	196	—
MMSE			
Mean (SD)	22.32 (4.13)	29.43 (0.88)	<0.01
Range	8–30	26–30	
CDR sum of boxes			
Mean (SD)	5.15 (2.30)	0.00 (0.04)	<0.01
Range	0.5–13.0	0.0–0.5	
Trails A			
Mean (SD)	6.89 (2.86)	10.31 (2.68)	<0.01
Range	2–14	2–17	
Trails B			
Mean (SD)	5.27 (3.42)	10.96 (2.53)	<0.01
Range	2–18	3–18	
WMS-III Logical Memory I			
Mean (SD)	4.64 (2.46)	13.57 (2.76)	<0.01
Range	1–10	6–18	
WMS-III Logical Memory II			
Mean (SD)	3.69 (1.89)	13.99 (2.63)	<0.01
Range	1–11	5–19	
WMS-III Visual Reproduction I			
Mean (SD)	4.97 (2.77)	12.41 (3.19)	<0.01
Range	1–13	5–18	
WMS-III Visual Reproduction II			
Mean (SD)	4.82 (2.27)	13.57 (3.14)	<0.01
Range	2–12	5–19	
Boston naming test			
Mean (SD)	7.32 (3.79)	11.94 (3.04)	<0.01
Range	2–16	2–17	
COWAT			
Mean (SD)	7.91 (2.89)	11.64 (2.75)	<0.01
Range	2–18	4–18	
AMNART			
Mean (SD)	9.28 (3.55)	12.11 (3.37)	<0.01
Range	2–18	2–18	
Estimated VIQ			
Mean (SD)	108.32 (15.05)	117.12 (9.88)	<0.01
Range	38.66–127.44	42.11–132.28	

verbal fluency, and global cognition. The anti-inflammatory markers were found to be significantly positively and negatively associated with a measure of global cognition and disease severity (CDR), and immediate and delayed visual and verbal memory. These findings are not surprising as such conflicting results have been documented previously in the literature. These findings also make sense biologically as these immune-related markers serve a purpose that only becomes detrimental when the system gets out of balance, which has been observed in AD. Our findings also point to the fact that the relationship between inflammation and neuropsychological functioning varies according to disease status and cognitive domain. In fact, the majority of our significant findings were for AD cases rather than controls.

When examined by case status, proinflammatory markers were negatively associated with a measure of verbal fluency and positively associated with immediate verbal memory for the AD group. Within the normal control group, proinflammatory markers were positively associated with processing speed. In addition, a positive association with information processing speed was observed among individuals in the 3rd tertile of proinflammatory markers.

These findings suggest the importance of examining profiles of the inflammatory response. As suggested in prior research, [30] those with cognitive impairment may possess different proinflammatory and anti-inflammatory profiles compared to those without cognitive impairment. For example, the Leiden 85+ study examined the ratio of TNFalpha,

Table 4: Biomarkers by diagnosis.

	AD	Control	*P* value
Number of subjects	124	196	—
TNF-alpha Log			
Mean (SD)	0.55 (0.30)	0.62 (0.37)	0.05
Range	−0.30–1.04	−0.30–1.79	
Alpha-1 Antitrypsin Log			
Mean (SD)	0.36 (0.16)	0.32 (0.17)	0.09
Range	−0.23–0.71	−0.43–0.71	
IL-8 Log			
Mean (SD)	1.36 (0.14)	1.31 (0.16)	<0.01
Range	1.04–1.78	0.98–1.84	
C-Reactive Protein Log			
Mean (SD)	0.14 (0.52)	0.33 (0.46)	<0.01
Range	−1.34–1.36	−1.19–1.40	
IL-1ra Log			
Mean (SD)	1.93 (0.30)	2.05 (0.29)	<0.01
Range	0.90–2.61	0.90–2.62	
IL-10 Log			
Mean (SD)	0.93 (0.26)	0.94 (0.31)	0.83
Range	0.04–1.43	0.04–1.62	
Sum of markers			
Mean (SD)	2.40 (1.85)	2.76 (1.95)	0.11
Range	0–8	0–8	

a proinflammatory cytokine and IL-10, an anti-inflammatory cytokine. The ratio was higher in patients diagnosed with AD compared with normal controls which suggests a net "proinflammatory profile". The findings of the current study also indicate the importance of examining the combination of upper median levels of pro- and anti-inflammatory biomarkers.

The current findings are limited by the cross-sectional nature of the analyses; however, the TARC cohort is being evaluated annually and follow-up analyses examining the profiles of anti- and proinflammatory markers in those with and without a diagnosis of AD will be completed. The current findings point towards the use of profiles of biomarkers as a possible way to understand the relationship between inflammation and neuropsychological functioning versus the standard approach of examining biomarkers individually.

Authors' Contributions

VB conceived the study, drafted the paper and took lead on design and coordination of the paper. RB, SO, and JH provided considerable aid in conceiving this study, drafting the paper and providing edits. LH carried out all statistical analyses and provided edits of paper. RDA participated in the design of the study and provided edits to the paper.

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Review Article

Will Posttranslational Modifications of Brain Proteins Provide Novel Serological Markers for Dementias?

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Drug development for dementias is significantly hampered by the lack of easily accessible biomarkers. Fluid biomarkers of dementias provide indications of disease stage, but have little prognostic value, cannot detect early pathological changes, and can only be measured in CSF (cerebrospinal fluid) which significantly limits their applicability. In contrast, imaging based biomarkers can provide indications of probability of disease progression, yet are limited in applicability due to cost, radiation and radio-tracers. These aspects highlight the need for other approaches to the development of biomarkers of dementia, which should focus on not only providing information about pathological changes, but also on being measured easily and reproducibly. For other diseases, focus on development of assays monitoring highly specific protease-generated cleavage fragments of proteins has provided assays, which in serum or plasma have the ability to predict early pathological changes. Proteolytic processing of brain proteins, such as tau, APP, and α-synuclein, is a key pathological event in dementias. Here, we speculate that aiming biomarker development for dementias at detecting small brain protein degradation fragments of generated by brain-derived proteases specifically in blood samples could lead to the development of novel markers of disease progression, stage and importantly of treatment efficacy.

1. Introduction

Dementias are a group of disorders characterized by declining cognitive function, usually with increasing prevalence as a function of aging. Several types of dementia exist, with Alzheimer's disease (AD) being the most prominent, followed by dementia with Lewy bodies (DLB), frontotemporal dementia (FTD) also known as frontotemporal lobar degeneration (FTLD), corticobasal degeneration (CBD), and vascular dementia (VaD) [1]. According to the World Alzheimer Report 2009, it has been estimated that 36 million people worldwide are affected by dementia, with numbers doubling every 20 years [2]. Women are overrepresented in terms of incidence of dementias, with 3.4 of 5.4 million Americans living with Alzheimer being females [3].

By the time dementia can be clinically diagnosed, neuropathologic features are already extensive in some regions of brain, and hence some of the pathological changes appear irreversible. Therefore, the general consensus is that dementia should be diagnosed as early as possible, and thereby improving the possibility for intervention [4–7].

Genetic studies have shown that individuals with mutations in the genes for *APP*, *PSEN1*, or *PSEN2* genes are predisposed for early onset AD; however, only a very low percentage of AD patients have these mutations [3]. In contrast, between 40 and 80% of AD cases have the APOE $\varepsilon 4$ allele [8, 9]. Having this allele either as a heterozygotic or homozygotic allele raises the probability for Alzheimer's by three and 15 times respectively [3].

Mutations in several other genes are known to predispose the carriers for different forms of dementia, and these include mutations in the *MAPT* gene, which encodes tau, and these mutations can lead FTD, CBD, and other forms of dementia [10]. In addition, mutations in the *PGRN* gene encoding progranulin can cause FTD [11, 12]. Several other gene mutations are known to predispose carriers for different forms of dementia; however, these are beyond the scope of this paper, and we refer to [10].

Besides aging, risk factors for dementia are hypercholesterolaemia, hypertension, atherosclerosis, coronary heart disease, head injuries, smoking, obesity, and diabetes; however, whether they are causal factors or not is still undergoing investigation [3, 13, 14].

At present, a diagnosis of dementia can be reached through neuropsychological testing, imaging-based analysis, and through the use of CSF biomarkers [15, 16]. However, while a positive diagnosis of AD can be obtained with a rather large certainty, the picture is more complicated for other dementias, where misdiagnosis and mixed pathologies are complicating factors [17]. Although imaging-based analysis can allow early diagnosis as well as provide some prognostic value, the limitations are still many [15]. Furthermore, for several of the dementias, it is well known that the pathological changes start appearing markedly earlier than the cognitive decline [4–7, 15], and hence there is a substantial need for a novel approach to biomarkers of dementia.

With respect to treatment of dementias, the presently available treatment possibilities only provide symptomatic relief or slow the cognitive decline moderately, but temporarily, and following the reduction in progression neuronal degradation accelerates again [3, 15, 18]. Despite failures in drug development for dementias, including prominent AD phase III trial failure semagacestat [19], there are still numerous clinical trials ongoing.

A major issue in the development of drugs for dementias is the lack of biomarkers allowing the selection of the cohort, that is, patients who have not yet reached a point-of-no-return and will show disease progression during the study. Secondly, biomarkers of treatment efficacy are lacking, although this could also be a result of the lack of useful biomarkers for inclusion of patients in the studies [15, 20–25].

In this paper, we ask the following question: is it possible to develop a biomarker system allowing the detection of neuronal pathology in the circulation by focusing on protease-generated protein fragments? This would allow easier and more frequent sampling and analysis and potentially promote earlier diagnosis and prognosis and thereby allow monitoring of treatment efficacy.

2. Pathological Proteins in Dementia

Converging lines of investigation have revealed potential common pathogenic mechanisms underlying many diverse dementias [10, 26], and, interestingly, dementias are in general characterized by faulty protein processing mechanisms, resulting in accumulation of pathological forms of brain proteins. The pathological processing is a crucial step towards induction of neuronal apoptosis, and hence monitoring these processes would be of great interest as biomarkers of neuronal status.

Most dementias are characterized by similar molecular mechanisms, including protein cleavage, protein aggregation, and inclusion body formation in selected regions of brain and thereby the formation of numerous alterations in the brain proteins resulting in the formation of posttranslational modifications (PTMs), also referred to as neoepitopes, that is, novel sites not previously presented in the body [27–29].

In AD, there are two well-described processes, namely, (1) the formation of Aβ senile plaques, through an imbalance in the processing of APP (see Table 1), and APP processing appears to be an initiating factor for AD [29], (2) the formation of neurofibrillary tangles (NFTs) through pathological processing of tau involving phosphorylations and protease cleavage, and subsequent aggregation [28], a process that appears to be directly involved in triggering neuronal death [30, 31]. Interestingly, recent data indicate that Aβ is involved in inducing cleavage of tau and subsequent neuronal apoptosis [30, 32], thus providing some mechanistic insight into the pathology of AD.

Dementia with Lewy bodies on the other hand is characterized not only by having NFTs but also inclusions consisting of aggregations of pathologically processed α-synuclein (Lewy bodies), while the presence of amyloid plaques is very limited [33–35].

In FTD, accumulations of pathologically processed proteins are also observed, and in these cases the proteins TAR DNA-binding protein 43 (TDP-43) and FUsed in Sarcoma (FUS) are highly relevant and are known to undergo several pathological changes, which all contribute to neuronal toxicity [37, 38]. The accumulations of these proteins are an important part of the subclassification of FTD into more specific categories [39].

Interestingly, CBD and VaD are primarily tauopathies and are characterized by accumulations of NFTs in the neurons, and while other pathological changes are known to occur, these do not appear to include other protein aggregations, although some AD-like traits are observed [40, 41].

These mechanisms are interrelated in complex vicious cycles which lead to cell dysfunction and death. However, in relation to development of novel biomarkers, the processing steps the individual proteins undergo are of great interest [27].

3. Presently Available Biomarkers and Why Alternatives Are Needed

Biomarkers for the different forms of dementia, and especially AD, have been investigated for a long time, and hence numerous biomarkers exist. These are based on imaging and CSF-based technologies, which can detect and monitor dementia, but they are limited by aspects such as the need for lumbar puncture, use of radiotracers, and lack of predictive value in terms of both disease progression, but also response to treatments in development [15, 43].

As the cognitive decline only can be observed several years after the onset of neuropathological changes, cognitive tests are mainly useful at a diagnostic level [15, 44]. The most commonly used imaging modality is MRI, which allows assessment of both static and dynamic parameters in the brain [15]. These techniques have predictive value and can

Table 1: Relevant proteins for each dementia (see text for references).

Type of dementia	Relevant protein
Alzheimer's disease (AD)	Tau, Aβ
Dementia with Lewy bodies (DLB)	α-Synuclein, tau
Frontotemporal dementia (FTD)	Tau, TDP-43, FUS
Vascular dementia (VaD)	Tau
Corticobasal degeneration (CBD)	Tau

Table 2: BIPED classification adopted from [36].

Burden of disease:	Burden-of-disease markers assess the severity or extent of disease
Investigative:	A marker which does not have a clear-cut pathological relevance, but is used exploratively
Prognostic:	The key feature of a prognostic marker is the ability to predict the future onset of disease
Efficacy of intervention:	An efficacy-of-intervention biomarker provides information about the efficacy of treatment or those at high risk for its development
Diagnostic:	Diagnostic markers are defined by the ability to classify individuals as either having or not having a disease

measure many of the desired changes in the brain, such as cortical atrophy, neuronal integrity, and more [15].

In addition, methods such as FDG-PET and ^{18}C-PIB scans also provide essential information about brain status and progression of disease [15, 43, 45]; however, they are significantly limited by the number of scans allowed due to radiation and costs as well as patient discomfort/unruliness associated with being placed in the scanner [16, 46, 47].

CSF-derived biomarkers for dementias, include Aβ(1–42), total tau protein, and hyperphosphorylated tau, and these protein species have well-established patterns in dementias [21, 48–51]. However, while they are of use for diagnosis and segregation of disease, their use is still somewhat limited by the need for lumbar puncture, a procedure perceived as unpleasant and risky. Lumbar puncture cannot be performed as often as desired when monitoring treatment efficacy [51, 52]. Furthermore, the CSF biomarkers have limited prognostic value [15].

As illustrated in Figure 1, novel biomarkers of dementias should focus on detection of very early stages, which will allow treatment at the right time and assist in selection of patients at risk for progression [15, 27]. In addition, biomarkers monitoring treatment efficacy, especially at the level of neuronal integrity would be of great use [15, 27].

In summary, early diagnosis before the cognitive decline accelerates, selection of those who will show progression of disease, and monitoring of treatment efficacy, all those aspects ideally require the identification of effective biomarkers. These biomarkers should preferably be monitored in serum and/or plasma, as this would allow a more frequent sampling and hence a more detailed understanding of both pathology and effects of treatments.

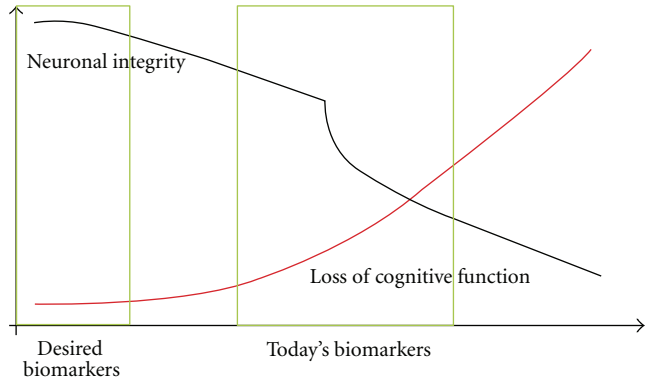

Figure 1: Schematic illustration of the alterations in neuronal integrity (black line) and subsequent loss of cognitive function (red line). The green boxes indicate at what level the presently available biomarkers have diagnostic/prognostic value, that is, once cognitive decline has begun, and at which level it is desired to be able to provide a prognosis/diagnosis, that is, biomarkers monitoring very early changes in neuronal integrity.

4. Present Fluid Biomarkers for AD and Their Classification and Utility

Correction application of biomarkers is facilitated by a useful classification, and, within the field of osteoarthritis, five useful categories have been proposed. These categories are referred to as the BIPED criteria and they introduce a simple and useful way of separating biomarkers (Table 2 and [36]).

As illustrated in Table 3, the presently available fluid biomarkers for dementias primarily belong in the burden of disease and diagnostic categories [48], and while these are highly useful in terms of strengthening diagnosis, can provide a modest segregation into different dementia types, and are applied across this field, and fluid biomarkers of treatment efficacy, and importantly with predictive value, are still lacking to a great extent [15, 42, 53]. One major reason for these limitations is most likely the need for collection of CSF, which requires a lumbar puncture and which cannot be performed frequently [16].

Several studies have examined the potential of measuring Aβ and tau in plasma/serum samples, and Aβ is present in plasma but the levels are not related to pathology [54–56], a finding likely explained by the large level of Aβ bound to immunoglobulins in plasma [57]. On the other hand, plasma Aβ appears to respond to γ-secretase inhibitor treatment and hence appears to be relevant as an efficacy marker for drugs affecting the γ-secretase [58]. In addition, a study has monitored switches in isoforms of APP in plasma as a function of AD, and it appears that there is a switch towards lower molecular weight isoforms in AD [59].

Serum/plasma tau levels have been explored extensively, but tau is virtually undetectable in MCI and/or AD [60];

Table 3: Present fluid biomarkers for dementias from [15, 33, 42].

Analysis sample	Biomarker	BIPED classification	Relationship with pathology
CSF	t-tau	B,D	Increased in AD, indicates the neuronal degeneration
	p-tau	B,D	Increased in AD, reflects the formation of tangles
	Aβ42	B,D	Reduced in the onset stage of AD, it remains unchanged after onset of AD
	Aβ oligomers	B,D	Increased in AD
	APPs-a	B,D	Soluble APPa is decreased in AD
	APPs-β	B,D	APPs-β is a product of APP cleavage by BACE-1; it cannot discriminate normal from AD
	APLIβ	B,D	Fragments generated by β- and r-secretase are increased in AD
	α-Synuclein	B,D	There is an inverse relationship between severity of disease and α-synuclein, it increases rapidly after neuron death in DLB
	BACE-1	B,D	Increased activity in MCI but not AD
Plasma	Aβ40,42	E	Plasma Aβ is in large amount bound to plasma protein, it cannot discriminate normal from AD, but may have a role as an efficacy marker

however, serum tau levels are elevated in a series of other pathologies with a markedly different pathology, such as ischemic stroke [61], Creutzfeldt-Jacob's disease [60], and traumatic brain injury [62]. For hyperphosphorylated tau, there are no studies clearly showing any relevance of this marker in serum/plasma [15, 63].

Hence, monitoring in serum/plasma at present is limited to experimental markers, such as the 18 peptide profile described which initially was thought to be useful for segregating different dementias [64], or the blood-based algorithm by O'Bryant and colleagues [65]. However, underscoring the complex nature of monitoring brain pathologies in blood specimens is a recent study finding that the 18 peptide profile cannot segregate dementias and only have limited diagnostic value in another cohort [66]. These points clearly illustrate the need for novel approaches for identification of fluid biomarkers for dementias.

5. Critical Considerations for the Design of Serum Biomarkers for Dementia

A major issue in relation to serum detection of brain-derived proteins is the blood-brain barrier (BBB), which does not allow large proteins to cross. The (BBB) exists between the peripheral circulation and brain, and its primary function is to protect the brain from potentially harmful substances present in blood [67, 68]. However, in addition to reducing entry into the brain, the BBB also reduces exit of molecules from the brain [68, 69], a function which has complicated the biomarker development process significantly and which is the main reason for the lack of useful serum biomarkers for dementias.

Of importance is that CSF is absorbed into blood every day, and some exchanges of peptides occur, meaning that a protein fragment of sufficiently small size may have the possibility to pass BBB potentially allowing detection in serum or plasma [16]. An example of this is that Aβ is present in plasma, but is bound to immunoglobulins, and thus cannot be reliably used for diagnosis [57].

All of the dementias are characterized by pathological protein processing, of which fragmentation and other posttranslational modifications (PTMs) appear to be key pathological events [31, 70–72]. With these aspects in mind, we speculate that protease-mediated cleavage of brain proteins will lead to the generation of small fragments which can be released into the serum, and which in serum will represent neoepitopes of potential use for serum detection (Figure 2).

6. Neuronal Proteins and Proteases of Interest for Development of Dementia Biomarkers

Dementias are characterized by aberrant protein processing, and the processed proteins have for a long time been explored and used as biomarkers for dementias [15, 42]. These include Aβ(1–42) and hyperphosphorylated tau, both of which represent pathological processes ongoing in the brain; however, data generated using these markers is only meaningful in CSF as the free levels of these present in serum are extremely low [15, 57].

When examining the forms of dementia, several proteins and proteases are known to show alterations [28, 29].

Classical examples of pathological processing steps in neuronal proteins include γ-secretase cleaved amyloid precursor protein (APP) to generate the 1–42 amino acid polypeptide (Aβ), which forms toxic oligomers and eventually deposits as plaques [28].

Microtubule-associated protein tau undergoes several posttranslational modifications, and recent data have indicated that the caspase cleavage at the C-terminal is a key early event occurring, which appears to occur only in the absence of phosphorylation and which causes aggregation into the neurofibrillary tangles [30, 73–75] (Table 2).

In addition to tau and APP processing, processing of α-synuclein and TDP-43 and several other proteins with high specificity for the brain are known to be processes by different classes of proteases during pathological events, and these known processing steps related to dementia are listed in Table 4.

Table 4: Proteins, proteases, and the consequences in relation to dementia.

Protein	Normal function	Protease	Alteration and consequence	Disease	Reference
APP	Lipid metabolism, axonal transport??	α,β,γ-Secretases ADAMs MMPs	Fragmentation, generation of Aβ, formation of amyloid plaques	AD	[28, 94, 95]
tau	Microtubule stabilizing protein	Caspase Calpain	C-terminal truncation in AD and aggregation causing NFTs	AD	[28, 73–75]
α-Synuclein	Molecular chaperone	MMPs calpain cathepsins	Truncation and aggregation leading to Lewy bodies	DLB	[34, 35, 96, 97]
TDP-43	Transcription and splicing regulation, apoptosis, cell division, and stabilisation of messenger RNA	Caspase?	C-terminal truncation, aggregation formation of Lewy bodies	FTLD-TDPAD	[37, 39, 98, 99]
FUS	Transcription factor	??	??	FTLD-FUS	[39]
GFAP	Neurofilament	Caspase	Truncation and neuronal death	Alexander disease	[100]

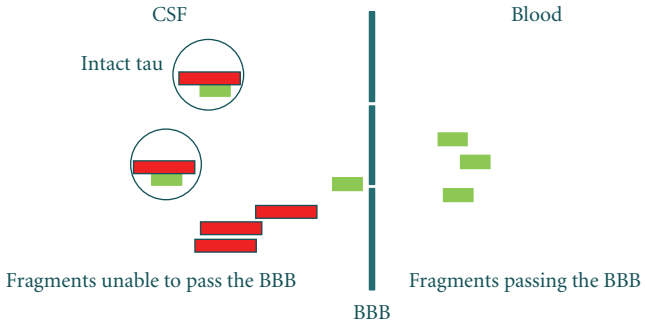

Figure 2: Schematic illustration of proteins present in the CSF, and the possibility that small fragments can cross.

We speculate that in addition to these truncations several others will take place and that selective searching for fragments of the proteins of interest in serum/plasma could provide useful biomarkers of neuronal pathologies.

7. The Potential of Posttranslational Modifications (PTMs) as Biomarkers

Importantly, several different posttranslational modifications (PTMs) exist, and these include the aforementioned generation of novel protease cleavage sites, isomerizations, crosslinks, phosphorylations, nitrosylations, glycosylations, glycations, hydroxylations, and more [27].

The dementia field has for many years taken advantage of the fact that pathologies introduce PTMs in proteins, and this approach has been duplicated across many research fields [27].

Within the dementia field, the best characterized PTM-based biomarkers are the Aβ(1–42) fragment and hyperphosphorylated tau, but of which, as mentioned previously, are used diagnostically for AD [15, 42]. However, as described earlier, despite the application of PTM approach, there are still several limitations to the fluid biomarkers [16].

Hence, other peripheral biomarkers, such as serological biomarkers which could provide us noninvasive, inexpensive, convenient, and frequent samples, are intensely sought [16]. One promising approach towards this goal is to focus on the size of fragments generated by protease cleavage and then work selectively on identifying small fragments of proteins in circulation, and these could then be explored for their biomarker potential. Interestingly, recent studies have highlighted the C-terminal truncation of tau causing generation of the protein species referred to as tau-C3 as a key initiator of tau processing ultimately causing NFTs and death [31, 72–76]; however, whether the tau-C3 can be measured in fluids such as CSF or even serum has not yet been explored.

If successful, this approach could be followed by searching for other PTMs present in the identified fragments, and as both phosphorylation and glycations are known to occur in dementia pathologies these are candidates of interest [77–79].

8. An Example of What a PTM-Based Biomarker Can Do

Aβ is one of the most used PTM/neoepitope biomarkers, and as described previously in this paper, it is highly useful for diagnosis of AD. However, as also described there are also significant limitations to its use [15, 42].

There is in particular one PTM biomarker in serum, which has been used extensively, and this is the bone resorption marker β-CTX-I, and the use of this marker has illustrated many of the benefits and a few of the challenges of this class of biomarkers [80].

In bone, the ECM consists of 90% type I collagen, and this matrix is degraded by the bone resorbing osteoclast [81]. The osteoclasts degrade type I collagen using the cysteine proteinase cathepsin K, and this has been shown to lead to

the generation of the CTX fragment (^{1207}EKAHDDGR^{1214}) [82–84].

The CTX fragment hence contains a cathepsin K cleavage site as its primary PTM; however, in addition, it is a dipeptide linked together via a lysine crosslink adding another PTM [27, 85]. Finally, it contains a DG amino acid sequence, and this site undergoes isomerization with time, and the β-CTX-I system measures the isomerized, hence aged, form, thereby including one more PTM in this system [85].

Studies of b-CTX have shown that it is elevated in postmenopausal women, it has predictive value for osteoporotic fractures, it not only responds to treatment but also predicts effect of the antiresorptive treatment on BMD [80], and hence it fits into all the BIPED categories [86].

In relation to the issues of β-CTX-I, measurements of β-CTX-I in studies have highlighted that it is very rapidly removed from circulation, which allows the measurement of changes in levels within less than one hour after treatment in human studies [87–89]. Furthermore, it exhibits a pronounced diurnal variation, which correlates directly to observation of osteoclast activity and which appears to be controlled almost completely by food intake [87–89]. The sensitivity of CTX-I to food intake/diurnal variation is often seen as a limitation; however, on the other hand, when knowing how to handle this variation, CTX-I is a highly sensitive and rapidly responsive biomarker, and, therefore, this unique triple PTM biomarker is among the most used biomarkers in both in vitro preclinical and clinical studies within the bone field as well as in studies of the bone safety of other drugs, that is, glitazones and Serotonin Reuptake Inhibitors [90, 91].

We speculate that utilizing the knowledge about PTMs/neoepitopes from the bone field may provide possibilities for biomarkers development within the dementia fields, although this still remains to be demonstrated conclusively.

9. Conclusions and Challenges

While there are imaging- and CSF-based technologies for the diagnosis of dementias, there is still a large unmet need for novel markers, especially markers which can predict disease progression and treatment response, and preferably in serum or plasma as this allows more frequent sampling at a markedly lower risk for complications.

The major hurdle in the development of serum biomarkers for dementias is the limited passage of proteins or fragments through the BBB, as underlined by the difficulties in detecting intact and phosphorylated tau in serum samples [68]. This is where the protease-generated protein fragments, often referred to as neoepitopes, are of great interest, as these provide the possibility for getting a protein fragment of sufficiently small size to allow crossing of the BBB, but also with pathological relevance and a high level of specificity due to the possibility of combining specific brain proteins with specific brain proteases. Interestingly, there are already studies showing that specific pathological processing of tau results in the generation of a highly interesting fragment [31, 71–75], and although it still is unclear whether this fragment can be utilized as a serum marker, this is a promising finding.

If successful, this approach could also allow differential diagnosis of the different dementias, as they are characterized by different enzymatic processing steps, as well as a series of other PTMs, all which can be explored in the context of biomarker development, and thus potentially result in a biomarker panel with the ability to clearly separate different forms based on their protein degradation profile. An example could be a caspase-generated tau fragment specific for AD versus a calpain-generated α-synuclein fragment to separate AD from DLB [48].

However, the probability of success is to a large extent defined by the ability to identify small brain-derived fragments in serum, and even if these are identified they are likely to be present at low concentrations, which will increase the demand for a highly sensitive and highly specific detection system. Furthermore, as illustrated by the β-CTX-I example, numerous parameters need to be investigated in detail in order to validate the relevance of a potential marker [80], and whether this approach or other approaches, that is, mass spectrometry-based analyses of CSF and/or plasma samples, will end up providing a fluid biomarker with a broader application remains to be seen.

Another interesting aspect in relation to dementias is the possibility of monitoring comorbidities, such as diabetes and/or cardiovascular disease [92, 93]. As biomarkers of these pathologies exist, combining them with novel dementia biomarkers could provide additional strength to the analysis of the patients, and thus ultimately help segregation of patients for clinical trials, for the right type of treatment, and so forth.

In summary, although the dementia field has been working on biomarker development for several years, there is still a large area that has not been explored, namely, the small degradation fragments in serum. Based on the success of this approach within other diseases where biomarkers have been lacking, we are enthusiastic about this possibility, despite the numerous hurdles that will need crossing.

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