Research article

Brain-derived proteins in the CSF, do they correlate with brain pathology in CJD?

Constanze Boesenberg-Grosse¹, Walter J Schulz-Schaeffer², Monika Bodemer¹, Barbara Ciesielczyk¹, Bettina Meissner², Anna Krasnianski¹, Mario Bartl¹, Uta Heinemann¹, Daniela Varges¹, Sabina Eigenbrod³, Hans A Kretzschmar³, Alison Green⁴ and Inga Zerr*¹

Address: ¹National Reference Center for TSE Surveillance at the Dept. of Neurology, Georg-August-University Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany, ²Dept. of Neuropathology, Georg-August-University Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany, ³Institute of Neuropathology, LMU München, Föedor-Lyén-Str. 23, 81377 München, Germany and ⁴National CJD Surveillance Unit, The University of Edinburgh, EH4 2XJ Edinburgh, UK

Email: Constanze Boesenberg-Grosse - yynoc2001@web.de; Walter J Schulz-Schaeffer - wjschulz@med.uni-goettingen.de; Monika Bodemer - mbodemer@med.uni-goettingen.de; Barbara Ciesielczyk - bciesiel@med.uni-goettingen.de; Bettina Meissner - betti.meissner@med.uni-goettingen.de; Anna Krasnianski - anna.krasnianski@med.uni-goettingen.de; Mario Bartl - m.bartl@web.de; Uta Heinemann - uta.heinemann@med.uni-goettingen.de; Daniela Varges - d.varges@gmx.de; Sabina Eigenbrod - s.eigenbrod@med.unimuenchen.de; Hans A Kretzschmar - hans.kretzschmar@med.uni-muenchen.de; Alison Green - alison.green@ed.ac.uk; Inga Zerr* - inga.zerr@med.uni-goettingen.de

* Corresponding author

Abstract

Background: Brain derived proteins such as 14-3-3, neuron-specific enolase (NSE), S 100b, tau, phosphorylated tau and Aβ₁₋₄₂ were found to be altered in the cerebrospinal fluid (CSF) in Creutzfeldt-Jakob disease (CJD) patients. The pathogenic mechanisms leading to these abnormalities are not known, but a relation to rapid neuronal damage is assumed. No systematic analysis on brain-derived proteins in the CSF and neuropathological lesion profiles has been performed.

Methods: CSF protein levels of brain-derived proteins and the degree of spongiform changes, neuronal loss and gliosis in various brain areas were analyzed in 57 CJD patients.

Results: We observed three different patterns of CSF alteration associated with the degree of cortical and subcortical changes. NSE levels increased with lesion severity of subcortical areas. Tau and 14-3-3 levels increased with minor pathological changes, a negative correlation was observed with severity of cortical lesions. Levels of the physiological form of the prion protein (PrPc) and Aβ₁₋₄₂ levels correlated negatively with cortical pathology, most clearly with temporal and occipital lesions.

Conclusion: Our results indicate that the alteration of levels of brain-derived proteins in the CSF does not only reflect the degree of neuronal damage, but it is also modified by the localization on the brain pathology. Brain specific lesion patterns have to be considered when analyzing CSF neuronal proteins.
Background
In recent years, the analysis of the cerebrospinal fluid (CSF) has become increasingly important to support the clinical diagnosis in patients with sporadic Creutzfeldt-Jakob disease (sCJD). Various brain-derived proteins have been studied in the CSF to date, such as 14-3-3 proteins, gamma-enolase (neuron-specific enolase, NSE), tau, phosphorylated tau, S-100b and Aβ1-42 [1-11]. These studies were mainly focused on diagnostic aspects. Elevated levels of these proteins were used as surrogate parameters for neuronal damage (14-3-3, tau, gamma-enolase) or astrocytic gliosis (S-100b), following the idea that CSF proteins reflect changes in pathological brain conditions.

Although a lot of information has been gained about abnormal CSF levels of these proteins, only few publications report on levels of particular proteins and disease stage or even brain pathology [12-16]. It was assumed that those protein levels correlate with neuronal damage or astrocytic gliosis. However, the question of whether particular protein level abnormalities reflect changes in certain brain areas affected has not been addressed so far.

In this study, we have investigated brain-derived proteins such as the physiological form of the prion protein (PrPC), 14-3-3 proteins, tau, phosphorylated tau, NSE and S-100b in cerebrospinal fluid (CSF) in patients with sCJD with respect to the neuropathological lesion profile in these patients.

Methods
Study design
Patients
The study population comprised 57 sporadic CJD according to criteria who were registered at the National Reference Center for TSE Surveillance in Göttingen from June 1993 to August 2003 with clinical data, CSF samples and brain lesion profile scoring available (see below) [17-19]. Iatrogenic and familial or genetic cases were excluded.

The study was approved by the Ethic Committee of the Medical Faculty in Göttingen, Germany, (approvals 11/11/93 from 18th September 1996, amendments from 11/11/93 from 12th September 2002 and 30/1/05 from 18th February 2005). The informed consent of the relatives was obtained.

Clinical data concerning sex, date of birth/death, age at disease onset, disease duration, date of lumbar puncture, were collected from examination protocols and medical charts.

The analysis of the codon 129 genotype of the prion protein gene (PRNP) was performed after isolation of genomic DNA from blood according to standard methods [20]. None of the cases carried a mutation (n = 55). Because the primary goal of the study was to analyze the correlation between brain lesion profiles and CSF markers and because only of the limited numbers of PrPSc-typing data were not stratified by different molecular subtypes.

Neuropathology
Nine brain regions were investigated for the degree of spongiform changes, gliosis and nerve cell loss. They comprised five cortical regions (medial frontal gyrus, cingulate gyrus, inferior temporal gyrus, inferior parietal gyrus and area striata and parastriatata), three subcortical regions (caudate nucleus, putamen and medio-dorsal thalamus) and vermis cerebelli. An investigator blinded for clinical data classified the pathological changes semiquantitatively for each section (0–4 points for no change, mild, moderate, severe or maximal changes) [21,22]. The intensity was estimated for spongiform changes and nerve cell loss. For gliosis, the quantity of glial proliferation, including nuclear pleomorphy and gemistocytic changes of the cytoplasm, was assessed. A status spongiosus with collapsing tissue matrix, severe astrocytic gliosis and nearly complete nerve cell loss was considered as the maximal change. The reliability of the neuropathological scoring profiles was assessed before and revealed comparable results between the investigators in former investigations [22].

Biochemical CSF analysis
The routine investigations of the CSF did not reveal any abnormalities with respect to cell count and protein profile.

All CSF samples were analyzed with respect to 14-3-3 proteins, neuron specific enolase (NSE), tau, phosphorylated tau, S-100b, PrPc and Aβ1-42 in the reference laboratory of the Surveillance Unit in Göttingen according to standards classified previously [2,23-26]

The 14-3-3 capture kit, Repairgenics, Bioproducts (Mainz, Germany, until July 2003, now Schwerin, Germany), was used for 14-3-3 detection, LIAISON® NSE for NSE detection, LIAISON® SANGTEC® 100 for S-100b detection, INNOTEST™ hTauAg by INNOGENETICS for tau detection, INNOTEST™ Phospho Tau for the detection of the phosphorylated tau at residue 181, INNOTEST™ β-Amyloid (1–42) for β-Amyloid. PrPc (comprising PrPSc and potentially to minor degree PrPSc) concentrations were determined by using a commercially available ELISA for detection of the abnormal PrPSc (Platelia BSE detection kit, BIO-RAD Laboratories GmbH, Munich, Germany). The following modifications were made to the test protocol: the proteinase K digestion step, which is used to degrade the normal, but not the abnormal form of the prion pro-
tein, was omitted, since we were interested in detecting all PrPc which was present in the sample. To quantify levels of PrP, a standard curve using recombinant human prion protein (Prionics AG, Zurich, Switzerland) was used in each experiment, according to another PrP-ELISA protocol [25,27].

NSE was determined in all 57 patients, tau protein in 56, phosphorylated tau in 33, S-100b protein in 54, Aβ1–42 in 41, PrPc in 45 and 14-3-3 in 51 cases.

Statistical analysis
A regression analysis with respect to the degree of neuropathological changes in all nine examined brain regions and the concentrations of the CSF markers was performed.

The statistics software package we used for our calculations was SigmaStat 3.1 and SigmaPlot 9.0 by Systat Software Inc., Point Richmond, USA. A linear regression and the calculation of the Pearson Product Moment Correlation Coefficient were performed for the analysis of any trends between neuropathological lesion profiles and concentrations of CSF markers.

Results
Study population
Clinical data on 57 sCJD cases and the CSF concentration of tau, phosphorylated tau, 14-3-3, S100b, NSE, PrPc and Aβ1–42 are given in Tables 1 and 2. Looking at the time of the lumbar puncture within the whole disease course, 6 patients had their CSF taken in the first third, 12 patients in the second third and 39 patients during the last third of the disease course (Table 1). The stratification of the data by time of lumbar puncture and the exclusion of single cases with extremely long disease duration or a lumbar puncture very early at onset did not change the results presented here (data not shown).

Correlation of neuropathological lesion profiles and concentrations of brain-derived proteins in the CSF
The degree of neuropathological changes (spongiform changes, neuronal loss and gliosis) in nine defined brain areas was analyzed. The severity of spongiform changes correlates with neuronal loss and gliosis (data not shown).

A regression analysis of the neuropathological lesion profiles and the concentrations of the CSF markers was carried out. The correlation coefficients were calculated and significant results (\( p \leq 0.05 \)) are indicated (Tables 3, 4, 5).

We analyzed if there is a correlation between the NSE concentration in the CSF and neuropathological changes. The NSE concentrations showed no correlation with the degree of spongiform change, gliosis and nerve cell loss in the cortical regions. However, elevated NSE levels correlated significantly with the degree of gliosis in the basal ganglia and cerebellum and with the degree of spongiform changes in the thalamus.

The tau protein levels correlated negatively with the degree of neuropathological changes in cortical regions. A positive correlation was also found between the degree of spongiform changes and gliosis in the cerebellum. The tau concentrations showed no correlation with the degree of spongiform changes and neuronal loss in the basal ganglia, in contrast to the degree of gliosis in the basal ganglia, where a positive correlation was found.

The levels of phosphorylated tau concentrations show negative correlations with the degree of neuropathological changes in cortical regions. A negative correlation was also found between the degree of neuronal loss and gliosis in the thalamic region, whereas a positive correlation was found between spongiform changes and the thalamus. Spongiform changes in the cerebellum correlated in a positive way with phosphorylated tau levels. No correlation was found between the degree of neuropathological changes and phosphorylated tau levels.

14-3-3 protein levels correlated negatively with the degree of spongiform changes in the cortical regions and with the degree of neuronal loss in the thalamus. The 14-3-3 protein levels showed a slightly positive correlation with the degree of spongiosis in the thalamus. For other brain regions, no correlation was observed.

The S 100b concentrations correlated negatively with neuropathological changes cortical regions and with the degree of gliosis and nerve cell loss in the thalamus. In contrast to this, a positive correlation was found with the degree of spongiform change in the thalamus and the degree of gliosis in the basal ganglia and cerebellum.

PrPc concentrations in the CSF were negatively correlated with the degree of neuropathological changes in most cortical regions. A negative correlation was also found with the degree of gliosis and neuronal loss in the basal ganglia. The PrPc levels correlated negatively with the degree of neuronal loss in the cerebellum and the degree of gliosis in the thalamus. However, PrPc levels correlated significantly in a positive way with the degree of spongiform changes in the cerebellum.

The Aβ1–42 concentrations were negatively correlated with the degree of neuropathological changes in all cortical regions and the basal ganglia. A negative correlation was also found between the degree of neuronal loss and gliosis.
in the thalamus. Aβ1–42 concentrations correlated positively with spongiform changes in the cerebellum.

A synopsis on correlation of various brain-derived proteins and degree of pathological changes in various cortical and subcortical regions is shown in Table 6. In general, for cortical lesions, NSE levels do not correlate with severity of pathological changes (they do not increase further with lesion severity), whereas all other proteins analyzed showed a tendency towards lower levels with the increasing severity of pathological changes. This effect was more pronounced for Aβ1–42 and neuronal loss in temporal regions, cingulated gyrus and parietal regions. The analysis of the pathological changes in subcortical areas showed more diverse results. The degree of pathological changes in the basal ganglia correlated with a tendency of NSE levels to increase and PrPc and Aβ1–42 levels to decrease. The severity of neuronal loss and gliosis in the thalamus correlated negatively with concentrations of Aβ1–42, S-100b and phosphorylated tau levels. A positive correlation was seen for spongiform changes and levels of 14-3-3, NSE, S 100b, and phosphorylated tau.

Figures 1, 2, 3, 4, 5 show the correlation between degree of neuronal loss in various brain areas and CSF levels of NSE, tau, 14-3-3, Aβ1–42 and PrPc.

**Discussion**

In CJD patients, most attention has been concentrated on investigating the role and the biochemical properties of the pathological form of the prion protein (PrPsc) in central nervous system tissue. Brain derived proteins were mainly studied with respect to their clinical diagnostic potential rather than to reflect the severity of brain lesions in CJD. The mechanisms of elevation of brain-derived proteins in the CSF in patients with CJD and other acute neurological diseases are not known in detail and the current (yet unproven) hypothesis suggests a leakage into the CSF following rapid neuronal damage [1,2,28-30].

The earliest markers studied in CJD were NSE and S 100b proteins, which were shown to be elevated in the CSF during the disease progression, but a subsequent study was done only in one patient with repeated lumbar punctures [13]. In other neurological diseases, high NSE levels in CSF and serum were used as a prognostic marker of acute neuronal damage (e.g. hypoxia or ischemia) and disease progression [31,32]. In CJD, NSE concentrations increased to a maximum when the disease activity was most prominent and returned to normal or mildly elevated levels in the terminal stage [13]. These results imply that these protein levels can serve as biochemical markers for the presence of an acute neuronal loss in CJD brain [13]. In addition, other studies assumed that measurement of the NSE might correlate with the disease progression and NSE levels decrease with reduced numbers of remaining neuronal cells [15].

We observed that NSE levels increase with severity of neuronal lesions already when only minor changes take place and they are clearly correlated to the damage of subcortical grey matter nuclei, in particular the thalamus, but also...
Table 3: Severity of lesion and concentrations of CSF markers (correlation coefficient): Spongiform changes

| Cortical regions | Subcortical regions | Cerebellum |
|------------------|---------------------|------------|
|                  | CG°                 | MFG°       | ITG°       | IPG°       | AS°      | Putamen | Caudate ncl. | MDT° |
| NSE              | 0.0709              | 0.0731     | -0.0351    | -0.0485    | -0.0267  | 0.164   | 0.14       | 0.305* | 0.034    |
| Tau              | -0.173              | -0.313*    | -0.219     | -0.309*    | -0.217   | -0.0158 | -0.0963   | 0.024  | 0.239    |
| Tau phosphorylated | -0.302              | -0.0729    | -0.227     | -0.440*    | -0.195   | -0.0431 | 0.0466     | 0.282  | 0.375    |
| 14-3-3           | -0.142              | -0.0968    | -0.275     | -0.317     | -0.198   | 0.0631  | 0.190      | 0.246  | 0.179    |
| S 100b           | -0.0686             | -0.0459    | -0.303*    | -0.282     | -0.359*  | 0.0893  | 0.0864     | 0.242  | -0.005   |
| PrP              | -0.276              | -0.0955    | -0.307     | -0.364*    | -0.335*  | 0.0109  | -0.0289    | 0.00314 | 0.451*   |
| $A\beta_{1-42}$  | -0.453*             | -0.233     | -0.560*    | -0.356*    | -0.426*  | -0.159  | -0.248     | 0.0489 | 0.465*   |

* MDT: medio-dorsal thalamus
CG: cingulate gyrus
MFG: medial frontal gyrus
ITG: inferior temporal gyrus
IPG: inferior parietal gyrus
AS: area striata
* significant, p \(\leq 0.05\)

Table 4: Severity of lesion and concentrations of CSF markers (correlation coefficient): Neuronal loss

| Cortical regions | Subcortical regions | Cerebellum |
|------------------|---------------------|------------|
|                  | CG°                 | MFG°       | ITG°       | IPG°       | AS°      | Putamen | Caudate ncl. | MDT° |
| NSE              | 0.0183              | -0.0846    | 0.0428     | -0.0769    | -0.0586  | 0.216   | 0.216      | -0.114 | 0.202    |
| Tau              | -0.233              | -0.229     | -0.159     | -0.276     | -0.158   | 0.121   | 0.0516     | -0.257 | -0.140   |
| Tau phosphorylated | -0.216              | -0.236     | -0.337     | -0.454*    | -0.505*  | -0.128  | -0.130     | -0.235 | -0.058   |
| 14-3-3           | -0.146              | -0.170     | -0.103     | -0.154     | -0.118   | 0.0184  | 0.0698     | -0.266 | -0.127   |
| S 100b           | -0.169              | -0.203     | -0.192     | -0.344*    | -0.282*  | 0.195   | 0.199      | -0.254 | -0.0115  |
| PrP              | -0.240              | -0.219     | -0.457*    | -0.318     | -0.511*  | -0.260  | -0.144     | -0.159 | -0.279   |
| $A\beta_{1-42}$  | -0.386              | -0.295     | -0.661*    | -0.315     | -0.488*  | -0.387  | -0.302     | -0.321 | -0.0136  |

* MDT: medio-dorsal thalamus
CG: cingulate gyrus
MFG: medial frontal gyrus
ITG: inferior temporal gyrus
IPG: inferior parietal gyrus
AS: area striata
* significant, p \(\leq 0.05\)

Table 5: Severity of lesion and concentrations of CSF markers (correlation coefficient): Gliosis

| Cortical regions | Subcortical regions | Cerebellum |
|------------------|---------------------|------------|
|                  | CG°                 | MFG°       | ITG°       | IPG°       | AS°      | Putamen | Caudate ncl. | MDT° |
| NSE              | -0.0553             | -0.0280    | 0.0104     | -0.0345    | -0.0445  | 0.298*  | 0.265*      | 0.0413 | 0.386*   |
| Tau              | -0.242              | -0.174     | -0.178     | -0.160     | -0.207   | 0.141   | 0.254      | -0.0726 | 0.236    |
| Tau phosphorylated | -0.423*             | -0.175     | -0.496*    | -0.479*    | -0.536*  | -0.131  | -0.111     | -0.260  | -0.090   |
| 14-3-3           | -0.189              | -0.185     | -0.185     | -0.0348    | -0.114   | 0.0775  | 0.198      | -0.132  | -0.110   |
| S 100b           | -0.261              | -0.188     | -0.195     | -0.271     | -0.245   | 0.262   | 0.323*     | -0.236  | 0.321    |
| PrP              | -0.344*             | -0.260     | -0.426*    | -0.383*    | -0.511*  | -0.250  | -0.168     | -0.409* | -0.026   |
| $A\beta_{1-42}$  | -0.517*             | -0.387*    | -0.495*    | -0.396*    | -0.462*  | -0.306  | -0.357*    | -0.387* | 0.038    |

* MDT: medio-dorsal thalamus
CG: cingulate gyrus
MFG: medial frontal gyrus
ITG: inferior temporal gyrus
IPG: inferior parietal gyrus
AS: area striata
* significant, p \(\leq 0.05\).
The PrP\textsuperscript{c} concentrations showed a high correlation with the degree of gliosis and nerve cell loss in the area striata and the inferior temporal gyrus and with spongiform changes in the cerebellum. Levels of PrP\textsuperscript{c} and A\textsubscript{β}\textsubscript{1-42} clearly correlated with the degree of pathology in cortical structures, but not with basal ganglia and thalamic pathology in CJD. This pattern was consistent in the analysis of various cortical regions and the most striking effect was seen for A\textsubscript{β}\textsubscript{1-42} when compared to the degree of lesion severity in the temporal lobe and cingulate gyrus.

**Conclusion**

Taken together, we observed three different patterns of CSF protein levels associated with the degree of cortical and subcortical changes. The first one was seen for NSE. Levels increased with only mild cortical and subcortical changes and increased further with the degree of subcortical changes.

---

**Table 6: Synopsis of the degree of pathological changes and levels of various brain-derived proteins in the CSF**

| Spongiform change/neuronal loss/gliosis * | Cortical Regions | Subcortical Regions | Cerebellum |
|------------------------------------------|------------------|---------------------|-----------|
|                                          | basal ganglia    | thalamus            |           |
| NSE                                      | ↔/↔/↔/↔         | ←/↑/↑/↑           | ←/↑/↑/↑   |
| tau                                      | ↓/↓/↑/↑         | ←/↑/↑/↑           | ↑/↑/↑/↑   |
| phosphorylated tau                       | ↓/↑/↑/↑         | ↑/↑/↑/↑           | ↑/↑/↑/↑   |
| 14-3-3                                   | ↓/↓/↓/↓         | ↑/↑/↑/↑           | ↑/↑/↑/↑   |
| 14-3-3                                   | ↓/↓/↓/↓         | ↑/↑/↑/↑           | ↑/↑/↑/↑   |
| S 100b                                   | ↓/↓/↓/↓         | ↑/↑/↑/↑           | ↑/↑/↑/↑   |
| PrP                                      | ↓/↑/↓/↓         | ↑/↑/↑/↑           | ↑/↑/↑/↑   |
| A\textsubscript{β}1-42                   | ↓/↓/↓/↓         | ↑/↑/↑/↑           | ↑/↑/↑/↑   |

*: within the cells in the table the first arrow shows spongiform change, the second shows nerve cell loss and the third gliosis; only the strongest neuropathological changes among the different regions are shown in the table

****: strong correlation

 ↔ correlation coefficient from -0.2 to +0.2: no correlation

 ↑ correlation coefficient from +0.2 to +0.6: weak correlation

 ↓ correlation coefficient from -0.2 to -0.6: weak correlation

↑↑ correlation coefficient from +0.2 to +0.6: strong correlation

---
Figure 1
Correlation between CSF levels of neuron-specific enolase and degree of neuronal loss in various brain areas. (- - - - - cut-off 12.5 ng/ml).
Figure 2
Correlation between tau CSF levels and degree of neuronal loss in various brain areas. (-- - - - cut-off 195 pg/ml).
Figure 3
Correlation between 14-3-3 CSF levels and degree of neuronal loss in various brain areas. (--- --- cut-off 200 ng/ml).
Correlation between PrP CSF levels and degree of neuronal loss in various brain areas. (--- cut-off 22 ng/100 ml).

Figure 4
Correlation between Aβ₁₋₄₂ CSF levels and degree of neuronal loss in various brain areas. (--- cut-off 849 pg/ml).

Figure 5
Correlation between Aβ₁₋₄₂ CSF levels and degree of neuronal loss in various brain areas. (--- cut-off 849 pg/ml).
The second pattern was seen for tau and 14-3-3. There is a clear elevation of the levels of these proteins in the CSF in disease stages when the degree of spongiform changes, neuronal loss and gliosis in cortical areas are relatively small. In later stages, the levels correlate with the degree of neuronal damage in cortical structures and decline. One can only speculate on the mechanisms for this finding: a potential for increased synthesis or upregulation at early stages when the brain damage is relatively small cannot completely be excluded [37,38].

A completely different mechanism must be discussed for PrPc and Aβ1–42. Normal levels are measured in the CSF for those proteins at initial stages. With increasing severity of cortical lesions they decrease. Levels of PrPc and Aβ1–42 levels are independent of the degree of the brain damage in subcortical areas. Since PrPc is mainly synthesized in neurons, one can assume that PrPc CSF levels are determined by the degree of neuronal loss, mainly in cortical areas [39]. Among the latter, pathological changes in the inferior temporal lobe seem to have the most effect on PrPc and Aβ1–42 levels in the CSF.

To conclude, brain specific lesion patterns have to be considered when analyzing CSF neuronal proteins.

Competing interests
The author(s) declare that they have no competing interests.

Authors' contributions
CB-G, WJS-S and IZ conceived of the study concept and design. CB-G, WJS-S, MB, AK, BM, UH, DV and AG were responsible for acquisition of data. CB-G, AK, UH, BM and IZ carried out the analysis and interpretation of data. CB-G, WJS-S and IZ participated in the drafting of the manuscript. IZ analysed and revised the manuscript for important intellectual content. HAK and IZ obtained funding. MB, BC and SE provided administrative, technical and material support. IZ supervised the study.

All authors read and approved the final manuscript.

Acknowledgements
The study was supported by grants from the Bundesministerium für Gesundheit und Soziale Sicherung (BMGS) (GZ: 325-4471-02/15) to HAK and IZ, from the Bundesministerium für Bildung und Forschung (BMBF) (01GI0301 and KZ: 0312720) to IZ and by the European Commission (EC) (QLG3-CT-2002-81606) to IZ.

References
1. Hisch G, Kenney K, Gibbs Jr, Cj, Lee KH, Harrington MG: The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. N Engl J Med 1996, 335(13):924-930.
2. Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, Windl O, Kretzschmar HA, Weber T: Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 1998, 43(1):32-40.
3. Groth A: Use of 14-3-3 in the diagnosis of Creutzfeldt-Jakob disease. Biochem Soc Symp 2002, 30:382-386.
4. Kohira I, Tsuji T, Ishizu H, Takao Y, Wake A, Abe K, Kuroda S: Elevation of neuron-specific enolase in serum and cerebrospinal fluid of early stage Creutzfeldt-Jakob disease. Acta Neurol Scand 2000, 102:385-387.
5. Van Everbroeck B, Green A, Martenj, Cras P: Decreased levels of amyloid-beta 1-42 in cerebrospinal fluid of Creutzfeldt-Jakob disease patients. J Alzheimers Dis 1999, 1:419-424.
6. Van Everbroeck B, Green A, Vanmechelen E, Vanderstichele H, Pals P, Sanchez-Valle R, Corrales NC, Martin Jj, Cras P: Phosphorylated tau in cerebrospinal fluid as a marker for Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 2002, 73:79-81.
7. Otto M, Esselmann H, Schulz-Schaeffer W, Neumann M, Schroeter A, Ratzka P, Cepok L, Zerr I, Steinacker P, Windl O, Kornhuber J, Kretzschmar HA, Poser S, Wiltfang J: Decreased beta-amylloid 1-42 in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Neurology 2000, 54(5):1099-1102.
8. Van Everbroeck B, Boons J, Cras P: Cerebrospinal fluid biomarkers in Creutzfeldt-Jakob disease. Clin Neurol Neurosurg 2005, 107:255-360.
9. Van Everbroeck BR, Boons J, Cras P: 14-3-3 gamma-isomform detection distinguishes sporadic Creutzfeldt-Jakob disease from other dementias. J Neurol Neurosurg Psychiatry 2005, 76:100-102.
10. Piubelli C, Fiorini M, Zanusso G, Milli A, Fasoli E, Monaco S, Righetti PG: Searching for markers of Creutzfeldt-Jakob disease in cerebrospinal fluid by two-dimensional mapping. Proteomics 2006, 6 Suppl 1:256-261.
11. Goodall CA, Heaf MW, Everington D, Ironside JW, Knight RS, Green AJ: Raised CSF phospho-tau concentrations in variant Creutzfeldt-Jakob disease: diagnostic and pathological implications. J Neurol Neurosurg Psychiatry 2006, 77:89-91.
12. Van Everbroeck B, Quolin S, Boons J, Martin Jj, Cras P: A prospective study of CSF markers in 250 patients with possible Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 2003, 74:1210-1214.
13. Jinn T, Wakayama Y, Shibuya S, Nakata H, Tomaru T, Takahashi Y, Kosaka K, Asano T, Kato K: High levels of nervous system-specific proteins in cerebrospinal fluid in patients with early stage Creutzfeldt-Jakob disease. Clin Chim Acta 1992, 211(1-2):37-46.
14. Brandel JP, Pech K, Beaudry P, Welaratne A, Bottos C, Agid Y, Laplanche JL: 14-3-3 protein cerebrospinal fluid detection in human growth hormone-treated Creutzfeldt-Jakob disease patients. Ann Neurol 2001, 49(2):257-260.
15. Kropp S, Zerr I, Schulz-Schaeffer WJ, Riedemann C, Bodemer M, Laske C, Kretzschmar HA, Poser S: Increase of neuron-specific enolase in patients with Creutzfeldt-Jakob disease. Neurosci Lett 1999, 261:124-126.
16. Mollenhauer B, Serafin S, Zerr I, Steinhofl BJ, Otto M, Scherer M, Schul-Schaeffer W, Poser S: Diagnostic problems during late course in Creutzfeldt-Jakob disease. J Neurol 2003, 250:629-630.
17. Zerr I, Pochianni M, Collins S, Brandel JP, de Pedro Cuesta J, Knight RS, Bernheimer H, Cardone F, Delasnerie-Laupretrêe N, Cuadrado Corrales N, Ladogana A, Fletcher A, Bodemer M, Awan T, Ruiz Bremón A, Budka H, Laplanche JL, Will RG, Poser S: Analysis of EEG and CSF 14-3-3 proteins as aids to the diagnosis of Creutzfeldt-Jakob disease. Neurology 2000, 55:811-813.
18. WHO: Human transmissible spongiform encephalopathies. Weekly Epidemiological Record 1998, 47:361-365.
19. Poser S, Mollenhauer B, Krauss A, Zerr I, Steinhoff BJ, Schröter A, Finkenstaedt M, Schulz-Schaeffer W, Kretzschmar HA, Felgenhauer K: How to improve the clinical diagnosis of Creutzfeldt-Jakob disease. Brain 1999, 122:2345-2351.
20. Windl O, Giese A, Schulz-Schaeffer W, Zerr I, Skowrc K, Arends S, Oberdieck C, Bodemer M, Poser S, Kretzschmar HA: Molecular genetics of human prion diseases in Germany. Hum Genet 1999, 105:244-252.
21. Parchi P, Castellani R, Capellini S, Gheti B, Young K, Chen SG, Farlow M, Dickson DW, Sima AAF, Trojanowski JQ, Petersen RB, Gambetti P: Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 1996, 39(6):767-778.
22. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, Poser S, Rojiani A, Streichenberger N, Julian J, Vital C, Ghezzi B, Gambetti P, Kretzschmar HA. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46:224-233.

23. Otto M, Wiltfang J, Cepek L, Neumann M, Mollenhauer B, Steinaecker P, Ciesielczyk B, Schulz-Schaeffer W, Poser S. Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 2002; 58:192-197.

24. Otto M, Stein H, Szuadra A, Zerr I, Bodemer M, Gefeller O, Poser S, Kretzschmar HA, Maeder M, Weber T. S-100 protein concentration in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. J Neurol 1997; 244(9):566-570.

25. Zerr I, Bodemer M, Kaboith U, Kretzschmar H, Oellerich M, Armstrong J, Wiltfang J, Nutten M, Vanmechelen E, Forstl H, Kurz A. Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. Molecular Psychiatry 2003; 8:343-347.

26. Geschwind M, Martindale J, Miller D, De Armond SJ, Uyehara-Lock J, Gaskin D, Kramer JH, Barbaro NM, Miller BL. Challenging the clinical utility of the 14-3-3 protein for the diagnosis of sporadic Creutzfeldt-Jakob disease. Arch Neurol 2003; 60:813-816.

27. Pasternak D, Zimmermann K, Zerr I, Bodemer M, Lindner T, Turecek PL, Poser S, Schwarz HP. Immunochemical determination of cellular prion protein in plasma from healthy subjects and patients with sporadic CJD or other neurologic diseases. Transfusion 2001, 41(4):441-448.

28. Green AJ, Thompson EJ, Stewart GE, Zeidler M, McKenzie JM, MacLeod MA, Ironside JW, Will RG, Knight RS. Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 2001; 70:744-748.

29. Geschwind M, Martindale J, Miller D, De Armond SJ, Uyehara-Lock J, Gaskin D, Kramer JH, Barbaro NM, Miller BL. Challenging the clinical utility of the 14-3-3 protein for the diagnosis of sporadic Creutzfeldt-Jakob disease. Arch Neurol 2003; 60:813-816.

30. Schraes JF, Prange HW, Reiber H. Neuron-specific enolase concentration in blood as a prognostic parameter in cerebrovascular diseases. Stroke 1994; 25:558-565.

31. Wunderlich MT, Wallesch CW, Goerdeler M. Release of neurobiochemical markers of brain damage is related to the neurovascular status on admission and the site of arterial occlusion in acute ischemic stroke. J Neurol Sci 2004; 227:49-53.

32. MacLeod MA, Ironside JW, Will RG, Knight RS. Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 2001; 70:744-748.

33. Grossman M, Farmer J, Leight S, Work M, Moore P, Van Deelen V, Prat M, Clark CM, Branch Coslett H, Chatterjee A, Gee J, Trojanowska M, Lee M. Cerebrospinal fluid profile in frontotemporal dementia and Alzheimer's disease. Ann Neurol 2005, 57(5):721-729.

34. Castellani RJ, Colucci M, Xie Z, Zou W, Li C, Parchi P, Capellari S, Pasternak D, Rahbar MH, Chen SG, Gambetti P. Sensitivity of 14-3-3 protein test varies in subtypes of sporadic Creutzfeldt-Jakob disease. Neurology 2004, 63:436-442.

35. Chen XQ, Fung YW, Yu AC. Association of 14-3-3 gamma and phosphorylated bad attenuates injury in ischemic astrocytes. J Cereb Blood Flow Metab 2005; 25:338-347.

36. Kawamoto Y, Akiguchi I, Jarius C, Budka H. Enhanced expression of 14-3-3 proteins in reactive astrocytes in Creutzfeldt-Jakob disease brains. Acta Neuropathol (Berl) 2004; 108:302-308.

37. Jensen GH, Vogelaar CF, Elshof SM. Distribution of cellular prion protein in normal human cerebral cortex — does it have relevance to Creutzfeldt-Jakob disease? Clin Chem Lab Med 2001, 39(4):294-298.