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ORIGINAL COMMUNICATION

Quantification of CSF biomarkers using an electrochemiluminescence-based detection system in the differential diagnosis of AD and sCJD

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Abstract The identification of reliable diagnostic tools for the differential diagnosis between sporadic Creutzfeldt–Jakob Disease (sCJD) and Alzheimer's disease (AD) remains impeded by the existing clinical, neuropathological and molecular overlap between both diseases. The development of new tools for the quantitative measurement of biomarkers is gaining experimental momentum due to recent advances in high-throughput screening analysis and with the optimization of assays for their quantification in biological fluids, including cerebrospinal fluid (CSF). Electrochemiluminescence (ECL)-based immunoassays have demonstrated to achieve clinical quality performance in a variety of sample types due to its high sensitivity and dynamic range. Here, we quantified the CSF levels of Tau-protein, β-amyloid 1-42 (Aβ42) and α-synuclein, as important biomarkers in CSF used in the differential diagnosis of neurodegenerative disorders in 12 AD, 12 sCJD and 12 control cases by singleplex ECL-based technology. Its performance has been compared to classical enzyme-linked immunosorbent assays (ELISA) to confront their clinical accuracy. ECL-based technology validates previous data obtained with ELISA and presents a higher performance in the discrimination of three analysed groups as determined by increased area under the curve (AUC) values for the three biomarkers. Importantly, α-synuclein levels detected by ECL allow an excellent discrimination between sCJD cases and AD and control cases, unveiling a new clinical approach for the differential diagnosis of sCJD.

Keywords Cerebrospinal fluid · Biomarkers · Neurodegeneration · Alzheimer's disease · Creutzfeldt–Jakob disease · Electrochemiluminescence-based detection system · ELISA

Introduction

Alzheimer's disease (AD) and Creutzfeldt–Jakob disease (CJD) are neurodegenerative diseases characterized by the presence of pathogenic protein aggregates leading to an alteration of brain functions and to profound dementia. AD usually displays a slow progression rate, although rapid progressive AD forms (known as rapid progressive AD) have been also described [28]. A broad range of pharmacological treatments can temporarily alleviate the symptoms of AD patients slowing down disease progression. On the contrary, CJD is a fatal progressive disease with an invariable rapid progression rate for which there is no current treatment [1, 7].

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Therefore, biomarkers to discriminate between CJD and AD are required to generate an appropriate therapeutic intervention before pathological changes spread throughout the brain.

CSF analyses have demonstrated that the ratio between phosphorylated and total Tau levels [25], the presence or absence of the 14-3-3 protein [39, 41] and the ability of PrPsc to induce the seeding of a template PrPc [3, 8, 9] can discriminate between CJD and AD cases. However, different disease duration, progression rates, disease subtypes, blood contamination, CSF storage conditions as well as the presence of co-pathology significantly alter the sensitivity and specificity of these biomarkers. In addition, an overlap exists between CJD and AD in the levels of main biomarkers used for clinical diagnosis such as Tau and Aβ42 [21, 36, 37].

Different approaches are explored to overcome the inherent limitations of a differential diagnosis. On one side, the screening of new molecules with better clinical performance to those currently in use and, on the other side, the development of new techniques and methodologies able to detect the presence of well-known biomarkers with higher sensitivity and larger dynamic ranges are two of the main research focus in the field of biomarkers development.

In this context, the use of an electrochemiluminescence (ECL)-based detection system developed by Meso Scale Discovery (MSD, Gaithersburg, MD, USA) has been shown to present high sensitivity, reproducibility, recovery rates and low background in the detection of several biomarkers in biological fluids [4, 5, 24]. However, the analytical and clinical performance of ECL technology in CSF samples has not been explored in detail for the differential diagnosis of neurodegenerative dementias.

In the present study, we have analysed in sCJD, AD and control samples the levels of the CSF biomarkers Tau, Aβ42 and α-synuclein, previously reported to be deregulated in several neurodegenerative diseases using ECL technology. Details about the comparative performance between MSD's ECL and conventional ELISA are reported.

Methods

Demographics

This diagnostic study is based on data from an ongoing surveillance study of the German National Reference Centre for Transmissible Spongiform Encephalopathies [11].

Lumbar puncture was performed for diagnostic purposes with analysis of CSF standard parameters (e.g. cell count, proteins and immunoglobulins). CSF was centrifuged and stored at −80 °C until analysis. All patients with sCJD were classified as definite cases by neuropathological examinations or as probable CJD cases according to diagnostic consensus criteria [40, 41]. All sCJD cases were tested positive for 14-3-3 CSF protein. AD diagnosis was based on the ICD-10 definition (F.00 G.30). Controls are patients with neurological disorders that were diagnosed according to clinical syndrome, neuroimaging and standard neurological clinical and paraclinical findings. The presence of neurodegenerative disease in the control cohort was excluded in the follow-up clinical diagnosis. In addition, CSF biomarkers proposed to predict development of dementia (p-Tau, Tau, Aβ42/Aβ40) were negative in controls cases. A total of 12 CSF samples from age- and sex-matched cases per each group were analysed. Mean and Standard deviation from ECL measurements, as well as mean of patients' age are reported (Suppl. Table 1). For comparisons between detection methods for Tau, Aβ42 and α-synuclein, AUC values derived from ROC curves from ELISA and ECL measurements were calculated. AUC values for ELISA measurements reported in the present study are in line with those previously reported for the German National Reference Centre for Transmissible Spongiform Encephalopathies cohort [14, 15, 31].

ECL-based analysis

Quantification of Tau and Aβ42 was performed using Human Total Tau Kit V-PLEX^{TM} (Meso Scale Discovery$^{®}$) and Human Aβ42 kit V-PLEX^{TM} (Meso Scale Discovery$^{®}$), respectively, following manufacturer's instructions. Quantification of α-synuclein was performed as described before [17, 18]. Tau and α-synuclein values were analysed logarithmically to the base of 10 when necessary for clear visualization of differences between studied groups.

ELISA analysis

Total tau levels were also measured using a commercially available ELISA according to the manufacturer's instructions (Innogenetics) [23]. Aβ42 was detected with a commercially available ELISA kit [INNOTEST® ß-AMYLOID(1–42) Innogenetics] following manufacturer's instructions. α-synuclein was analysed as described before [19, 35]. In addition, S100B protein was analysed as reported before [26, 27].

Statistical analysis

The ANOVA test followed by post-test Tukey's Multiple Comparison Test was used to compare the values from

different groups; $*p < 0.05$; $**p < 0.01$ $***p < 0.001$. The box plot was used for the graphs. ROC curves and statistical analyses were performed using Graph Pad Prism 5 software. Correlations (Pearson r) and statistical significance (p value) between data obtained from ELISA and ECL methodologies in the same set of samples were calculated.

Ethics

The present study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and has been approved by the local ethics committee in Göttingen (No. 9/6/0). Informed consent was given by all study participants or their legal next of kin. All samples were anonymized.

Results

CSF levels of neurodegenerative diseases biomarkers in AD and sCJD

First, we examined whether Tau, Aβ42 and α-synuclein levels were altered in the CSF of AD and sCJD cases using the ECL-based MSD system (Fig. 1). ROC analysis was performed and AUC was calculated for each group in a comparative analysis between ECL and ELISA technologies (Table 1).

There was a statistically significant difference between Tau levels in both clinical groups when compared to control samples in agreement with previous reports [36]. While Tau levels are able to discriminate sCJD from control (AUC = 0.9931) and AD samples (AUC = 0.9583) with an excellent accuracy ($p < 0.001$), some overlap is observed between control and AD cases (AUC = 0.8125 and $p < 0.01$) (Fig. 1a; Table 1).

Low levels of Aβ42 were detected in AD ($p < 0.001$) and sCJD ($p < 0.05$) samples when compared to control cases (Fig. 1b) in agreement with previous data [33, 37]. Although no differences were observed between both clinical groups, Aβ42 levels can discriminate with higher accuracy controls from AD cases (AUC = 0.9097) than from sCJD samples (AUC = 0.7813) (Table 1).

Increased α-synuclein levels were detected in sCJD (AUC = 0.9306) when compared to control cases. No changes in α-synuclein levels could be detected between AD cases and control donors (AUC = 0.5030). Intriguingly, α-synuclein levels allow an excellent discrimination rate between AD and sCJD samples ($p < 0.001$, AUC = 0.9861) due to decreased levels, although not statistically significant, of α-synuclein in AD cases when compared to controls (Suppl. Table 1).

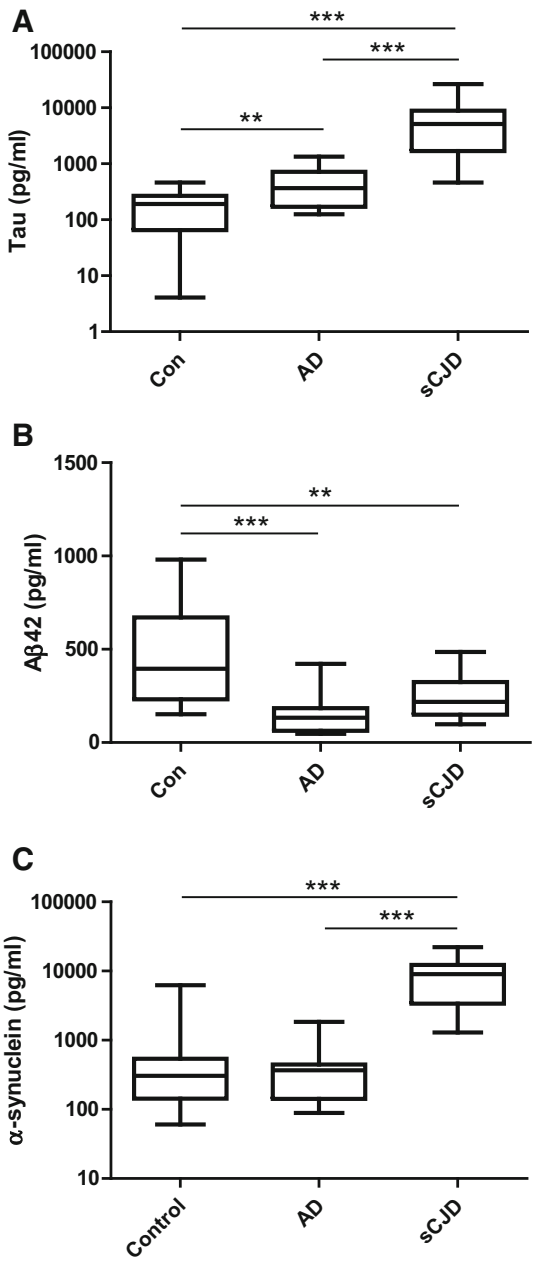

Fig. 1 ECL-based analysis of Tau, Aβ42 and α-synuclein in control, AD and sCJD cases. *Box plots* showing (**a**) Tau levels (**b**) Aβ42 levels and (**c**) α-synuclein levels in the CSF of Control, AD and sCJD patients. Tau and α-synuclein levels are displayed on a logarithmic scale (*y*-axis). Whiskers represent min to max for each group. ANOVA test followed by post-test Tukey's Multiple Comparison Test was used to compare the values from different groups. *P* values for the comparisons of the three groups are indicated in the figure: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

By comparing both detection systems, we found a good correlation between ECL and ELISA methods for Tau ($r = 0.81$) and Aβ42 ($r = 0.78$), while a weaker but positive correlation was observed for α-synuclein ($r = 0.61$) between both methods (Table 1). Increased AUC values

Table 1 Comparison of the clinical value of Tau, Aβ42 and α-synuclein biomarkers between ECL and ELISA methodologies. Regression values (*r*) correlating ECL and ELISA methodologies in the combined analysis of Control, AD and sCJD cases and AUC values for ELISA and ECL methodologies for each pair of groups

	r value	AUC					
		Con vs AD		Con vs sCJD		sCJD vs AD	
	ELISA vs MSD	ELISA	MSD	ELISA	MSD	ELISA	MSD
Tau	0.81	**0.8002**	**0.8125**	**0.9858**	**0.9931**	**0.9557**	**0.9653**
Aβ42	0.79	**0.8155**	**0.9097**	0.7058	0.7951	0.5489	0.7632
α-synuclein	0.61	0.6843	0.5139	**0.8269**	**0.9306**	0.7666	**0.9861**

AUC values greater than 0.8 are indicated in bold

are observed when using ECL methodology in comparison to ELISA kits. Increased AUC values are modest for Tau, but highly significant for Aβ42 and α-synuclein (Table 1).

Correlation between α-synuclein and Tau levels

Among the biomarkers analysed herein by ECL, Tau and α-synuclein levels present optimal discrimination rates between AD and sCJD patients. Thus, we explored a potential correlation between α-synuclein and Tau levels in sCJD and AD cases. A strong and significant positive relationship between the levels of both biomarkers was observed in sCJD patients using α-synuclein concentrations as measured by ECL and Tau concentrations as measured in both systems [ECL ($r = 0.85$) and ELISA ($r = 0.73$)] (Fig. 2a). A weaker but positive and significant correlation between α-synuclein and Tau ECL ($r = 0.85$) or ELISA ($r = 0.60$) data was also detected in AD cases where the levels of both proteins are significantly lower than those detected in sCJD (Fig. 2b). In addition, α-synuclein levels in sCJD significantly correlate with the levels of S100B ($r = 0.54$) (Suppl. Figure 1). S100B is an astroglial marker widely reported as a CSF biomarker for prion diseases, whose levels are highly upregulated in the CSF of CJD cases when compared to controls and AD [13]. α-synuclein levels were unrelated to the age of control and diseased patients (data not shown).

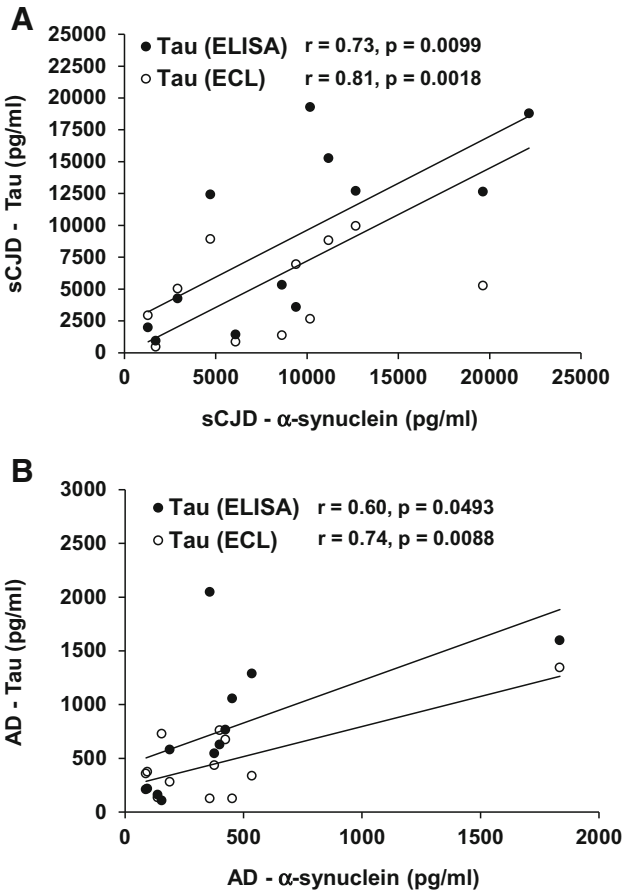

Fig. 2 Correlations between Tau and α-synuclein levels in AD and sCJD cases. Graphs showing the correlation between Tau and α-synuclein levels in the CSF of **a** sCJD and **b** AD cases. *R* values are indicated for each analysis. For Tau measurements, data obtained from ECL analysis (*empty circles*) and ELISA analysis (*full circles*) are plotted

Discussion

In the field of neurodegenerative diseases, the need for improved diagnostic accuracy urges for the identification of more reliable biomarkers as well as for the development of new optimized assays and methodologies for their quantification.

Development of quantitative assays for CSF biomarkers on the MSD platform has been discussed in several publications [5, 24] but detailed descriptions about their clinical performance for the differential diagnosis of neurodegenerative diseases are very limited.

The aim of the present work was to study whether the MSD platform presents a better performance in discriminating control, AD and sCJD cases than the well-established colorimetric ELISA platforms.

ECL-based quantification proves to be slightly superior (Tau) or significantly superior (Aβ42 and α-synuclein) in the discrimination of controls, AD and sCJD groups on

these groups of biomarkers, whose levels are deregulated in both diseases and currently used for their clinical diagnosis.

Low Aβ42 and high Tau levels are shown to be a characteristic feature in AD and sCJD cases, when compared to control samples and thus, an overlap between the detection of these biomarkers is commonly observed [32, 33]. The increased AUC values between the analysed groups for Tau and Aβ42 using ECL indicate that implementation of the ECL-based technologies system in the clinical work-up for both biomarkers could improve the diagnostic discrimination between AD and sCJD.

A new promising clinical outcome is derived from analysis of α-synuclein. The presence of an increased α-synuclein level in sCJD patients has already been reported during the last years. However, a large discrepancy on its clinical accuracy, most likely due to the different measurement tests used impeded the implementation in the clinical practice [16, 19, 20].

ECL shows superior performance to the established ELISA methods for the differential detection of α-synuclein in sCJD cases when compared to control and AD samples. This is in line with a previous report using a second generation of ELISA methodology, where only sCJD cases presented upregulated levels of α-synuclein in a cohort of several neurodegenerative diseases [20]. Interestingly, α-synuclein, contrary to Tau and Aβ42, is specifically regulated in sCJD. Although increased levels of α-synuclein have been reported in AD cases when compared to controls, these increases range from low to moderate and present no relevant clinical significance [34, 38].

Therefore, the introduction of α-synuclein in the clinical work-up of dementia diseases could improve the diagnostic accuracy and possibly monitor disease progression. Indeed, α-synuclein levels correlate with those observed for Tau, especially in sCJD. Since Tau has been extensively reported to be a marker of neuronal/axonal injury [6] and sCJD presents increased neuronal damage when compared to AD, it is tempting to speculate that α-synuclein could reflect the extent of synaptic damage in a similar manner as Tau reflects the extent of neuronal damage. In this line, we recently described that human α-synuclein in the CSF is mainly derived from neurons of the brain and spinal cord [22].

Since S100B levels in the CSF have been reported to reflect the disease-specific pathological mechanisms between sCJD and AD [13], the strong correlation between S100B levels and α-synuclein supports the idea that the differential α-synuclein levels in the CSF of sCJD and AD patients reflect the specific aetiology between both diseases.

On the other hand, CSF-Tau levels increased in parallel with CJD progression [29], while Tau levels remained stable along disease duration in patients suffering from AD and other non-prion dementias [2]. Thus, it will be interesting to study in larger cohorts of AD and sCJD samples if α-synuclein is able to track disease progression. In this regard, although α-synuclein is not clinically useful as a biomarker for α-synuclein-related disorders [10] and no association between α-synuclein levels in the CSF and PD severity has been observed [12], α-synuclein has been proposed to predict PD cognitive decline in one study [30]. New studies should also consider the presence of underlying modifying factors such as the presence of preclinical AD cases and co-morbidities (such as vascular events) which could influence the levels of the three CSF biomarkers analysed in the present study.

Major advantages of the ECL-based technology described here are: (1) Reduction in laboratory time: The ECL-based assay is performed in approximately 4 h. Conventional ELISAs sometimes take two days or longer due to overnight incubations, (2) Low volumes required for sample input, (3) Excellent reproducibility of quantification results: Compared to convention ELISA assays where up to 100 µl or more of precious samples are needed, the MSD platform requires only 25–50 µl diluted CSF, in our case diluted 1 in 8. Excellent reproducibility of the ECL-based system has also been reported (Kruse et al., manuscript in preparation).

Further studies on larger independent CSF cohorts of defined AD and sCJD patients, as well as in samples from patients suffering from other dementia types, are recommended to establish cut-off definitions and to validate the definitive performance of ECL measurements for Tau, Aβ42 and α-synuclein in the clinical routine for the differential diagnostic of neurodegenerative dementias.

However, the present data present a step forward toward their differential diagnosis. On one hand, from a methodological point of view, ECL-based MSD technology presents a high discrimination potential between control and diseased groups for Tau, Aβ42 and α-synuclein. On the other hand, we describe the gained value of α-synuclein measurements in the CSF as new sCJD biomarker which overcomes the overlap observed in classical dementia biomarkers (Tau, p-Tau and Aβ42) between AD and sCJD.

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Compliance with ethical standards

Conflicts of interest The authors declare that there are no conflicts of interest.

Ethical standard The study has been approved by the appropriate ethics committee and have therefore been performed in accordance

with the ethical standards laid down in the 1964 Declaration of Helsinki. As stated in the text, all persons gave their informed consent prior to their inclusion in the study and samples were anonymised.

References

1. Aguzzi A, Sigurdson C, Heikenwaelder M (2008) Molecular mechanisms of prion pathogenesis. Annu Rev Pathol 3:11–40
2. Andreasen N, Vanmechelen E, Van d V, Davidsson P, Hesse C, Tarvonen S, Raiha I, Sourander L, Winblad B, Blennow K (1998) Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow up study. J Neurol Neurosurg Psychiatry 64:298–305
3. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, Yamada M, Mizusawa H, Kitamoto T, Klug G, McGlade A, Collins SJ, Nishida N (2011) Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nat Med 17:175–178
4. Bibl M, Lewczuk P, Esselmann H, Mollenhauer B, Klafki HW, Welge V, Wolf S, Trenkwalder C, Otto M, Kornhuber J, Wiltfang J (2008) CSF amyloid-beta 1-38 and 1-42 in FTD and AD: biomarker performance critically depends on the detergent accessible fraction. Proteomics Clin Appl 2:1548–1556
5. Bibl M, Mollenhauer B, Lewczuk P, Esselmann H, Wolf S, Trenkwalder C, Otto M, Stiens G, Ruther E, Kornhuber J, Wiltfang J (2007) Validation of amyloid-beta peptides in CSF diagnosis of neurodegenerative dementias. Mol Psychiatry 12:671–680
6. Blennow K, Hampel H (2003) CSF markers for incipient Alzheimer's disease. Lancet Neurol 2:605–613
7. Colby DW, Prusiner SB (2011) Prions. Cold Spring Harb Perspect. Biol 3:a006833
8. Cramm M, Schmitz M, Karch A, Mitrova E, Kuhn F, Schroeder B, Raeber A, Varges D, Kim Y-S, Satoh K, Collins S, Zerr I (2015) Stability and reproducibility underscore utility of RT-QuIC for diagnosis of Creutzfeldt–Jakob disease. Mol Neurobiol. doi:10.1007/s12035-015-9133-2
9. Cramm M, Schmitz M, Karch A, Zafar S, Varges D, Mitrova E, Schroeder B, Raeber A, Kuhn F, Zerr I (2015) Characteristic CSF prion seeding efficiency in humans with prion diseases. Mol Neurobiol 51:396–405
10. Gao L, Tang H, Nie K, Wang L, Zhao J, Gan R, Huang J, Zhu R, Feng S, Duan Z, Zhang Y, Wang L (2014) Cerebrospinal fluid alpha-synuclein as a biomarker for Parkinson's disease diagnosis: a systematic review and meta-analysis. Int J Neurosci [Epub ahead of print]
11. Heinemann U, Krasnianski A, Meissner B, Varges D, Kallenberg K, Schulz-Schaeffer WJ, Steinhoff BJ, Grasbon-Frodl EM, Kretzschmar HA, Zerr I (2007) Creutzfeldt–Jakob disease in Germany: a prospective 12-year surveillance. Brain 130:1350–1359
12. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, Jankovic J, Zabetian CP, Leverenz JB, Baird G, Montine TJ, Hancock AM, Hwang H, Pan C, Bradner J, Kang UJ, Jensen PH, Zhang J (2010) DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. Brain 133:713–726
13. Jesse S, Steinacker P, Cepek L, von Arnim CA, Tumani H, Lehnert S, Kretzschmar HA, Baier M, Otto M (2009) Glial fibrillary acidic protein and protein S-100B: different concentration pattern of glial proteins in cerebrospinal fluid of patients with Alzheimer's disease and Creutzfeldt–Jakob disease. J Alzheimers Dis 17:541–551
14. Kaerst L, Kuhlmann A, Wedekind D, Stoeck K, Lange P, Zerr I (2013) Cerebrospinal fluid biomarkers in Alzheimer's disease, vascular dementia and ischemic stroke patients: a critical analysis. J Neurol 260:2722–2727
15. Kaerst L, Kuhlmann A, Wedekind D, Stoeck K, Lange P, Zerr I (2014) Using cerebrospinal fluid marker profiles in clinical diagnosis of dementia with Lewy bodies, Parkinson's disease, and Alzheimer's disease. J Alzheimers Dis 38:63–73
16. Kasai T, Tokuda T, Ishii R, Ishigami N, Tsuboi Y, Nakagawa M, Mizuno T, El-Agnaf OM (2014) Increased alpha-synuclein levels in the cerebrospinal fluid of patients with Creutzfeldt–Jakob disease. J Neurol 261:1203–1209
17. Kruse N, Schulz-Schaeffer WJ, Schlossmacher MG, Mollenhauer B (2012) Development of electrochemiluminescence-based singleplex and multiplex assays for the quantification of alpha-synuclein and other proteins in cerebrospinal fluid. Methods 56:514–518
18. Lazaro DF, Rodrigues EF, Langohr R, Shahpasandzadeh H, Ribeiro T, Guerreiro P, Gerhardt E, Krohnert K, Klucken J, Pereira MD, Popova B, Kruse N, Mollenhauer B, Rizzoli SO, Braus GH, Danzer KM, Outeiro TF (2014) Systematic comparison of the effects of alpha-synuclein mutations on its oligomerization and aggregation. PLoS Genet 10:e1004741
19. Llorens F, Zafar S, Ansoleaga B, Shafiq M, Blanco R, Carmona M, Grau-Rivera O, Nos C, Gelpí E, Del Río JA, Zerr I, Ferrer I (2014) Subtype and regional regulation of prion biomarkers in sporadic Creutzfeldt–Jakob disease. Neuropathol Appl Neurobiol. doi:10.1111/nan.12175
20. Mollenhauer B, Cullen V, Kahn I, Krastins B, Outeiro TF, Pepivani I, Ng J, Schulz-Schaeffer W, Kretzschmar HA, McLean PJ, Trenkwalder C, Sarracino DA, Vonsattel JP, Locascio JJ, El-Agnaf OM, Schlossmacher MG (2008) Direct quantification of CSF alpha-synuclein by ELISA and first cross-sectional study in patients with neurodegeneration. Exp Neurol 213:315–325
21. Mollenhauer B, Esselmann H, Roeber S, Schulz-Schaeffer WJ, Trenkwalder C, Bibl M, Steinacker P, Kretzschmar HA, Wiltfang J, Otto M (2011) Different CSF beta-amyloid processing in Alzheimer's and Creutzfeldt–Jakob disease. J Neural Transm 118:691–697
22. Mollenhauer B, Trautmann E, Otte B, Ng J, Spreer A, Lange P, Sixel-Doring F, Hakimi M, Vonsattel JP, Nussbaum R, Trenkwalder C, Schlossmacher MG (2012) alpha-Synuclein in human cerebrospinal fluid is principally derived from neurons of the central nervous system. J Neural Transm 119:739–746
23. Otto M, Wiltfang J, Cepek L, Neumann M, Mollenhauer B, Steinacker P, Ciesielczyk B, Schulz-Schaeffer W, Kretzschmar HA, Poser S (2002) Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt–Jakob disease. Neurology 58:192–197
24. Pan C, Korff A, Galasko D, Ginghina C, Peskind E, Li G, Quinn J, Montine TJ, Cain K, Shi M, Zhang J (2015) Diagnostic values of Cerebrospinal Fluid T-Tau and Abeta42 using meso scale discovery assays for Alzheimer's disease. J Alzheimers Dis 45:709–719
25. Riemenschneider M, Wagenpfeil S, Vanderstichele H, Otto M, Wiltfang J, Kretzschmar H, Vanmechelen E, Forstl H, Kurz A (2003) Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt–Jakob disease from other dementias. Mol Psychiatry 8:343–347
26. Sanchez-Juan P, Green A, Ladogana A, Cuadrado-Corrales N, Saanchez-Valle R, Mitrovaa E, Stoeck K, Sklaviadis T, Kulczycki J, Hess K, Bodemer M, Slivarichova D, Saiz A, Calero M, Ingrosso L, Knight R, Janssens AC, van Duijn CM, Zerr I (2006) CSF tests in the differential diagnosis of Creutzfeldt–Jakob disease. Neurology 67:637–643

27. Sanchez-Juan P, Sanchez-Valle R, Green A, Ladogana A, Cuadrado-Corrales N, Mitrova E, Stoeck K, Sklaviadis T, Kulczycki J, Hess K, Krasnianski A, Equestre M, Slivarichova D, Saiz A, Calero M, Pocchiari M, Knight R, van Duijn CM, Zerr I (2007) Influence of timing on CSF tests value for Creutzfeldt–Jakob disease diagnosis. J Neurol 254:901–906
28. Schmidt C, Wolff M, Weitz M, Bartlau T, Korth C, Zerr I (2011) Rapidly progressive Alzheimer disease. Arch Neurol 68:1124–1130
29. Skillback T, Rosen C, Asztely F, Mattsson N, Blennow K, Zetterberg H (2014) Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt–Jakob disease: results from the Swedish Mortality Registry. JAMA Neurol 71:476–483
30. Stewart T, Liu C, Ginghina C, Cain KC, Auinger P, Cholerton B, Shi M, Zhang J (2014) Cerebrospinal fluid alpha-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort. Am J Pathol 184:966–975
31. Stoeck K, Sanchez-Juan P, Gawinecka J, Green A, Ladogana A, Pocchiari M, Sanchez-Valle R, Mitrova E, Sklaviadis T, Kulczycki J, Slivarichova D, Saiz A, Calero M, Knight R, Aguzzi A, Laplanche JL, Peoc'h K, Schelzke G, Karch A, van Duijn CM, Zerr I (2012) Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt–Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. Brain 135:3051–3061
32. Stoeck K, Sanchez-Juan P, Gawinecka J, Green A, Ladogana A, Pocchiari M, Sanchez-Valle R, Mitrova E, Sklaviadis T, Kulczycki J, Slivarichova D, Saiz A, Calero M, Knight R, Aguzzi A, Laplanche JL, Peoc'h K, Schelzke G, Karch A, van Duijn CM, Zerr I (2012) Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt–Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. Brain 135:3051–3061
33. Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, Bergeson J, Manetti GJ, Zimmermann M, Tang B, Bartko JJ, Cohen RM (2003) Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. JAMA 289:2094–2103
34. Tateno F, Sakakibara R, Kawai T, Kishi M, Murano T (2012) Alpha-synuclein in the cerebrospinal fluid differentiates synucleinopathies (Parkinson disease, dementia with Lewy bodies, multiple system atrophy) from Alzheimer disease. Alzheimer Dis Assoc Disord 26:213–216
35. Tokuda T, Salem SA, Allsop D, Mizuno T, Nakagawa M, Qureshi MM, Locascio JJ, Schlossmacher MG, El-Agnaf OM (2006) Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. Biochem Biophys Res Commun 349:162–166
36. van Harten AC, Kester MI, Visser PJ, Blankenstein MA, Pijnenburg YA, van der Flier WM, Scheltens P (2011) Tau and p-tau as CSF biomarkers in dementia: a meta-analysis. Clin Chem Lab Med 49:353–366
37. Van EB, Green AJ, Pals P, Martin JJ, Cras P (1999) Decreased levels of Amyloid-beta 1-42 in cerebrospinal fluid of Creutzfeldt–Jakob disease patients. J Alzheimers Dis 1:419–424
38. Wennstrom M, Surova Y, Hall S, Nilsson C, Minthon L, Bostrom F, Hansson O, Nielsen HM (2013) Low CSF levels of both alpha-synuclein and the alpha-synuclein cleaving enzyme neurosin in patients with synucleinopathy. PLoS ONE 8:e53250
39. Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, Windl O, Kretzschmar HA, Weber T (1998) Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt–Jakob disease. Ann Neurol 43:32–40
40. Zerr I, Kallenberg K, Summers DM, Romero C, Taratuto A, Heinemann U, Breithaupt M, Varges D, Meissner B, Ladogana A, Schuur M, Haik S, Collins SJ, Jansen GH, Stokin GB, Pimentel J, Hewer E, Collie D, Smith P, Roberts H, Brandel JP, van DC, Pocchiari M, Begue C, Cras P, Will RG, Sanchez-Juan P (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt–Jakob disease. Brain 132:2659–2668
41. Zerr I, Pocchiari M, Collins S, Brandel JP, de Pedro CJ, Knight RS, Bernheimer H, Cardone F, Delasnerie-Laupretre N, Cuadrado CN, Ladogana A, Bodemer M, Fletcher A, Awan T, Ruiz BA, Budka H, Laplanche JL, Will RG, Poser S (2000) Analysis of EEG and CSF 14-3-3 proteins as aids to the diagnosis of Creutzfeldt–Jakob disease. Neurology 55:811–815