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Serum Heart-Type Fatty Acid-Binding Protein and Cerebrospinal Fluid Tau: Marker Candidates for Dementia with Lewy Bodies

Brit Mollenhauer[a, c] Petra Steinacker[c, f] Erik Bahn[d] Mirko Bibl[e] Peter Brechlin[c] Michael G. Schlossmacher[a] Joseph J. Locascio[a, b] Jens Wiltfang[g] Hans A. Kretzschmar[h] Sigrid Poser[c] Claudia Trenkwalder[i] Markus Otto[c, f]

[a]Center for Neurologic Diseases, Brigham and Women's Hospital and [b]Memory and Movement Disorder Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Mass., USA; Departments of [c]Neurology, [d]Pathology and [e]Psychiatry, Georg August University, Göttingen, [f]Department of Neurology, University of Ulm, Ulm, [g]Department of Psychiatry, Friedrich Alexander University, Erlangen/Nuremberg, [h]Department of Neuropathology, Ludwig Maximilians University Munich, Munich, and [i]Georg August University Göttingen, Paracelsus-Elena Klinik, Kassel, Germany

Key Words
Heart-type fatty acid-binding protein · Tau protein · Alzheimer's disease · Dementia with Lewy bodies · Parkinson disease dementia · Cerebrospinal fluid

Abstract
Background: The measurement of biomarkers in cerebrospinal fluid (CSF) has gained increasing acceptance in establishing the diagnosis of some neurodegenerative diseases. Heart-type fatty acid-binding protein (H-FABP) was recently discovered in CSF and serum of patients with neurodegenerative diseases. ***Objective:*** We investigated H-FABP in CSF and serum alone and in combination with CSF tau protein to evaluate these as potential biomarkers for the differentiation between dementia with Lewy bodies (DLB) and Alzheimer's disease (AD). ***Methods:*** We established H-FABP and tau protein values in a set of 144 persons with DLB (n = 33), Parkinson disease with dementia (PDD; n = 25), AD (n = 35) and nondemented neurological controls (NNC; n = 51). Additionally, serum H-FABP levels were analyzed in idiopathic Parkinson disease patients without evidence of cognitive decline (n = 45) using commercially available enzyme-linked immunosorbent assays. We calculated absolute values of H-FABP and tau protein in CSF and serum and established relative ratios between the two to obtain the best possible match for the clinical working diagnosis. ***Results:*** Serum H-FABP levels were elevated in DLB and PDD patients compared with NNC and AD subjects. To better discriminate between DLB and AD, we calculated the ratio of serum H-FABP to CSF tau protein levels. At the arbitrary chosen cutoff ratio ≥ 8 this quotient reached a sensitivity of 91% and a specificity of 66%. ***Conclusion:*** Our results suggest that the measurement of CSF tau protein, together with H-FABP quantification in serum and CSF, and the ratio of serum H-FABP to CSF tau protein represent marker candidates for the differentiation between AD and DLB.

B.M. and P.S. contributed equally to this work. B.M. and M.O. were the principal investigators and together with P.S. designed, organized, and oversaw the trial and interpreted the data. C.T., M.B. and B.M. were responsible for all field- and hospital-based activities and diagnoses. M.G.S. was involved in the analysis and editing of the paper. J.J.L. was responsible for statistical analysis. H.A.K. and S.P. were involved in the CJD study. J.W., E.B. and P.B. were responsible for the quality control of the analyses.

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Brit Mollenhauer
Center for Neurologic Diseases, Department of Neurology
Brigham and Women's Hospital, 77, Avenue Louis Pasteur
Harvard Institutes of Medicine (HIM) 764, Boston, MA 02115 (USA)
Tel. +1 617 525 5123, Fax +1 617 525 5252, E-Mail bmollenhauer@rics.bwh.harvard.edu

Introduction

The distinction between dementia with Lewy bodies (DLB) and Alzheimer's disease (AD) remains challenging due to the significant overlap in clinical signs [1]. Today a definite diagnosis of the underlying neurodegenerative syndrome still requires neuropathological confirmation [2–4]. Early and accurate diagnosis becomes more and more important, since novel and disease-specific intervention trials are being planned, since unnecessary medication and tests are to be avoided, and since the costs for dementia care soar [5]. Biological markers supporting the clinical working diagnosis are often missing or do not meet requirements for routine standardization [6, 7].

So far the differentiation between AD and DLB based on the quantification of select constituents in cerebrospinal fluid (CSF) as potential surrogate markers, including of tau protein and β-amyloid 1–42, has shown unsatisfactory results owing to the large overlap in values between the two syndromes [8–10]. Nonetheless, total tau protein may contribute to the clinical distinction between the tauopathy of AD and the synucleinopathy of DLB [8, 9].

Recently, heart-type fatty acid-binding protein (H-FABP) was identified as a potential CSF biomarker for Creutzfeldt-Jakob disease (CJD) [11]. Fatty acid-binding proteins (FABPs) are cytosolic 14- to 16-kDa proteins involved in the uptake, transport and metabolism of fatty acids. FABPs are found in all cells that utilize fatty acids, and are known to be rapidly released into the extracellular space following cellular damage. H-FABP was initially purified from heart muscle and previously evaluated as a biochemical marker of cardiac ischemia and brain injury [12–14]. H-FABP has been found to display a wide tissue distribution including the expression in brain [15]. Another FABP is α-synuclein, which is genetically and neuropathologically linked to Parkinson disease (PD) and DLB [16], and was recently shown to function as a FABP-like constituent of neurons, and to affect lipid metabolism in vivo [15–21]. α-Synuclein-positive Lewy bodies request a pathological hallmark of DLB and PD, and are also found in sympathetic neurons of the peripheral nervous system including that of heart [22]. Based on these findings, myocardial scintigraphy using [^{123}I]metaiodobenzyl guanine has been investigated to help differentiate between synucleinopathies and tauopathies [23, 24].

In an independent pilot study of several degenerative dementias, high levels of CSF H-FABP were found in sporadic CJD patients and the highest number of H-FABP reactivity was observed in the serum of a small cohort of DLB patients [25].

Accordingly, we carried out a cross-sectional study of H-FABP and tau protein levels in biological fluids of a cohort of patients with neurodegenerative diseases to evaluate their individual diagnostic value individually and to explore combinatorial ratios thereof for the possible distinction between AD and DLB.

We hypothesized that H-FABP is a biomarker candidate not only for DLB but also possibly for Parkinson disease with dementia (PDD) given their virtually identical phenotype of central nervous system pathology. The biochemical parallels between α-synuclein and H-FABP as well as several links between tau protein, α-synuclein and parkinsonism provided further impetus for our study [19–21]. Here, we demonstrate that H-FABP together with CSF tau protein has the potential to be of use in the differential diagnosis of several neurodegenerative diseases, especially the distinction between DLB and AD.

Methods

Patients

We analyzed CSF and serum of 144 patients with DLB, PDD, AD and nondemented neurological controls (NNC). Additionally, we examined serum of 45 patients with idiopathic Parkinson disease without dementia (IPD). All samples were drawn in the morning under fasting conditions. Routine laboratory investigations (e.g. liver enzymes, creatinine, creatine phosphokinase, lactate dehydrogenase, total protein content in serum as well as complete cell count and protein level in the CSF) were normal, and no patient showed signs of acute myocardial infarction (clinically or by laboratory investigations and electrocardiogram) or of overt congestive heart failure. In all patients either computed tomography or magnetic resonance imaging of the brain was carried out to exclude structural entities.

None of the patients included received medication known to potentially affect the lipid metabolism. In the case of evidence for another cause of dementia (e.g. recent or acute cardiac or cerebral infarction) subjects were excluded.

Dementia with Lewy Bodies

Patients diagnosed as 'probable DLB' at a neurological university hospital (according to the clinical classification criteria of McKeith et al. [2, 3]) were hospitalized for ≥24 h to evaluate them for fluctuating cognition, extrapyramidal symptoms and visual hallucinations. All DLB patients developed dementia before extrapyramidal symptoms and were thus differentiated from PDD according to DLB guidelines [2, 3]. We included 10 male and 23 female DLB patients with a mean age of 70 ± 9 years (±SD; range 66–86 years) (table 1). Disease duration at the time of lumbar puncture (LP) was 24 ± 21 months (range: 6–72 months). Mean MMSE was 14 ± 5 points (range: 9 and 21 points; normal: 30).

PDD and IPD

All PD patients were evaluated and their course followed in a specialized clinic for parkinsonism and movement disorders (Paracelsus-Elena Klinik, Kassel, Germany). The diagnosis was

Table 1. Demographics and phenotypic characterization of study participants

	DLB (n = 33)	PDD (n = 25)	AD (n = 35)	IPD (n = 45)	NNC (n = 51)
Age, years	70 ± 9	74 ± 6	68 ± 11	69 ± 10	67 ± 12
Range	66 – 86	56 – 84	63 – 85	45 – 84	32 – 94
Median	72	74	65	68	66
Sex (male/total)	0.3	0.8	0.4	0.6	0.4
Duration of disease	24 ± 21 months	156 ± 72 months	44 ± 25 months	132 ± 96 months	n.a.
Range	6 – 72 months	48 – 336 months	8 – 84 months	24 – 444 months	
Median	14 months	132 months	40 months	120 months	
MMSE	14 ± 5	18 ± 7	16 ± 6	>28	30
Range	9 – 21	5 – 25	3 – 24		
Median	14	11	40		
UPDRS	n.a.	49 ± 13	n.a.	40 ± 18	n.a.
Range		30 – 83		10 – 69	
Median		48		46	
Hoehn and Yahr	n.a.	4 ± 1	n.a.	3.5 ± 1	n.a.
Range		2 – 5		1 – 5	
Median		4		3.75	

MMSE = Mini-Mental Status Examination; n.a. = not available.

made according to the UK Brain Bank diagnostic criteria for clinically definite PD [26]. All PD patients had suffered from idiopathic PD for at least 3 years prior to the occurrence of cognitive decline (in PDD cases) or showed no change in cognition (IPD).

The Hoehn and Yahr (H&Y) score was used for classification of clinical disability [27]. All PD patients were also evaluated using the motor subscale of the Unified Parkinson Disease Rating Scale (UPDRS part III, items 18–31) during their on-state [28]. They all received treatment according to widely used practice guidelines [29, 30].

PD patients were divided into two groups:

(1) IPD patients: This group comprised 45 patients. All IPD patients had an MMSE >28 points and showed no signs of psychosis or depression.

We included 45 patients (17 males and 28 females) with IPD of whom blood samples were available for H-FABP quantification (table 1). No CSF samples were available from these IPD patients. Mean age in this group was 69 ± 10 years (range: 45–84 years). The duration of IPD was 231 ± 96 months and ranged between 24 and 444 months; classification according to H&Y was 3.5 ± 1 and ranged between 1 and 5, and the mean UPDRS score was 40 ± 18 and ranged between 10 and 69.

(2) PDD patients: This group comprised 25 patients. All PDD fulfilled the DSM-IV criteria for dementia and presented with an MMSE ≤25 points, thereby defining dementia. All patients were examined by a neurologist and either by a psychologist and/or psychiatrist to exclude the diagnosis of depression. Subjects with MMSE ≥25 were excluded as were those with signs of depression.

We included 20 male and 5 female PDD patients with a mean age of 74 ± 6 years (range: 56–84 years) (table 1). Mean duration of PDD at the time of LP was 156 ± 72 months (range: 48–336 months). Mean MMSE was 18 ± 7 points (range: 5–25 points). H&Y classification was 4 ± 1 and ranged between 2 and 4; mean UPDRS (part III) was 49 ± 13 and ranged between 30 and 83.

Alzheimer's Disease

We included patients with the diagnosis of AD recruited in the departments of psychiatry and neurology. The diagnosis of 'probable AD' in all 34 cases was made according the DSM-IV criteria for dementia and the established NINCDS-ADRDA criteria [31]. For 1 patient, neuropathological verification of AD was obtained postmortem using paraffin-embedded formalin fixation of brain tissue and immunohistochemistry.

We included 15 male and 20 female patients with AD (table 1). Their mean age was 68 ± 11 years and ranged between 63 and 85 years. MMSE was 16 ± 6 points (range: 3–24 points). Disease duration was 44 ± 25 months (range: 8–84 months).

Nondemented Neurological Controls

NNC patients underwent LP during routine workup of nondementing illnesses. These patients suffered from the following clinical working diagnoses: headache (n = 12), peripheral neurological diseases (n = 10), depression (n = 8), focal cerebral ischemia (n = 5), epileptic seizures (n = 3), amyotrophic lateral sclerosis (n = 2), dizziness (n = 2), myelopathy or radiculopathy (n = 6), and multiple sclerosis, small cell lung carcinoma without metastasis to the central nervous system, and transient global amnesia (n = 3).

Patients with cognitive changes and/or extrapyramidal signs were excluded from our study. There were 20 male and 31 female patients In the NNC group with a mean age of 67 ± 12 years (range: 32–94 years) (table 1).

Marker Measurement

Tau Protein

All specimens were obtained by LP, aliquoted and stored at –80°C. All samples were analyzed using a commercially available enzyme-linked immunosorbent assay for tau protein (INNOTEST hTAU Antigen, Innogenetics, Gent, Belgium) [32–34].

Table 2. Laboratory data according to working diagnosis

	DLB	PDD	AD	IPD	NNC
Tau protein in CSF, pg/ml	285 ± 196	248 ± 141	945 ± 1,821	n.a.	150 ± 109
Range	75 – 973	99 – 702	75 – 11,152		75 – 583
Median	220	192	691		116
H-FABP in CSF, pg/ml	1,511 ± 843	1,278 ± 1,207	1,607 ± 950	n.a.	861 ± 506
Range	341 – 3,476	100 – 6,400	399 – 4,980		100 – 2,507
Median	1,283	1,283	1,542		800
H-FABP in serum, pg/ml	10,199 ± 15,526	6,699 ± 9,271	3,299 ± 2,090	2,714 ± 2,406	3,734 ± 6,824
Range	510 – 89,688	1,824 – 40,675	581 – 9,029	288 – 11,021	361 – 35,683
Median	7,337	3,121	3,055	2,250	1,983

n.a. = Not available.

The signal corresponding to the lowest values for tau protein was equalized to the lowest standard concentration at 75 pg/ml.

H-FABP in CSF and Serum

H-FABP levels were measured in 25-μl aliquots of serum and 50-μl aliquots of CSF, using a commercially available solid-phase enzyme-linked immunoassay based on the sandwich principle (HyCult Biotechnology, The Netherlands). Specificity of the antibodies to H-FABP was previously established by Pelsers et al. [35], as cross-reactivity with non-H-FABP had been excluded. No performance differences were observed in the FABP assay when applying serum or plasma; samples subjected to freeze and thaw cycles showed no loss of immunoreactivity [36]. Signals corresponding to the lowest values of H-FABP reactivity were set at the lowest standard concentration of 200 pg/ml of recombinant protein.

The study was approved by the Ethics Committee of the University of Göttingen and the local board of registration of Hessen, Germany. Investigations were carried out with the informed consent of all patients or their next of kin in the case of persons with dementia.

Statistical Analysis

Statistical analysis was performed applying the Mann-Whitney test, when values from two cohorts were compared. For more than two groups the Kruskal-Wallis test was applied. To illustrate the variability of sensitivity and specificity for different cutoff levels of the different marker combinations the receiver-operating characteristic (ROC) curve was computed. In addition, the area under the curve (AUC) for ROC curves was calculated. There, higher levels represented a better test performance [37]. Only the best discriminating cutoff values alone or in combination are presented. Spearman correlations are given with p value and correlations (c).

To confirm results from our exploratory statistical approach, we conducted linear discriminant and logistic regression analyses. This statistical approach was carried out by an experienced statistician (J.J.L.) using SAS. Logarithmic transformations of values (log) were used to meet assumptions of normality and homogeneity of variances for some of the statistical tests.

Results

Tau Protein Levels in CSF

In diseased brains the mean CSF tau protein was 285 ± 196 pg/ml (range: 75–973 pg/ml) for DLB, 248 ± 141 pg/ml (range: 99–702 pg/ml) in PDD and 949 ± 1,821 pg/ml (range: 75–11,152 pg/ml) in AD. Tau protein levels were 150 ± 109 pg/ml (range: 75–583 pg/ml) in the control group (NNC) (table 2, fig. 1).

Mean tau protein levels were significantly different between the cohorts of AD and DLB and AD and PDD ($p < 0.001$) as well as between both DLB and AD and NNC ($p < 0.001$) as reported [9]. At a reduced level of statistical significance, tau protein levels differed between PDD and NNC ($p = 0.01$). No difference was seen between PDD and DLB ($p = 0.18$) as expected, as they share nearly identical neuropathological phenotypes [38, 39].

Total CSF tau protein was the most useful discriminating parameter at the best fit cutoff of 240 pg/ml between AD and NNC, with a sensitivity of 86% and a specificity of 92%.

The patient with neuropathologically verified AD in our cohort had undergone LP 2 months after the onset of disease (4 years prior to death), and his CSF tau level was 1,126 pg/ml.

H-FABP Levels in CSF

To further discriminate between PDD, DLB and AD, we next examined CSF H-FABP levels (tables 2, fig. 1). In DLB, H-FABP was 1,511 ± 843 pg/ml (range: 341–3,476 pg/ml), in PDD 1,278 ± 1,207 pg/ml (range: 100–6,400 pg/ml) and in AD 1,607 ± 950 pg/ml (range: 399–4,980 pg/ml). In the NNC group, CSF H-FABP levels were 861 ± 506 pg/ml (range: 100–2,507 pg/ml).

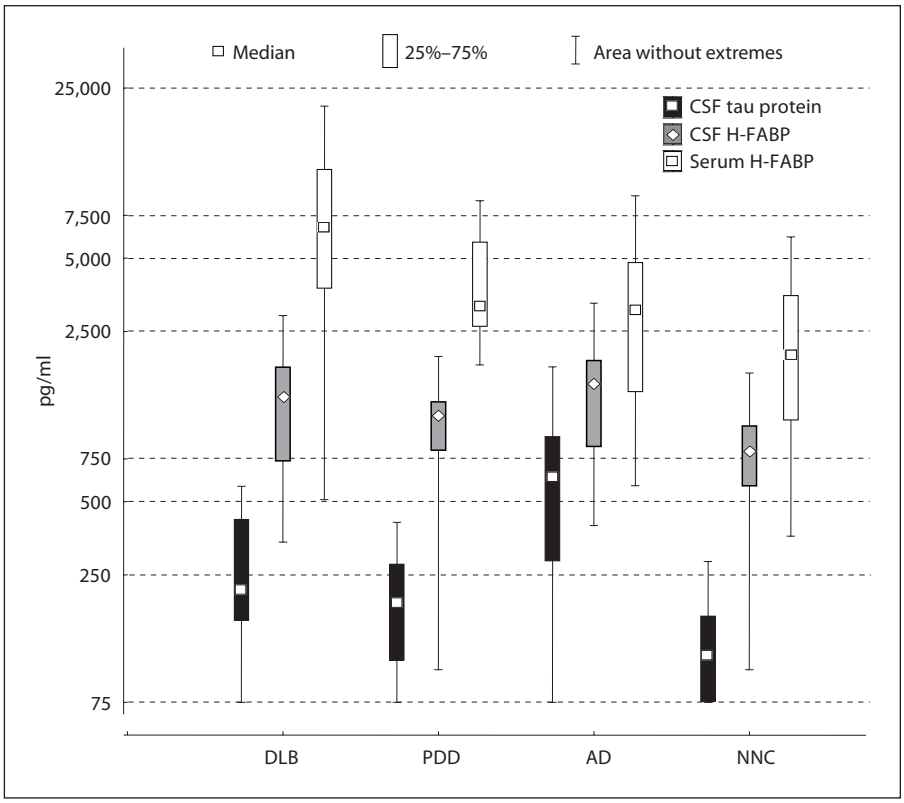

Fig. 1. Logarithmic box plot of CSF tau protein and H-FABP in serum and CSF in DLB, PDD, AD and NNC.

Accordingly, H-FABP levels were highly significantly different between DLB as well as AD and NNC ($p < 0.001$). Statistical significance was also observed between PDD and NNC ($p = 0.03$), but did not differ between AD and DLB ($p = 0.64$), PDD and DLB ($p = 0.16$), and PDD and AD ($p = 0.08$).

H-FABP Levels in Serum

To pursue a higher order of diagnostic accuracy, we next determined serum H-FABP levels in our subjects. These were higher (mean: 10,199 ± 15,526 pg/ml) and ranged between 510 and 89,688 pg/ml in DLB. In PDD, the mean serum H-FABP levels were 6,699 ± 9,271 pg/ml (range: 1,824–40,675 pg/ml) and in AD cases 3,299 ± 2,090 pg/ml (range: 581–9,029 pg/ml). The NNC showed mean serum H-FABP levels of 3,734 ± 6,824 pg/ml (range: 361–35,683 pg/ml) (table 2, fig. 1, 2). Thus, serum levels of H-FABP significantly differed between DLB and AD ($p < 0.001$) and between both PDD and DLB over NNC ($p < 0.001$).

The one patient with neuropathological verification of the clinical diagnosis of AD showed a serum H-FABP of 5,923 pg/ml and a CSF H-FABP level of 2,419 pg/ml.

Suspected Synucleinopathies

To further examine cohorts of clinically suspected synucleinopathies, we also investigated a group of IPD patients. Mean serum H-FABP levels were 2,714 ± 2,406 pg/ml (range: 288–11,021 pg/ml) (table 2, fig. 2).

Intriguingly, serum H-FABP levels were significantly different between PDD and DLB versus IPD ($p < 0.001$); however, no difference was seen between PDD and DLB ($p = 0.14$) as expected. Thus, in our cohort analyses, serum H-FABP alone could discriminate between parkinsonism with dementia versus classical PD without cognitive impairment (e.g. PDD and DLB vs. IPD) (fig. 2). Best cutoff points regarding the AUC varied among the clinical working diagnoses and reached sensitivities between 69 and 92%, and specificities between 64 and 88% (table 3).

Combinational Analysis

To achieve a higher degree of specificity and sensitivity, and possibly allow a laboratory value-based distinction between our various cohorts, we next examined the usefulness of various combined test values.

Ratios of H-FABP serum to CSF levels showed significant differences between both the PDD and DLB groups

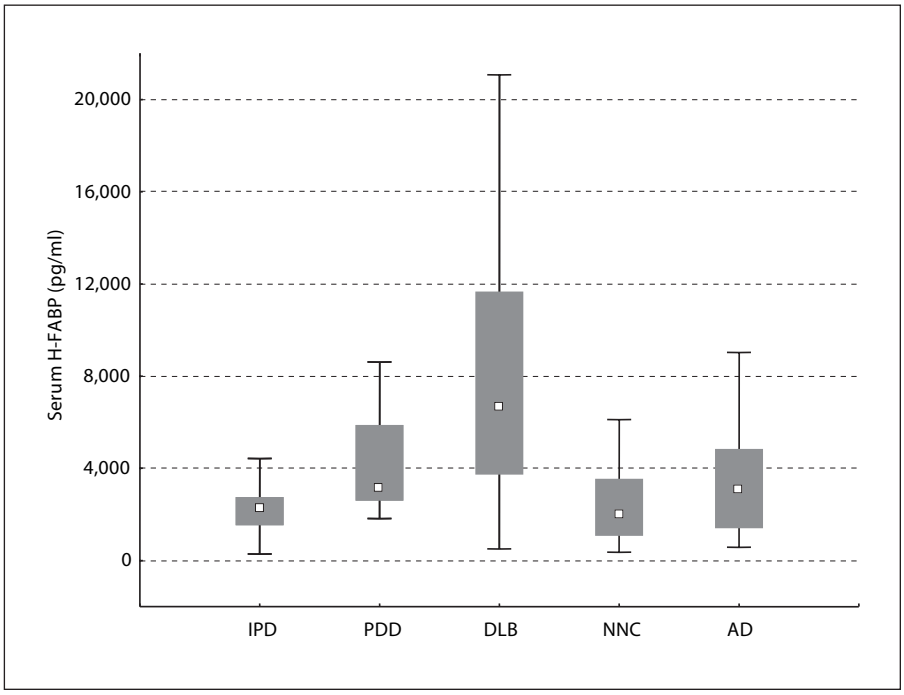

Fig. 2. Box plot of H-FABP in serum in IPD, PDD, DLB, NNC and AD.

Table 3. Overview of the most informative parameters and chosen cutoff levels in correlation with working diagnosis

Working diagnosis	Most informative parameter	Best cutoff	Sensitivity %	Specificity %	AUC[a]
DLB vs. AD	serum H-FABP	>4,000 pg/ml	71	69	0.799*
	serum H-FABP/CSF H-FABP	>3.3	80	74	0.779*
	serum H-FABP/CSF tau protein	>8.0	91	66	0.866*
DLB vs. PDD	serum H-FABP	>3,500 pg/ml	81	65	0.693
DLB vs. IPD	serum H-FABP[b]	>3,200 pg/ml	84	82	0.836*
DLB vs. NNC	serum H-FABP	>4,500 pg/ml	74	88	0.819*
PDD vs. AD	serum H-FABP/CSF tau protein	>9.0	88	74	0.840*
PDD vs. IPD	serum H-FABP[b]	>2,800 pg/ml	69	80	0.783*
PDD vs. NNC	serum H-FABP	>2,350 pg/ml	92	64	0.750*
AD vs. NNC	CSF tau protein	>240 pg/ml[c]	86	92	0.925*

* p values <0.001.
[a] AUC according to the ROC curves [36].
[b] In 45 patients with IPD only serum was analyzed.
[c] At cutoff 450 pg/ml [32, 33] sensitivity was 60% and specificity was 96%.

versus AD ($p = 0.003$ and $p < 0.001$, respectively) (table 3, fig. 3). Accordingly, the ratio of serum H-FABP to CSF tau showed highly significant differences between AD and DLB and successfully discriminated between both diseases at the chosen best-fit cutoff of ≥8.0, with a sensitivity of 91% and a specificity of 66% (table 3, fig. 4). At a chosen cutoff of ≥9, this quotient discriminated between AD and PDD with a sensitivity of 88% and a specificity of 74% (table 3, fig. 3).

Consistent with these results, logistic regression analyses of individual and combinations of marker variables showed significant ($p < 0.001$) and optimal separation of

DLB and AD with a linear combination of log CSF tau (coefficient = –1.58) and log serum H-FABP (1.35), each marker providing significant (p = 0.001) classification power beyond that of the other. This combination produced an AUC of 0.869 and reflected well the ratio between serum H-FABP and CSF tau protein with an AUC of 0.866 we introduced for practical reasons.

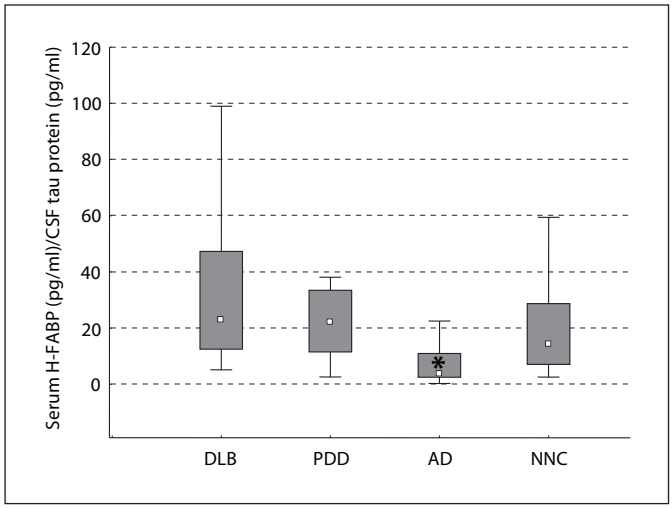

Fig. 3. Box plot of quotient serum H-FABP/CSF tau protein in DLB, PDD, AD and NNC. * Neuropathologically verified AD patient.

Discussion

We evaluated CSF tau protein together with H-FABP in CSF and serum in a larger group of patients suffering from neurodegenerative dementia, and compared these markers with NNC including subjects with idiopathic PD. Among the latter, we included subjects with and without dementia according to widely used clinical criteria (see Methods section). Tau protein is an accepted marker in the differential diagnosis of neurodegenerative diseases [40], whereas H-FABP was only recently described [11]. Giving the intriguing parallels between H-FABP and α-synuclein biology as well as the pathological links between α-synuclein and tau protein [41], we analyzed both H-FABP and tau-protein for their best-differential diagnostic potential for discriminating between DLB and AD by evaluating practical cutoff levels and ratios.

Based on data with elevated CSF H-FABP and normal serum H-FABP values in 30 CJD patients (data not shown), we concluded that H-FABP proteins in CSF from CJD subjects are likely to originate from the brain and not from a peripheral source [25]. In contrast, the high levels of serum H-FABP in DLB and PDD compared to IPD, NNC and AD (without corresponding H-FABP elevation in CSF) suggested that H-FABP dysregulation

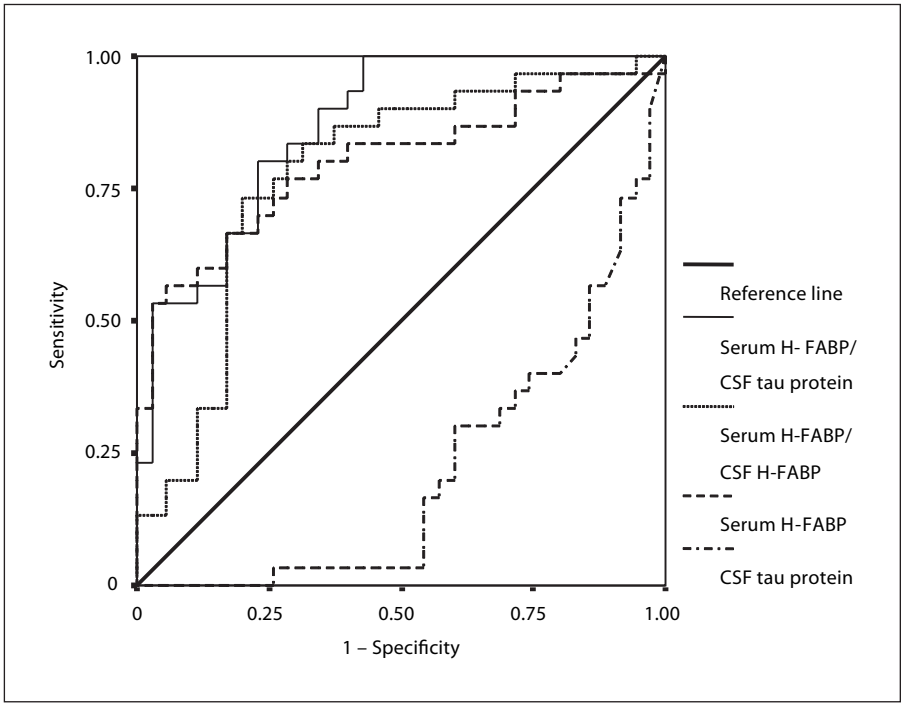

Fig. 4. ROC curves for the differential diagnosis of DLB versus AD using cutoff points and marker combinations. Best results (e.g. highest AUC) were obtained for the quotient of serum H-FABP/CSF tau protein.

was more likely related to a peripheral rather than neural source.

In none of our patients, was clinically or laboratory-based cardiac dysfunction recognizable and no other organ dysfunction could be identified.

The generally held distinction between PDD and DLB rests on the observation that the disease begins with either motor or cognitive signs, respectively, and that progression is more rapid and more widespread in DLB than in PDD. Neuropathologically, there is no substantive qualitative difference in their α-synucleinopathy phenotypes [39].

H-FABP is a member of a protein family found in brain as well as in heart tissue (from where H-FABP was initially purified) [15]. Upon detection of release of H-FABP from myocardial tissue following cellular damage, plasma H-FABP levels were explored as candidate marker for myocardial ischemia [12, 13, 35]. Elevated levels of brain-derived serum H-FABP were seen in patients following brain injury including cerebral ischemia and hemorrhage [42–44]. However, these acute disease entities are less relevant in the case of chronically evolving neurodegenerative disease, and are therefore unlikely to present a diagnostic dilemma in the neurological practice setting.

It is currently unknown whether elevated serum H-FABP levels seen in DLB and PDD are the result of cardiac involvement in the disease process or of systemic upregulation. Of note, α-synuclein-positive Lewy bodies are found in sympathetic neurons of the peripheral nervous system including the conduction system of the heart, and these are frequently found abnormal in DLB and PD [22, 23, 45]. These peripheral nervous system changes gave rise to the hypothesis of a peripheral starting point for the disease [38]. Importantly, future studies will further explore H-FABP levels in serum and CSF of synucleinopathy subjects, and will include the recently developed quantification of α-synuclein by ELISA [46–48].

A primary or secondary role of H-FABP in the widespread synucleinopathies of PDD and DLB could underlie our observed laboratory changes, as α-synuclein has fatty acid-binding capacities, and a reciprocal interaction between polyunsaturated fatty acids and α-synuclein has recently been demonstrated in vivo [19–21]. Therefore, H-FABP might be upregulated in concert with dysregulated α-synuclein processing [17, 18, 21]. It was also shown that H-FABP is associated with the dompamine D2 receptor [49]. Future studies will address the systemic mRNA levels of H-FABP in our patient cohorts, and H-FABP levels in cellular and animal models of synucleinopathies.

Aside from the important pathophysiological question, we have demonstrated a potential relevance of serum and CSF H-FABP levels for clinical practice. The best discriminator for the clinically often challenging distinction between DLB and PDD over AD in our study was the ratio of serum H-FABP to CSF tau, where a larger number suggested a higher probability of synucleinopathy in the clinical context of dementia (fig. 3). Such a degree of discrimination is not yet possible with currently investigated markers [8–10]. To date, CSF markers (such as tau protein alone and in combination with β-amyloid 1–42) fail to discriminate between AD and DLB because of a sizeable overlap and thus do not fulfil the requirements of a reliable biomarker [7]. Future examination of additional cohorts may support the clinical separation of AD from DLB (and PDD subjects) using serum H-FABP/CSF tau ratios.

We are aware of several potential weaknesses of our study: (1) its cross-sectional design and (2) the partial overlap seen between PDD and DLB groups versus our NNC cohort (fig. 3). We will seek to address these in a prospective study with phenotypically well-characterized cohorts and include the quantification of CSF and blood α-synuclein levels [46–48]. In contrast to other ongoing biomarker development efforts (e.g. functional and structural neuroimaging studies), our CSF and serum-based assays for tau and H-FABP reactivities carry with them the potential benefit of a more universally applicable and less expensive marker of DLB and PDD pathology, provided they can be validated in future studies.

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