Heart fatty acid binding protein as a potential diagnostic marker for neurodegenerative diseases

Petra Steinacker a, Brit Mollenhauer a, Mirko Bibl b, Lukas Cepek a, Hermann Esselmann b, Peter Brechlin a, Piotr Lewczuk c, Sigrid Poser a, Hans A. Kretzschmar d, Jens Wiltfang c, Claudia Trenkwalder e, Markus Otto a,∗

a Department of Neurology, University Hospital, Robert-Koch-Str. 40, 37075 Goettingen, Germany
b Department of Psychiatry, University Hospital, Goettingen, Germany
c Department of Psychiatry, University Hospital, Erlangen/Nuremberg, Germany
d Department of Neuropathology, University of Munich, Munich, Germany
e Paracelsus-Elena Hospital, Kassel, Germany

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Abstract

The diagnosis of neurodegenerative diseases with dementias requires several different test approaches and often remains uncertain. Using a proteomic approach it was shown in nine patients that heart fatty acid binding protein (H-FABP) might be a biomarker for Creutzfeldt-Jakob disease (CJD).

The aim of our study was to evaluate whether H-FABP is a biomarker for the differential diagnosis of dementias. Therefore we measured H-FABP in cerebrospinal fluid (CSF) and serum of patients having CJD, dementia with Lewy-bodies (DLB), Alzheimer’s disease (AD) and in non-demented control (NDC) patients.

H-FABP levels in CSF and serum of CJD patients are increased compared to non-demented controls. Levels of H-FABP were significantly higher in CJD patients compared to AD and DLB in CSF. However, discrimination between CJD and AD was not possible in serum. Interestingly, highest levels of H-FABP were found in serum of DLB patients.

Our results suggest that H-FABP might be a useful biomarker for the differentiation between the dementias examined if levels in CSF and serum are determined in parallel.

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The differential diagnosis of dementias is usually based upon clinical criteria, while neurochemical data are rarely included as diagnostic algorithms. In the case of sporadic Creutzfeldt-Jakob disease (CJD) the measurement of 14-3-3 proteins in cerebrospinal fluid (CSF) gives a high diagnostic sensitivity and specificity and has been included in the criteria by the WHO [17]. Other biomarkers have been reported to have a high diagnostic value in the differential diagnosis of neurodegenerative diseases, but they have yet to be accepted as clinical criteria. Tau protein levels in CSF are elevated in CJD and Alzheimer’s disease (AD) and a differentiation is possible by applying suitable cut-off levels [1,6,10,12]. Abeta1–42 peptides are decreased in the CSF of AD patients and can also be decreased in CJD patients [2,9,11]. However, by introducing specific quotients (e.g. Abeta1–42 to Abeta1–39), a discrimination of these two diseases can be achieved [18]. S-100B is significantly increased in CJD CSF as well as in serum, but elevated serum levels are also found in other dementias (review: [13]). Nevertheless, the final diagnosis of a dementia
Heart-type fatty acid binding protein (H-FABP) is often uncertain and there is need for additional sensitive and specific biomarkers in CSF or in serum to allow an early diagnosis and to improve diagnostic certainty [13]. Recently, by 2D-PAGE analysis of CSF and subsequent sequencing, heart-type fatty acid binding protein (H-FABP) was shown to be a potential biomarker for CJD. In a small sample size of CJD patients, elevated levels of H-FABP were detected in plasma and CSF by ELISA measurement in comparison to non-demented control patients and to a group of patients with other dementias [5].

Fatty acid binding proteins are cytosolic proteins. They belong to a multigene family of low molecular weight proteins (Mr, 14–16 kDa) which are the key fatty acid carrier proteins in cytosol. Fatty acid-binding proteins are found in all cells utilizing fatty acids and are expected to be rapidly released from cells into the circulation shortly after the onset of cell damage. H-FABP was initially purified from heart muscle, but has since been found to have a widespread tissue distribution [16]. In serum/plasma of healthy individuals, approximately 1.6 ng/ml of H-FABP is present and a slight increase with age has been reported. Clinically, H-FABP can be used as an additional marker in serum after acute myocardial infarction [4]. Elevated levels are found within 3 h after the acute myocardial infarction which generally return to normal values within 12–24 h. High levels of H-FABP have also been seen in stroke patients [20]. The aim of our study was the determination of H-FABP levels in CSF and serum samples of patients who are under a differential diagnosis of a dementia, to evaluate whether H-FABP measurement can be used to improve the differential diagnosis.

We analyzed samples of 62 patients. Serum concentrations were determined in all patients. CSF levels were measured in 45 patients. The CJD group comprised 14 individuals. Five of these patients were neuropathologically verified. Clinically, all CJD patients were classified as “probable” CJD according to the WHO criteria (1998). The group of other dementias included 16 patients with dementia with Lewy-bodies (DLB) and 18 patients with Alzheimer’s disease (AD) [7,8]. As a fourth group we investigated 16 patients with Lewy-bodies (DLB) and non-demented control (NDC) patients. Plot shows 10th, 25th, 75th and 90th percentiles and outliers.

The best results for sensitivity and specificity were obtained at a cut off of 3500 pg/ml (Youden index: 0.93). At this cut-off the sensitivity is 93% and the specificity is 100%. The optimal cut-off level for dichotomising tau-protein values was selected as the situation maximising the Youden index [19].

The levels of H-FABP in CSF were increased in the CJD samples compared to the NDC samples (P < 0.0001) and to other dementias AD and DLB (P < 0.0001). H-FABP levels are summarized in Table 1 and were illustrated by the boxplot in Fig. 1.

Statistical analysis was performed applying the Mann–Whitney test, if values from two collectives are compared. For more groups the Kruskal–Wallis test was applied. P-values below 0.05 were considered to be significant. The optimal cut-off level for dichotomising tau-protein values was selected as the situation maximising the Youden index [19].

The levels of H-FABP in CSF were increased in the CJD samples compared to the NDC samples (P < 0.0001) and to other dementias AD and DLB (P < 0.0001). H-FABP levels are summarized in Table 1 and were illustrated by the boxplot in Fig. 1.

The best results for sensitivity and specificity were obtained at a cut off of 3500 pg/ml (Youden index: 0.93). At this cut-off the sensitivity is 93% and the specificity is 100%. AD and DLB patients had higher H-FABP levels in CSF than NDC patients (P = 0.005). AD and DLB did not differ in their H-FABP levels (P = 0.24).

Different levels were observed when determining H-FABP amounts in serum (P < 0.0001). H-FABP serum levels are summarized in Table 1 and illustrated in Fig. 1.

Patients with CJD had higher levels than NDC patients (P = 0.0002), but not compared to the other groups (CJD versus AD + DLB, P = 0.8322). The highest serum levels were measured in the DLB group. They differ significantly from the levels in the NDC group (P < 0.0001) and the AD group (P = 0.0064).
Table 1

<table>
<thead>
<tr>
<th>Age (range; median) (years)</th>
<th>Male/female</th>
<th>Serum: n (range; median) (pg/ml)</th>
<th>CSF: n (range; median) (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CJD 57–78 (73)</td>
<td>6/8</td>
<td>14 (1836–25000; 3979)</td>
<td>14 (2050–25000; 7294)</td>
</tr>
<tr>
<td>DLB 55–86 (73)</td>
<td>4/12</td>
<td>16 (1292–25000; 5220)</td>
<td>7 (341–2667; 1040)</td>
</tr>
<tr>
<td>AD 47–65 (65)</td>
<td>5/13</td>
<td>18 (581–9029; 2037)</td>
<td>8 (643–3043; 1911)</td>
</tr>
<tr>
<td>NDC 32–76 (63)</td>
<td>7/9</td>
<td>16 (445–3543; 1423)</td>
<td>16 (0–1729; 561)</td>
</tr>
</tbody>
</table>

Number of individuals tested in each group, with measured range of H-FABP and median.

The best differentiation of DLB and CJD patients was observed by an introduction of H-FABP-serum to H-FABP-CSF ratio (Fig. 2). Using the cut-off value 1.2, the DLB and CJD patients can be differentiated with 100% sensitivity and 75% specificity, DLB and AD patients can be differentiated with 100% sensitivity and 63% specificity.

H-FABP, measured in the CSF of nine CJD patients in comparison to a heterogeneous control group comprising 13 demented patients, was reported to be a potential biomarker for CJD [5]. In our study we quantified H-FABP in the CSF of 14 CJD patients in comparison to 8 AD and 7 DLB patients and also to 16 NDC’s. We confirmed that H-FABP levels are significantly elevated in the CSF of CJD patients. High levels – on a lower level – were also found for AD and DLB patients when compared to NDC’s. These results suggest that H-FABP might be a useful biomarker not only in the diagnosis of CJD, but also, if suitable cut-off values are applied, for the separation of AD and DLB, respectively.

In a pilot study with seven CJD patients and seven healthy controls, it was suggested that H-FABP might be a potential plasma biomarker for CJD [5]. We also confirmed higher levels of H-FABP in serum in comparison to NDC. However, as there is an enormous overlap between CJD an AD levels in serum, this H-FABP will not be useful as a serum marker. Surprisingly, we found highly elevated H-FABP levels in serum of DLB patients. Given the low hydrodynamic radius of H-FABP, it is most likely that almost all of H-FABP measured in the CSF is brain and not serum derived [3]. We would expect levels of protein with the size of H-FABH be low 100 pg/ml in CSF if these were serum derived [14,15].

As CSF levels of H-FABP were higher for CJD than DLB patients, the unexpectedly high serum levels of H-FABP point to a partial systemic source of this protein in DLB. Further investigations will have to clarify whether patients with DLB might have a general disturbance of fatty acid metabolism. However, our data show that H-FABP in serum might be a potential biomarker for DLB. This certainly has to be evaluated in a larger group. Best results for the differentiation of DLB and CJD patients were obtained by the introduction of a serum H-FABP to CSF H-FABP ratio.

Taken together, H-FABP seems to be a useful biomarker for the differential diagnosis of dementias, especially when serum and CSF are determined in parallel.

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