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Featured Article

Evaluation of α-synuclein as a novel cerebrospinal fluid biomarker in different forms of prion diseases

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Abstract

Introduction: Accurate diagnosis of prion diseases and discrimination from alternative dementias gain importance in the clinical routine, but partial overlap in cerebrospinal fluid (CSF) biomarkers impedes absolute discrimination in the differential diagnostic context.
Methods: We established the clinical parameters for prion disease diagnosis for the quantification of CSF α-synuclein in patients with sporadic (n = 234) and genetic (n = 56) prion diseases, in cases with cognitive impairment/dementia or neurodegenerative disease (n = 278), and in the neurologic control group (n = 111).
Results: An optimal cutoff value of 680 pg/mL α-synuclein results in 94% sensitivity and 96% specificity when diagnosing sporadic Creutzfeldt-Jakob disease (CJD). Genetic CJD cases showed increased CSF α-synuclein values. No increased α-synuclein levels were detected in non-CJD cases with rapid progression course.
Discussion: Detection of α-synuclein in the CSF of patients with suspected CJD is a valuable diagnostic test reaching almost full discrimination from non-prion disease cases. These data highlight the utility of CSF α-synuclein quantification in front of classical CSF biomarkers in clinical routine.

Keywords: Cerebrospinal fluid; α-Synuclein; Biomarker; ELISA; Neurodegenerative diseases; Sporadic Creutzfeldt-Jakob disease; Genetic Creutzfeldt-Jakob disease; Prion diseases

1. Introduction

Prion diseases represent a group of fatal progressive neurodegenerative diseases (NDs) caused by the abnormal folding of normal cellular prion protein (PrP^{c}) in the brain into its pathologic form (PrP^{Sc}) [1]. They are characterized by an invariable rapidly progressive dementia leading to memory loss, behavioral alterations, and a heterogeneous range of clinical symptoms. In humans, development of prion disease usually leads to the death of the individual within the first year after the onset of the disease. Cardinal neuropathologic lesions are spongiform change, neuronal loss, gliosis, and PrP^{Sc} deposits [2,3].

Despite the recent advances in their diagnosis, there is no single test that can conclusively diagnose prion disease in a

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living person. Thus, histopathologic examination of biopsied or autopsied brain tissue is still required for definite diagnosis [4].

Creutzfeldt-Jakob disease (CJD) is the most common form of prion disease in humans, and its clinical diagnosis is achieved by a combination of neuroimaging methods, electroencephalography patterns, and cerebrospinal fluid (CSF) tests [5–7]. Most CJD cases are sporadic (sporadic CJD [sCJD]) (85%), developing in patients without known risk factors, whereas about 10% to 15% of the cases test positive for mutations associated with the prion protein gene (*PRNP*). Inherited prion diseases are genetic or familial CJD (genetic CJD [gCJD]), fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker (GSS) syndrome [8–10].

The clinical diagnosis is supported by various CSF biomarkers and recently established real-time quaking-induced conversion (RT-QuIC) protein aggregation assay [11,12,21]. Differential diagnosis from other NDs, especially those with rapid progressive forms, can be challenging because neurodegenerative conditions such as dementia with Lewy bodies (DLBs) or rapid progressive forms of Alzheimer's disease (rpAD) might present with a similar clinical syndrome [13–18].

Already established CSF prion biomarkers are proteins 14-3-3 and total tau (tau), which are tested in the routine clinical workup in suspected CJD [19–20]. Slight to moderate alterations in amyloid β-42 (Aβ42) and phosphorylated tau (p-tau) have also been reported [22,23], although their clinical values are limited. The major limitation of prion disease biomarkers results from the occurrence of increased tau and p-tau values and low Aβ42 levels in alternative neurologic and NDs [24–26]. In addition, increased 14-3-3 levels in CSF, considered to reflect severe neuronal damage and tissue destruction, can also be found in some neurologic disorders such as acute stroke, meningoencephalitis, subarachnoidal hemorrhage, and due to blood contamination [16–18]. Altogether this leads to a partial biomarker overlap among different neurologic and neurodegenerative conditions, impairing their clinical accuracy. Therefore, there is an urgent need to find further CSF biomarkers that may play an important role in the diagnostic workup. In this regard, the search for CSF biomarker patterns able to discriminate sCJD from neurodegenerative dementias with heterogeneous presentations in their progressive course and available symptomatic treatment is gaining experimental momentum.

The presence of increased CSF α-synuclein levels in sCJD cases has been previously reported, although the clinical significance of these differences was limited when using classical colorimetric assays [27,28]. In a small cohort of cases, using a well-characterized enhanced chemiluminescence (ECL)-based enzyme-linked immunosorbent assay (ELISA) system [29,30] (Kruse et al., manuscript in preparation), we recently reported the presence of increased CSF α-synuclein levels in sCJD patients without major overlap with control subjects and Alzheimer's disease (AD) cases [29]. The observation of decreased α-synuclein levels in synuclein aggregation disorders [31,32], slightly increased or unchanged in AD, and unaltered levels in frontotemporal dementia, vascular dementia, progressive supranuclear palsy, and corticobasal degeneration [29,33–35] makes α-synuclein measurements gain importance in the differential diagnosis of neurodegenerative dementias.

In the present study, we have analyzed CSF α-synuclein levels in a large cohort of CJD patients in comparison to neurologic and neurodegenerative cases from different etiologies. Increased α-synuclein levels have been found exclusively in CJD cases, allowing valuable diagnostic discrimination from non-CJD cases. These findings, validated in two alternative cohorts, highlight the importance of α-synuclein measurement as an sCJD biomarker and facilitate its incorporation in the differential diagnosis of neurodegenerative dementias in laboratory routine.

2. Methods

2.1. Demographics

To establish the potential diagnostic utility of CSF α-synuclein quantification in sCJD, the study was based on cases from following three independent cohorts: (1) from an ongoing surveillance study of the German National Reference Centre for Transmissible Spongiform Encephalopathies (study cohort); (2) from cases recruited at Polish neurologic and psychiatric hospital departments, collected in the Department of Molecular Pathology and Neuropathology, Medical University of Lodz (validation cohort 1); and (3) from cases presented in neurologic clinics of Northern Greece hospitals that were further processed at the Laboratory of Pharmacology (School of Pharmacy, Aristotle University of Thessaloniki) (validation cohort 2). Lumbar puncture was performed for diagnostic purposes with analysis of CSF standard parameters. α-Synuclein, tau, p-tau, and 14-3-3 were analyzed at the time point of first diagnostic workup. In addition, Aβ42 levels were analyzed for the study cohort and validation cohort 1. The interval between clinical onset and diagnostic workup was not the same across all groups in our cohorts because of the different disease progression rates between groups. However, we did not observe differences in α-synuclein levels between time from disease onset to lumbar puncture when clinical data were known.

For the study cohort, samples were routinely analyzed for cell counts, total protein, and immunoglobulins to rule out inflammatory processes. Routine investigation of the CSF did not reveal any abnormalities with respect to these parameters. The neurologic control (NC) group in study cohort was composed of patients with either clinically or pathologically defined alternative diagnosis [24]. This group included cases of psychiatric disorders (psychosis, bipolar disorder, depression, and schizophrenia), ischemic stroke, epilepsy,