THE ROLE OF MEASUREMENT OF TAU PROTEIN IN THE DIAGNOSIS OF CREUTZFELDT-JAKOB DISEASE: COMPARISON OF DATA OBTAINED AT THE DEPARTMENT OF NEUROLOGY, UNIVERSITY OF SZEGED WITH THE SCIENTIFIC LITERATURE

A tau fehérje meghatározás szerepe a Creutzfeldt-Jakob betegség diagnosztikájában: A Szegedi Tudományegyetem Neurológiai Klinikáján mért eredmények összevetése a szakirodalmi adatokkal

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Abstract

Since the definite diagnosis of Creutzfeldt-Jakob disease (CJD) can currently only be provided by autopsy, there is a special need for fine diagnostic tools in live patients to achieve accurate diagnosis as early as possible. The aim of this study was to perform a preliminary retrospective analysis on the utility of the measurement of total Tau (tTau) and phosphorylated Tau (pTau) proteins, and β-amyloid peptide 1-42 ($A\beta_{1-42}$) from the cerebrospinal fluid (CSF) of patients with rapidly progressive dementia in the diagnostic work up of CJD.

Beside the assessment of relevant clinical data and the findings of electroencephalography and brain magnetic resonance imaging, the presence of 14-3-3 protein and the levels of tTau, pTau, and $A\beta_{1-42}$ were determined by Western blot technique and enzyme-linked immunosorbent assay from the CSF of 19 patients diagnosed with rapidly progressive dementia between the period of 2004-2017 at the Department of Neurology, University of Szeged.

This preliminary study provided 100% sensitivity and, interestingly, only 40% specificity values for the positivity of 14-3-3. Regarding tTau, the sensitivity values were calculated to be 100% or 83%, whereas the specificity values were 71% or 86%, depending on the applied cut-off levels. With regard to pTau and $A\beta_{1-42}$, all the calculated parameters were considerably poor, except for a relatively good specificity (86%) of pTau.

The results of the current study tend to be in line with the literature data. The combined application of these and novel chemical biomarkers may increase both sensitivity and specificity to a desired level.

Absztrakt

Mivel jelenleg csak a pathológiai vizsgálat nyújt biztos diagnózist a Creutzfeldt-Jakob betegség (CJB) vonatkozásában, ezért különösen nagy szükség van olyan tesztekre, melyek még élő állapotban szolgáltatnak megfelelő diagnózist lehetőleg a betegség kezdeti stádiumában. Jelen tanulmány célja egy előzetes retrospektív analízis végzése volt a gyorsan progrediáló demenciával diagnosztizált betegek liquor totál tau (tTau), foszforilált tau (pTau) és a β-amiloid peptid 1-42 ($A\beta_{1-42}$) szintjének CJB diagnosztikájában való használhatóságáról.

A releváns klinikai adatok, valamint elektroenkefalográfiás és agyi mágneses magrezonanciás vizsgálati eredmények feldolgozása mellett a 14-3-3 fehérje kimutatása, továbbá a tTau, pTau és $A\beta_{1-42}$ szintek meghatározása Western blot, illetve enzimhez kapcsolt immunoszorbens vizsgálatokkal történt 19, a Szegedi Tudományegyetem Neurológiai Klinikáján 2004-2017 között gyorsan progrediáló demenciával diagnosztizált beteg liquorából.

A jelen előzetes tanulmány 100%-os szenzitivitást és meglepő módon, csak 40%-os specificitást mutatott a 14-3-3 fehérjére a CJB diagnosztikájában. A tTau esetén a szenzitivitás 100%-nak, illetve 83%-nak, míg a specificitás 71%-nak, illetve 86%-nak adódott a használt referenciaértéktől függően. A pTau és $A\beta_{1-42}$ vonatkozásában a fenti kalkulált paraméterek meglehetősen alacsonyak voltak, kivéve a pTau jónak mondható specificitását (86%).

Jelen vizsgálat eredményei jó összhangban vannak a szakirodalmi adatokkal. Valószínűleg a fenti anyagok és néhány új kémiai biomarker együttes alkalmazása a megfelelő szintre emelheti mind a szenzitivitást, mind a specificitást a CJB diagnosztikájában.

1. Introduction

Human transmissible spongiform encephalopathies, which include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI), and kuru, are rare neurodegenerative disorders, out of which CJD is by far the most common (1-2/1.000.000 person/year)[1]. Beside classic prion diseases, the prion-like propagation of the pathological process was raised in other neurodegenerative conditions, such as Alzheimer's disease (AD) and Parkinson's disease as well[2]. CJD is characterized by a rapid disease course associated with the conformational transformation of the human cellular prion protein (PrP^{c}) into the neurotoxic, protease-resistant scrapie form of this protein (PrP^{Sc})[3]. Considering the etiology of CJD, sporadic, familial, and acquired (variant and iatrogenic) forms are specified[3,4]. The sporadic form (sCJD) is the most common type, representing approximately 85% of all CJD cases with an unknown mechanism of infection[5]. It has 6 phenotypes, obtained from the combination of the methionine/valine (M/V) polymorphism in the coding region of the prion protein gene (*PRNP*) with the two isoforms of the PrP^{Sc}[6–8]. The genetic form of CJD (gCJD) accounts for 10-15% of the cases internationally[9,10]. The variant form of CJD (vCJD) shows a regional accumulation, mainly restricted to the United Kingdom and France, and it is known to be caused by consuming bovine spongiform encephalopathy-infected food[11]. The principal sources of iatrogenic CJD (iCJD) include contaminated growth hormone extracts and dura mater grafts derived from human cadavers with undiagnosed CJD infections, and it has an incidence of 1% of all CJD cases[12].

In addition to the presence of characteristic rapidly progressive dementia, the clinical picture may include the following clinical features: myoclonus, visual or cerebellar disturbance, pyramidal/extrapyramidal dysfunction, and akinetic mutism[13]. Along with typical electroencephalographic (EEG) findings during an illness of any duration AND/OR a positive 14-3-3 cerebrospinal fluid (CSF) assay with a clinical duration to death < 2 years AND/OR high signal abnormalities in the caudate nucleus and putamen or at least two cortical regions (temporal-parietal-occipital) either in DWI or FLAIR sequences IF routine investigations do not suggest an alternative diagnosis, the diagnosis of probable or possible CJD can be set up depending on the number of matching factors[13,14]. The diagnosis of definite CJD requires comprehensive post-mortem neuropathological assessment[13]. Although the current diagnostic criteria list only 14-3-3 as a biomarker, total tau (tTau), phosphorylated tau (pTau), α-synuclein, S100B protein[15,16], prion proteins[4], neuron-specific enolase (NSE)[17,18], neurofilament light chain (NF-L), phosphorylated neurofilament heavy chain (pNF-H)[19,20], and β-amyloid peptide[20,21] were also assessed for their suitability as biomarkers (Fig. 1.).

The presence of 14-3-3 protein in the CSF, which is characteristic of almost 95% of sCJD cases[17], is usually determined by Western blot test. From the 7 known isomers (β, γ, ε, ζ, η, σ, and τ) only the presence of the β, γ, ε, and η forms has been demonstrated in CJD[22]. The tTau protein proved to be a useful biomarker in the diagnostic work up of different neurological diseases, including AD[23] and CJD, and together with the presence of 14-3-3 protein, they can reliably support the clinical diagnosis[15]. The tTau protein is highly increased in CJD, whereas this characteristic elevation cannot be observed for pTau[24,25]. In addition to the above-mentioned proteins, the levels of NF-L and pNF-H were found increased as well in both serum and CSF samples[19,20,26–28]. With regard to β-amyloid concentrations, similarly to AD, decreased values were reported compared to control groups[25,29]. It was demonstrated that S100B protein may have a role in the diagnosis of genetic prion diseases, including gCJD, GSS, and FFI, showing elevated concentrations beside the presence of the positivity of 14-3-3 and the increased level of NSE[30]. The level of α-synuclein protein, measured by electrochemiluminescence-based human enzyme-linked immunosorbent assay (ELISA) kit, was considerably elevated in sCJD cases compared to controls[31,32]. Furthermore, the confirmation of the presence of PrP^{Sc} in live patients was proposed to be included in the criteria for an *in vivo* definite diagnosis in the future[33]. There are three different techniques for the detection of PrP^{Sc} from different body fluids[34], including 1) the real-time quaking-induced conversion for the detection of PrP^{Sc} from the CSF[35] or specimen obtained from nasal brushing[36], 2) the protein misfolding cyclic amplification (PMCA) method for the detection of PrP^{Sc} from blood samples[37], or 3) a specific ELISA test[21,38].

The measurement of β-amyloid peptide 1-42 ($A\beta_{1-42}$), tTau, and pTau proteins by ELISA method is available since 2009 in our institute for scientific purposes. Although the assessment of these 3 biomarkers may mainly have a supporting role in the clinical diagnosis of AD, but the above data may raise the possibility of the usefulness of particularly tTau protein in the diagnostic work up of CJD as well.

The aim of the present preliminary retrospective study was to measure the levels of tTau, pTau, and $A\beta_{1-42}$ in the CSF samples of our patients with the presumptive clinical diagnosis of CJD from 2004 to 2017, and to assess their potential supporting diagnostic role in the context of other available test results (such as EEG, brain MRI, 14-3-3 protein measurement, genetic analysis, and neuropathological assessment). The findings of descriptive statistical analysis were compared with data from the biomarker literature.

2. Patients, materials, and methods

2.1 Sample collection and the work-up of clinical data

All patients with relatively rapidly progressive dementia with suspected CJD who underwent a lumbar puncture since 2004 at the Department of Neurology, University of Szeged were enrolled to this retrospective study, following the approval of the local Ethical Committee of the University of Szeged (46/2014), adhering to the tenets of the most recent revision of the Declaration of Helsinki. For the clinical diagnosis of CJD according to the World Health

Organization (WHO) criteria[13], in addition to the progressive dementia, the presence of the following 4 clinical features were assessed: myoclonus, visual or cerebellar disturbance, pyramidal/extrapyramidal dysfunction, and akinetic mutism. Furthermore, to be able to distinguish between possible and probable CJD, the available EEG and MRI findings were analyzed as well looking for periodic sharp wave complexes and high signal abnormalities in caudate nucleus and putamen or at least two cortical regions (temporal-parietal-occipital) either in DWI or FLAIR[39], respectively, along with the qualitative determination of CSF 14-3-3 protein with Western blot technique. The diagnosis of definite CJD was based on post-mortem neuropathological findings. The test results were supplemented with the data of genetic analysis (either *PRNP* gene mutation or codon 129 polymorphism) where available as well. Patients with relatively rapidly progressive dementia who did not meet the diagnostic criteria for CJD were designated as non-prion rapidly progressive dementia (npRPD).

2.2 Determination of CSF total tau, phosphorylated tau, and beta-amyloid levels

Following the lumbar puncture, the CSF samples were centrifuged at 8.000 RPM for 10 min. The supernatants were stored in sterile polypropylene tubes in −80°C until use, distributed into aliquots to avoid repeated freeze–thaw cycles. We utilized commercially available ELISA kits (Innogenetics N.V., now Fujirebio Europe N.V., Ghent, Belgium) for the quantitative determination of $A\beta_{1-42}$, tTau, and pTau levels according to the manufacturers' instruction, as described previously[40]. Briefly, all samples and standards were run in duplicates, the optical density values were detected at 450/560 nm with a plate reader (Awareness Technology Inc., Palm City, FL, USA) and the respective concentrations were read from the standard curves fitted by Sigmaplot 10.0 software (Systat Software Inc., Richmond, CA, USA). The lower limit of detection of the assays was 87, 87 and 15 pg/ml for $A\beta_{1-42}$, tTau, and pTau, respectively. The normal values provided by the reference manual are presented in the footnotes of Table 1. Where the measured values of tTau exceeded that of the highest standard, the actual highest standard values were given. Values were accepted if the respective coefficients of variation were less than 15%.

Our laboratory is approved for the diagnostic analyses of tTau, pTau, and $A\beta_{1-42}$, by the applied kits, for which we could use samples running in the 'Alzheimer's Association QC program for CSF biomarkers' as quality control samples to rule out analytical bias.

2.3 Statistical analyses

The sensitivity (i.e., the number of CJD patients with alteration in the observed parameter / (the number of CJD patients with alteration in the observed parameter + the number of CJD patients without alteration in the observed parameter)) and specificity (the number of npRPD patients without alteration in the observed parameter / (the number of npRPD patients without alteration in the observed parameter + the number of npRPD patients with alteration in the observed parameter)) values were calculated for all the laboratory biomarkers, and for the presence of characteristic findings on the EEG and MRI as well. Alteration in a chemical biomarker was considered if the CSF level was higher than the established cut-off for tTau and pTau and lower than that for $A\beta_{1-42}$, whereas CSF 14-3-3 was considered altered when it was positive. The cut-off values used and the method of their calculation are presented in the footnotes of Table 2. A descriptive statistical analysis was carried out for the sensitivity and specificity values found in the scientific literature for all the above-mentioned and some other biomarkers.

3. Results

The clinical data (in light of the diagnostic criteria of CJD), the test results, and the autopsy findings (where applicable) of patients presenting with relatively rapidly progressive dementia in the period of 2004-2017 at the University of Szeged, Department of Neurology are demonstrated in Table 1. The proportion of genetically determined CJD among CJD cases that underwent genetic analysis was remarkably high (6/7), which is in line with previous data reporting the gCJD form to predominate in this geographical area[41,42], contrasting with international data[10]. This is most probably not merely attributable to selection bias secondary to an influence by a positive family history, as this incidence is substantially high even if related to all consecutive CJD cases identified (6/12). Although, due to the limited number of cases in this preliminary study, a receiver operating characteristic (ROC) curve analysis cannot be carried out, the respective crude sensitivity and specificity values could be easily calculated by using the equations above, and these data are presented in Table 2. The specificity for CJD was found to be as surprisingly high as 100% in the cases of both the PSWCs in EEG and the characteristic alterations in MRI, but the sensitivity was only fair (75% and 80%, respectively). Among CSF biomarkers, only 14-3-3 and tTau provided 100% sensitivity (if mean + 1 SD of the reference values was applied as cut-off for tTau). However, the specificity of 14-3-3-positivity was considerably poor (40%), whereas that of tTau elevation was found to be fair (71%). If cut-off values were changed to > 1200 pg/ml for tTau (consistent with many of the studies in the literature), both good sensitivity (83%) and specificity (86%) values could be achieved. Due to the very limited number of cases where both measurement data were available, only an approximation could be carried out on the utility of their combined use, providing a sensitivity of 87.5% and a specificity of 80%. The sensitivity values for pTau (25% in the case of both cut-off values) and $A\beta_{1-42}$ (50% and 17%, respectively) remained considerably low irrespective of the application of the upper quartile and the mean - 1 SD of the reference values as cut-offs, respectively, or > 60 pg/ml and < 400 pg/ml values (consistent with many of the studies in the literature), respectively. Surprisingly, the specificity values for pTau were found to be good (86% in both cases), irrespective of the applied cut-off values. However, the specificity values for $A\beta_{1\text{-}42}$ remained considerably low, irrespective of the applied cut-off values as well (29% and 57%, respectively).

4. Discussion

CJD is a rapidly progressive, fatal neurological disorder, where only autopsy provides definite diagnosis. Accordingly, there is a special need for fine diagnostic tools in live patients to achieve accurate diagnosis as early as possible. In addition to the prognostic importance of the early diagnosis for the patients and their relatives, the possibility of disease transmission may raise hygienic considerations as well[43], despite the fact that CJD cannot be transmitted through direct contact or airborne spread[44]. Accordingly, only the standard precautions are to be complied with by the caregivers. However, in case of iatrogenic and variant forms, the direct implantation or transplantation of materials of human-origin or the consumption of infected bovine meat may result in disease transmission[44,45,46].

As mentioned above, at present, the definite diagnosis of CJD can only be established by confirming pathological prion protein deposition in the brain[47]. However, the probabilistic clinical diagnosis can be supported by the presence of PSWCs in EEG and/or abnormal signal changes on DWI or FLAIR MRI evaluated by a qualified neuroradiologist, trained to detect the characteristic signs for CJD. Both methods provide good specificity (74–100%[1,14,48–50] and 85.1–100%[49,51–55], respectively), confirmed by the results of the current study as well (Table 2), but the sensitivity values are often only fair (32–66%[1,14,48–50] and 62.9–100%[49,51–55], respectively). Accordingly, the additional utility of several chemical biomarkers of neuronal damage were assessed to achieve a more precise diagnosis. The most commonly used chemical biomarker in CJD is the 14-3-3 protein[14,17,19,29,30,34,38,56–80], the only chemical biomarker applied in the WHO diagnostic criteria for CJD[13], with sensitivity and specificity values ranging between 50% and 100% (median: 89.8%) and between 43% and 100% (median: 87%), respectively (for more details see Table 3). Keeping in mind the heterogeneous nature of literature data, mainly as regards the composition of control groups, when studies using npRPDs as controls were selected to decrease the heterogeneity, the values remained almost the same (sensitivity with a median of 93.5% and specificity with a median of 83.2%). Surprisingly, data of the present preliminary study demonstrated a sensitivity of 100% and a specificity of 40% for 14-3-3 protein positivity, the opposite ends of the range found in the literature. The second most commonly applied biomarker is the tTau protein[16,17,19,20,26,29,38,59,61,63–66,68–70,72,74,75,77–79,81–85], which is also considered to have a possible diagnostic value in light of its relatively high sensitivity (ranging between 59.68% and 100% with a median of 89.2%) and specificity (ranging between 75.31% and 100% with a median of 92%; for more details see Table 3). The similar reduction of the variability of control groups to that carried out in the case of 14-3-3 also did not considerably affect the sensitivity (median: 89.7%) and specificity (median: 93.55%) values. With regard to the current study, if we applied the mean + 1 SD of the control values provided by the manufacturer as cut-off level, a considerably high (100%) sensitivity value was achieved but the specificity remained only fair (71%). Although the limited number of samples did not allow the use of ROC analysis, the increase of the cut-off value to 1200 pg/ml, the cut-off reported in the majority of literature, resulted in the increase of specificity (86%) with a consequent decrease of sensitivity (83%), but in that case, both of them could be considered to be good. The utility of the combined use of these 2 biomarkers was assessed as well in some studies, resulting in a slightly decreased sensitivity (49–93%[59,65,68,69,75]) but a considerable increase in specificity (92–98%[59,65,68,69,75]). Due to the reduced number of data for this analysis, the current study could not replicate this change. The statistical comparison of sensitivity and specificity values obtained in these complex studies demonstrated a significantly ($p < 0.05$) better specificity for tTau than 14-3-3.

Less data are available about the utility of other chemical biomarkers in the diagnostic work-up of CJD. The assessment of S100B[16,17,30,59,66,68,69,72,76], NSE[17,18,30,59,65,75,76], and PrP protein[21,36,38,73,86–88] for that purpose provided sensitivity values ranging between 65% and 98% (median: 84.2%), 53% and 90% (median: 76.35%), and 73% and 94% (median: 81.2%), respectively, with specificity values ranging between 29% and 93% (median: 86.2%), 79% and 98% (median: 92%), and 75% and 100% (median: 99.5%), respectively (for more details see Table 4). In light of these ranges, the diagnostic utility of these chemical biomarkers seemingly does not exceed those of 14-3-3 and tTau proteins; however, the considerably high median specificity values draw attention to more and more widespread application of the determination of PrP protein.

The availability of literature data is limited with regard to the application of $A\beta_{1\text{-}42}$ peptide and pTau protein as chemical biomarkers in CJD, and it was proposed that these substances alone are not suitable as diagnostic markers for CJD, as their concentration values overlap with other neurological diseases[29,81]. Accordingly, with regard to $A\beta_{1\text{-}42}$ peptide, the comparison of CJD and respective control groups provided controversial results[29,78,89–91]. In line with these findings, the present study demonstrated poor sensitivity (50% or 17% if mean - 1 SD of the reference values provided by the manufacturer or 400 pg/ml levels were applied as cut-offs, respectively) and specificity values (29% or 57% if mean - 1 SD of the reference values provided by the manufacturer or 400 pg/ml levels were applied as cut-offs, respectively). Although the current study showed good specificity values for pTau (86%, either the cut-off value was calculated as the upper quartile of the reference values according to the manual or a cut-off value of > 60 pg/ml was applied) similarly to those reported in the literature (ranging between 70% and 92.13%[21,82,83,85]), the sensitivity values remained considerably poor (25%, either the cut-off value was calculated as the upper quartile of the reference values according to the manual or a cut-off value of > 60 pg/ml was applied), in line with literature data ranging between 19.35% and 81%[21,82,83,85].

Beside the above chemical biomarkers, some promising novel ones have been recently reported as well, including NF-L and pNF-H[27,28]. The sensitivity values for NF-L and pNF-H vary between 80% and 100%[19,20,26] and 90% and 100%, respectively[19,20,26], whereas specificities vary between 85% and 100%, respectively[19,20,26]. Some recent studies[31,32] have reported larger than 95% sensitivity and specificity values for α-synuclein when comparing sCJD with the control group.

It can be concluded that despite the low number of cases, the present preliminary study replicated well the results of previous studies with large subject numbers. In light of the presented literature review, probably the measurement of 14-3-3 and tTau protein levels are the most widely used approaches to detect the characteristic pronounced neuronal damage in CJD. Recently, several attempts have been made by the application of novel chemical biomarkers to increase the sensitivity and specificity for the diagnosis of CJD; however, except for the excellent specificity values for PrP, neither of them provided reliably high values near 100%. Accordingly, the combination of these tests may yield the best diagnostic value, keeping in mind the availability and preferably low cost of the methods as well.

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7. Figure legend

Fig. 1. Some major physiological functions of certain chemical biomarkers, most frequently assessed in Creutzfeldt-Jakob disease