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Anti-cytoskeletal antibodies in patients with multiple sclerosis and other neurological diseases

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Contents

Abstract

This thesis focuses on the role of anti-cytoskeletal antibodies in various neurodegenerative diseases. Autoantibodies against different axonal cytoskeletal proteins, such as the light (NF-L) and medium (NF-M) subunits of neurofilament (NF) and tubulin (TU), in serum and cerebrospinal fluid may be generated in response to the release of cytoskeleton from damaged neurons.

The aim of this study was to assess autoimmune involvement in amyotrophic lateral sclerosis (ALS) as well as to evaluate antibody light and medium neurofilament subunit in serum and cerebrospinal fluid (CSF) of patients with ALS. Furthermore, we were interested in the relationships among these antibodies in the serum and in the CSF as well as between the two anti-NF antibody subtypes. Secondly, the aim was to compare the levels of anti-tubulin antibodies (anti-TU) in cerebrospinal fluid and serum in multiple sclerosis (MS) disease, using bovine tubulin as the antigen in one enzyme-linked immunosorbent assay (ELISA) method (anti-TUb antibodies) and a synthetic neuron-specific octapeptide of tubulin in a second ELISA method (anti-TUs antibodies).

In the observed groups of ALS patients, serum levels of anti-NF-L antibodies were higher in ALS patients than in controls and serum anti-NF-L antibodies and intrathecal anti-NF-M antibodies were related to patient disability. Positive correlation was found between anti-NF-L levels and anti-NF-M levels in ALS patients and the controls in the CSF.

Levels of CSF anti-TUs and anti-TUb antibodies were significantly higher in the MS patients compared to normal control group. The intrathecal synthesis of anti-TUs antibodies was higher compared to anti-TUb in all groups. Positive correlation was found between anti-TUb and anti-TUs antibodies in the CSF of all examined groups.

Our results point to opportunities anti-cytoskeletal antibodies as using marker of axonal damage.

Abstrakt

Předkládaná práce se zabývá úlohou anti-cytoskeletálních protilátek u různých neurodegenerativních onemocnění. Při postupné demyelinizaci dochází k uvolňování strukturních částí cytoskeletu, jako jsou např. lehké (NF-L) a středně těžké (NF-M) podjednotky neurofilament (NF) či tubulin (TU). Proti těmto uvolněným strukturám se mohou vytvářet protilátky, jejichž stanovení slouží jako marker axonálního poškození.

Cílem této studie bylo posoudit, zda autoimunitní mechanizmy proti cytoskeletálním strukturám mohou hrát roli v onemocnění amyotrofickou laterální sklerózou (ALS). Humorální autoimunita byla hodnocena pomocí stanovení protilátek třídy IgG proti lehké a střední podjednotce neurofilament v séru a mozkomíšním moku (MMM) pacientů s ALS. Dále nás zajímalo, zda existují nějaké souvislosti mezi těmito typy protilátek v séru a v MMM. Dalším cílem bylo porovnat různé populace anti-tubulinových (anti-TU) protilátek v mozkomíšním moku a séru pacientů s roztroušenou sklerózou (RS). Anti-cytoskeletální protilátky byly stanovovány pomocí enzymoimunoanalytických (ELISA) metod. V případě anti-TU protilátek byly testovány dva druhy antigenu: 1) hovězí tubulin (anti-TUb protilátky), 2) syntetický neuron-specifický oktapeptid tubulinu (anti-TUs protilátky).

U pacientů s ALS byly pozorovány zvýšené hladiny sérových anti-NF-L protilátek ve srovnání s kontrolní skupinou. Sérové hladiny anti-NF-L protilátek a intratekální anti-NF-M protilátky úzce souvisely se stupněm postižení. Pozitivní korelace anti-NF-L protilátek s hladinami anti-NF-M protilátek byla nalezena u pacientů s ALS v MMM.

Ve skupině RS pacientů byly hladiny anti-TUs a anti-TUb protilátek v MMM významně zvýšené oproti skupině zdravých kontrol. Intratekální syntéza anti-TUs protilátek byla signifikantně zvýšena ve všech sledovaných skupinách. U všech sledovaných skupin byla také nalezena pozitivní korelace hladin anti-TUb a anti-TUs protilátek.

Naše výsledky tak ukazují na možné využití anti-cytoskeletálních protilátek u pacientů s různými neurodegenerativními onemocněními.

1. Introduction

1.1 Multiple sclerosis

Multiple sclerosis (MS) is the most common neurological disease among young adults, with onset at an age range 20 – 40 years. MS is an inflammatory demyelinating autoimmune disease of the central nervous system (CNS) characterized by both inflammation and axonal degeneration.

The clinical symptoms in MS are heterogeneous. MS can be classified into three clinical subtypes according to the disease course: relapsing-remitting (RR), secondary progressive (SP) and primary progressive (PP) [1].

The prevailing etiological hypothesis for MS is that it is a multifactorial disorders, affecting individuals predisposed by a combination of several susceptibility genes and environmental factors [2, 3]. Experimental autoimmune encephalomyelitis (EAE) is an animal model induced in mammals by active immunization with either whole brain tissue or specific myelin proteins.

However, there is increasing evidence that axonal degeneration plays an important role in the pathogenesis of neurological disability [4]. Axonal damage is seen in both acute and chronic MS lesions, and this damage appears to correlate with the degree of clinical disability [5]. The cause of axonal damage in MS is unknown, but it is likely that a destructive process directed against specific components of the axonal cytoskeleton may contribute to the accumulation of disability [6].

1.2 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease, characterized by progressive degeneration of upper and lower motor neurons [7]. The exact mechanisms underlying the selective motor neuron degeneration in ALS remain elusive, but experimental evidence implicates many potential factors, including oxidative damage [8], excitotoxicity [9], apoptosis [10], abnormal neurofilament function [10, 11], defects in axonal transport, aberrant protein processing and degradation [12], increased inflammation, and mitochondrial dysfunction [11].

Approximately 10 % of ALS cases are inherited, with the remainder of cases being sporadic in origin. This distribution of familial (FALS) and sporadic (SALS) disease is similar to other adult-onset neurodegenerative diseases, such as Parkinson disease and Alzheimer

disease (ED370). Except for atypical variants of ALS, the familial form of disease is clinically indistinguishable from the sporadic cases [13, 14].

1.3 The axonal cytoskeleton

The axonal cytoskeleton is a highly regulated system that plays a central role in maintaining the integrity of axons. The physiological functions of neuronal cytoskeleton are dependent upon several interconnected filaments: the actin microfilaments, microtubules, and intermediate filaments (IF) [15]. The major types of IF proteins expressed in mature neurons, are the neurofilaments [16]. The neurofilament protein (NF) is most abundant cytoskeletal element in large myelinated axons that preserves axonal caliber and transport [4]. NFs consist of three components: a light chain (NF-L), intermediate chain (NF-M), and a heavy chain (NF-H). Tubulin, the basic building block of microtubules is the heterodimeric protein αβ-tubulin, comprises between 15 and 20% of cellular protein in the brain [17]. The neuron-associated class III β-tubulin isotype is most abundant in cell of neuronal origin [18].

The abnormal accumulations of these filaments are pathological hallmark of many human neurodegenerative disorders including MS or ALS [16]. The structures of cytoskeleton may be released from the damaged neurons into the extracellular space. The interaction of cytoskeletal proteins with immunocompetent cells can result in the synthesis of autoantibodies [19, 20].

2 Aim of the study

1. **To evaluate anti-cytoskeletal antibodies using ELISA (Enzyme-Linked Immuno-Sorbent Assay) method**
    - To evaluate anti-tubulin antibodies in the serum and cerebrospinal fluid (CSF) in patients with MS and in patients with other neurological diseases.
    - To determine if various populations of anti-tubulin IgG antibodies could be differentiated using two distinct, yet similar antigens: 1. whole intact tubulin from bovine brain; 2. synthetic tubulin fragment specific for neuronal tissue.
    - To determine if the relationship between neuron-specific and whole-spectrum anti-tubulin antibodies exists.
    - To determine if the anti-tubulin responses differed between CSF and serum, and also to estimate the proportion of neuron-specific antibodies (anti-TUs) relative to the whole spectrum of anti-tubulin antibodies (anti-TUb) as well as their intrathecal synthesis
    - To evaluate antibodies against light (anti-NFL) and medium (anti-NF-M) subunits of neurofilaments in serum and CSF in patients with ALS.
    - To determine if the relationship between anti-NF antibodies in serum and in CSF exist as well as between the two anti-NF antibody subtypes.

2. **Clinical use of antibodies**
    - To evaluate the significance of anti-TU antibodies in patients with MS, unlike in other neurological patients.
    - To assess autoimmune involvement in ALS

3 Materials and methods

3.1 Parameters of clinical study

A MS patients

Paired CSF and serum samples were obtained from 34 MS patients, 13 patients with various other neurological diseases (control diseases, CD) and 17 normal control patients (CN). The diagnosis and course of MS at the time of lumbar puncture (LP) were determined using established criteria [21, 22]. 15 patients were classified as having relapsing-remitting MS, 8 patients had secondary progressive MS and 6 patients had primary progressive MS. 5 patients had a clinically isolated syndrome (CIS) as the first manifestation of MS [23]. The disability score for all MS patients was evaluated using the Expanded Disability Status Scale (EDSS) [24]. The CD group included patients with a variety of conditions (e.g. stroke, polyneuropathy, meningitis, and hemidysesthesia). The CN patients presented with conditions such as vertigo, headache (spondylogenic, non-specific, and migraine), psychogenic syndrome and fatigue syndrome. A thorough assessment of these patients did not provide any alternative explanations for their complaints. There was no evidence of any structural, hemorrhagic or inflammatory etiology in the CN patients.

B ALS patients

Paired CSF and serum samples were obtained from 38 amyotrophic lateral sclerosis patients. The diagnoses of ALS (done at the time of the lumbar puncture) were classified using the revised El Escorial criteria [25, 26]. Disability of patients was assessed according to the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) by an experienced neurologist [27]. Patients with ALS were divided into a bulbar onset (BO) subgroup and a limb onset (LO) subgroup. The control group of 20 individuals included predominantly patients with headache (spondylogenic or non-specific), vertebrogenic syndromes, cranial facial or oculomotor palsy and neurotic or fatigue syndromes. There was no evidence of any structural, hemorrhagic or inflammatory etiology in control patients.

All subjects gave a written informed consents regarding study participation. The Postgraduate Medical Education Ethics Committee, Prague, approved the study. Specimens were stored at -80 °C until analysis.

3.2 Methods

Anti-cytoskeletal antibodies – ELISA methods

Antibodies to whole natural bovine brain tubulin (anti-TUb) as well as anti-NF-L and anti-NF-M antibodies were measured by adapted ELISA method, described previously [28, 29].

Antibodies to neuron specific octapeptide (anti-TUs) were determined using commercial ELISA - Viditest kit (VIDIA, spol. s r. o.).

Biochemical analysis

The concentrations of albumin and total IgG in serum and CSF were assayed by commercially available kits using certified biochemical techniques with automated analyzer Immage (Beckman Coulter, USA).

Calculations – Assessment of intrathecal antibody production

To distinguish between leakage of serum IgG through compromised blood-brain barriers (BBB) and intrathecal synthesis (IT) of IgG was calculated the IgG index as well as the intrathecal synthesis of each anti-cytoskeletal IgG antibodies was estimated using the formula for antibody specificity index (ASI) [28, 29]:

IgG index: *(CSF units IgG/serum units IgG) / (CSF albumin/serum albumin)*

ASI:

(CSF antigen-specific IgG/CSF IgG total) / (serum antigen-specific IgG /serum IgG total)

An index ≥ 1.5 is strong evidence that intrathecal synthesis of autoantigen-specificic IgG is occurring in the CNS.

The function blood-brain barrier was considered by the Albumin quotient (Q_{Alb}) [29, 30]:

Q_{Alb} = *(Albumin in CSF / albumin in serum)*

Values > 7 are indicative of disturbed BBB permeability.

In case of anti-tubulin antibodies, the proportion of neuron-specific antibodies to all antibodies against tubulin was expressed as the anti-TUs/anti-TUb quotient:

antibodies to neuron-specific tubulin octapeptide / antibodies to bovine tubulin.

This quotient was calculated for serum (serum quotient) and for CSF (CSF quotient) using anti-tubulin levels expressed as absorbance according to the formula:

serum quotient = *(serum anti-TUs · 100) / (serum anti-TUb · 400)*

CSF quotient = *CSF anti-TUs / CSF anti-TUb*

Statistical analysis

Statistical analysis of group differences was performed by Kruskal-Wallis test followed by the Mann-Whitney U-test. Relationships between anti-TU or anti-NF antibodies were evaluated using the Spearman's correlation coefficient. For statistical analysis of comparison serum and CSF quotient and ASI, the Wilcoxon pair test was used.
The results were considered as statistically significant at $p < 0.05$.

4 Results

A Anti-tubulin antibodies

Relationships between anti-TUb and anti-TUs levels of antibodies in serum and CSF

The levels of antibodies to bovine tubulin (anti-TUb) were significantly related to those against neuron-specific tubulin octapeptide (anti-TUs) in the CSF (MS: $r = 0.8$, $P < 0.0001$; CD: $r = 0.8$, $P < 0.005$; CN: $r = 0.5$, $P < 0.05$). We also found a weak correlation between both types of anti-TU antibodies in the serum of the MS group (MS: $r = 0.4$, $P < 0.05$; CD: $r = 0.2$, n.s.; CN: $r = 0.4$, n.s.).

Levels of CSF and serum anti-TU antibodies

The figure 4.1(A, B) shows levels anti-TUb antibodies in CSF (A) and in serum (B) in patients and controls. The CSF levels of anti-TUb antibodies in the MS and CD patients were significantly higher than in the CN group (MS and CN $p < 0.001$; CD and CN $p < 0.05$). There were observed no differences CSF levels of anti-TUb between MS and CD groups (A). No differences in serum anti-TUb antibodies were found amongst the three groups (B).

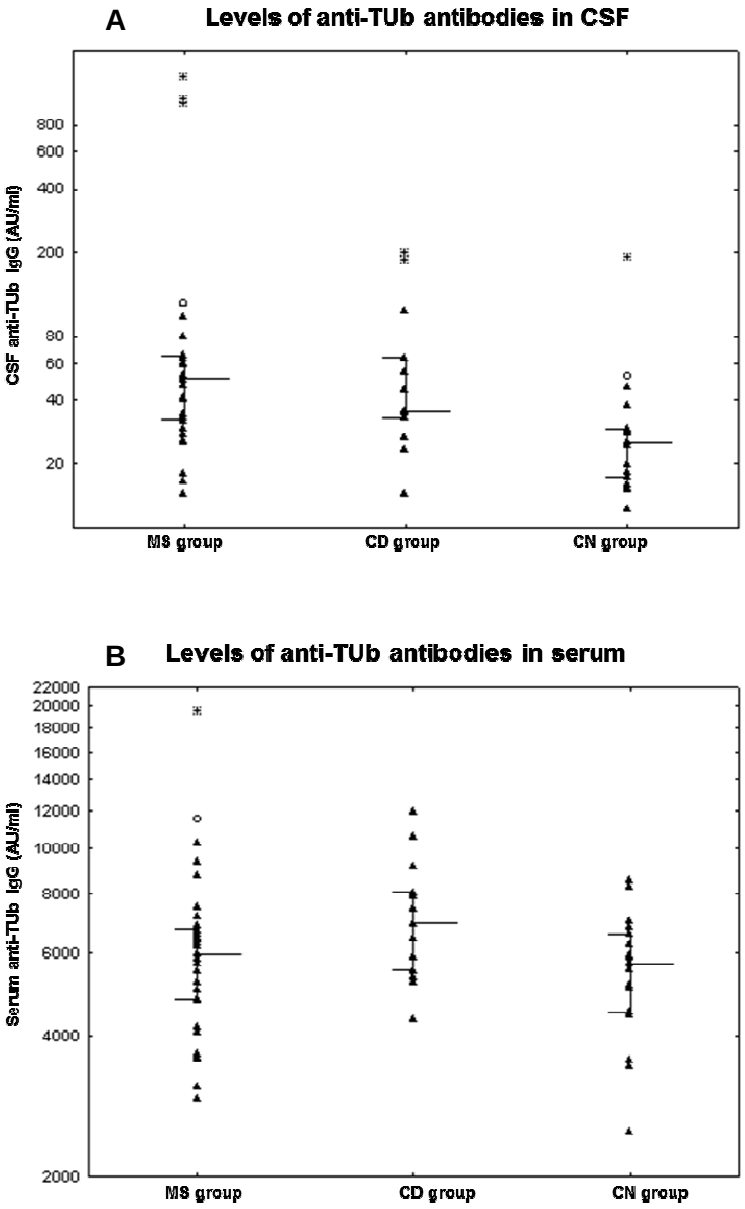


Figure 4.1(A, B): Levels of cerebrospinal fluid (CSF) **(A)** and serum **(B)** anti-tubulin antibodies (anti-TUb) using the bovine tubulin antigen in patients and controls

The symbol]- represents the median and the 25th and the 75th percentile. The adjacent scatter plot represents the individual values of patients.

The figure 4.2(A, B) similarly depicts levels anti-TUs antibodies in CSF (A) and in serum (B) in MS patients and controls groups. The CSF anti-TUs antibodies levels in the MS patients were significantly higher than in the CN group (MS and CN $p < 0.005$). The levels of CSF anti-TUs did not differ between the MS and the CD groups and between the CD and the

CN groups (A). No differences in serum anti-TUs antibodies were found among the three groups (B).

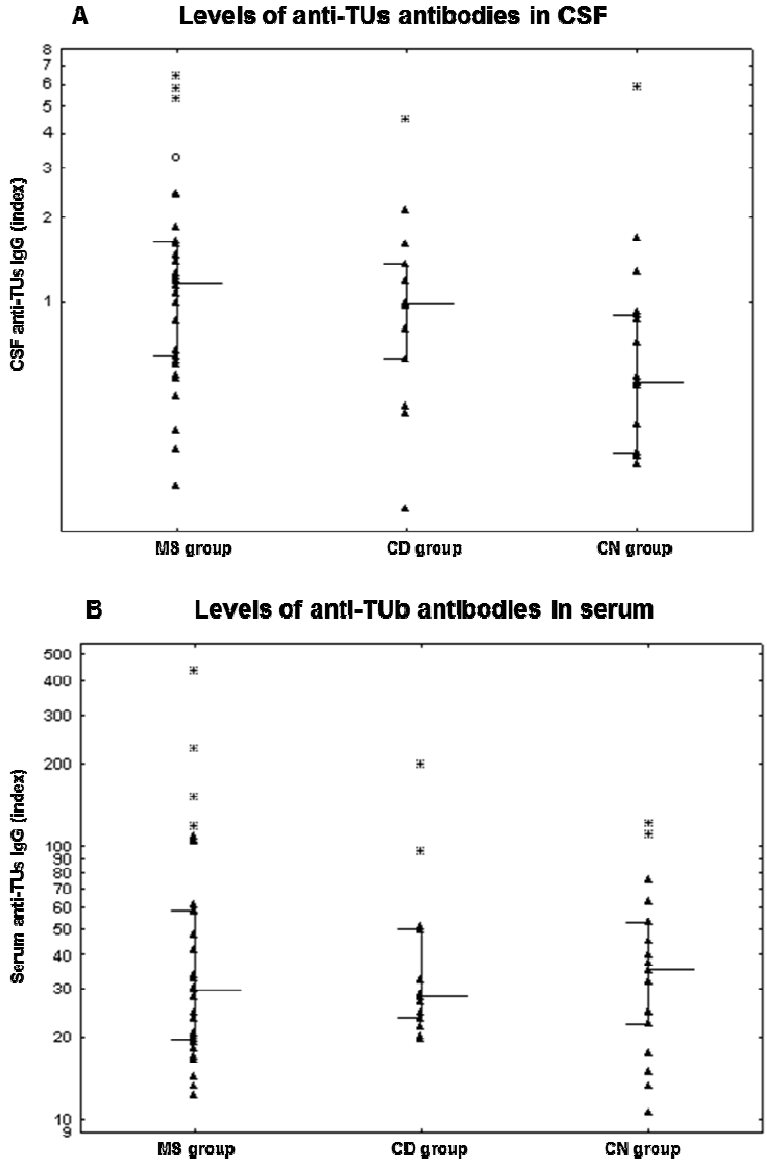


Figure 4.2(A, B): Levels of cerebrospinal fluid (CSF) **(A)** and serum **(B)** anti-tubulin antibodies (anti-TUs) using the synthetic fragment of neuron-specific octapeptide tubulin as the antigen in patients and controls.

The symbol]- represents the median and the 25th and the 75th percentile. The adjacent scatter plot represents the individual values of patients.

Intrathecal synthesis of anti-tubulin antibodies

The intrathecal synthesis of anti-TU antibodies was expressed as ASI for each type of antigens. We observed that IT synthesis of anti-TUs antibodies was significantly higher than the IT synthesis of anti-TUb antibodies in all groups (table 1).

Table 1: Antibody specifity indices (ASI) for anti-TUb and anti-TUs antibodies

	Antibody specifity index		
Diagnostic group	anti-TUb, median (25th-75th percentile)	anti-TUs, median (25th-75th percentile)	p
MS	1.6 (1.3-2.8)	7.7 (2.5-14.3)	< 0.001
CD	1.8 (1.4-2.7)	6.7 (4.9-13.6)	< 0.005
CN	1.8 (1.6-2.01)	9.4 (5.1-21.7)	< 0.001

Relationships between anti-tubulin IgG antibodies and total IgG in CSF and serum

Correlation analysis showed significant relationship between serum anti-TU to bovine tubulin and total serum IgG in all groups. We did not find any relationships between anti-TU antibodies and total IgG antibodies when the neuron-specific octapeptide was used in serum or CSF. For all three groups, serum anti-tubulin antibodies, regardless of antigen, were unrelated to those found in the CSF (table 2).

Table2: Correlations amongst levels of anti-TUb to anti-TUs antibodies and total IgG antibodies in CSF and serum.

		Anti-TUb		Anti-TUs	
Diagnostic group	**Correlations between**	r	p	r	p
MS	S-anti-TU x CSF-anti-TU	0.3	n.s.	0.2	n.s.
	S-anti-TU x S-IgG total	**0.5**	**< 0.005**	− 0.1	n.s.
	CSF-anti-TU x CSF-IgG total	0.3	n.s.	0.2	n.s.
CD	S-anti-TU x CSF-anti-TU	0.4	n.s.	− 0.03	n.s.
	S-anti-TU x S-IgG total	**0.7**	**< 0.01**	0.4	n.s.
	CSF-anti-TU x CSF-IgG total	0.2	n.s.	− 0.03	n.s.
CN	S-anti-TU x CSF-anti-TU	0.3	n.s.	− 0.05	n.s.
	S-anti-TU x S-IgG total	**0.6**	**< 0.05**	0.4	n.s.
	CSF-anti-TU x CSF-IgG total	**0.6**	**< 0.05**	0.4	n.s.

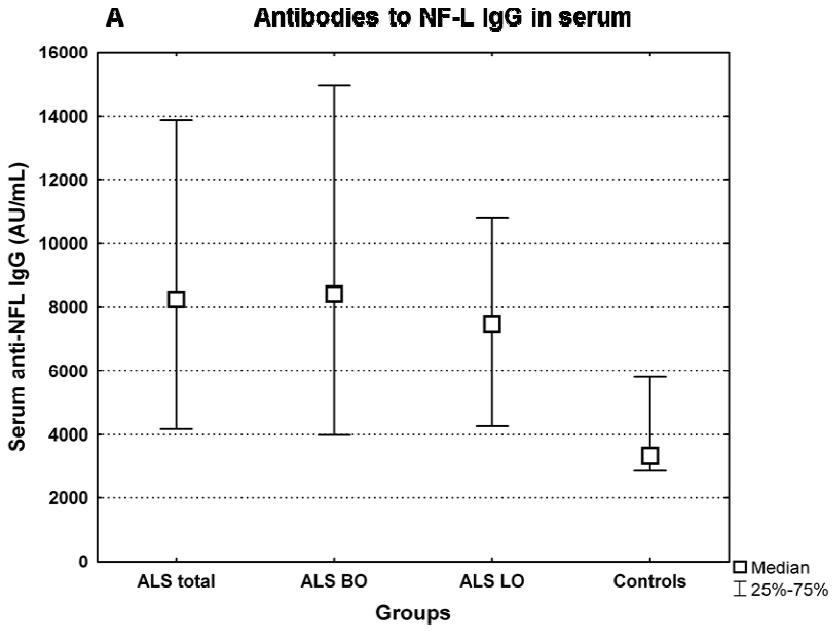
n.s. – not significant; r – Spearman correlation coefficient

Others correlations

The inverse relationship was found between IT synthesis of anti-TUb antibodies and patient age in the CN group ($r = -0.73$; $p < 0.001$). Anti-tubulin antibodies were not related to clinical parameters of the disease, subject's age, disease duration, and the EDSS score.

B Anti-neurofilaments antibodies

Figure 4.3(A, B) depicts the levels of anti-NF-L (A) and anti-NF-M (B) antibodies in serum. Serum anti-NF-L levels were significantly elevated in both subgroups ALS patients compared with controls (ALS BO versus controls $p < 0.05$; ALS LO versus controls $p < 0.005$) (A). Serum anti-NF-M levels were significantly elevated only in ALS BO patients compared with controls (ALS BO versus control $p < 0.05$) (B).



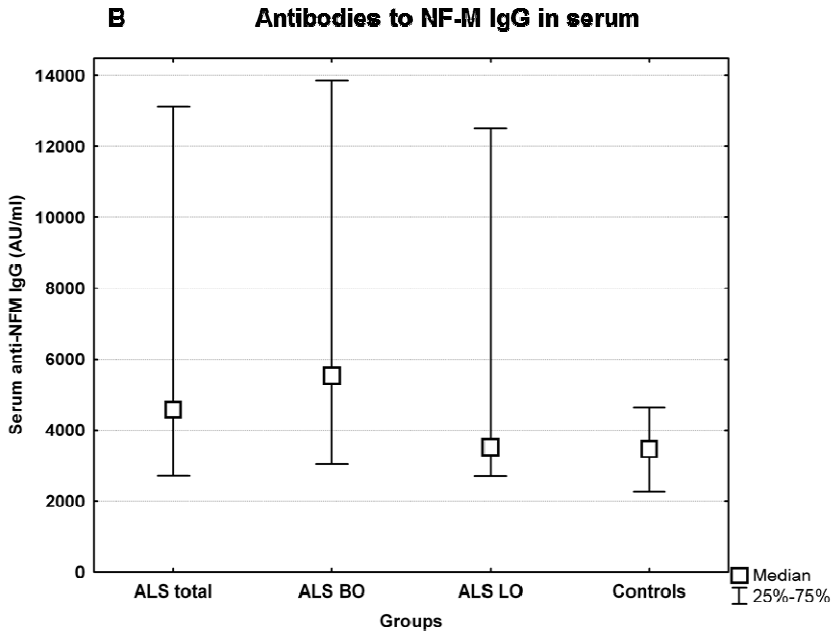


Figure 4.3(A, B): Serum levels of anti-NF-L IgG **(A)** and anti-NF-M IgG **(B)** antibodies in ALS patients and controls.

No statistical significance of antibodies levels was found in CSF. The CSF levels of both types of antibodies were only higher in ALS group.

Intrathecal synthesis and other correlations

Intrathecal synthesis of anti-NF-L IgG and anti-NF-M IgG antibodies was similar in all groups and did not vary between ALS patients in comparison with the control group. It was found a relationship between the levels of anti-NF antibodies in cerebrospinal fluid and the blood–CSF barrier function in ALS patients (anti-NF-L: $r = 0.5$, $p < 0.005$; anti-NF-M: $r = 0.4$; $p < 0.05$), but not in the control group.

No correlation was found between total IgG and antibodies to NF-L and NF-M in serum, with the exception of a significant relationship between total IgG and anti-NF-L in the control group ($r = 0.5$; $p < 0.05$). A significant correlation between total IgG and anti-NF-L in the CSF was observed in the ALS group ($r = 0.5$; $p < 0.005$). We observed a significant difference in age between ALS patients and controls ($p < 0.01$). However, we found no relationship between age and anti-NF-L and anti-NF-M levels in serum and CSF in ALS patients or in the control group. Intrathecal synthesis of anti-neurofilament antibodies also failed to correlate with age in any group.

ALSFRS and serum anti-NFL levels were related in the ALS group ($r = -0.3$; $p < 0.05$). Additionally, a relationship between ALSFRS and intrathecal synthesis of anti-NF-M was found in the ALS group ($r = -0.4$; $p < 0.05$). We did not observe any association between disease duration and anti-NF in serum or CSF.

5 Conclusions

In our work, we optimized the ELISA method for measurement selected anti-cytoskeletal antibodies in serum and CSF.

In case in study of anti-tubulin antibodies we compared various populations of anti-TU IgG antibodies using two distinct, yet similar antigens – whole bovine tubulin isolated from bovine brain and synthetic tubulin octapeptide fragment specific for neuronal tissue. We found a close relationship between levels of antibodies to whole tubulin and to its synthetic fragment in the CSF of all studied groups and in the serum of the MS group. We observed too similar differences amongst groups of subjects in the levels of anti-TU antibodies regardless of antigen. These findings suggest that the natural tubulin isolated from bovine brain as well as neuron-specific octapeptide fragment of tubulin can be used interchangeably for determination of anti-TU antibodies in the CSF by ELISA method.

The serum levels of both types of anti-TU antibodies were similar in all subjects but no relationships between anti-TUs and anti-TUb levels of antibodies were observed with the exception of a weak correlation in the MS group. Levels of antibodies to bovine tubulin in serum corresponded to the total serum IgG levels in all three groups, but similar relationship was not found for antibodies to neuron-specific tubulin octapeptide. We assume that divergent anti-TU antibodies which include those to extraneuronal tubulins as well as to non-specific neuronal epitopes of brain tubulins are present in serum.

The spectrum of anti-tubulin antibodies in serum and CSF is different. The evaluation of ASI demonstrated that the synthesis of antibodies against neuron-specific octapeptide predominates in the intrathecal compartment. The results obtained by comparison of tubulin octapeptide/antibodies to bovine tubulin quotients (anti-TUs/anti-TUb quotients) in CSF and serum corroborate that neuron-specific anti-tubulin antibodies significantly prevail in CSF, especially in the MS group.

We did not find any relationships between serum and CSF anti-tubulin antibodies. It seems that anti-TU levels in serum and CSF proved to be independent. Therefore, determination of anti-TU antibodies in serum cannot replace CSF analysis.

Clinically, CSF anti-TU antibodies were elevated in patients with MS compared with anti-TU antibody levels of presumably normal subjects. We found statistically significant differences only between MS and CN groups and not between CD and CN in levels of anti-TUs antibodies. It seems that antibodies to neuron-specific fragment of tubulin should be more specific for patients with MS. It is possible that a part of tubulin which should induce the synthesis of anti-tubulin antibodies to neuron-specific octapeptide may release more intensively during the process of axonal damage in MS.

The estimation of anti-tubulin antibodies in CSF can contribute to the overall assessment of axonal damage. Serum anti-tubulin antibodies were divergent and not useful for differential purposes in MS.

In study of anti-NF antibodies in patients with ALS we observed higher levels of anti-NF in serum ALS patients. Tangibly, we found increased serum levels of anti-NF-L in ALS patients and anti-NF-M in ALS BO patients, but not in the CSF or intrathecally. Serum levels of anti-NF-L antibodies inversely correlated with patient disability.

We did not find any relationship between anti-NF-L and anti-NF-M levels in serum. It can be caused by different antigen stimulation depending on the individual neurofilament subunits in the serum and CSF. This view may be supported by the finding of a non-significant correlation between total IgG and anti-NF-L and anti-NF-M in the serum of ALS patients in contrast to a significant correlation between total IgG and anti-NF-L in the CSF. This particular relationship seems to be a common feature.

The correlation of ALSFRS with serum anti-NF-L and intrathecal anti-NF-M levels indicates that disability is reflected by antibody status.

These findings contribute to the growing body of knowledge associated with the immune humoral response in ALS patients.

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7 List of original articles

Publications *in extenso* related to the thesis (with IF):

1. Švarcová J, Fialová L, Bartoš A, Šteinbachová M, Malbohan I. Cerebrospinal fluid antibodies to tubulin are elevated in the patients with multiple sclerosis. Eur J Neurol. 2008; 15: 1173-1179. **IF = 2,732**

2. Fialová L, Bartoš A, Soukupová J, Švarcová J, Ridzoň P, Malbohan I. Synergy of serum and cerebrospinal fluid antibodies against axonal cytoskeletal proteins in patients with different neurological diseases. Folia Biologica 2009; 55: 23-26. **IF = 0,924**

3. Fialová L, Švarcová J, Bartoš A, Ridzoň P, Malbohan I, Keller O, Rusina R. Cerebrospinal fluid and serum antibodies against neurofilaments in patients with amyotrophic lateral sclerosis. Eur J Neurol. 2010; 17: 562-566. **IF = 3,765**

Publications *in extenso* with different objectives (with IF):

1. Fialová L, Bartoš A, Švarcová J, Malbohan I. Increased intrathecal high-avidity anti-tau antibodies in patients with multiple sclerosis. PLoS One. 2011; 6(11): e27476. **IF = 4,41**

2. Fialová L, Švarcová J, Bartoš A, Malbohan I. Avidity of antineurocytoskeletal antibodies in cerebrospinal fluid and serum. Folia Microbiol 2012, *in press*.

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4. Muravská A, Germanová A, Jáchymová M, Hájek Z, Švarcová J, Zima T, Kalousová M. Association of Pregnancy-associated plasma protein A polymorphism with preeclampsia – A pilot study. Clin Biochem. 2011; 44: 1380-1384.

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Publications *in extenso* with different objectives (without IF)

1. Fialová L, Bartoš A, Švarcová J, Doležil D, Malbohan I. Stanovení tau proteinu v mozkomíšním moku pacientů s roztroušenou sklerózou dvěma soupravami ELISA. Klinická biochemie a metabolismus 2011; 19/40(2): 113-118.