

Cerebrospinal Fluid Biomarkers in the Diagnosis of Creutzfeldt-Jakob Disease in Slovak Patients: over 10-Year Period Review

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Abstract Creutzfeldt-Jakob disease is a rare, but rapidly progressive, up to now untreatable and fatal neurodegenerative disorder. Clinical diagnosis of Creutzfeldt-Jakob disease (CJD) is difficult; however, it can be facilitated by suitable biomarkers. Aim of the present study is to compare levels of cerebrospinal fluid biomarkers (total tau protein, phosphorylated-tau protein, protein 14-3-3 and amyloid beta) in Slovak population of CJD suspect cases, retrospectively in over a 10-year period. One thousand three hundred sixty-four CSF samples from patients with suspect CJD, forming a homogenous group in terms of geographical as well as of equal transport conditions, storage and laboratory processing, were analysed. Definite diagnosis of Creutzfeldt-Jakob disease was confirmed in 101 patients with genetic form, and 60 patients with its sporadic form of the disease. Specificity of protein 14-3-3 and total tau in both forms CJD was similar (87 % for P14-3-3/85 % for total tau), sensitivity to P 14-3-3 and total tau was higher in sporadic Creutzfeldt-Jakob disease (sCJD) (90/95 %) than in genetic Creutzfeldt-Jakob disease (gCJD) (89/74 %). As expected, the total tau levels were significantly higher in CJD patients than in controls, but there was also significant difference between gCJD and sCJD (levels in gCJD were lower; $p = 0.003$). There was no significant difference in p-tau and A β 1-42 levels neither between both CJD forms nor between CJD patients and control group.

Keywords Cerebrospinal fluid · Creutzfeldt-Jakob disease · E200K mutation · Protein 14-3-3 · Total tau · Biomarker

Introduction

Human prion diseases are rare, transmissible, neurodegenerative lethal diseases. They are characterized by spongiform changes, neuronal loss and deposits of non-degradable, misfolded prion proteins (PrP^{Sc}) in the brain tissue. The conformationally altered protease-resistant proteins are considered to constitute the transmissible agent [1]. The most frequent human prion disease is Creutzfeldt-Jakob disease (CJD), which occurs in sporadic (sCJD), genetic (gCJD) and iatrogenic (iCJD) forms. While worldwide is the most frequent sCJD, (gCJD represents only 10–15 % of all CJD cases), Slovakia is peculiar by exceptionally high incidence (65–75 %) of the gCJD cases with the E200K mutation (gCJD^{E200K}) [2] accumulated in geographic clusters [3]. Increased number of gCJD with the same mutation in Israel has an ethnical origin [4, 5].

Diagnostic criteria of WHO define possible, probable and definite diagnosis of CJD [6]. Definite diagnosis is confirmed by post-mortem histopathological examination of the brain tissue and by detection of PrP^{Sc}. Probable diagnosis of CJD is based on the typical clinical symptoms and results of clinical diagnosis such as EEG, analysis of the cerebrospinal fluid (CSF) and brain MRI [6–9]. CSF marker analysis is an important part of the probable CJD diagnosis. Investigated CSF biomarkers, such as protein 14-3-3 (P 14-3-3), total tau (t-tau) protein, phosphorylated form of tau protein (p-tau), and amyloid beta (A β), indicate neuronal degeneration and/or activation of glial cells.

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P 14-3-3 belongs to the family of proteins which are found in eukaryotic cells, with the highest expression in neuronal synapses [10]. It is a non-specific marker, and an increased CSF value indicates a pathological process with a massive neuronal death. The P 14-3-3 level is increased in the CSF of CJD-affected patients, but it can also be detected in inflammatory diseases and tumours of the CNS. Factors influencing the false-positive results of the P 14-3-3 in the CSF are blood cells and pleocytosis in the CSF, caused by inflammatory processes of the CNS [10].

Tau protein is a natural, structural protein of neuronal microtubules. Abnormal, misfolded tubular protein has a tendency to form thick fibres and to aggregate into neurofibrillary tangles in the patient's brain. Abnormal tau protein has a tendency to transform into phosphorylated-tau protein (p-tau). The cutoff value for the t-tau protein in CJD diagnosis, based on international criteria, was determined to 1300 pg/ml [11, 12]. Tau protein analysis is not a part of the current diagnostic criteria. The t-tau value correlates with the patient's age. This fact could be important for differentiation of the normal ageing from pathologic, neurodegenerative processes. According to published data, only the t-tau protein is the highly increased in CJD [13, 14], not its phosphorylated form. In CJD, the neuronal destruction and increasing level of t-tau are relatively fast, representing a significant difference to Alzheimer's disease (AD) with a slower destruction process.

The pathological A β protein is the basis of senile or neuritic plaques in AD [15], and its level in the CSF is reduced. Differentiation is made between two main isoforms, shorter form A β 1-40 and longer one A β 1-42. Under normal circumstances, the soluble A β 1-40 isoform prevails, and A β 1-42 isoform aggregates faster. The A β 1-42 isoform exhibits the highest degree for adherence in plaques and has the most significant decline in values in AD patients [15]. In affected area, it participates in destruction of synapses, degenerative processes and progressive neuronal death.

While the WHO diagnostic criteria of probable CJD includes semi-quantitative P 14-3-3 test, it does not include the quantitative determination of the tau protein. Discrepancies in experience with detection of these CSF biomarkers resulted in some criticism concerning P 14-3-3 [12, 16], as well as in different view on their diagnostic value.

The aim of this study is to analyse values of the investigated CSF biomarkers in the up to now largest homogenous investigated group of gCJD^{E200K} patients, to compare them with data obtained in the Slovak sporadic CJD cases, as well as with previously published results in the literature, considering their diagnostic value and implementation in differential diagnosis of CJD.

Material and Methods

Samples Collection

The study includes 1364 samples of CSF, 1364 blood and 71 sera collected in the Department of Prion Diseases (National Reference Centrum for Prion Diseases in Slovakia) in the course of 2002–2014.

CSF samples were obtained from suspect cases of CJD from regional hospitals and transferred to our laboratory. The samples were shipped refrigerated or frozen, and prior to analysis, they were stored at -20°C , while after analysis, they are stored in freezers at -80°C .

Totally, 101 confirmed gCJD^{E200K} were obtained from the analysed 1364 CSF samples, and 60 samples were from patients affected by sCJD. Investigating the influence of the storage interval on tested samples, 20 CSF samples from gCJD^{E200K} and 18 samples of sCJD with duration of storage (at -80°C) for more than 10 years have been analysed. A control group was formed by 50 samples from previously suspected CJD cases. In which 25 of them were CJD excluded histopathologically, additional 25 patients were added in the control group retrospectively after having the negative results of the early in vivo diagnostic methods (DNA analysis for the presence of specific mutation and P14-3-3 detection) and the patient's typical CJD clinical symptoms did not persist.

The same number (1364) of blood samples were examined for detection of the E200K mutation and distribution of the polymorphism M129 V. Possible age influence on obtained results was investigated comparing three groups of patients, consisting of those less than 50 years old, patients 51 to 65 years old and the ones older than 65 years old. Besides described investigations of CSF samples, t-tau determination has been performed also in 71 sera from patients with confirmed CJD.

The present study has been in line with rules approved by the ethical committee acting on the Slovak Medical University.

Protein Analysis in CSF

P 14-3-3 (β isoform) was determined in CSF samples by Western blot according to Zerr et al. [17]. Heating up the samples in a buffer was followed by separation of the proteins based on different molecular weight by SDS/polyacrylamide gel electrophoresis. This was followed by a semi-dry blotting of proteins from the gel to a PVDF membrane, and blocking of free binding sites of the membrane in PBS containing 5 % low-fat milk. Detection was carried out by overnight incubation with primary antibody (pan 14-3-3 (K19) monoclonal antibody Santa Cruz Biotechnologies), and with the secondary antibody (Goat anti-rabbit IgG (H + L) HRP Jackson ImmunoResearch) the following day. Membranes were

developed by enhanced chemiluminescence (SuperSignal West Pico, PIERCE). Each Western blot was analysed by semi-quantitative method (negative result = no signal present; positive result = strong signal/band; weakly positive result = weaker signal compared to positive control). Each analysis was performed with positive and negative control. The sensitivity (% of positive results in patients with a confirmed diagnosis of CJD) and specificity (% of false-positive results in patients with other types of diagnosis than CJD) of the used method for detection of the P 14-3-3 were evaluated. A possible age influence on achieved results was investigated comparing three groups of patients consisting of patients less than 50 years old, 51 to 65 years old patients and those older than 65 years old.

T-tau, p-tau and A β 1-42 were measured in CSF sample collection retrospectively, using a commercially available sandwich ELISA kit (INNOTEST hTAU Ag, INNOTEST phospho-TAU (181P), INNOTEST amyloid β (1-42) manufactured by Innogenetics) following the manufacturer's instructions. A positive t-tau test for CJD was considered at cutoff level >1300 pg/ml [11, 18, 19]. According to the manufacturer's instructions, aimed at the diagnosis of AD, a pathological elevated p-tau level was considered at >61 pg/ml and pathological decreased A β 1-42 was considered at <450 pg/ml [11]. Besides individual t-tau and p-tau proteins, combined CSF levels of 1/t-tau and p-tau (pg/ml) and 2/t-tau and A β (pg/ml) were compared in sCJD with gCJD^{E200K}, as well as between CJD with non-CJD patients.

Molecular Biological Analysis

For the sake of molecular biological analysis of the prion protein gene (PRNP), DNA was isolated from blood. Subsequently, genotype was determined by polymerase chain reaction followed by restriction (PCR-RFLP) assay using cleavage enzymes (enzyme BsmAI for codon 200, and enzyme HpyCH4 for codon 129). Cleavage fragments were visualized by electrophoresis. All samples (101) from the genetic CJD group had PRNP point mutation at codon 200 (E200K mutation) (gCJD^{E200K}).

Statistical Analyses

Statistical analysis was performed using the program SPSS16.0. The Kolmogorov-Smirnov test showed that the results obtained with t-tau, p-tau and A β 1-42 proteins did not have a normal distribution, and therefore, the Mann-Whitney *U* test was used to compare these variables between diagnostic groups. For comparison of quantitative parameters between different genotypes, the non-parametric Kruskal-Wallis test was used; χ^2 test was used to determine differences between categorical variables. Spearman's test was used for correlation between individual parameters. The text presents

average data; value $p < 0.05$ was considered as statistically significant.

Results

Age Distribution of CJD Patients

Comparison of gCJD and sCJD cases in three age categories (younger than 50 years old, 51 to 65 years old, older than 65 years old) showed significantly different age distribution ($p = 0.004$). Sporadic CJD patients were older ($p < 0.001$) than gCJD cases.

Protein 14-3-3

Specificity of the method varied between 75 and 90 % in the course of 2004–2014. Increasing age of the patients had no influence on specificity of the method, since the lowest specificity was observed in the group of 51 to 65 years old patients. Sensitivity of P14-3-3 in sCJD (90 %) was similar to gCJD^{E200K} (89 %). P 14-3-3 signal intensity was stronger in sCJD than in the genetic form, where 19 % of the samples showed weak positive signal, while in case of sCJD, it was weak in only 0.6 % of the samples.

T-Tau Protein

It was retrospectively examined in the CSF samples stored in the Department of Prion Diseases since 2000. Obtained results showed significantly ($p = 0.003$) higher values of t-tau protein in sCJD (4718.7 pg/ml, with a range from 100 to 24,000 pg/ml) than in gCJD^{E200K} (2115.9 pg/ml, with a range from 50 to 16,000 pg/ml) (Fig. 1a, Table 1). Sensitivity of the t-tau assay was lower in gCJD^{E200K} (74 %) than in sCJD (95 %). Sensitivity of the method in gCJD^{E200K} could be increased by reducing the threshold on 1000 pg/ml (84 %).

Out of 18 tested samples of clinically suspect CJD, carriers of the E200K mutation without definite diagnosis confirmed by autopsy, total tau values were above the cutoff value of 1300 pg/ml in 10 cases (56 %). In case of eight of these patients, also the P 14 3-3 test was positive. However, values of t-tau were significantly higher in the confirmed cases of CJD ($p = 0.016$). In the group of 25 patients with histopathologically excluded CJD, 6 CSF samples have a t-tau above 1300 pg/ml (24 %). These are patients with AD, paraneoplastic neurological syndrome associated with cancer and stroke (based on data provided by clinical departments). Patients with t-tau < 1000 pg/ml from control group have AD, stroke, metabolic encephalopathy and paraneoplastic neurological syndrome.

The specificity for t-tau protein was 85 %, similar as for P 14-3-3 (87 %).

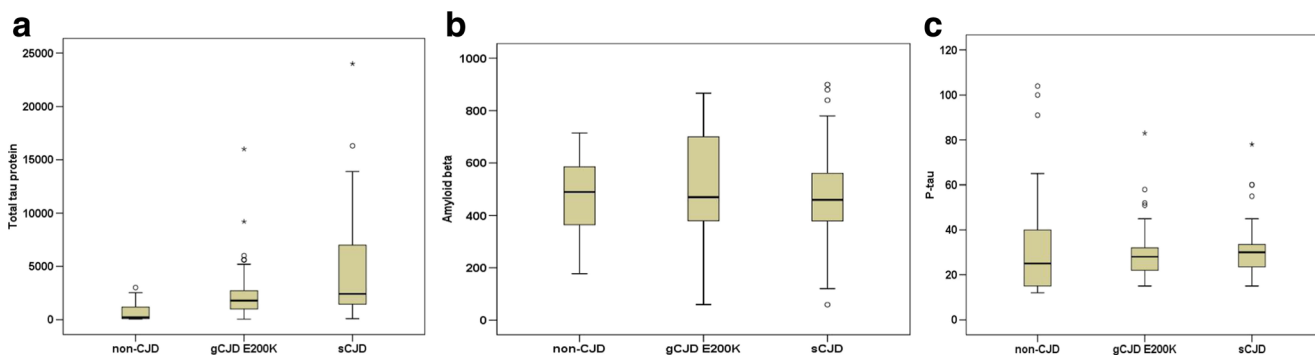


Fig. 1 a–c Box plots of CSF **a** total tau, **b** amyloid beta, **c** p-tau in sCJD, gCJD^{E200K}, non-CJD patients. Plots show 10, 25, 50, 75 and 90th percentiles and outliers, with significant difference in average CSF total tau protein between non-CJD and both forms of CJD, and no difference in

average CSF amyloid beta and p-tau in non-CJD and CJD patients. t-tau: *n* (sCJD) = 60, *n* (gCJD) = 99, *n* (non-CJD) = 50. p-tau and Aβ1–42: *n* (sCJD) = 40, *n* (gCJD) = 72, *n* (non-CJD) = 50

Combined CSF levels of both 1/t-tau and p-tau (pg/ml) (Fig. 2a) and 2/t-tau and Aβ (pg/ml) (Fig. 2b) compared in sCJD with gCJD^{E200K}, as well as between CJD with non-CJD patients, showed statistically significant difference (*p* < 0.05). To compare biomarkers, ratio of p-tau/t-tau and Aβ 1-42/t-tau non-parametric Man-Whitney test was used and had shown statistically significant difference between genetic and sporadic CJD (*p* < 0.001). The group is small to calculate cutoff value. For the (p-tau/t-tau) × 100, ratio is mean value in gCJD 4.09 (95 % confidence interval 2.67–5.51), in group of sCJD 1.6 (95 % confidence interval 0.52–2.68) and in non-CJD 16.1 (95 % confidence interval 9.53–22.62).

Codon 129

Distribution of M129 V polymorphism in examined patients showed in both sporadic and genetic CJD forms, a significant (75 %) prevalence of the M/M homozygosity (Table 1).

Homozygous M/M patients had a higher values of t-tau protein compared to M/V patients in both gCJD^{E200K} (*p* = 0.007)

and sCJD (*p* = 0.01). Homozygosity M/M on prion gene (PRNP) at codon 129 is considered as a risk factor for CJD [20]. Between the patients with sporadic CJD, there were homozygotes as well as heterozygotes with disease duration from 1 to 3 months from lumbar puncture (LP) until their death (Table 1). Patients with gCJD^{E200K} died within 3 months after LP in a homozygous form M/M 70 %, and in heterozygous form M/V 11 % (Table 1).

Detection of Unspecific Biomarkers

In our study, Aβ 1-42 and p-tau did not show significant differences between CJD patients and patients with other neurodegenerative diseases. Average value of Aβ 1-42 amyloid in the CSF samples of 112 CJD patients was 498 pg/ml (with a range from 100 to 867), and in 50 control samples, it was 458 pg/ml (with a range from 180 to 890) (Fig. 1b, Table 2). Values in sCJD and gCJD^{E200K} were not significantly different. Average values of p-tau in CSF samples of 112 patients with CJD were 41 pg/ml (20 to 83), and in 50 control samples, they were 48 pg/ml (20 to 120) (Fig. 1c, Table 2). Values in sCJD and gCJD^{E200K} were not

Table 1 Characterization of tested groups and principal biomarker results

		gCJD (<i>n</i> = 101)	sCJD (<i>n</i> = 60)	Non-CJD (<i>n</i> = 25)
Gender (M/F)		48/53	24/36	16/9
Age at LP, years (min-max)		58 (28–77)	64 (43–83)	60(33–89)
P14-3-3 in CSF (+/–)	Positive	90	54	12
	Negative	11	6	13
Total tau in CSF (pg/ml)		2115.9	4718.7	720.8
		(1697–2534) ^a	(3457.4–5980.0) ^a	(438.8–1002.8) ^a
PRNP codon 129 genotype	MM	79	38	16
	MV	22	9	6
	VV	0	10	3
Total		101	60	
Up to 2004		20	18	
Since 2004		81	42	

^a 95 % confidence interval

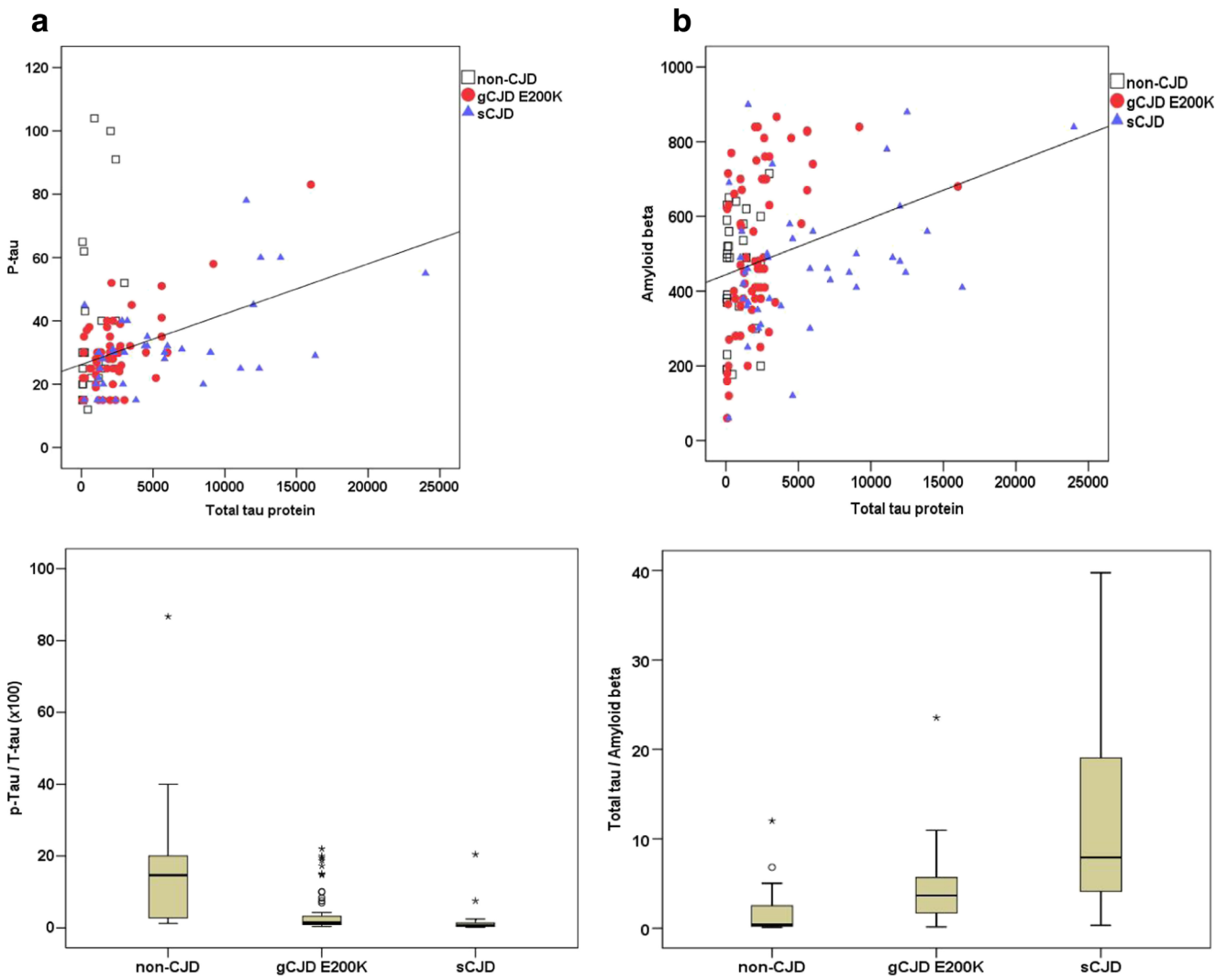


Fig. 2 **a, b** Correlation CSF levels (pg/ml) of total tau and p-tau (**a**), total tau and amyloid beta (**b**) in sCJD, gCJD^{E200K} and non-CJD patients (Spearman’s $r = 0.451$ for p-tau/t-tau, $p < 0.001$; Spearman’s $r = 0.323$ for A β /t-tau, $p < 0.001$) and comparison of ratio CSF levels of total tau and p-tau (**c**), total tau and amyloid beta (**d**), with statistically difference not only between non-CJD and CJD patients but also between both forms of CJD (Mann-Whitney test). n (gCJD) = 69 p-tau/t-tau($\times 100$) mean

$v. = 4.09$ (CI 95 % = 2.67–5.51); $p < 0.001$. t-tau/A β 1–42 mean $v. = 4.25$ (CI 95 % = 3.40–5.1); $p < 0.001$. n (sCJD) = 40 p-tau/t-tau($\times 100$) mean $v. = 1.6$ (CI 95 % = 0.52–2.68); $p < 0.001$. t-tau/A β 1–42 mean $v. = 12.17$ (CI 95 % = 8.97–15.37); $p < 0.001$. n (non-CJD) = 30 p-tau/t-tau($\times 100$) mean $v. = 16.08$ (CI 95 % = 9.53–22.62); $p < 0.001$. t-tau/A β 1–42 mean $v. = 1.85$ (CI 95 % = 0.82–2.88); $p < 0.001$

Table 2 Biomarker values in investigated subgroups

Biomarker	sCJD $n = 60$	gCJD E200K $n = 101$	Non-CJD (histopat.) $n = 25$	Non-CJD (clinically) $n = 25$
Total tau	<1000 pg/ml	6	27	19
	1000–1300 pg/ml	0	15	0
	>1300 pg/ml	54	59	6
A β 1-42	<450 pg/ml	17	30	12
	>450 pg/ml	23	42	13
P-tau	<60 pg/ml	37	70	22
	>60 pg/ml	3	2	3

significantly different. In two gCJD^{E200K} cases, in addition to the positive P 14-3-3 and high levels of t-tau, levels of p-tau were also significantly increased, while those of A β 1-42 were reduced. There was suspected parallel course of AD and CJD in these two patients based on the clinical symptoms. Both patients were demented with memory impairment, cognitive difficulties, hallucinations, impulsive behaviour and from the other clinical symptoms are observed myoclonus, aphasia, ataxia and gait disturbance. Diagnosis of CJD was confirmed histopathologically and by Western blot. Examination to establish AD diagnosis has not been performed.

Age of Patients

Comparison of biomarkers with the age of patients (Fig. 3a–f) (Fig. 3a–c) shows that age has not a significant effect on t-tau and A β 1-42 in gCJD group; p-tau level is even slightly declining. T-tau and p-tau levels have in sCJD rising tendency; A β 1-42 is decreasing (Fig. 3d–f) which is probably related to ageing processes.

Total Tau Protein Levels in Sera

Total tau protein was tested also in 51 sera from gCJD^{E200K} patients and 20 sera from patients with sCJD. Most of the

values (87 %) of the t-tau (using a commercial kit from the company INNOTEST hTau. INNOGENETICS) were lower than 100 pg/ml (average value was 67.5, with range from 20 to 450). Since the measuring accuracy indicated by the kit producer is within range from 125 to 1000 pg/ml, the detection limit is 87 pg/ml; the serum does not seem to be an appropriate biological material for this kit.

Discussion

With respect to the predominant occurrence of sCJD worldwide, investigations of CSF biomarkers in patients with gCJD were done mostly as multicentre studies. [11, 19, 21]. A group of 54 gCJD^{E200K} patients analysed in Israel [5] makes an exception. In our study, results of CSF and blood samples from genetic and sporadic CJD patients analysed for more than 10 years are presented, containing 101 samples from patients with gCJD^{E200K}, which represent a valuable homogeneous group with the same conditions of transport, storage and samples processing. Considering the achieved results, diagnostic value of tested biomarkers for gCJD^{E200K} and sCJD was analysed, compared and correlated with relevant data in the literature. Detection of the P14-3-3 is included into WHO diagnostic criteria of CJD [6]. Quality of the biological

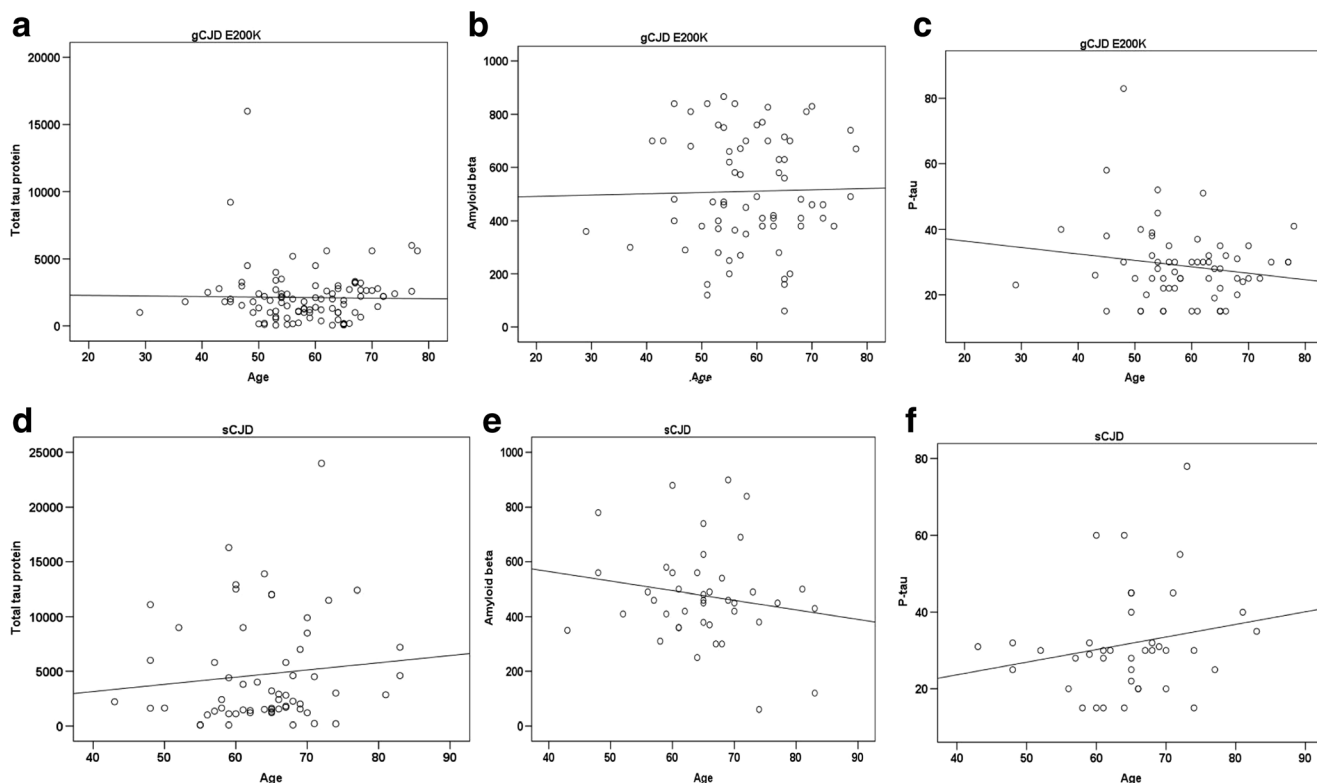


Fig. 3 a–f Correlation CSF levels (pg/ml) total tau, amyloid beta, p-tau and age in time of LP (samples delivery) in gCJD^{E200K} (a–c) and sCJD (d–f) patients without statistical significance. T-tau: n (sCJD) = 60, n (gCJD) = 99, p-tau and A β (1-42), n (sCJD) = 40, n (gCJD) = 72; t-tau/

age Spearman's $r = 0.64$ (a) $p = 0.53$; Spearman's $r = 0.157$ (d), $p = 0.226$; A β (1-42)/age Spearman's $r = 0.017$ (b) $p = 0.892$; Spearman's $r = -0.096$ (e), $p = 0.549$; p-tau/age Spearman's $r = -0.103$ (c) $p = 0.417$; Spearman's $r = 0.202$ (f), $p = 0.218$

materials, especially the presence of blood in the CSF, significantly affects quality of the obtained results. According to our experience, red blood cells above 250 ery/ml may cause a false-positive result. Influence of blood contamination studied by Schmitz et al. [10] also confirmed that inoculation of CSF with erythrocytes (2500 cell/ml) has significantly increased the percentage of positive P14-3-3 results. Sensitivity of biomarkers in the CSF can be affected also by other factors such as age in time of the CJD onset, duration of the disease, lumbar puncture timing or the molecular subtype [5, 12, 21, 22]. Some limitation of the P 14-3-3 assay results from the visual inspection of immunoblots, leading to a subjective interpretation of bands [23]. When the signal in patients was too weak or negative, and the clinical symptoms consistent with CJD were persisting, another lumbar puncture was recommended after at least 2 months, since previous studies shows that repeated CSF analysis could be useful [24, 25].

In case of our patients, the sensitivity of P 14-3-3 in sCJD (90 %) and gCJD^{E200K} (89 %) was similar and comparable with published data, where sensitivity ranged from 77 to 97 % in sCJD [5, 12, 17, 19, 26], and from 77 to 97 % in gCJD [5, 21, 27]. Regardless of the similar sensitivity in our compared CJD forms, the intensity of P 14-3-3 signal was stronger in sCJD. Specificity of the P14-3-3 test in the Slovak CJD group was 87 % in average, varying between 75 and 90 % in the course of 2004–2014. Specificity could be influenced by the occurrence of atypical forms of CJD (e.g. age less than 50 years of age in sCJD [12]) and by selection of suspect patients over-evaluating their clinical symptoms, which might have increased number of false-positive results. Suggested influence of the number of examined samples was not confirmed, since despite the increasing annual number of tested CSFs in European countries (including Slovakia, where 120 CSFs were examined in 2011 and 196 CSFs in 2015), the specificity for P 14-3-3 remains generally stable, up to 92 % [11]. Assumed influence of age was not confirmed in our CJD patients. While the compared age groups showed significantly higher age in sCJD as in gCJD^{E200K}, correlating with our previous data [3], no influence of the ageing on the P 14-3-3 specificity was observed, since the lowest values were found in the 51 to 65-year-old patient group. T-tau was examined in one of the largest dataset of patients with gCJD^{E200K} from a single country. Sensitivity of t-tau in our genetic group was lower in comparison to sCJD, as well as to European studies [19, 21, 27]. T-tau (p-tau and A β 1-42) in our patients were tested retrospectively, in CSF samples collected since 2000, so the quality of results could probably be influenced by duration of the sample storage. This statement is supported by observations of Schmitz et al. [10], that about 25 % of effectiveness of methods is decreasing in samples older than 9 years. Because of significantly higher values of t-tau, this influence is less evident in sCJD. Influence of the optimal cutoff value is demonstrated by Meiner et al., Satoh et al. [5, 28] and

Coulthard et al. [16], who reported considerably increased t-tau sensitivity after reduced threshold value to 1000 pg/ml [5], or more precisely to 976 pg/ml [16].

Reducing the t-tau cutoff values from 1300 to 1000 pg/ml in the Slovak gCJD^{E200K} cases, we have also observed that the t-tau sensitivity has increased from 74 to 84 %. That is comparable with the published data claiming 82 [19] and 86 % [21].

In our group of suspect CJD patients, there were 18 carriers of the E200K mutation; however, autopsy was not done to any of them. High t-tau protein was present in 10 (56 %) of them, signaling a considerable number of not confirmed genetic CJD cases. Evaluation of CSF tau proteins is not included in diagnostic criteria of CJD, but described observation along with results of the two compared methods indicate that testing of CSF tau protein could represent a valuable contribution to the differential CJD diagnosis.

Specificity of t-tau in our CJD patients was 85 %, but it was calculated in 1 year and in smaller set of samples. Chohan, Meiner and Coulthart et al. [5, 12, 16] have reported higher specificity for t-tau when compared to P14-3-3; our observation showed similar specificity for P14-3-3 and t-tau (87/85 %). Comparing the sensitivity of both methods for gCJD^{E200K}, we have observed that it was higher to P 14-3-3 than to t-tau protein; both methods have decreased sensitivity in gCJD in comparison with sCJD. The reason is unclear, but maybe it is related to lower age of gCJD patients. Sensitivity of t-tau protein in our sCJD group was similar to the sensitivity of P 14-3-3 and comparable with the published findings [5, 12, 17, 21].

Analysing a possible influence of the M129 V polymorphism of the PRNP gene, our results were different compared to those described by Meiner et al. [5]. While in their study, correlation neither between t-tau values nor between P14-3-3 positivity and M129 V polymorphism was observed; our study has observed significantly higher t-tau values in methionine homozygous patients with both gCJD^{E200K} ($P = 0.007$) and sCJD ($p = 0.01$). There was also another contrast to Meiner et al. [5] recorded previously, specifically in relation to methionine homozygotes and shorter duration of the disease in our group of gCJD^{E200K} [3].

Moeko Noguchi-Shinohara et al. [29] reported significantly higher values of t-tau in CJD sera when compared to other neurological diseases. In our serum samples, as much as 87 % of t-tau values were under the measuring accuracy indicated by the producer of kits. At the moment, we have found methodical limitation for detection of serum t-tau protein in asymptomatic carriers of the E200K mutation as an early pre-clinical indicator of the disease development and progress. CJD and AD have several clinical and neuropathological features, resulting in diagnostic difficulties [26]. It is well known that reduced levels of A β 1-42 and increased levels of total tau protein are characteristic for numerous different types of

dementia. According to Otto et al. [30], reduced levels of A β 1-42 in the CSF do not exclude diagnosis of CJD, although the value of A β 1-42 levels in the CSF of patients with CJD and AD do not show significant differences. However, these values are significantly different from those in other types of dementia or in patients without dementia. Cutoff values for A β 1-42 and the p-tau in CJD are not defined. Increased p-tau suggests a risk for AD [7]. In our tested group, we have observed decreased A β 1-42 values in both CJD and non-CJD patients, being in agreement with the assumption that A β 1-42 is not a specific CJD marker [11]. Since not only absolute values, but also ratio of their combinations are important for biomarkers, we have correlated t-tau with A β 1-42 and t-tau with p-tau. We have found statistically significant differences in the ratio of t-tau/A β 1-42 not only between CJD and non-CJD patients but also between gCJD^{E200K} and sCJD. Alcolea et al. [31] have reported correlation between values of t-tau and A β 1-42, reflecting normal ageing process and depending on the A β 42 status. Significantly, increased t-tau, p-tau values and decreased A β 1-42 according to the ageing can be detected neither in sCJD nor in gCJD^{E200K}. Zanusso et al. [32] refer that ratio of p-tau/t-tau or of A β 1-42/p-tau may be helpful for distinguishing CJD from other dementias (AD, FDT). Lorens et al. [33] recommended introduction of p-tau/t-tau ratio into clinical investigations, which can be helpful to avoid misdiagnosis between sCJD and AD. Our observations have also confirmed the statistically significant difference in p-tau/t-tau ratio (Fig. 2a) not only between CJD and non-CJD patients but also between both investigated forms of CJD.

Conclusion

Comparison of CSF biomarkers in sCJD and gCJD has been done in a homogenous group of CJD patients with up to now largest number of gCJD^{E200K} cases. Achieved results demonstrate that while the detection of absolute values of unspecific biomarkers as p-tau and A β 1-42 did not show significant differences between CJD and other neurodegenerative diseases, their combinations have differential diagnostic value.

Evaluation of specific t-tau and P 14-3-3 biomarkers have confirmed significant differences between sCJD or gCJD when compared to non-CJD control groups. The specificity of both diagnostic methods in our CJD patients was comparable, but sensitivity of these specific biomarkers was significantly higher in sCJD. Efficiency of t-tau method could be increased by reducing cutoff value from 1300 to 1000 pg/ml.

Detection of P 14-3-3 protein is unlike t-tau included in WHO diagnostic criteria. Achieved results demonstrate that detection of both compared biomarkers has an important role in the differential diagnosis of CJD. In genetic cases, confirmed CJD-specific mutation and positive results of both

methods may decidedly contribute to the diagnosis of transmissible dementia and to protective preventive measures.

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Compliance with Ethical Standards

Conflict of Interests The authors declare that they have no conflicts of interests.

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