EDITOR'S OFFICE

SCRIPTA MEDICA
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Publisher: Faculty of Medicine,
Masaryk University, Brno

Periodicity: 4 issues per volume
The journal Scripta Medica is printed
in 500 copies.

Printed by Tiskárna Helbich, a.s., Brno

Art studio: Metoda, s.r.o., Brno

Photos: Libor Teplý (pages 2, 3, 57)
The Faculty of Medicine uses the traditional symbol of the serpent-entwined staff of Asclepius. This also forms the head of its mace.
Photos: Jaroslav Winkler, Faculty of Medicine, Masaryk University

Advertising is accepted in the journal.
Further information including rates, specification, etc. is available on request.

ISSN 1211-3395

MK ČR E 4996

Scripta Medica is available free online at
http:/www.med.muni.cz/biomedjournal/

SCRIPTA MEDICA is indexed in

EMBASE/Excerpta Medica

DIALOG DataStar

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Bibliographia Medica Čechoslovaca

Online Computer Library Center, Inc.

Deutsches Institut für Medizinische Dokumentation und Information

Ladies and gentlemen,

Scripta Medica, the scientific journal of the Faculty of Medicine of Masaryk University, has been published since 1922. The journal has been an important source of scientific literature based on exchange services with medical schools and other scientific institutes abroad. The history of the journal is too long to be able to describe everything important. During the history of the journal we have learnt that a good communication strategy is an absolute condition of success for a scientific journal.

Today the journal Scripta Medica enters a new period of its existence. We have learnt from the mistakes and found out that we have established tradition, research experience, work skills and practice, and reasonable chances to be successful. We are aware of the heritage of our past and we choose the best of it. We do not want to focus on publishing scientific information only, but we want to pay attention to scientific communication, scientific research, scientific congresses, and all events leading up to better understanding of medical science research. We appreciate your comments as well as we appreciate good communication and cooperation with you. We believe in closer cooperation in the international field of science.

It is with great pleasure that we introduce to you a new layout of the journal but also a new communication strategy. We wish you were our readers, authors, reviewers, and critics. The journal is quarterly, with four issues in a year. The scientific standard of published papers is guaranteed by the Editorial Board. The journal is addressed to scientists not only in the Czech Republic but all over the world. We use the English language as a scientific language and we believe that we will be able to speak to scientists from different disciplines in all aspects. We wish to be a journal widely opened to all research workers for scientific investigation – medical and biomedical research, laboratory research, and experimental work. We are prepared to become a communication channel joining research workers from every branch of biological medicine. Our targets are ambitious. Our journal is now changing into a modern scientific journal in all aspects, with changes in its title page, size and graphic layout, taking heed of the quality of its papers and reviews, and also changing its distribution. The journal is distributed free again, but we have given priority to explicit addressing. The journal Scripta Medica is printed in a number of 500 copies.

Ladies and gentlemen,
We are honoured by the fact that in 2008 the journal Scripta Medica Facultatis Medicae Universitatis Brunensis Masarykianae was included by the Research and Development Council in the List of Reviewed Non-impacted Periodicals published in the Czech Republic.

Marie Korcová

Prof. MUDr. Josef Bednařík, CSc.

MUDr. Stanislav Voháňka, CSc., MBA

Department of Neurology:
Twenty years' history and present state

The Department of Neurology was founded and started its work on 1st September 1989 as one of the core departments of the new Faculty Hospital of Brno-Bohunice, when the new 17-floor building was opened. The first head of the department was Assoc. Prof. MUDr. Pavel Strnad, CSc. The new department was equipped with modern technology, especially for electrodiagnostics. An intensive care unit as part of a neurological department was very rare at that time and its focus on vascular neurology was pioneering. After a short period of provisional heading by the deputy head, MUDr. Ladislav Ondroušek, the department had been headed by Prof. MUDr. Zdeněk Kadaňka, CSc., since 1st January 1993, and after his retirement it has been headed by Prof. MUDr. Josef Bednařík, CSc., since September 2007. The current deputy heads are MUDr. Stanislav Voháňka, CSc. (deputy for treatment) and Assoc Prof. MUDr. Pavel Štourač, Ph.D. (deputy for science and education).

The Department of Neurology nowadays has one 6-bed intensive care unit, one 6-bed stroke unit and two general wards, each with 24 beds, specialised electrophysiological laboratories for electromyography, evoked potentials, electroencephalography, ultrasonography and sleep analysis, and an outpatient neurological department with 5 ambulances. Specialised care is concentrated into several dedicated centres for the treatment of multiple sclerosis, sleep disorders, neuromuscular disorders, spondylogenic disorders, and botulotoxin treatment for movement disorders.

The working team consists of 28 doctors, 2 psychologists, one speech therapist – aphasiologist, 53 nurses, 9 hospital attendants, 2 physiotherapists, 2 secretaries, and 1 data manager.

The Department of Neurology is involved in several national and international grants and research projects. The neurologists are members of several society committees including the committee of the Czech Neurological Society (Prof. Bednařík is the vice-chairman). Dr. Voháňka is the current chairman of the Czech Neuromuscular Society and Prof. Bednařík is the editor-in-chief of the Czech and Slovak Neurology and Neurosurgery (the impacted official journal of the Czech and Slovak Neurological and Neurosurgical Societies).

Prof. MUDr. Josef Bednařík, CSc.
MUDr. Stanislav Voháňka, CSc., MBA

SPONDYLOTIC CERVICAL MYELOPATHY: WHAT IS THE BEST APPROACH TO TREATMENT?

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Received after revision February 2009

KEY WORDS

Spondylotic cervical myelopathy
Spinal cord compression
MR hyperintensities
Cross-sectional transverse area

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ABSTRACT

The authors address the daunting challenge of the treatment of patients with spondylotic cervical myelopathy (SCM). It is emphasised that clinical diagnosis must be made in the light of all available information from the history of the disease and from objective neurological investigation, and that everything must be done with reference to the paraclinical findings (imaging, electrophysiological, serological, etc.). Decision-making must then be based on evaluation of the cross-sectional transverse area of the compressed spinal cord, the number of compressed levels and its significance, hyperintensities in the spinal cord at the level of maximal compression, duration of symptoms, and the overall clinical status (including mental condition). Practical recommendations for the treatment of SCM conclude the article.

INTRODUCTION

Although a large amount of information has been published about spondylotic cervical myelopathy (SCM), in everyday clinical practice many doctors are still hesitant to commit themselves to a treatment regime. The radiological finding of a narrow spinal canal, or even spinal cord compression, often proves too strong a temptation to solve the problem radically "as soon as possible", "not wasting valuable time" regardless of any facts arising out of evidence-based medicine, or even of common sense. Overemphasis on one finding irrespective of a non-corresponding clinical picture often verges on desperate endeavour to disclose immediately the cause of the patient's signs and symptoms. How is this challenge best addressed?

The clinical diagnosis must take into full account all the information contained in the history of the disease and the objective neurological investigation, and everything must be done in concordance with the paraclinical findings (imaging, electrophysiological, serological, etc.). If this is done, however, other difficulties must be overcome in the process of making a decision as to which treatment to opt for. The following items should be evaluated:

1. The cross-sectional transverse area of the compressed spinal cord
2. The number of compressed levels and its significance
3. Hyperintensities versus normal MR spinal cord image at the level of maximum compression
4. Duration of symptoms
5. Overall clinical status (including mental condition)

Clinical features

Symptoms of spondylotic cervical myelopathy (SCM) most frequently begin in patients between 50 and 70 years of age, but may occur much earlier or in more advanced years. The patients tend to visit their doctor at 50–55 (1–4). Men are affected more frequently (2–3:1) than women [1,3,4].

Symptoms may develop progressively or in stepwise fashion, with remissions between periods of deterioration. Symptoms and signs may arise for the first time or be aggravated following injuries such as carrying an excessive weight, a fall on a slippery surface, a motor vehicle accident, or forced hyperextension of the neck.

The most frequent and characteristic symptoms are gait disorders (82 %), clumsy hands (84 %), and neck and back pain (95 %). Pain in the neck (67 %), shoulder, and arms is a common presenting complaint [1]. Pain may radiate in a radicular distribution and is usually dermatomic. However, it may occasionally occur in the distribution of the affected myotome. Paresthesias (78 %), fasciculations in the upper extremities (32 %), and muscle weakness in the distribution of the affected nerve roots (30 %) are often encountered. Reduction in the reflexes may be observed in the upper extremities, but in the lower extremities it may be associated with hyperactive reflexes (60 %) [1]. Although the patient may complain of only unilateral lower extremity symptoms, neurological examination usually reveals signs of bilateral disturbance of long tract function. Spasticity (62 %) is one typical sign, and suddenly stretching legs may be reported. Sensory complaints, especially pain in the lower extremities, may be misleading. Lhermitte's sign is very specific, though of low sensitivity (14–27 %) [5,1]. Disturbances of sphincter function (24 %) are late phenomena; they are usually mild and generally do not occur in the absence of advanced spinal cord dysfunction, which manifests itself earlier by dysfunction of other modalities [1].

The duration of signs and symptoms varies considerably with personal history. It may last from several hours through many (25–35) years, but typically occurs over one or several years (1–4).

Hyperintensities

MRI is an excellent means to visualise the narrowed spinal canal associated with spinal cord compression due to degeneration of the spine (Figure 1). New technology, such as multi-array coils, provides high-resolution sagittal images of the entire cord in a single scan and fast spin-echo sequences produce high-resolution T2WI within a short time [12].

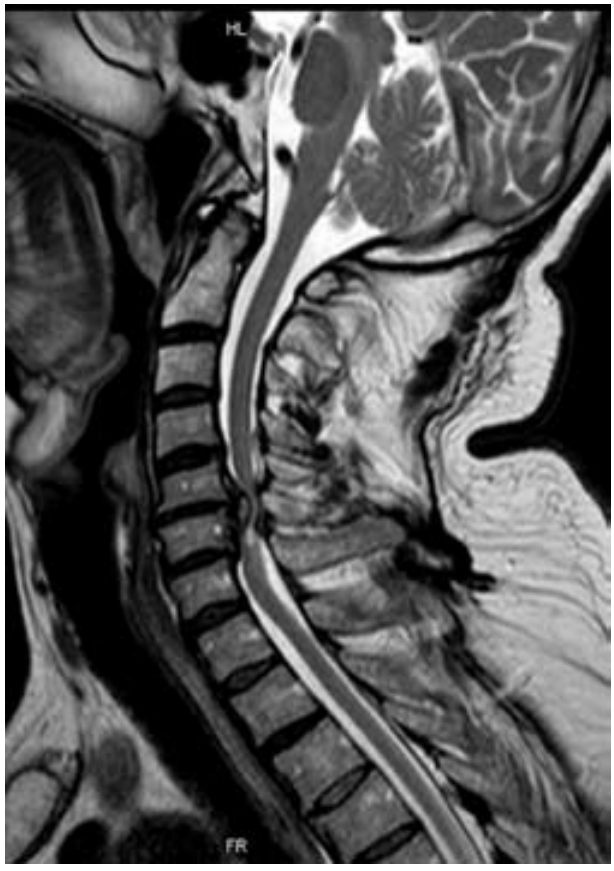

Figure 1
An MR proton density image in the sagittal plane; spondylogenic multistage spinal cord compression of CD-7 by massive protrusion of intervertebral disc

MRI can reveal cord signal abnormalities on T2WI at (or near) the site of compression, which may signal evidence of oedema, demyelinisation, gliosis, or myelomalacia.

The clinical significance of **increased signal intensity on MR scans** is controversial. Some studies have found a negative correlation between the outcome of surgery or conservative treatment and increased signal intensity (6–9), but others have not [11], or have even noted a positive correlation (11). Some found this relation predictive only in cases with multilevel manifestation [13] or when a low signal on T1WI and a high signal on T2WI were combined [14]. Those that found no correlation consider the reason to be that hyperintensities chiefly indicate lesions in the grey matter that do not give rise to spastic paralysis and are, therefore, not significantly related to patients' disabilities. Decompressive surgery can result in rapid

Inpatient clinic - doctors room

resolution of imaging abnormalities, but clinical improvement does not always accompany these changes [15]. In our studies comparing conservative treatment with surgery the appearance of increased signal intensity in the cervical spinal cord did not predict the outcome in either type of treatment [16, 17].

Cross-sectional transverse area at the level of maximal compression

In some cases, the spinal cord does not lose its function even under severe compression. It has been well known from animal experiments that spinal cord function may be considerably resistant to compression [18]. Moreover, the critical degree of spinal cord compression required to induce pathological changes in clinical practice remains unknown [19]. This renders matters difficult, since radiological findings for spinal cord compression, particularly if mild, may be clinically relevant or merely a coincidental anomaly that has little or nothing to do with the symptoms reported by the individual patient. This situation is far from unfamiliar in healthy subjects aged 18 to 72 years; degenerative changes, especially in the cervical region, are present on MRI in 64 % of them and are associated with cord impingement in only 11 % [12]. If only subjects aged 64 or more are taken into account, cord impingement has been observed in 26 %, and cord compression in 7 % [12]. The percentage of cord area reduction, however, has never exceeded 16 %. No significant difference in cord cross-sectional areas between those with degenerative changes and those without them has been observed. This could mean that direct and pronounced compression is necessary to evoke signs of spinal cord dysfunction [12].

To identify a clinically significant degree of compression, already associated with corresponding clinical symptoms or for the identification of patients suitable for active treatment, it is essential to establish the critical value of such compression, or other circumstances such as hyperintensities, number of compressed levels, etc.

Although in population studies there exists a correlation between a narrow sagittal diameter of the spinal canal and SCM, there is a considerable degree of overlap between the frequency histograms for the minimum anteroposterior diameter of the asymptomatic population and those with SCM [21]. An acquired anteroposterior diameter of the spinal

canal of less than 11–12 mm results in deformation of the cord (the normal sagittal diameter of the spinal cord at C5 segment is around x = 9.6 mm (range 8.5–11) taken from myelograms and from cadaver cord specimens). A more versatile measurement is, however, the cross-sectional area of the cord. The spinal cord is reported to lose its functional tolerance if the transverse area, as measured by computed tomographic myelography and MRI, is less than 55–75 % of the normal value [22,23]. These studies were first performed in 12 post-mortem specimens and then in animal models. However, a minimum spinal cord cross-sectional area of 0.44 cm^2 in a non-myelopathic group and a maximum measurement of 0.86 cm^2 in a myelopathic group (20 patients with cervical spondylosis) has been demonstrated [24]. The cross-sectional area at the level of maximum compression is not the only factor in the estimation of myelopathy, but is very important and easy to measure. In one group of 20 patients it has been demonstrated that narrowness of the cross-sectional area of the spinal cord was an independent prognosticator for the severity of myelopathy ($p<0.05$), accounting for 63 % of the variations in the myelopathy score [24].

We studied the impact of the degree of spinal cord compression on clinical picture in a group of 246 patients with spondylogenic cervical cord compression. A significant difference in mJOA score was found in those with a transverse spinal cord area of below 50 mm^2 in comparison with patients with a transverse spinal cord area of above 60 mm^2. The critical degree of spinal cord compression needed to induce clinically pathological changes is between 50 and 60 mm^2. This relationship is valid for compression accompanied by the presence of hyperintensities on MR T2WI and was not found in patients with small cross-sectional area lacking hyperintensities in the spinal cord.

The **duration of symptoms** is frequently considered a significant factor with predictive value for long-term results [25–27]. However, it is necessary to approach the evaluation of this factor with care, because recognition of the precise onset of the disease may prove to be a difficult task.

The **number of compression levels** can affect the outcome of treatment or the natural history. A single compression level produces a mild functional deficit in comparison with multiple compression, as has been shown experimentally [28]. According to some studies, the results of surgery are better in single-level than in multilevel compressions [29–30].

The overall clinical status (including mental condition)

For surgical treatment to be indicated, the patient must be of a status that allows such an approach, i.e. normal internal (cardiopulmonary and metabolic) condition combined with a good mental approach to co-operation and the willpower necessary for rehabilitation in the postoperative period. Psychological condition is important and it is necessary to be aware of the fact that depression is not a significant factor in the pathogenesis of the neurological clinical picture. Patients must be informed that, often contrary to expectations, surgery may stop the progression of the disease but not actually ameliorate their symptoms.

Conclusions for clinical practice

The crucial question in the treatment of mild and moderate non-progressing SCM is not "to operate or not to operate", because both the conservative and the surgical approaches are potentially useful. Considering the results of our studies of SCM and taking the relevant literature into account, we feel that we can recommend the following guidelines:

1. Surgery is indicated in patients with clinically severe (mJOA -modified Japanese Orthopaedic Association- score less than 12 points –Score) or progressing clinical compressive spondylotic cervical myelopathy (a minority of the patients).
2. Surgery is indicated in patients with severe spinal cord compression (50 % or more) in spite of minimal symptoms and signs.
3. Conservative treatment is appropriate for patients with a stable, non-progressing, mild-to-moderate clinical picture (mJOA score >12 points) and mild spinal cord compression (30 % or less), regardless of the presence of hyperintensities at the level of maximum compression. It is, however, necessary to check them at regular intervals (6–12 months), both clinically and with imaging (MR every 3–4 years) and electrophysiological approaches (every 3–4 years), and to reconsider indications for surgery in the event of progression.

Our results may serve as a contribution to the theory that conservative treatment has some advantages over surgery in a carefully selected group of patients. The most promising candidates for high predictive values for good results in either conservative treatment or surgery could be the transverse area of the stenotic cord, duration of the disease [30], the osseous or cartilaginous compression, the developmental diameter of the canal, positivity of electrophysiological findings, low-signal intensity changes on T1-weighted sequences [18], and severity of the neurological deficit and its dynamics.

ACKNOWLEDGEMENT

This study was supported by the Czech Ministry of Education Research Plan No. MSM0021622404.

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THE PROMOTOR POLYMORPHISM OF MACROPHAGE METALLOELASTASE IN MULTIPLE SCLEROSIS

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Received after revision February 2009

KEY WORDS

Matrix metalloproteinases
Macrophage metalloelastase
Multiple sclerosis
Polymorphism

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ABSTRACT

Multiple sclerosis is a chronic demyelinating disease of the central nervous system in which the genetic background plays an important role. Its pathophysiology is characterised by two major processes: neuroinflammation and neurodegeneration. Matrix metalloproteinases are involved in both of them. Macrophage metalloelastase is one of the three matrix metalloproteinases the common elevation of which has been confirmed in multiple sclerosis and also in animal models with experimental allergic encephalomyelitis. To assess the association between its promotor polymorphism and demyelinating disease we genotyped a total of 92 patients (23 men, 69 women, mean age 37 years) with definite multiple sclerosis (according to the McDonald criteria) and 51 healthy controls (17 men, 34 women) matched for age and sex. Genotyping was performed by means of polymerase chain reaction with restriction analysis. We observed no statistically significant differences in genotype or allele distribution of –82 A/G polymorphism between the groups examined (OR=2.6, p=0.026, pcorr=0.078). Due to insufficient numbers of patients with the progressive form [9], no statistically significant differences in genotype or allele frequencies emerged among the patients with variant forms of multiple sclerosis. Nevertheless, all patients with the progressive form (which is associated with a more severe course and higher disability) were of the same genotype: homozygotes AA. This genotype is connected with higher promotor activity and a higher expression of the final protein. It may represent a variant genotype base for a different course of multiple sclerosis in the polymorphism under investigation.

ABBREVIATIONS USED

MS – multiple sclerosis
MMP – matrix metalloproteinase
CNS – central nervous system
EAE – experimental allergic encephalomyelitis

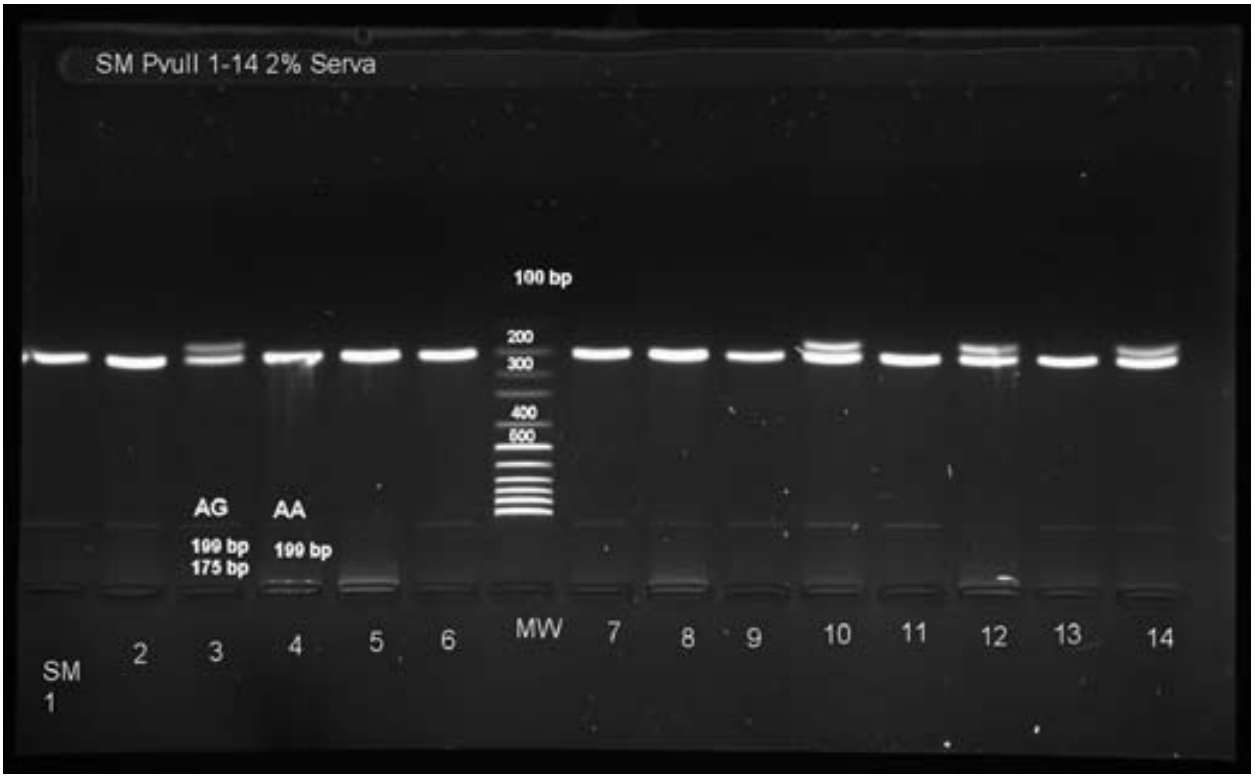

Figure 1
Final genotypes visualised in UV light after ELFO on 2% Serva agarose gel with ethidium bromide

RR – relapsing remitting
SP – secondary progressive
PP – primary progressive
DNA – deoxyribonucleic acid
PCR – polymerase chain reaction
ELFO – electrophoresis
AP–1 – activator protein–1
HW – Hardy-Weinberg equilibrium

INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). It is one of the most frequent causes of young adult disability in our geographical zone [1]. MS is a multifactorial disease in which the genetic background plays an important role. Its pathophysiology is characterised by neuroinflammation and neurodegeneration. Matrix metalloproteinases (MMPs) – a family of Zn-dependent endopeptidases [2] – are involved in both these processes. Some 26 MMPs are known to date, of which 23 may be present in humans. The expression of MMPs is usually low in the adult body and is typically observed in a local manner in the course of specific processes [2–4]. In the pathogenesis of multiple sclerosis, MMPs participate in blood-brain barrier disruption, leukocyte perivascular infiltration, myelin destruction, and conversion of the proforms of inflammatory molecules; they may also be responsible for neurotoxicity [4]. Because of their heavy destructive effect, their activity is under very tight regulation [2, 3]. The first step in this path is regulation through the gene polymorphisms.

Elevated levels of nine MMPs have been found in spinal cord samples taken at the peak of disease in experimental allergic encephalomyelitis (EAE), the accepted animal model of MS [5–7]. One of them was macrophage metalloelastase (MMP12), which up-regulated by a factor of more than three in one of these studies [8]. Increased expression of MMP12 was also confirmed in vitro in cultured rat astrocytes [9]. It has been found that the macrophages of MM12-deficient mice have a markedly diminished capacity to degrade extracellular matrix components. In addition, these macrophages were essentially unable to penetrate reconstituted basement membranes both in vitro and in vivo. MMP12 is therefore required for macrophage-mediated extracellular matrix proteolysis and tissue invasion [10]. Elevated MMPs have also been

Table 1
Primers and PCR conditions

Left primer (5´-3´) **Right primer (5´-3´)**	**PCR** *(temperature °C, time)*
GAGATAGTCAAGGGATGATATCAGC AAGAGCTCCAGAAGCAGTGG	95˚C/5´ 95˚/45´´-55˚/30´´-72˚/45´´ (30x) 72˚/7´ 10˚/10

Table 2
Restriction analysis

Restriction enzyme	**Conditions**	**Fragment length (bp)**
Pvu II, 5´CAG↓CTG 3´GTC↓GAC	37°C /4h: PCR product 15µl, H2O 2.7µl, buffer NEB 2 2µl, Pvu II 3U	AA 199 / 199 bp GG 24,175 / 24,175 bp AG 199 / 24,175 bp

Table 3
Genotypes and alleles

MMP12 -82 A/G	**PATIENTS** *number (frequence %)*			**CONTROLS** *number (frequence %)*		
Genotypes	**All (n=92)**	**Men (n=23)**	**Women (n=69)**	**All (n=51)**	**Men (n=17)**	**Women (n=34)**
AA	64 (69.57)	16	48	42 (82.35)	13	29
AG	27 (29.35)	7	20	7 (13.73)	3	4
GG	1 (1.09)	0	1	2 (3.92)	1	1
Alleles						
A	155 (0.84)	39	116	91 (0.89)	29	62
G	29 (0.16)	7	22	11 (0.11)	5	6

detected in serum and cerebrospinal fluid and the brains of MS patients on autopsy. Prominent MMP12 staining has been reported in macrophages found in active demyelinating MS lesions [11]. A lower proportion of phagocytes was positive for MMP12 in chronic active and inactive plaques [11]. Thus, MMP12 is one of the three MMPs (together with MMP9 and 3) the common elevation of which has been confirmed in MS and also in mouse and rat EAE models [4,12].

The MMP12 gene is located on the eleventh chromosome (11q22.2-q22.3) [13]. By single-strand polymorphism confirmation analysis of deoxyribonucleic acid (DNA) from healthy individuals a common polymorphism within the MMP12 gene promotor has been detected. This –82 A/G polymorphism of MMP12 presents a functional polymorphism in which allele A shows a higher affinity for the transcription factor activator protein–1 (AP–1) and is thus associated with a higher promotor activity and a higher MMP12 expression in assays [14].

In the present study we examined the relationship between –82 A/G MMP12 polymorphism and MS.

MATERIALS AND METHODS

Patients and control subjects

In a case-control study a total of 92 unrelated patients (23 men and 69 women, mean patient age 37.3 ± 9.0 years, mean EDSS score 3.71 ± 1.4, and mean disease duration 9.9 ± 5.1

years) with definite MS according to the McDonald criteria were recruited from the MS centre at the Department of Neurology, Faculty Hospital and Faculty of Medicine, Masaryk University, Brno. The cohort included 83 patients with the remitting relapsing form of MS – RRMS, 8 patients with the secondary progressive form – SPMS (5 women and 3 men, mean EDSS score 5.8 ± 1.4, mean disease duration 10.4 ± 4.3 years), and one patient with the primary progressive form – PPMS (a man, EDSS 4.0, disease duration 4 years). The control group consisted of 51 healthy controls (17 men and 34 women) with no history of MS or other autoimmune disease, and the subjects were matched for age and sex. All patients and controls were of Czech ancestry. Written informed consent was obtained from all the subjects examined. The study was approved by the Committee for the Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University, Brno [ref. No. 48/ 2003].

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes by a standard technique using proteinase K digestion of cells. The resulting DNA was used as a template for polymerase chain reaction (PCR). The method used to type the MMP12 –82A/G promotor polymorphism has been described previously [14]. Primers and conditions for amplification are shown in Table 1. PCR was performed at a final volume of 25µl containing 3µl of genomic DNA, 0.5µl dNTP, 1.25µl of each primer, 2.5µl buffer, 0.14µl Taq polymerase, and 2µl 25mM MgCl2. Equal amounts of 15µl of the PCR products were digested with specific endonucleases to obtain final fragments (Table 2). The final genotypes were visualised in UV light after ELFO on 2% Serva agarose gel with ethidium bromide (Figure 1).

Statistical analysis

Hardy-Weinberg equilibria (HW) of the study polymorphism in all cases and controls were chi-square tested. Comparisons of the allele variant frequencies as well as comparisons of genotype incidence in the case-control study were calculated using the Fisher exact test. Statistical significance was considered as $p<0.05$. The Holm test for multiple comparisons was employed where appropriate.

RESULTS

Differences were evident in genotype distribution between our study groups, in which the AG heterozygotes were more frequent in MS patients (OR=2.6, p=0.026). Using the Holm test for multiple comparison, the results indicated only a trend, lacking statistical significance (pcorr=0.078). Even after the stratification of the subjects by sex, no allele or genotype differences were observed between the groups examined. The representation of the appropriate genotypes and alleles is shown in Table 3. The control group was not in HW equilibrium. No differences were found in genotypes or alleles among MS patients with variant forms of the disease. Nevertheless, all the patients with the progressive form (which is associated with a more severe course and higher disability) were of the same genotype: homozygotes AA (data not shown).

DISCUSSION

It is assumed that different genetic backgrounds exist for the various forms of MS and also that genetic backgrounds vary from population to population. The Czech Republic is a country with both a high genetic homogeneity and a high prevalence of MS. The group examined therefore constitutes a proper and representative sample. On the other hand, the study group is too small to assess susceptibility to the different forms of MS. Despite this, all the patients with the progressive form of MS were of the same genotype, homozygotes AA. This genotype is associated with a higher promotor activity, a higher expression, and a higher final MMP12 plasma level. This corresponds with the idea of different genetic backgrounds for the various courses of MS.

In animal models elevated MMP12 has repeatedly been observed not only at the peak disease activity but also in the late phase of infection [11, 15–17]. MMP12 protein has been localised by immunohistochemistry in intralesional microglia/macrophages and astrocytes and might account for ongoing demyelination [15]. One of these studies demonstrated that MMP12 was the most highly up-regulated MMP. However, in contrast to previously published findings, this increase was associated with protection, as MMP12-null mice had a significantly worse maximum severity and EAE disease burden compared with wild-type controls [17]. MMP12 can therefore play both negative and positive roles in the pathophysiology of MS. At the peak phase of the disease during relapses, it may participate in blood-brain barrier disruption and potentiation of leukocyte infiltration, but in the late phase of the neuroinflammation the increased MMP12 level may be connected, for example, with some remyelination. Elevated levels of MMP12 have been observed at the time of myelination in mouse brain maturation [18].

CONCLUSIONS

There can be no doubt about MMP participation in the pathophysiology of MS, but the exact function of specific MMPs is not sufficiently understood. This results from the high degree of interaction among most MMPs, not only at the gene level but

also at further stages, where they may activate or inhibit each other. For this reason, further and more extensive investigations into the genotypes, and especially the haplotypes of MMPs, are indicated. They may well disclose more profound effects on susceptibility to MS or on tendencies to different courses of MS.

ACKNOWLEDGEMENT

This study was supported by the Czech Ministry of Education Research Plan No. MSM0021622404.

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PAINFUL SENSORY NEUROPATHIES IN THE ELDERLY

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Received after revision February 2009

KEY WORDS

Elderly
Intraepidermal nerve fibre density
Painful neuropathy
Quantitative sensory testing
Small and large nerve fibres

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ABSTRACT

Introduction: Painful sensory neuropathies are frequently encountered in elderly individuals. The pathophysiology of peripheral nerve dysfunction as well as the clinical picture and applicable diagnostic methods in senescence may be at least partly different from those in the general neuropathy population with respect to the potential role of age-related neurodegeneration as well as to some specific features of the elderly population in general.

Patients and methods: Thermal quantitative sensory testing (T-QST), evaluation of intra- (IENFD) and subepidermal nerve fibre densities in skin biopsy samples, nerve conduction studies, autonomic nervous system testing, and clinical neurological examination including detailed assessment of neuropathic symptoms and pain intensity were performed in a group of 25 elderly (≥65 years) and 74 non-elderly patients with painful sensory neuropathy. For comparison, data from 37 age-matched healthy individuals (10 elderly and 27 non-elderly) were used.

Results: The involvement of small nerve fibres documented by T-QST or IENFD was almost invariably found in both elderly and non-elderly patients and the sensitivity as well as applicability of both these methods was similar between the age groups. Nevertheless, an obvious trend to decrease in nerve fibre counts with age was observed in the healthy control group. The dysfunction of large nerve fibres (assessed by nerve conduction studies or clinical examination) as well as of the autonomic ones was significantly more frequent in elderly neuropathy patients compared to younger age groups. Moreover, evaluation of the autonomic nervous system could frequently not be performed in elderly patients with respect to associated heart diseases or medication.

Conclusions: Painful neuropathy patients almost invariably display involvement of small nerve fibres regardless of the patient's age. In elderly painful neuropathy patients, however, a more frequent and more extensive dysfunction of large myelinated fibres and autonomic fibres was found suggesting the increasing role of age-related neurodegeneration in the development of peripheral neuropathies in old age and

implying the need of age-stratified reference data of most of the diagnostic tests of small- and large-fibre dysfunction in peripheral neuropathies.

ABBREVIATIONS USED

AUDIT – alcohol use disorders identification test
IENFD – intraepidermal nerve fibre density
MNSI – Michigan neuropathy screening instrument questionnaire (subscales i and ii)
NDS – neuropathy disability score
NSS – neuropathy symptom score
PGP – protein gene product
T-QST – thermal quantitative sensory testing
SENPD – subepidermal nerve plexus density
SNAP – sensory nerve action potential
VAS – visual analogue scale

INTRODUCTION

Painful sensory neuropathies are frequently encountered in elderly individuals and may have an important impact on their sleep and quality of life. Besides an increasing incidence of neuropathy risk factors in senescence, degeneration of the peripheral nervous system in old age has repeatedly been shown in humans as well as in animal models of nervous system aging, and seems to play a role in the development of peripheral neuropathies in the elderly [1, 2]. Both the age-related degeneration and the involvement of peripheral nerves by some pathological processes may involve various types of nerve fibres, and the pattern and proportion (and probably also the time sequence) of the fibres affected are crucial for the clinical picture of peripheral neuropathy. Experimental models [1, 2, 3] suggest that, in contrast to e.g. metabolic neuropathies, where initial and more pronounced involvement of so-called small non-myelinated and low myelinated nerve fibres of the classes A-delta and C is usually described, the age-related degeneration process affects predominantly large myelinated fibres of A-beta class. The suggested different pattern of the affected nerve fibres in the elderly therefore indicates that the resulting clinical picture and diagnostic methods of peripheral neuropathies in senescence may be at least partly different from those in younger age groups.
Besides more frequent affection of large nerve fibres in senescence, age-related loss of small nerve fibres has also been described in rodents [2, 3] as well as in some of the human studies on epidermal innervation reflecting the age-related changes of small nerve fibre status [4, 5]. The involvement of these fibres (mediating pain and temperature, and also serving autonomic functions) has repeatedly been shown to play an important role in the pathophysiology of neuropathic pain [6, 7], and their dysfunction has been found in most of the painful neuropathies [8, 9, 10]. A-delta and C fibres can even be the only nerve fibres affected in some of the painful neuropathy patients (so-called small fibre neuropathies) [8, 9, 10]. Their involvement, however, is usually underdiagnosed in clinical practice, because common clinical examination and nerve conduction studies fail in the verification of their dysfunction [8], and special diagnostic methods (e.g. examination of thermal and/or pain sensation on quantitative sensory testing (T-QST) or quantification of intraepidermal nerve fibre density (IENFD)) have to be used for this purpose [8]. Both of these methods, however, may have some limitations, particularly in elderly patients. Together with some specific characteristics of senescence in general, all these facts point out the possibility that the pathophysiology, the clinical picture and applicable diagnostic methods of neuropathies in the elderly are at least partly different from the general neuropathy population, in particular when pain is a leading clinical symptom. Only few studies, however, deal with clinical, neurophysiological, and morphological characteristics of painful peripheral neuropathies in senescence.
The aim of our study was therefore to evaluate selected clinical, morphological, and electro- and psychophysical findings in older adults with painful sensory neuropathy compared to younger patients to reveal possible specific features of this diagnostic unit in the elderly and to assess the diagnostic validity of several methods used to confirm a peripheral nerve dysfunction in these patients.

PATIENTS AND METHODS

Twenty-five elderly patients (older than 65 years) with painful sensory neuropathy and a prominent complaint of "burning feet" were included in the study (Table 1). For comparison, data from seventy-four non-elderly individuals with painful neuropathy complying with the same inclusion and exclusion criteria (see below) were used (Table 1). All the patients from both these groups were prospectively recruited from the Peripheral Neuropathy Outpatient Clinic of the Brno Faculty Hospital between September 1999 and March 2005. The protocol was approved by the institutional ethics committee of the University of Brno; written informed consent was obtained from all patients and volunteers before inclusion into the study.
The following inclusion criteria had to be met: [1] Positive sensory symptoms (pain or painful dysesthesias described as electric shock-like, burning, cold, prickling, tingling, or itching) in a distal symmetrical distribution in the lower extremities for more than 3 months and with an intensity of at least 3 on a visual analogue scale (VAS) of 0–10. [2] Abnormal thresholds for at least one thermal modality as assessed by T-QST.

Table 1
Clinical characteristics of patients and controls. Abbreviations are defined in the text

Demographic characteristics	Neuropathy group		Controls	
	Elderly	Non-elderly	Elderly	Non-elderly
Number of subjects studied	25	74	10	27
Males	13	45	6	11
Signs of large fibre affection	17	24		
Age				
Median (min/max) (years)	71 (65/83)	55 (25/64)	70 (67/86)	54 (27/64)
Symptom duration				
Median (min/max) (years)	1.50 (0.25/6)	2.00 (0.25/13)		
Associated relevant diseases (number of subjects)				
Diabetes mellitus or impaired glucose tolerance	5	27		
Alcohol abuse	2	13		
Others[1]	7	14		
Undetermined	11	20		

[1]Others: potential aetiological factors or co-factors include toxins (anticancer chemotherapy or antituberculous drugs), paraneoplastic syndromes, monoclonal gammopathy, hyperlipidaemia, and amyloidosis

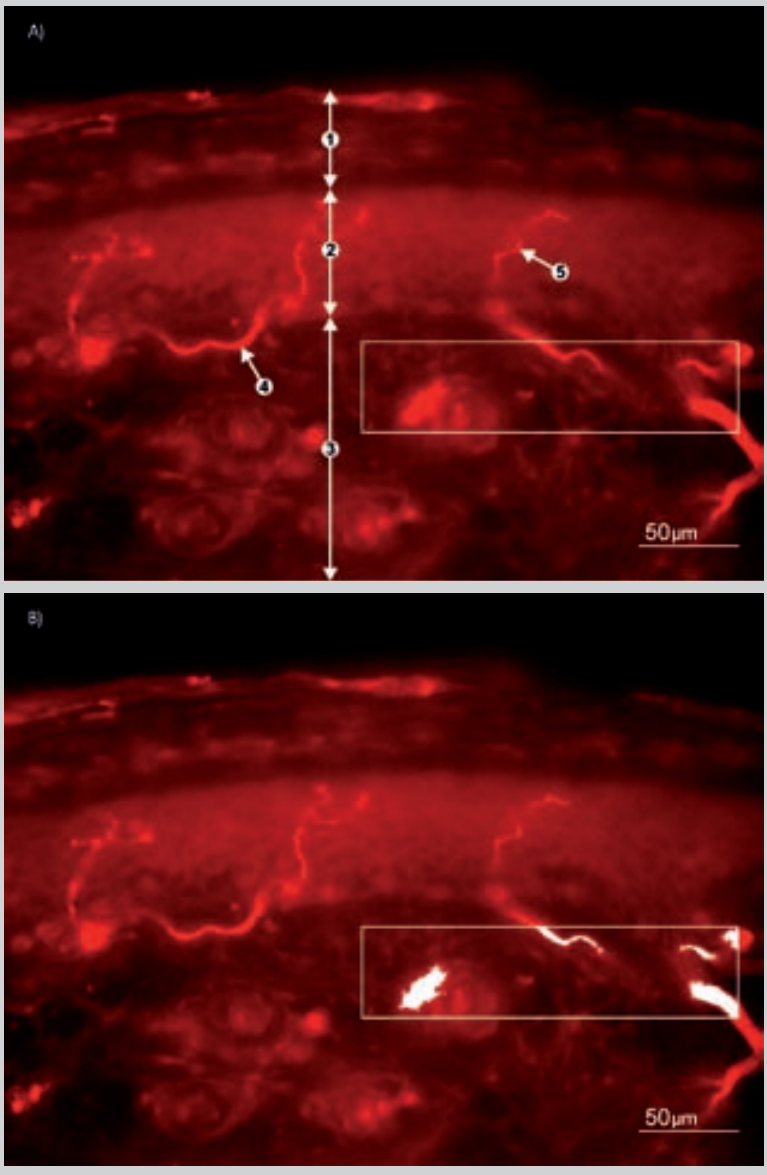
Exclusion criteria were signs of central (brain or spinal cord) involvement and any overt clinical motor signs (weakness, distal muscle atrophy, fasciculations). Other diseases or conditions leading to foot pain (both neuropathic and non-neuropathic) such as plantar fasciitis, Charcot's joints, osteoarthritis, peripheral vascular disease, central nervous system dysfunction, tarsal tunnel syndrome, and other peripheral mononeuropathies were excluded by history and clinical examination.

For comparison of skin nerve fibre densities with the non-neuropathy population, data from a group of thirty-seven healthy volunteers matched for age and body mass index (10 elderly and 27 non-elderly subjects) were used (Table 1). These individuals were recruited at the Brno Faculty Hospital, mainly from among hospital employees and their relatives, and the details about their history and clinical status as well as their laboratory findings of the parameters with a potential aetiological relation to peripheral neuropathies were published previously in detail [9, 10].

Data from the patients and controls included into the present study were reported in part in a previous study evaluating in detail the diagnostic value of skin biopsy (including a newly validated parameter – subepidermal nerve plexus density) [9]. The previous study, however, dealt mainly with morphological findings and was focused neither on the specifications of painful neuropathies in the elderly nor on the differences between elderly patients and those of younger age groups. In contrast, the current study deals only with characteristic features of painful peripheral neuropathy in senescence, which are suggested to be partly different from the general neuropathy population.

A detailed medical history was taken from all patients, who underwent a thorough clinical neurological examination, including assessment of tendon reflexes, muscle strength, trophism, sensation to touch, pinprick, warm and cold stimuli, proprioception, and vibration sense. Associated diseases with potential aetiological relevance were explored by history and routine biochemical, haematological, and immunological blood tests including thyroid hormones, triglycerides, vitamin B_{12} and folate levels, serum protein electrophoresis, carbohydrate-deficient transferrin, and a set of autoantibodies (ANA, anti-Ro, anti-La, anti-DNA). Diabetes mellitus was confirmed by fasting plasma glucose levels and oral glucose tolerance tests. Quantification of alcohol consumption as well as further confirmation of harmful alcohol use and possible alcohol dependence was performed according to the WHO AUDIT (Alcohol Use Disorders Identification Test) manual [11].

For quantification of the presence and severity of clinical symptoms and neurological impairment, the Neuropathy Symptom Score (NSS) [12], the Neuropathy Disability Score (NDS) [12], and the Michigan Neuropathy Screening Instrument Questionnaire (MNSI) [13] were employed. The mean intensity of neuropathic pain during the week before clinical



Figures 1a, b
Images depicting the method for evaluation of the intraepidermal nerve fibre density (IENFD) and subepidermal nerve plexus density (SENPD) on PGP 9.5 immunoreacted 40-µm cryosections of the skin. SENPD was measured using a density threshold for PGP 9.5 immunoreactive structures and expressed as a percentage of the whole subepidermal area analysed (200 x 50 µm adjacent to the dermoepidermal junction in each optical field)

A: Original digitised image. Bar = 50 µm
1 + 2 = epidermis (1 = stratum corneum, 2 = stratum basale, spinosum, granulosum, and lucidum)
3 = dermis
4 = subepidermal nerve plexus
5 = intraepidermal nerve fibre

B: Density threshold set for morphometric determination of nerve fibre area. Bar = 50 µm

and electrophysiological testing was assessed using a 10-cm VAS, in which 0 cm represented "no pain" and 10 cm "the worst pain I can imagine". All the clinical and electrophysiological tests were performed at the time of admission to the clinic and no specific treatment for neuropathic pain had been delivered to these patients previously.

Quantitative sensory thermal threshold testing (T-QST) was performed using thermal sensory analyser software (Medoc Thermal Sensory Analyser 2001). Thermal thresholds were examined on the dorsum of the right foot; both the method of limits and levels were used. For evaluation we used our own normal limits [14]. The methodology was described elsewhere in detail [9, 14].

Electrophysiological examinations, sympathetic skin response, and cardiovascular tests based on heart rate variability examination were performed using a Keypoint type II electromyograph system (Dantec, Skovlunde, Denmark), following the published recommendations [15]. The results were processed according to the reference values related to age and height at the Brno laboratory.

Skin punch biopsies were taken from the distal calf. The details of skin specimen removal and further processing were published previously [9, 16] and follow standard recommendations [17, 18]. Sections of 40 µm thickness were immunostained with rabbit polyclonal antibodies to human protein gene product (PGP) 9.5 (Ultraclone, Wellow, UK; 1:800) as a primary antibody and goat anti-rabbit IgG labelled with a cyanine fluorescent probe as a secondary antibody (Amersham, Biosciences, Piscataway, NJ; 1:100). Using Image-Pro Plus 4.0 software (Media Cybernetics, Leiden, The Netherlands), the intraepidermal nerve fibre density (IENFD) was quantified by counting the nerve endings within an accurately measured length of epidermis [17, 18] (Figure 1).

Subepidermal nerve plexus density (SENPD) was measured using a density threshold for PGP9.5 immunoreactive structures and expressed as a percentage of the whole subepidermal area analysed (200 x 50 µm adjacent to the dermoepidermal junction in each optical field) [9] (Figure 1). The IENFD was defined as abnormal if lower than the cut-off value of 8.8 fibres/mm [9]. Similarly, the SENPD was defined as abnormal if lower than the cut-off value of 7.25 % [9]. The normal limits were established from the skin biopsy results in the group of normal individuals and the method settings were described in our previous publication [9].

Standard descriptive statistics were used to summarise the distribution of the data. Comparison of continuous data between both the subgroups was performed by a univariate t-test, while categorical data were compared using a chi-square test. The Pearson's correlation coefficient was used in correlation analyses. The value $\alpha < 0.05$ was taken as the universal limit for statistical significance.

RESULTS

As a matter of course, the age of elderly patients was higher in comparison with non-elderly ones ($p<0.001$), while the other demographic characteristics (i.e. sex and duration of symptoms) did not differ between the groups (Table 1). Prominent aetiological factors of both groups represented impaired glucose metabolism and chronic harmful alcohol use or possible alcohol dependence. The incidence and proportion of the particular aetiological factors did not differ significantly between the groups, though a trend towards lower incidence of glucose dysmetabolism ($p=0.12$) and a higher proportion of patients with undetermined (i.e. idiopathic) aetiology in elderly patients ($p=0.11$) was found (Table 1).

Clinically, impaired proprioception and vibration sense as signs of large-fibre sensory dysfunction were significantly more frequent in elderly patients ($p=0.001$ and 0.002, respectively; data not shown). For other sensory modalities, a trend towards higher incidence of abnormities in the elderly group was also found, though without statistical significance ($p= 0.07$, 0.05, and 0.10 for sensation to pinprick and pain and thermal perception, respectively; data not shown). In accordance with these findings, higher values and higher sensitivity of NDS and a second part of the MNSI questionnaire (as the scales reflecting the presence of clinical abnormities and in particular large-fibre function) were found in our group of elderly patients, while only less obvious and less significant differences were found in the Neuropathy Symptom Score values (reflecting both the small and large nerve fibre function) (Table 2).

The intensity of neuropathic pain as assessed by VAS as well as the presence of clinical symptoms of sensory dysfunction (evaluated either as particular items or as a summary score reflecting mainly the dysfunction of small nerve fibres, i.e. the first part of the MNSI questionnaire) were similar in both groups of neuropathy patients (Table 2). Accordingly, the sensitivity of this questionnaire was also comparable in elderly and non-elderly individuals.

Absolute values of cold and warm detection thresholds, the number of abnormities of thermal sensation found by the particular testing algorithms using the age-related normal values, and also the variability of the responses did not differ significantly between the groups (Table 2). The results obtained by the three T-QST methods were consistent and values did not differ significantly between the methods.

The reduction of sensory nerve action potential (SNAP) amplitudes represented the most significant difference of nerve conduction studies between the groups (Table 2). In general, electrophysiological signs of large fibre sensory neuropathy were more frequent in elderly patients ($p = 0.002$) (data not shown).

The elicitability of sympathetic skin response was similar in both groups of patients. In the elderly group, however, slightly

Table 2

Absolute values and number of abnormities (if applicable) revealed by selected psychophysical, neurophysiological, and clinical tests in elderly and non-elderly neuropathy patients. Values are mean ± standard deviation. Abbreviations are defined in the text

Test	Absolute values			Number of abnormities revealed		
	Elderly	Non-elderly	p-value (t-test)	Elderly	Non-elderly	p-value (chi²-test)
Thermal thresholds (dorsum of the right foot, °C)						
MLI – R CS	20.2 ± 10.4	21.5 ± 8.8	0.54	14	37	0.60
WS	47.0 ± 3.4	46.8 ± 2.8	0.73	22	56	0.19
MLE – CS	22.3 ± 10.6	23.3 ± 9.3	0.66	13	38	0.96
WS	44.4 ± 4.1	44.2 ± 3.5	0.76	22	67	0.72
Nerve conduction studies						
Sural SNAP amplitude (μV)	3.7 ± 3.3	8.7 ± 5.5	< 0.001	16	20	< 0.001
Sympathetic skin response (lower extremities)						
Mean latency (ms)*	2.23 ± 0.28	2.11 ± 0.22	0.04			
Mean amplitude (mV) *	0.41 ± 0.25	0.73 ± 0.72	0.04			
Elicitability (%)	58	66	0.27	4	8	0.50
Heart rate variability						
Heart-rate variability to						
deep breathing (%)	11.9 ± 5.8	18.8 ± 10.3	0.04	8	22	0.04
Clinical findings						
VAS*	6.14 ± 2.56	6.38 ± 2.0	0.62			
MNSI 1	7.60 ± 2.00	7.93 ± 2.29	0.52	19	57	0.91
MNSI 2	2.48 ± 1.41	1.35 ± 1.33	< 0.001	20	26	< 0.001
NSS*	2.56 ± 1.16	2.04 ± 0.83	0.02			
NDS*	15.7 ± 13.7	6.9 ± 7.9	< 0.001			

*No limit data for discrimination of normal and abnormal findings of this parameter are available

MLI – Method of Limits, MLE – Method of Levels, CS – cold sensation, WS – warm sensation, R – random variant of the test); other abbreviations are defined in the text

higher latencies and lower amplitudes of the response were found in the lower extremities (Table 2).

The heart rate variability testing could not be performed or evaluated in more than a half of the elderly group because of associated heart disease or medication with potential influence on the heart rate (56 % compared to 24 % of non-elderly individuals, p = 0.003). From the evaluable tests, the vast majority was abnormal in elderly patients, while in the non-elderly group abnormal results were much less frequent (72 and 36 %, respectively, p = 0.02). In most of the tests performed, significantly lower heart rate variability values were thus obtained were thus obtained in elderly individuals in comparison to younger ones (Table 2).

In the group of healthy individuals, a non-significant trend to the correlation of the IENFD and SENPD values with age as well as to higher values in the subgroup of elderly controls compared to non-elderly ones was found (Table 3). Comparison of healthy individuals and painful neuropathy patients demonstrated a clear reduction in both intra- and subepidermal nerve fibre/plexus density in both the elderly and non-elderly subgroups of patients with neuropathies compared to the particular age-related subgroups of healthy controls (Table 3). In neuropathy patients, lower values of the SENPD were found in elderly patients than in non-elderly ones, while the IENFD values did not differ significantly between the age groups (Table 3). A clear correlation of the IENFD values with warm thresholds in T-QST examination and with the scales reflecting particularly small nerve fibre involvement (i.e. MNSI I) and lower intraepidermal nerve fibre counts in the presence of clinical signs of small fibre dysfunction were found in both the age subgroups of neuropathy patients, while the SENPD correlated better with SNAP amplitude and MNSI II and NDS (as the scales showing mainly large nerve fibre function) in both elderly and non-elderly individuals (p from 0.02 to <0.001; data not shown).

DISCUSSION

The principal finding of this prospective study is that the involvement of small nerve fibres is almost invariably present in elderly patients with painful neuropathies. Despite this fact,

Table 3

Skin biopsy findings. IENFD (fibres/mm) and SENPD (% of the subepidermal area of the size 200 x 50 µm adjacent to the dermoepidermal junction): basic summary statistics, comparison of the neuropathy and control groups and elderly and non-elderly individuals, and correlation with age in the control group of healthy individuals. Abbreviations are defined in the text

	Groups of patients		Comparison of the groups	Correlation with age in control group	
	Elderly	Non-elderly	p_1-value	r	p-value
IENFD (fibres/mm)					
Group of patients	4.95 ± 3.02	5.69 ± 4.08	0.40		
Healthy individuals	9.81 ± 1.61	11.80 ± 3.30	0.08	–0.325	0.06
Comparison of the groups (p_2-value)	< 0.001	< 0.001			
SENPD (%)					
Group of patients	3.19 ± 1.91	4.88 ± 2.75	0.006		
Healthy individuals	8.20 ± 1.31	9.76 ± 2.76	0.10	–0.237	0.16
Comparison of the groups (p_2-value)	< 0.001	< 0.001			

p_1-value – comparison of nerve fibre density between elderly and non-elderly individuals in a group of patients (first row) or in a group of healthy individuals (second row)

p_2-value – comparison of nerve fibre density between elderly neuropathy patients and controls (first column) or non-elderly patients and controls (second column)

a partly different pattern of involvement of the particular nerve fibre types with a more severe and more frequent dysfunction of large nerve fibres compared to younger age groups was found in these individuals showing a probable role of age-related neurodegeneration in the development of peripheral neuropathies in the elderly. These findings imply an increasing significance of clinical examination and nerve conduction studies in the diagnostic algorithm of painful neuropathies in senescence. Almost all the elderly painful neuropathy patients however present an involvement of small nerve fibres and in a remarkable part of them (about 1/3) these fibres were shown to be the only affected ones. Appropriate diagnostic methods of small nerve fibre involvement (i.e. thermal quantitative sensory testing and examination of intraepidermal nerve fibre density) thus still play a pivotal role in the battery of diagnostic tests of painful neuropathies in the elderly, and our findings prove their applicability in older adults as well as their similar diagnostic validity compared to younger age groups, though some particularities (e.g. the need of age-related normal limit data) should be taken into account.

Painful sensory neuropathies are frequently encountered in older adults. As in the other types of peripheral nerve affections, both the clinical picture and applicable diagnostic methods of painful neuropathies in senescence depend in particular on the pattern and proportion of the affected types of nerve fibres. In accordance with the observations in the general painful neuropathy population [9, 19], dysfunction of small nerve fibres was almost invariably present in our group of elderly painful neuropathy patients. A comparison of the age groups showed a similar extent of small-fibre affection (proved both by examination of IENFD and T-QST) as well as an almost identical spectrum of corresponding clinical symptoms and pain intensity (as assessed by VAS) in elderly and non-elderly patients in our study. These findings suggest that, regardless of the patients' age, a similar extent of small fibre damage leads to a similar clinical picture, and thus indirectly confirm the role of small nerve fibre dysfunction in the development of pain and appropriate clinical symptoms of painful neuropathies.

In contrast to these findings, most of the diagnostic methods (in particular clinical examination and nerve conduction studies) proved a more severe and more frequent affection of large myelinated nerve fibres in our group of elderly neuropathy patients compared to the younger ones. A similar pattern of nerve fibre involvement was repeatedly shown in animal models of age-related degeneration of the peripheral nervous system [1, 2]. In aging rats, more symptoms of disturbed mechanosensation compared to disturbed nociception were found [2], and a more severe loss of large and myelinated fibres compared to smaller ones was shown in sural nerve studies of peripheral nervous system aging [1]. Our data thus confirm the observation of more severe age-related degeneration of large and myelinated fibres compared to smaller ones and imply that neurodegeneration plays an important role in the development of painful peripheral neuropathies in the elderly.

Despite these facts, age-related loss of epidermal and dermal innervation, involving both sensory and autonomic

components, was also described in rodents [2, 3]. In humans, some of the studies [4, 5] suggest a similar decrease of intraepidermal innervation with age, but there is no clear agreement on this field and not all the studies show such a significant negative correlation [20], probably due to the considerable interindividual variability of epidermal nerve counts in normal humans. Despite such controversial findings, only few studies are focused on normal skin innervation in senescence and provide separated limit data of intraepidermal nerve fibre counts in the elderly [21], which may complicate the evaluation and the validity of skin biopsy examination in older adults.

In our control group of healthy individuals, an obvious trend to inversed correlation of IENFD/SENPD with age was observed, as well as a trend to lower fibre counts in the subgroup of elderly controls compared to the younger ones. Both of these trends were apparent, but none of them was statistically significant, probably with respect to the small size of our control group and in particular to the small number of elderly individuals among our healthy controls (10 out of 37). Our findings nevertheless seem to confirm the age-related degeneration of intra- and subepidermal nerve fibres and suggest the need of setting particular normal limits for elderly and non-elderly individuals. An increased number of healthy controls and in particular a higher proportion of elderly individuals are therefore needed in future studies.

In comparison with the age-related subgroups of healthy controls, a highly significant decrease in IENFD/SENPD values was found in both the elderly and non-elderly painful neuropathy patients, confirming the high sensitivity of this method, regardless of the patients' age. The examination was well tolerated by all the individuals and no complications of wound healing were observed in any one of the elderly or non-elderly patients. Together with a good correlation of the IENFD with warm thermal thresholds in T-QST examination and with MNSI I as the methods reflecting mainly small fibre dysfunction, these findings show that the examination of the IENFD from skin biopsy is an applicable method in the evaluation of small fibre damage in older adults, and we can recommend the inclusion of this method to the diagnostic algorithm of painful neuropathies in the elderly.

Another method of evaluation of the small nerve fibre dysfunction involved in our study was the examination of thermal thresholds on quantitative sensory testing [8, 22]. In contrast to the morphological character of the previous test, T-QST is a psychophysiological method and therefore requires concentration, attention, and the ability of fast response [22], which may be decreased in the elderly. However, when using the age-stratified reference data, the number of abnormities revealed by the T-QST and the response variability of the methods used did not differ between younger and older neuropathy patients and the results obtained by the three T-QST methods were consistent and values did not differ significantly between the particular algorithms. These findings suggest that when using the age-stratified reference data, psychophysiological methods are useful in elderly patients and provide reliable and reproducible results, fully comparable with younger individuals.

Besides the methods evaluating sensory small nerve fibres, assessment of autonomic nervous system functions can also be used for the evaluation of small nerve fibre status in polyneuropathy patients. Our previous findings [9], however, suggest that the autonomic nervous system testing is less sensitive compared to T-QST and skin biopsy in the evaluation of painful neuropathies in general. In elderly patients, the use of these methods (in particular examination of heart rate variability) is furthermore complicated by the increasing number of patients with coincidental heart rate abnormalities (e.g. atrial fibrillation) and those with implanted pacemakers or using antiarrhythmics (e.g. beta-blockers) in their regular medication. Due to these conditions, the heart rate variability testing could not be performed or evaluated in up to 60 % of elderly patients in our study (but only in about 1/4 of younger individuals). Among the evaluable tests, a significantly higher proportion of abnormalities was found in older patients, suggesting that the autonomic nerves are more frequently involved in painful neuropathies in the elderly as compared with younger age groups. Despite this fact, the sensitivity of autonomic nervous system assessment did not reach the value of the T-QST or the IENFD examination and autonomic tests thus still remain only complementary methods in the diagnostic algorithm of painful neuropathies in senescence and we recommend their use in particular for the verification of autonomic dysfunction in patients with relevant clinical symptoms. The signs of more severe dysfunction of autonomic fibres in our elderly neuropathy patients compared to younger age groups are in agreement with the observation of a significant age-related degeneration of autonomic nerve fibres in experimental models of nervous system aging [3] and may again support the hypothesis of the important pathophysiological role of age-related degeneration in the development of peripheral neuropathies in the elderly, which is also corroborated by the diverse pattern of large and small nerve fibre affection and by the higher proportion of idiopathic neuropathies in senescence. Thus, in younger patients the development of peripheral nerve dysfunction seems to be in particular the result of peripheral nerve damage caused by various aetiological factors, while in the elderly, age-related nerve degeneration is suggested to play an increasing role in the development of peripheral neuropathies.

Finally we conclude that painful neuropathies in the elderly display a slightly different pattern of involvement of the

particular nerve fibre types with a more frequent and more extensive involvement of large myelinated fibres as well as of the autonomic ones in comparison with younger age groups, suggesting the increasing role of age-related neurodegeneration in the development of peripheral neuropathies in senescence. The involvement of small nerve fibres, however, represents the most frequent and most remarkable abnormality in elderly painful polyneuropathy patients, confirming the role of small nerve fibre dysfunction in the development of painful neuropathy symptoms regardless of the patient's age. The assessment of thermal thresholds on the T-QST and the examination of the IENFD on skin biopsy as methods of evaluation of small fibre status proved to be applicable and sensitive in older adults and continue to play a key role in the diagnostic algorithm of painful neuropathies in senescence, despite the increasing sensitivity of nerve conduction studies and autonomic nervous system testing in these age groups.

ACKNOWLEDGEMENTS

The study was supported by the Czech Ministry of Education Research Plan No. MSM0021622404. Dr. Vlčková-Moravcová performed the morphological studies at the University of Würzburg, supported by a Fellowship from the European Neurological Society (ENS).

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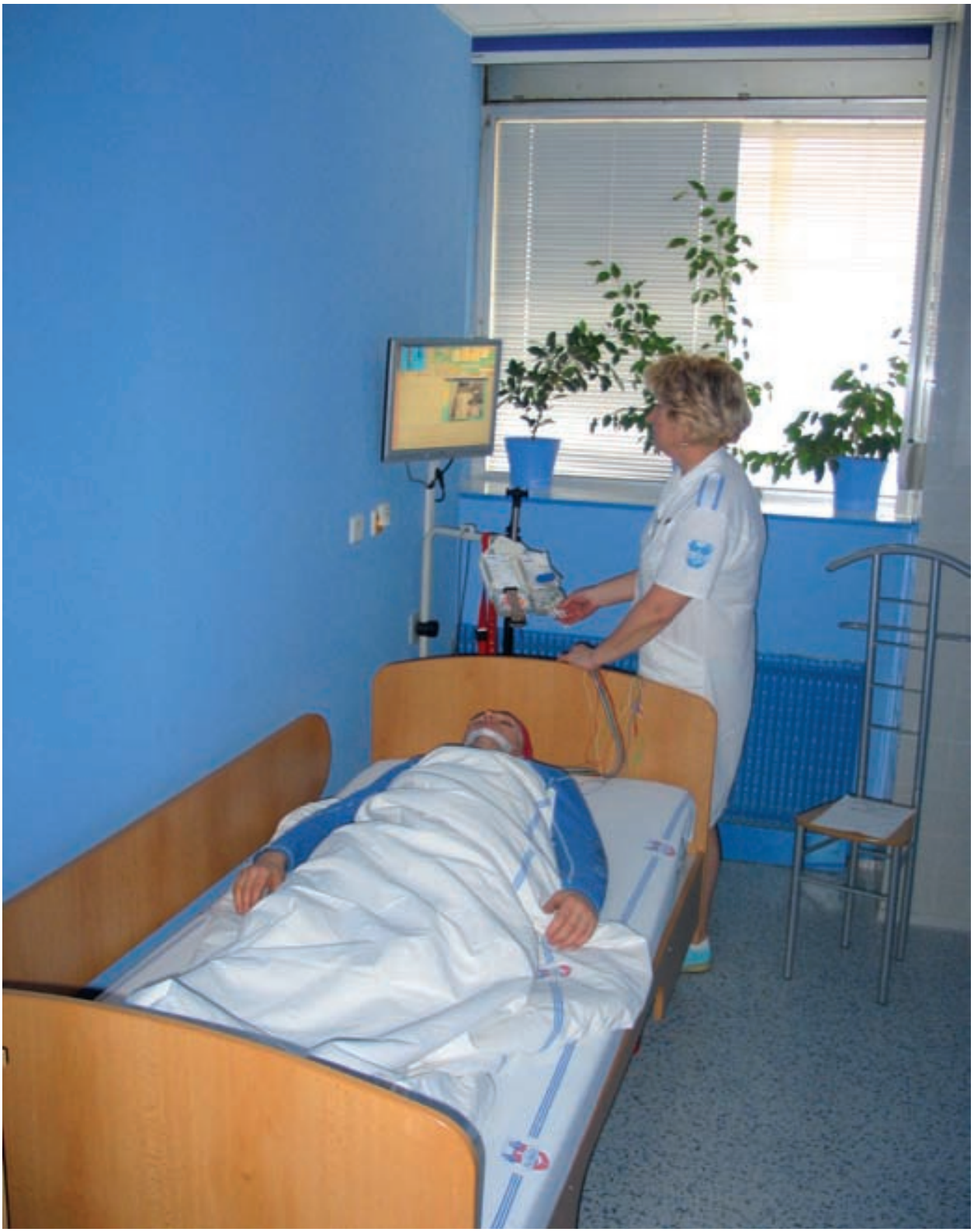

Sleep research laboratory

CRITICAL ILLNESS POLYNEUROMYOPATHY – A WELL-KNOWN BUT STILL CONTROVERSIAL ENTITY

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Received after revision February 2009

KEY WORDS

Critical illness
Polyneuropathy
Myopathy

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ABSTRACT

Two main clinical, pathological and electrophysiological types of acquired neuromuscular involvement in critically ill patients have been described in the past two decades: critical illness polyneuropathy and critical illness myopathy. However, there, still exist many controversies and unresolved questions regarding definition, terminology, diagnosis, and differentiation of what appears to be a spectrum of more or less overlapping neuromuscular disorders rather than distinct entities, and recently a new term – critical illness polyneuromyopathy (CIPM) has been coined. CIPM may present with muscle weakness and failure to wean from mechanical ventilation, but is discovered more often and earlier by electrophysiological examination. In this review, the incidence, clinical, electrophysiological and histopathological features, and risk factors of CIPM will be described. Among the most important risk factors for CIPM are sepsis or systemic inflammatory response syndrome and the severity of multi-organ failure. Acquired neuromuscular weakness in critically ill patients should be regarded as a part rather than a complication of critical illness – dysfunction or failure of a further (neuromuscular) system.

THE ICU WEAKNESS: TERMINOLOGY, DIAGNOSTIC CRITERIA AND DIFFERENTIAL DIAGNOSIS

Neuromuscular weakness is often encountered in patients in the intensive care unit (ICU). Patients can be admitted to the ICU because of increasing muscle weakness and threatening respiratory failure due to an underlying neuromuscular disorder, such as Guillain-Barré syndrome (GBS) and myasthenia gravis (MG). More frequently, a new weakness appears during the ICU stay. If weakness of central origin due to encephalopathy and rarely to myelopathy is excluded, a neuromuscular weakness could be caused by exacerbation of an underlying neuromuscular disease (such as MG, motor neuron disease or muscular dystrophy), or a weakness due to persistent neuromuscular blockade after administration

of non-depolarising muscle blocking agent (NDMBA) must be taken into consideration. By far the most frequent cause of new acquired weakness of neuromuscular origin in ICU patients is, however, critical illness polyneuropathy (CIP), critical illness myopathy (CIM), or both [1,2]. In this review, we will focus mainly on those aspects of acquired neuromuscular weakness in critically ill patients that we addressed in our previous research: epidemiology, diagnosis, and aetiology.

CIP was first systematically described in the early 1980s [3], while myopathy in critically ill patients was first reported in 1977 in association with the administration of corticosteroids [4]. The first paper focused on this problem in the Czech or Slovak literatures was published in 2000 [5], and subsequently also in paediatric patients [6]. The differentiation between CIP and CIM is based on the typical clinical, electrophysiological and histopathological signs of acute axonal sensorimotor polyneuropathy and myopathy, and on the assumption that most cases can be categorised as one type or the other [7,8]. There is, however, increasing evidence that myopathy may co-exist with neuropathy [9,10], and that the final diagnosis – CIP, CIM or both – is critically dependent on the method used [10]. Another factor complicating diagnosis is the fact that neither CIP nor CIM are homogenous entities. Although CIP is typically sensorimotor acute axonal polyneuropathy, pure motor and pure sensory forms of CIP have also been described [10–12]. CIM is an even more heterogeneous group of muscle disorders. At least four main types of CIM have been reported: myopathy with selective loss of myosin filaments ('myosin loss myopathy', 'thick filament myopathy'), acute necrotising myopathy, non-necrotising 'cachectic' myopathy, and decreased sarcolemmic excitability [13,14]. The term 'critical illness myopathy' has been coined for myopathy with loss of myosin [8]; for non-necrotising 'cachectic myopathy' with dominant type II atrophy [15]; or as a descriptive counterpart to CIP covering all types of myopathy in critically ill patients [16]. It is not known whether all these changes share the same aetiology and represent one disease.

CIP has been thought of as an easy diagnosis to make. Clinical diagnosis of CIP is based on the presence of flaccid areflexic quadriparesis with sensory involvement; this presence is, however, difficult to prove in comatose or unco-operative patients. Although some authors have reported different distributions of muscle weakness in CIP and CIM, differentiation between neuropathy and myopathy based on clinical grounds in comatose, sedated or encephalopathic critically ill patients is highly unreliable [8,17].

ELECTROPHYSIOLOGY

An electrophysiological pattern of acute axonal sensorimotor polyneuropathy is not difficult to discern, but differentiation between CIP and CIM is difficult or even impossible for numerous reasons not associated in other conditions [11,18]. Routine conduction studies and needle electromyography (EMG) provide only non-specific data. Abnormal spontaneous activity and decreased amplitudes of compound muscle action potentials can occur in different lesions of a motor unit, including myopathy. Diffuse tissue oedemas of the extremities of critically ill patients could lead to false-positive sensory conduction abnormalities. The assessment of recruitment and interference in the voluntary EMG pattern is obscured by severe weakness or poor voluntary effort in most patients. Some authors have reported the usefulness of sophisticated electrophysiological methods, such as single-fibre EMG [19], quantitative electromyography (QEMG), and motor unit number estimation [18,20]. CIM is a difficult diagnosis to make using electrophysiological methods [16]. If such diagnosis is based only on electrophysiological criteria, it is considerably underdiagnosed [10]. There are several reasons for this underestimation. Motor involvement in acute axonal neuropathy and myopathy shares the same pattern on conventional electroneurography (ENG) and needle EMG. In the presence of an abnormal sensory neurogram, motor abnormalities have invariably been attributed to neuropathy, irrespective of the possibility of concomitant myopathy associated with sensory or sensorimotor polyneuropathy [12]. Some additional techniques useful in the detection of myopathy, such as quantitative EMG, require patient co-operation, which is lacking in most cases. Nevertheless, some studies utilising these techniques have reported predominant myopathic involvement in critically ill patients [13,18,20]. Trojaborg et al. [18] carried out electrophysiological studies on 22 consecutive patients with critical illness-associated weakness and found electrophysiological signs of myopathy in all cases (confirmed by biopsy in nine of them), while only one patient showed electrophysiological signs of polyneuropathy. Lacomis et al. [20], studying 100 patients with new weakness, reported that electrophysiological signs of acute myopathy were three times more frequent (42 %) than those of acute polyneuropathy (13 %). Rich et al. [13] reported direct muscle stimulation (DMS) findings, indicative of decreased sarcolemmic excitability, in 11 out of 14 critically ill weak patients. The newly introduced DMS method [5,13,21] enables the investigator to distinguish decreased muscle membrane excitability from other causes of muscle weakness. It has, however, some methodological pitfalls [18,21]. One of them is the use of the ratio of compound muscle action potentials (CMAPs) obtained with direct muscle stimulation (dmCMAP) and nerve stimulation (neCMAP); where the ratio ne/dmCMAP is >0.5 it is seen as a sign of decreased muscle membrane excitability [13]. This value can be found in normal muscle, and electroclinical correlation would be rendered difficult or impossible by lack of co-operation or central palsy. The absolute value of

dmCMAP amplitude is a more valuable indicator of decreased sarcolemmic excitability [18,21], but it is an extremely variable parameter, prone to false-positive findings [21,22]. Another pitfall in the interpretation of DMS signs of decreased muscle membrane excitability is that the histopathological correlates of this condition are unknown in man. Recently, decreased muscle membrane excitability caused by increased fast inactivation of sodium channels was documented in an animal model of myosin loss myopathy in steroid-denervated rats [23]. In our material [21], decreased muscle membrane excitability was associated with various histopathological myopathic changes in all seven patients with this particular DMS pattern in whom muscle biopsy was performed. One can speculate that decreased muscle membrane excitability is probably a part or a phase of a complex pathological process involving muscle fibres during critical illness that does not usually remain isolated as a cause of muscle weakness. The interpretation of isolated electrophysiological motor abnormalities has been a matter of discussion for the last 15 years. A substantial proportion of weak, critically ill patients (46 % in our study sample, 30 % in a group studied by Coakley et al. [11]) show a pure motor abnormality on conventional ENG/EMG examination. Coakley [11] introduced the term 'pure motor syndrome' for this electrophysiological pattern. There are several reports of 'pure motor neuropathy', mostly attributed to the administration of non-depolarising muscle-blocking agents [23–25], but the evidence of neuropathy is based on non-specific neurophysiological motor abnormalities [24], on the absence of myopathic changes [23], or on the presence of neurogenic atrophy on muscle biopsy [25]. Interpretation of histopathological signs of 'denervation atrophy' is, however, somewhat controversial (see discussion below). Lacomis *et al*. [20] interpreted isolated motor abnormalities as signs of myopathy and we confirmed this assumption in our study: myopathic changes on muscle biopsy were found in all six patients with pure motor syndrome chosen for biopsy and decreased muscle excitability DMS pattern in eight out of 12 cases with pure motor syndrome in our group [21]. We can thus conclude that a motor form of critical illness polyneuropathy has not been adequately proved by pathological or electrophysiological studies. Recently, early electrophysiological detection of neuromuscular involvement in critically ill patients has been reported [26].

BIOPSY

Histopathological changes on muscle biopsy are used as a benchmark in the diagnosis of critical illness myopathy [4] and as a gold standard for evaluation of the validity of electrophysiological parameters [7,8,10,11,18]. Latronico et al. [10] studied 24 acutely ill neurological patients with clinical and ENG-EMG signs of CIP with muscle and nerve biopsy. They found that 23 patients (96 %) had a myopathy, and that 15 of these 'would have been diagnosed as having only a critical illness polyneuropathy', had they not performed muscle biopsy. Similar results were obtained in a study by DeJonghe et al. [27], in which all 10 patients with an ENG-EMG diagnosis of CIP also had signs of myopathy on muscle biopsy. The pathological features of myopathy in critically ill patients are somewhat complex, but most authors classify them into three main types:

- non-necrotising changes with atrophy of myofibres predominantly involving type II fibres (Figure 1), abnormal variation of muscle fibre size, angulated fibres, internalised nuclei, rimmed vacuoles, fatty degeneration of muscle fibres and fibrosis;
- necrotic changes with signs of regeneration (Figure 2);
- selective loss of thick myosin myofilaments (Figure 3) [8,10,15].

This classification is, however, somewhat arbitrary, with significant overlap of different changes in the same patient [8,18,28]. In contrast to the loss of myosin, considered to be a change that is pathognomic for CIM, atrophy of myofibres is less specific. Selective atrophy of type II myofibres is reported in myopathy, neuropathy, and disuse atrophy. Angular atrophic myofibres found multifocally or in small groups are traditionally interpreted as signs of denervation (Figure 4) [7,29,30]. Bolton and Breuer [7] stated that muscle biopsy in critically ill patients usually discloses 'denervation atrophy and mild muscle necrosis' and they interpret these findings as signs of critical illness polyneuropathy. Others, however, have described atrophic angular fibres as being characteristic signs of critical illness myopathy [15,31]. These discrepancies have resulted in striking differences in the classification of muscle biopsy changes in critically ill patients. Most authors who have systematically examined histopathological muscle changes in critically ill patients reported dominant myopathic changes [10,18,21,28,30]. The study carried out by Sander et al. [32] in eight quadriplegic areflexic patients with electrophysiological findings suggestive of CIP found normal nerve biopsy, but myopathic changes on muscle biopsy including myosin loss in all cases. Moreover, a significant proportion of cases examined has been interpreted as having a 'mixed' myopathic and neuropathic pattern. In particular, non-specific changes with dominant type II fibre atrophy were reported to be associated with CIP [15], but we found all types of myopathic changes to be associated with neuropathy [21]. Histopathological and immunopathological approaches are extremely valuable in the detection of various myopathic changes, but are less specific in the detection of acute denervation, especially in association with myopathic changes. The presence of muscle histopathological changes indicative of myosin loss has been proposed as one of four

major diagnostic features necessary to meet research diagnostic criteria for the diagnosis of definite critical illness myopathy [4]. Biopsy is, however, not a practical screening tool for larger observational epidemiological studies [18,21,33]. The methodological difficulties and reported coincidence of both types of neuromuscular involvement in the same critically ill patients led to the introduction of the descriptive term 'critical illness polyneuromyopathy' (CIPM) [5,6], critical illness polyneuropathy and myopathy [10,28,34], or CRIMYNE [35]. Some authors even maintain that the concept of critical illness polyneuropathy is outdated and have suggested the use of the clinical descriptive term 'critical illness weakness' followed by a description of myopathic, neuropathic, neuromuscular junction, metabolic and encephalopathic components [36]. Others have used a further descriptive term, 'ICU-acquired paresis' [27].

EPIDEMIOLOGY

At the beginning, the CIP–CIM interface seemed no more than a scientific curiosity, but research over the past 20 years has shown that CIP and CIM are the most frequent acute polyneuropathies and myopathies encountered in critically ill patients [37]. Although the exact incidence is unknown, due to wide variation in diagnostic criteria and patient case-mix, available data regarding the incidence of neuromuscular involvement in critically ill patients are rather impressive (Table 1): 25–57 % of critically ill patients (usually defined as >7 days in ICU (most or all mechanically ventilated, most or all with associated sepsis and multiple organ failure)) show clinically symptomatic weakness [21,28,34,38,39], and electrophysiological methods reveal abnormalities suggestive of CIP or CIPM in 21–100 % of these patients [11,21,26,34,38–43]. Critical illness myopathy is a primary myopathy that has only been characterised in recent years [10]. Data on its incidence are lacking; evidence is, however, mounting that CIM is at least as frequent as CIP [10,13,18,27,28]. Douglass et al. [44] reported myopathy detected clinically in 36 % of patients with status asthmaticus treated with corticosteroids and mostly with non-depolarising muscle-blocking agents (NDMBA). Campellone et al. [45], in a series of 100 consecutive patients after liver transplantation, detected clinical and electrophysiological signs of myopathy in seven cases (7 %); biopsy confirmed myosin loss myopathy in five cases.

AETIOLOGY

The concept of critical illness myopathy and neuropathy leads to a dichotomy in aetiological considerations of both types of involvement. Recently some authors have tended to replace this approach with the more descriptive concept of 'critical illness polyneuromyopathy' or 'ICU-acquired paresis' [27,28,34], and significant risk factors or predictors of CIPM have been sought. Sepsis or systemic inflammatory response syndrome (SIRS) is thought to be a leading cause of CIP [3,34,43,46,47]. Factors responsible for the systemic effects of sepsis, i.e. release of tumour necrosis factor (TNF), histamines and arachidonic acid metabolites, activation of the complement and cell adhesion systems, and formation of local free radicals may cause axonal degeneration [48]. As a mechanism, Bolton [48] suggested disturbances in the microcirculation of peripheral nerves. Using a bioassay by which toxic effects of patients' sera on motor neurons could be determined quantitatively, Hund et al. [49] demonstrated the presence of a low-molecular weight factor (<3 kDa) in the serum of patients with CIP that kills cultured motor neurons.

There exists a growing body of evidence that sepsis could also be responsible, at least in association with other factors, for the development of CIM or a myopathic component of CIPM [10,50–52]. Recently, the presence of SIRS has been found to increase the risk of clinically symptomatic CIPM development leading to artificial ventilation and this, together with the APACHE III score, has been used to estimate the risk [34]. Critical illness myopathy was originally reported as a complication of corticosteroid administration, either alone or in association with NDMBA [4,53,54]. Corticosteroids and NDMBA may serve as triggers, especially in necrotising and myosin-loss myopathies [15]. NDMBA may play a potentiating role, by virtue of a pharmacological denervation, that facilitates the toxic effect of other agents such as corticosteroids or inflammatory mediators. The functional denervation in CIP may provide a link between CIP and CIM. Pure motor neuropathy, on the other hand, has been attributed to the administration of NDMBA [55]. The causal role of NDMBA and corticosteroids in the development of CIM has been supported by numerous case reports, as well as by experimental data [56,57]. Recently, prospective studies reported controversial data on the significant independent influence of corticosteroids or NDMBA upon CIPM or ICU-acquired paresis development [27,34,40,58,59]. The differences between the studies might be explained by variable dosage of the corticosteroids administered, depending on the spectrum of diagnoses leading to critical illness. Another more general pitfall in the assessment of the aetiological influence of corticosteroids (in neuromuscular involvement of critically ill patients) could be a possibly predominant impact on the development of a myopathic component that is difficult to assess reliably in larger studies. Although administration of corticosteroids and NDMBAs has come to be avoided – or the drugs have been administered at the lowest possible doses – this practice does not seem to reduce the occurrence of neuromuscular involvement in critically ill patients. The influence of these drugs upon the development of CIPM and particular pathological subtypes of neuromuscular involvement thus remains to be established.

Table 1
Incidence of acquired neuromuscular involvement in critically ill patients (other than prolonged neuromuscular blockade): prospective observational studies

Incidence of neuromuscular involvement (%)	Group definition (inclusion criteria)	Diagnostic criteria	Study sample (No of cases)	Reference
57 % 28 %	>7 days in ICU, failure of at least 2 organs	Electrophysiological criteria of CIPM after 4 weeks Clinical signs of flaccid quadriparesis + electrophysiological criteria of CIPM after 4 weeks	61	Bednarik et al. 2005 [63]
84 %	>7 days in ICU, failure of at least 1 organ	Electrophysiological abnormalities	44	Coakley et al. 1998 [11]
25 %	Mechanical ventilation >7 days	Severe muscle weakness on day 7	95	DeJonghe et al. 2002 [27]
75 %	Mechanical ventilation, SIRS, MOF	Electrophysiological criteria of CIP after 3 weeks	73	Garnacho-Montero et al. 2001 [40]
33 %	Mechanical ventilation	Clinical and electrophysiological criteria of CIPM	98	DeLetter et al. 2001 [34]
57 %	Mechanical ventilation > 3 days	Clinical and electrophysiological criteria of CIP on day 14	28	Druschky et al. 2001 [39]
100 %	Mechanical ventilation, sepsis, MOF	Electrophysiological abnormality (decrease in CMAP amplitudes and/or fibrillations on days 2–5	9	Tennilä et al. 2000 [26]
21 %	MOF	Electrophysiological signs of CIP on discharge from ICU	33	Mohr et al. 1997 [41]
70 %	Sepsis, MOF	Electrophysiological signs of CIP	43	Witt et al. 1991 [42]
41 % 82 %	Sepsis, MOF	Clinical signs of CIP Electrophysiological signs of CIP	22	Berek et al. 1996 [38]
52 % (conventional treatment) 29 % (treated with insulin)	> 7 days in ICU	Electrophysiological signs of CIPM	206 157	Van den Berghe et al. 2001 [43]
36 %	Status asthmaticus, corticosteroid treatment	Clinical signs of CIM	25	Douglass et al. 1992 [44]
7 %	Liver transplantation	Clinical and electrophysiological signs of CIM	100	Campellone et al. 1998 [45]
30 %	> 10 days in ICU	Electrophysiological signs of CRIMYNE	92	Latronico et al. 2007 [35]

SIRS = systemic inflammatory response syndrome
MOF = multiple organ failure
CRIMYNE = critical illness myopathy and/or neuropathy

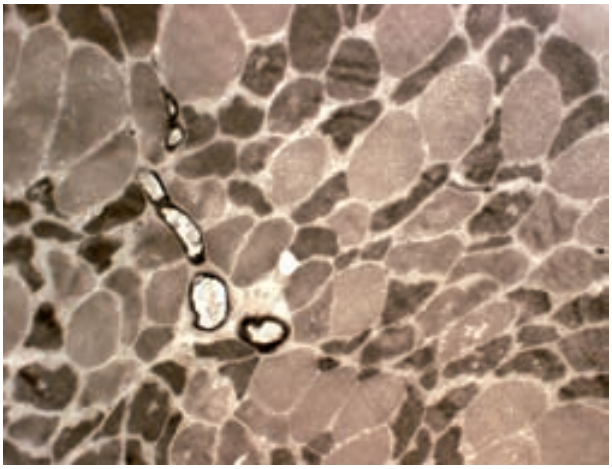

Figure 1
Myosin-ATPase: Atrophy of type 2 muscle fibres (dark fibres of type 2, light fibres of type 1)

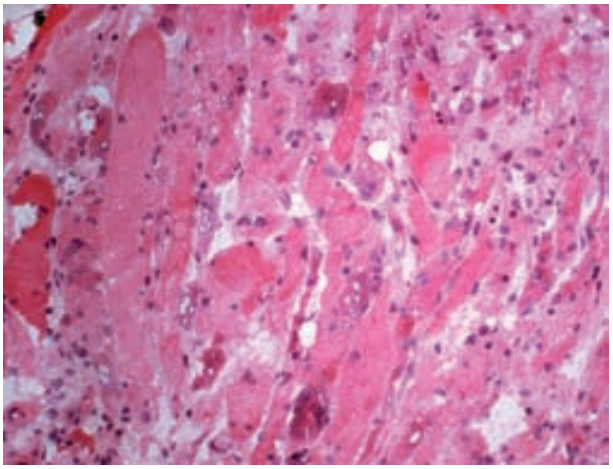

Figure 2
Hematoxylin-eosin staining: necrotising myopathy with regenerating muscle fibres

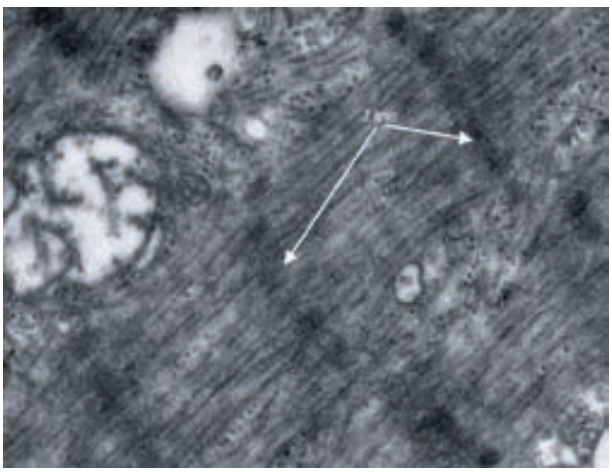

Figure 3
Electron microscopy from tibial anterior muscle: thick filament loss with relative preservation of thin filaments and Z-lines (arrows)

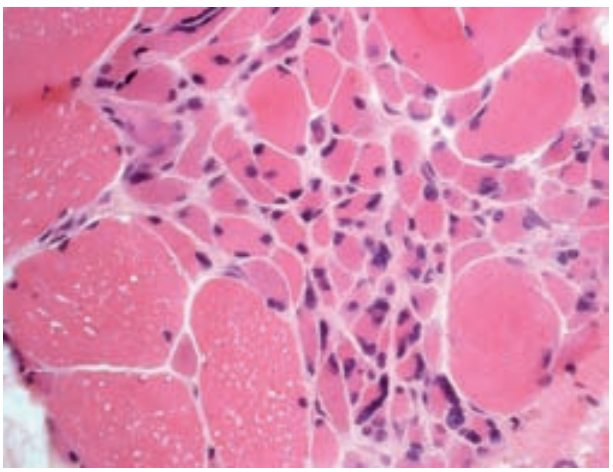

Figure 4
Hematoxylin-eosin staining: atrophic, polygonal, partially regenerating muscle fibres in groups as signs of denervation

Multiple organ failure is a hallmark of systemic critical illness and failures of individual organ systems have also been proposed as possible causative factors for the development of neuromuscular involvement. A significant association between CIP development and the duration of mechanical ventilation and neurological failure was found in a cohort of 73 critically ill patients with multiple organ dysfunction [40]. In contrast, Campellone et al. [45] found a significant association between CIM development and the severity of critical illness with the presence of renal failure. These associations between CIP or CIM and other organ failures, however, raise questions as to whether neuromuscular involvement is not simply a part of systemic critical illness and whether or not it represents just another organ failure. Among other variables reported as significantly and independently associated with increased risk of CIP development were hyperosmolality, parenteral nutrition [40], hypoalbuminaemia, hyperglycaemia [27,42,43], hyperpyrexia [60], NDMBA [40,55,61], aminoglycoside antibiotics [37], catecholamines/vasopressors [43,58], increased age [40], and female sex [27]. Most of these factors are intrinsically

related to sepsis and severity of critical illness and their causal relationship to CIPM is unclear [62,63]. In a recent prospective study [34], the development of critical illness polyneuromyopathy (CIPM) was significantly associated with APACHE III score (as a quantitative index of disease severity) and the presence of SIRS. These factors were used to estimate the risk of developing CIPM. In a prospective cohort study [63], we have demonstrated an increased risk of CIPM development in the presence of SIRS and especially in its longer duration. Another variable significantly associated with CIPM development was the severity and duration of multiple and some individual organ failures, namely neurological, cardiovascular and respiratory failures (and closely correlated duration of ventilation support).

The discrepancy between studies on the aetiology of neuromuscular disorders in critically ill patients could be explained by the varying severity of neuromuscular involvement in the cases studied and by different criteria for CIP, CIM or CIPM. Recent comparable prospective studies focused on clinically symptomatic CIPM in patients on mechanical ventilation [27,34], while others relied on more sensitive electrophysiological or histological criteria that are able somewhat to extend differentiation between neuropathy and myopathy.

CONCLUSIONS

Current knowledge of the incidence and pathophysiology of acquired neuromuscular involvement in critically ill patients is limited, which is a fact that has restrictive implications for clinical practice. Studies published to date are limited by a low number of analysed patient cases; however, inconsistent eligibility criteria and inconsistent case definitions preclude the combination of results by meta-analysis. Better animal models and improved epidemiological studies on the incidence of acquired neuromuscular involvement and on all potential risk factors with complete evaluation of all ICU patients at risk are required. Acquired neuromuscular weakness as a result of critical illness is more common than previously recognised and may result in substantial excess morbidity, mortality, and costs. It is questionable whether forthcoming aetiological studies on neuromuscular involvement in critically ill patients should adhere to the concept of CIPM or try to differentiate different components of neuromuscular component and assess them separately. The possibility of achieving reliable differentiation of myopathic and neuropathic components in larger prospective studies is limited not only by the inherent limitations of every method, but also by the frequent association between myopathy and neuropathy in critically ill patients. The neuromuscular system is probably diffusely, although patchily, involved in critical illness. Neuromuscular involvement in critically ill patients probably represents a continuum of neurogenic and myogenic changes of varying severity and progression over time. Studies utilising clinical and electrophysiological techniques should avoid strict categorisation of cases into CIP or CIM, but might do better to employ description of the main electrophysiological syndromes: isolated sensory neuropathy, isolated motor syndrome, mixed sensorimotor involvement, and decreased sarcolemmic excitability. Forced categorisation into myopathy and polyneuropathy frequently leads to misinterpretation of abnormalities and discrepancies between studies.

Muscle biopsy, as an invasive technique, is not a suitable diagnostic tool for large epidemiological studies. Nevertheless, it would be helpful to design a large multicentre study employing biopsy and differentiating all the histopathological types of CIM to address the dilemmas of the incidence of CIP and different types of CIM; of the correlation between electrophysiological findings and histopathological subtypes; and of the risk factors for different pathological subtypes of neuromuscular involvement. Acquired neuromuscular weakness in critically ill patients should be regarded as dysfunction or failure of a further (neuromuscular) system that could be detected early during critical illness. Records of neuromuscular function should be included as measures of outcome in all interventional ICU studies. Measurements to detect early signs of neuromuscular dysfunction (such as, possibly, decrease of CMAP amplitude, as shown recently by Latronico et al. [35], and decrease of sarcolemmic excitability) should be sought and validated.

ACKNOWLEDGEMENT

The authors thank Prof. MUDr. Zdeněk Lukáš, CSc., from the Department of Pathology, Faculty of Medicine, Masaryk University, and Faculty Hospital Brno, for kindly providing pictorial documentation.

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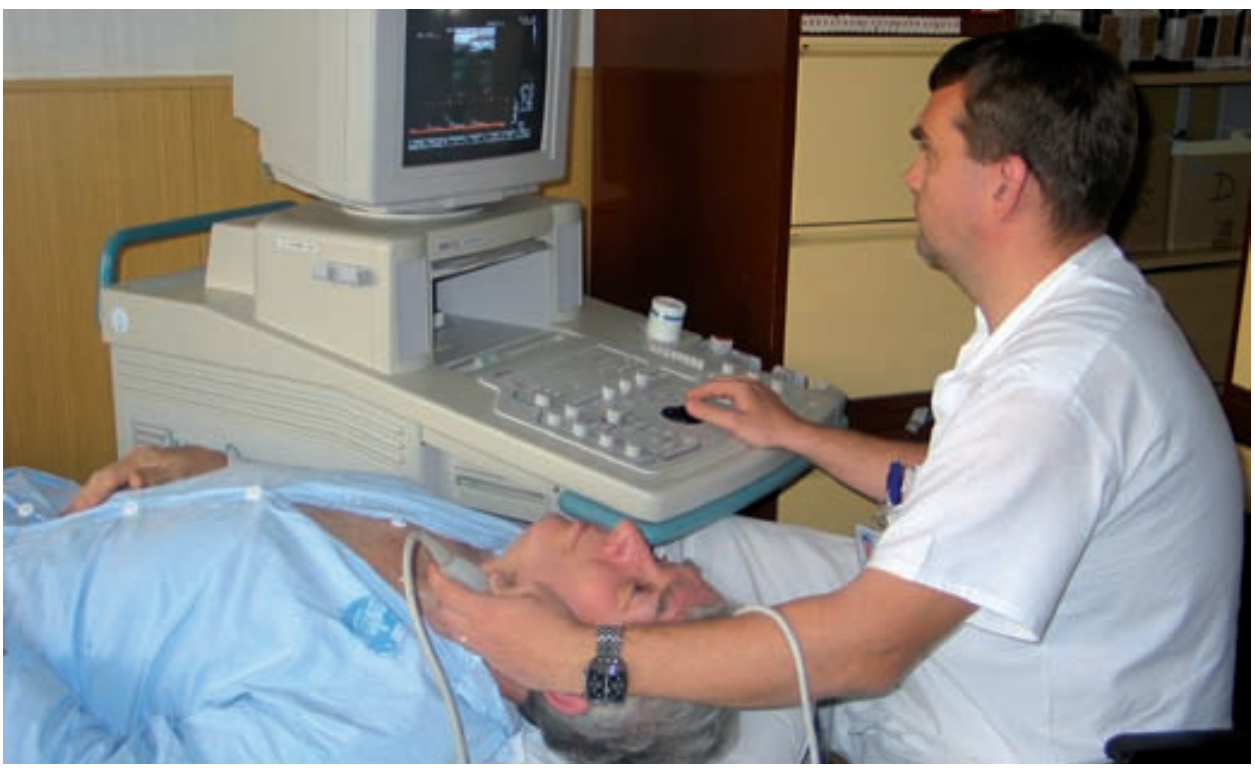
A meeting of the Department of Neurology management



Sonography laboratory

Lecture hall

Speech therapy examination

THE RESULTS AND CONTRIBUTION OF ELECTROPHYSIOLOGICAL EXAMINATION IN PATIENTS WITH LUMBAR SPINAL STENOSIS

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Received after revision February 2009

KEY WORDS

Lumbar spinal stenosis
Electrophysiological examination
Radiculopathy

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ABSTRACT

Monoradicular or polyradicular lumbosacral involvement is typical of lumbar spinal stenosis. Needle electrode examination is considered the most useful procedure for the evaluation of patients with suspected radiculopathy. The clinical relevance of motor and somatosensory evoked potentials is, however, uncertain.

The aim of this study was to evaluate the results of electrophysiological tests in patients with lumbar spinal stenosis and to assess the contribution of electrophysiological tests made to diagnosis.

The study group consisted of 102 patients (44 males, 58 females, aged 62 +/- 13 years) with clinically symptomatic lumbar spinal stenosis documented by computed tomography scan, all of whom volunteered to participate in the study. All patients underwent electrophysiological examination, which included needle electromyography and nerve conduction studies of the lower extremities, somatosensory evoked potentials of tibial, sural and superficial sensory peroneal nerves, and motor evoked potentials to the abductor hallucis muscle and the tibialis anterior muscle.

On the basis of nerve conduction studies and needle electromyography, the presence of radiculopathy was established in 70.6 % of patients with lumbar spinal stenosis; polyradicular involvement (46.1 % of the patients) was more common than monoradicular involvement (24.5 % of the patients). Involvement of the L4 root was established in 37.2 % of the patients, L5 root in 51.9 %, and S1 root in 50.9 % of the patients. Abnormal motor evoked potentials were found in 30.7 % of the patients and abnormal somatosensory evoked potentials in 58.8 % of the patients with lumbar spinal stenosis. Normal needle electromyography and nerve conduction studies were recorded in 18.6 % of the patients. When abnormalities of any evoked potentials were considered, the number of patients with normal electrophysiological findings was reduced to 12.7 %.

Nerve conduction studies and needle electromyography are the most useful electrophysiological examinations for the

evaluation of suspected radiculopathies in patients with lumbar spinal stenosis. Involvement of L5 and S1 roots is the most common. The diagnostic contribution of evoked potentials is of limited value in patients with lumbar spinal stenosis.

ABBREVIATIONS USED

LSS – lumbar spinal stenosis
NC – neurogenic claudication
MEP – motor evoked potential
SEP – somatosensory evoked potential
CT – computed tomography
SD – standard deviation
CMAP – compound muscle action potential
CMCT – central motor conduction time
MUAP – motor unit action potential
SNAP – sensory nerve action potential
EMG – electromyography
NCS – nerve conduction study
AH muscle – abductor hallucis muscle
TA muscle – tibialis anterior muscle

INTRODUCTION

Lumbar spinal stenosis is defined as any type of narrowing of the spinal canal, nerve root canals, or intervertebral foramina [1]. In the pathogenesis of LSS, the degenerative process in the lumbar spine is the main component producing compression of neural tissue in the spinal and/or nerve root canal [2]. LSS can give rise to several clinical syndromes: neurogenic claudication, low back pain and/or radiculopathy, and chronic cauda equina syndrome. Postural dependency is a hallmark of the symptoms of LSS. Spinal extension narrows the spinal canal and exacerbates symptoms, whereas spinal flexion increases the dimensions of the spinal canal and reduces symptoms [3, 4, 5]. NC is typical of LSS and is characterised by intermittent pain and paresthesia of the leg(s), most often in a lumbosacral root distribution, followed by weakness apparent on walking or standing [6, 7]. The incidence of NC is reported at 11 %–100 % in patients with LSS; the mean calculated from 32 studies is 62 %. LSS is currently the most common diagnosis for individuals over the age of 65 undergoing spinal surgery [8].

The diagnosis of LSS is based on the results of clinical examination and radiological documentation of narrowing of the lumbar spinal canal [9, 10]. Whereas clinical examination, even in severe LSS, has shown no specific sensory-motor deficit, electrophysiological recordings have indicated a neurogenic disorder within the lumbar spine of a majority of the patients. Electrophysiological recordings thus supplement neurological examination when the clinical relevance of a radiologically suspected LSS needs to be confirmed [11].

Electrophysiological examination in LSS is intended to detect lumbosacral root involvement (radiculopathy). EMG, especially needle electrode examination, is considered the most useful procedure in the detection of radiculopathy. Nerve conduction studies may yield abnormal results only rarely in radiculopathies, but are essential to the elimination of other conditions that may produce similar symptoms and signs [12]. Further, examination of SEPs and MEPs is used in different degree, but consensus on the contribution of evoked potentials in the diagnostics of LSS has not been established and the clinical relevance of MEPs and SEPs is uncertain [13]. Initially, the contribution of evoked potentials to the diagnostics of radiculopathy was overestimated. Electrophysiological examination in LSS patients is also used to exclude other peripheral neurogenic lesions (for example diabetic polyneuropathy). The clinical differentiation between lumbosacral polyradicular disease (typical of LSS) and generalised peripheral neuropathies of the distal-axonal type (typical of diabetic patients) can sometimes be difficult [14]. Electrodiagnostic testing is quite specific and reasonably sensitive in diagnosing spinal stenosis in comparison with low back pain or asymptomatic persons, and can detect neuromuscular diseases mimicking stenosis [15].

EMG findings may vary in patients with LSS. The most frequent finding (approximately 50 % of the patients) is bilateral multiple lumbosacral radiculopathy (cauda equina lesion). Polyradicular lumbosacral lesions are often asymmetrical, and usually involve the lower lumbosacral roots, especially S1. Chronic neurogenic MUAP changes tend to be prominent in needle EMG, whereas fibrillation potentials are often restricted to the more distal muscles of the myotomes. In some, two distinct lumbosacral radiculopathies occur either symmetrically or asymmetrically. In others, an isolated radiculopathy, almost always either an L5 or an S1 lesion, is found. Non-diagnostic abnormalities are encountered in some patients. Bilaterally absent H waves associated with normal sural SNAPs and a normal needle EMG of the lower extremities are common. Fibrillation potentials in a single limb muscle, most often one innervated by the S1 root, are sometimes encountered. In the remaining patients, extensive EMG examination is normal [16].

Magnetic transcranial and spinal stimulation of the motor pathway is a painless and safe neurophysiological technique for the examination of the central and proximal peripheral motor pathways [17]. Transcranial brain stimulation and paravertebral magnetic root stimulation are reliable diagnostic methods for the investigation of patients with radiculopathies. Their advantage is that disturbances of the motor functions in the nerve roots can be detected and, because of the different MEP patterns, localisation of the compression can often be established [18]. The clinical relevance of this examination is uncertain. False negative findings in MEP

examination in patients with LSS may be as high as 75 % [19, 20]. One source of such errors may be the fact that it is difficult to stimulate the nerve roots supramaximally.
No electrophysiological examination has attracted as much attention, or for that matter evoked so much controversy, as SEPs in radiculopathy. The theoretical advantage is that SEPs evaluate a sensitive part of the nerve root, including the preganglionic part of the sensitive pathway; abnormality is immediately present at the start of radiculopathy and images are provided of both axonal and demyelinating involvement. The normative data of latencies and amplitudes are heterogeneous. False-negative findings in SEP examination in patients with LSS may be as high as 65 % [19, 20]. In contrast, altered tibial SEPs were observed in 61.5 % of patients with LSS in another study [21].

The aim of the study was to evaluate the results of electrophysiological tests in patients with LSS and to assess the contribution of electrophysiological tests made to diagnosis.

MATERIALS AND METHODS

Patients with LSS
One hundred and two patients (44 males, 58 females, aged 62 +/- 13 years) were recruited consecutively from a total of 132 patients with LSS treated and observed in the Department of Neurology, Faculty Hospital, Brno, between 1998 and 2001.
Inclusion criteria for patients with LSS:
- clinically symptomatic LSS (NC and/or low back pain)
- at least one level LSS documented by CT scans
- absence of hip and/or knee joint degenerative arthritis limiting walking
- absence of diabetes mellitus or other disease causing polyneuropathy
- no serious cardiac disease

Electrophysiological examination
All patients underwent electrophysiological examination, which included:

- Needle electromyography and nerve conduction studies of the lower extremities (Table 1). Needle EMG centred on the muscles of L4, L5, S1 myotomes bilaterally, with evaluation of abnormal spontaneous activity and analysis of action potentials of motor units. A conventional EMG device was used to perform the nerve conduction studies from temperature-checked skin. Motor conduction studies of the peroneal nerve (muscle responses recorded via surface electrodes placed over the extensor digitorum brevis muscle) and the tibial nerve (muscle responses recorded via surface electrodes placed over the abductor hallucis muscle) were performed bilaterally. Sensory conduction studies of the sural and superficial peroneal nerves were carried out employing an antidromic surface technique. The F-wave responses of the tibial and peroneal nerves and the H-reflex of the soleus muscle were recorded bilaterally. Submaximal stimuli with increasing voltage were delivered and facilitation was used to provide the maximum H-reflex amplitude.
- Somatosensory evoked potentials of tibial, sural, and superficial sensory peroneal nerves. Electrical stimulation of the tibial nerve was applied posterior to the medial ankle, the sural nerve was stimulated posterior to the lateral ankle, and the superficial peroneal nerve was stimulated in the join of both ankles at a distance of one third from the lateral ankle. Scalp registration was used in all examined nerves, while registration over the spinosus process of vertebra L1 was used for the tibial nerve. The latency of potentials P40 and N45 and the amplitude of P40/N45 were evaluated in all examined nerves, while the latency of potential N22 was evaluated in the tibial nerve. Our electrophysiological laboratory has generated our own reference values for all the parameters, and latencies are correlated to body height. Prolongation of latencies of more than 3 SD above average, unelicited responses, side-to-side differences in latencies N22 or P40 of more than 3 SD above average, and reduction of amplitude P 40/N45 of more than 3 SD below average (after logarithmic transformation) were considered abnormal.
- Motor evoked potentials to the abductor hallucis muscle and tibialis anterior muscle. A Magstim 200 was used for transcranial and spinal stimulation. In the course of spinal stimulation, the coil was located one centimetre to one side of the centre of the low lumbar spine; for transcranial stimulation, the coil was located above the motor cortex and facilitation was used. Supramaximal stimulation was applied. Spinal latency, cortical latency, CMCT, and amplitude of cortical response were determined. Unelicited responses upon transcranial and spinal stimulation, prolongation of latencies of more than 3 SD above average, and an amplitude of cortical response of less than six per cent of the amplitude of CMAP (our own reference values) were considered abnormal.

Radiological examination
The patients were radiologically examined according to the following protocol:

1 A plain radiograph of the lumbar spine was taken, with assessment for the presence of spondylarthrosis, scoliosis, and degenerative or isthmic spondylolisthesis.
2. CT axial scans at three levels (L3-S1) were performed. The following standard parameters of the spinal canal were measured:

Table 1
Nerve conduction studies (NCS) of the lower extremities

Nerve conduction studies	Nerve
Motor conduction studies	peroneal nerve
	tibial nerve
Sensory conduction studies	superficial peroneal nerve
	sural nerve
Late responses	F-wave of tibial nerve
	F-wave of peroneal nerve
	soleus H-reflex

Table 2
Evaluation of the nerve conduction studies and needle EMG

Type of involvement	Normal finding	Radiculopathy			Polyneuropathy
		monoradiculopathy	polyradiculopathy	together	
Number of patients	19.0	25.0	47.0	72.0	12.0
In % of cases	18.6	24.5	46.1	70.6	11.7

- Anteroposterior diameter of the spinal canal at the level of the middle of the L3, L4, and L5 vertebrae.
- Transverse interarticular diameter (between ventral margins of facet joints) at the level of the upper margins of the L3/4, L4/5, and L5/S1 discs.
- Lateral recess diameter bilaterally at the same levels as the transverse diameter.

CT criteria of spinal stenosis were based on our own normal data [22]:
- Central stenosis: anteroposterior diameter <11.7 mm and/or transverse diameter <16.0 mm
- Lateral stenosis: lateral recess diameter <5.2 mm

RESULTS

Nerve conduction studies and needle EMG
The presence of radiculopathy and/or polyneuropathy in patients with LSS was established on the basis of NCS and needle EMG. The criteria for radiculopathy included the presence of abnormal spontaneous activity or chronic neurogenic MUAP changes in two or more muscles that receive innervation from the same root, preferably via different peripheral nerves. Abnormal values for the soleus H-reflex amplitude were also used to assess S1 radiculopathy. If clinical suspicion of radiculopathy existed and needle EMG of the muscles of this myotome was normal but the H-reflex of the soleus muscle was abnormal, it was considered sufficient to confirm radiculopathy S1. If the H-reflex proved impossible to elicit, or a side-to-side difference in the amplitude of more than 50 % in comparison with the healthy side emerged, the findings were considered abnormal. The criteria for polyneuropathy were non-elicitability or reduced amplitude in the sensory neurogram of the lower extremities, bilaterally abnormal values of the soleus H-reflex, and abnormal EMG needle findings in only the distal muscles of the lower extremities.
The presence of radiculopathy was established in 70.6 % of the patients with LSS; polyradicular involvement (46.1 % of the patients) was more common than monoradicular involvement (24.5 % of the patients). Normal needle EMG and NCS were recorded in 18.6 % of the LSS patients and polyneuropathy was established in 11.7 % of the patients (Table 2).
The involvement of the particular roots was also established in patients with LSS (number of patients with radiculopathy in at least one lower extremity). Involvement of the L4 root emerged in 37.2 % of the patients, L5 root in 51.9 %, and S1 root in 50.9 % (Table 3).

Evoked potentials (MEPs, SEPs)
Motor evoked potentials were evaluated in 101 patients with LSS (one patient was exempted from this examination because he had a pacemaker). Abnormal MEPs to the abductor hallucis muscle and/or to the tibialis anterior muscle were found in 31 patients (30.7 %) (Table 4). On detailed analysis of MEPs it was found that prolongation of spinal latency or non-elicitability of spinal response to AH muscle and/or TA

Table 3
The presence of involvement of lumbosacral roots based on nerve conduction studies and needle EMG

Distribution of radiculopathy	L_4	L_5	S_1	other
Number of patients with radiculopathy in at least one lower extremity	38.0	53.0	52.0	0.0
In % of cases	37.2	51.9	50.9	0.0

Table 4
Results of motor evoked potential examination

Type of MEP examination	MEP to AH muscle	MEP to TA muscle
Number of patients with abnormal MEP in at least one lower extremity	18.0	21.0
In % of cases	17.8	20.8

Table 5
Results of somatosensory evoked potential examination

Type of SEP examination	SEP of tibial nerve	SEP of superficial sensory peroneal nerve	SEP of sural nerve
Number of patients with abnormal SEP in at least one lower extremity	48.0	36.0	40.0
In % of cases	47.1	35.3	39.2

muscle had occurred in 27 patients. Three patients exhibited abnormal spinal response and an abnormal value of CMCT contemporaneously. One patient exhibited a low amplitude of cortical response in comparison with the CMAP amplitude. No isolated abnormality of CMCT to AH and/or TA muscle appeared. Contemporaneous abnormality of MEP to TA muscle and to AH muscle was found in 8 patients, only to TA muscle in 13 patients, and only to AH muscle in 10 patients.

Abnormal SEPs were found in 60 patients with LSS (58.8 %) (abnormality of SEPs in at least one of the three nerves evaluated) (Table 5). Unambiguous damage to the peripheral part of the somatosensory pathway predominated.

Abnormal SEPs of the tibial nerve in at least one lower extremity were found in 47.1 % of the patients, abnormal SEPs of the superficial sensory peroneal nerve in 35.3 %, and SEPs of the sural nerve in 39.2 %. Contemporaneous abnormality of all three types of SEP was found in 26 patients (25.5 %).

Of 19 patients with normal EMG, one patient showed abnormal MEPs, 4 patients abnormal SEPs, and one patient abnormal SEPs as well as MEPs. When abnormalities of any evoked potential (MEP, SEP) were considered, the number of patients with normal electrophysiological findings was reduced from 18.6 % to 12.7 % (Figure 1).

DISCUSSION

It is believed that the presence of radicular or (possibly) polyradicular involvement is a typical electrophysiological finding in patients with LSS. In this study, the presence of radiculopathy was established in 70.6 % of the patients with LSS, with polyradicular involvement predominating (46.1 % of the patients). These results do not disagree with the literature. In a prior study, needle EMG and nerve conduction studies revealed pathology in 75 % of patients with LSS [23]. The most frequent findings in LSS patients are considered bilateral, multiple lumbosacral radiculopathies (in about 50 % of the patients), and in approximately 20 % of the patients the presence of monoradiculopathy is determined. Lower lumbosacral roots, especially L5 and S1, are afflicted most often in patients with LSS [16]. In a study involving 200 patients with lumbosacral radiculopathy, L5 radiculopathy was recorded in 47.6 % of the patients, S1 radiculopathy in 30 %,

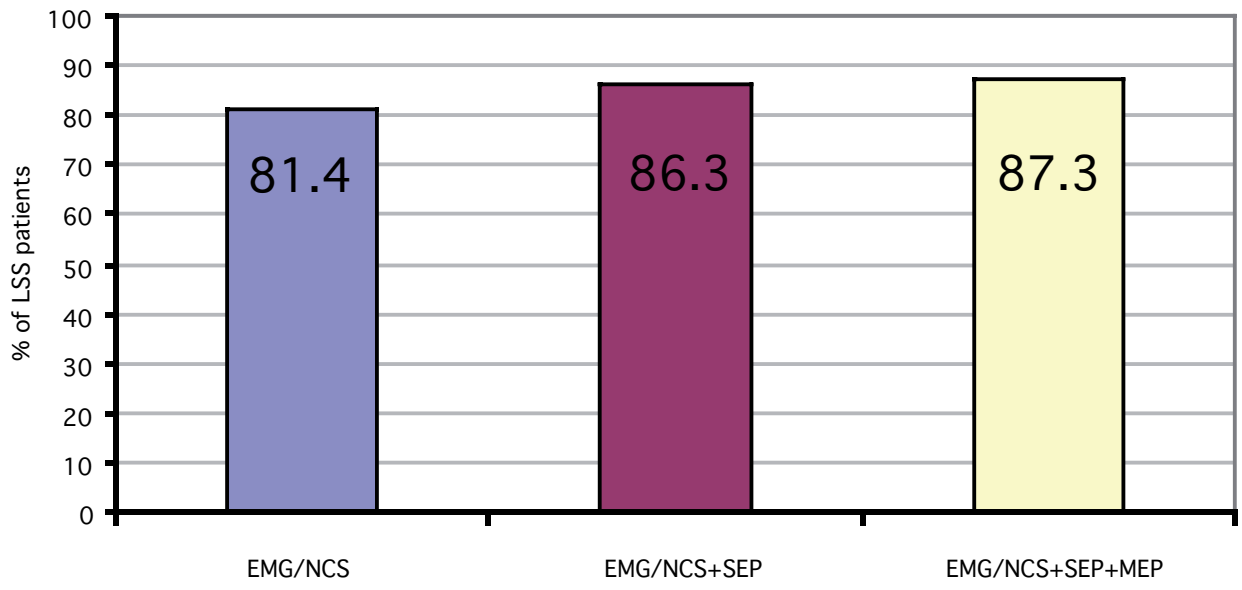

Figure 1
Diagnostic contribution of electrophysiological tests in patients with LSS

and L4 radiculopathy in 17.2 % [24]. This study found L5 radiculopathy in 51.9 % of the patients and S1 radiculopathy in 50.9 % of them; L4 root lesion was less common, in 37.2 % of the patients. The higher percentage of the incidence of L4, L5 and S1 radiculopathy in this paper may be due to the selected patients with LSS, in whom the presence of multiple radiculopathy is supposed.

One patient was unsuitable for MEP examination because of a pacemaker. Abnormal MEPs to the AH muscle and/or to the TA muscle were found in 31 patients (30.7 %). A study evaluating MEPs to the TA muscle in patients with LSS demonstrated abnormal findings in 42.3 % of its subjects [21]. In our study, abnormal MEPs to the TA muscle (separately or in combination with abnormal MEPs to the AH muscle) were found in 20.8 % of the patients. In another study, 65 % of the patients (of 43 patients with LSS) exhibited abnormal MEPs (prolongation of central motor conduction time – $CMCT^{M}$) [25]. The incidence of abnormal MEPs in our study was substantially lower. The difference in the results may be partially explained by the fact that the latter study evaluated MEPs in three muscles in the lower extremity (quadriceps femoris muscle, TA muscle, and extensor digitorum brevis muscle) rather than our two, and that a "normal range" of latencies was defined as mean $\pm$ 2 SD (in our study mean $\pm$ 3 SD), thus increasing the probability of abnormal findings. Furthermore, the difference with the results of the study that demonstrated abnormal MEPs in 52.6 % of the patients with acute radiculopathy is probably generated by different criteria for the definition of abnormal MEP findings (results that exceeded the mean + 2 SD values were considered pathological and right/left difference amplitudes of more than 50 % were also considered abnormal) [26]. When the MEP results in our study were analysed in detail, it was established that prolongation of spinal latency or non-elicitability of responses on spinal stimulation to AH muscle and/or to TA muscle (altogether 27 patients, i.e. 87 % of abnormal MEPs) predominated among the abnormal findings, which is thought of as an indicator of lateral compression of the nerve root. This finding is possibly to be expected in patients with LSS because of the frequent presence of lateral stenosis. Three patients exhibited abnormal spinal responses and contemporaneous abnormal CMCT, which may possibly be interpreted as a combination of medial and lateral compression of the nerve root. No isolated abnormality of CMCT to the AH muscle and/or to the TA muscle (implying medial compression of the root) appeared. The results of the current study did not confirm those of Bischoff et al., which appeared to demonstrate that there are no major differences between MEPs and EMG in terms of their sensitivity in detecting nerve root compression [18]. In the current study, the sensitivity of EMG exceeded that of MEPs. The different results of our study and that of Bischoff et al. can be elucidated by the different inclusion criteria. Bischoff investigated patients with clinically symptomatic radiculopathy with frequent occurrence of muscle weakness and the correlation between muscle weakness

and pathological MEP latency was demonstrated (that's why the sensitivity of MEPs was high). On the contrary, in the study of Bischoff only the presence of spontaneous EMG activity in muscles was regarded as a pathological finding but in our study the change of parameters of MUAPs was also regarded as a pathological finding (that's why the sensitivity of needle EMG was high in our study).

Abnormal SEPs were found in 60 patients with LSS (58.8 %) in our study (implying abnormality of SEPs in at least one of three nerves evaluated). In the literature, the positive SEP figures range from 20 % to 84 % in patients with radiculopathy [27, 28]. The results are substantially influenced by the type of stimulation and definitions of abnormal values. The literature indicates that SEPs make a major contribution in patients with LSS in comparison with the contribution made by SEPs in isolated radiculopathy, possibly because LSS is associated with multiple radiculopathy and a concomitant higher probability of abnormal SEPs [29, 30]. The results in the current study are similar to those of Leinonen, where altered tibial SEPs were observed in 16 of 26 patients with LSS evaluated (in our study, abnormal tibial SEPs were found in 47.1 % of the patients) [21].

Of 19 patients with normal EMG, abnormal MEPs appeared in one, abnormal SEPs in 4 patients, and abnormalities of both SEPs and MEPs appeared in one patient. When abnormalities of any evoked potentials (MEPs, SEPs) were considered, the number of patients with normal electrophysiological findings was reduced from 18.6 % to 12.7 %. We may conclude that a diagnostic contribution of evoked potentials was documented in LSS patients with normal needle EMG and conduction studies of the lower extremities.

CONCLUSIONS

Nerve conduction studies and needle EMG are the most useful electrophysiological examinations for the evaluation of suspected radiculopathies in patients with LSS. The involvement of L5 and S1 roots is the most common. The diagnostic contribution of evoked potentials (SEPs, MEPs) has limited value in patients with LSS.

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THE VALUE OF INTRATHECAL MRZ REACTION AND OLIGOCLONAL IgG BANDS FOR DISCRIMINATION BETWEEN THE PRIMARY PROGRESSIVE AND RELAPSING REMITTING COURSES OF MULTIPLE SCLEROSIS

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Received after revision February 2009

KEY WORDS

Multiple sclerosis
Cerebrospinal fluid
Oligoclonal IgG bands
MRZ reaction
Diagnostic significance

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ABSTRACT

Generally, we diagnose two different courses of multiple sclerosis: relapsing remitting course (RR-MS) and primary progressive (PP-MS) course. Differences in pathogenesis, immunology, and prognosis are supposed between these entities. A reliable surrogate marker in cerebrospinal fluid and serum for these courses is still missing. The aims of our work were to investigate the frequency of MRZ reaction and oligoclonal IgG bands (IgG-OB) in RR-MS and PP-MS subgroups of patients and to evaluate its diagnostic significance. We examined 29 patients (n–29) with RR-MS and 10 patients with PP-MS (n–10). The intrathecal synthesis of specific antibodies (MRZ reaction) was evaluated in the form of antibody indices calculated according to the Reiber's formula. Oligoclonal IgG bands were detected by isoelectric focusing using a commercial SEBIA kit. The MRZ reaction was positive in 2/10 patients with PP-MS and in 11/29 patients with RR-MS. IgG-OB were positive in 1/10 patients with PP-MS and in 20/29 patients with RR-MS. The calculated sensitivities for MRZ reaction were 20 % in PP-MS and 37 % in RR-MS; for IgG-OB they were 10 % in PP-MS and 69 % in RR-MS. The different frequencies of both parameters in PP-MS and RR-MS support the hypothesis about different pathogenesis and can be used as reliable surrogate markers for differential diagnosis in the context of clinical settings.

ABBREVIATIONS USED

CSF – cerebrospinal fluid
MS – multiple sclerosis
IgG – immunoglobulin G
OB – oligoclonal bands
IEF – isoelectric focusing
MRI – magnetic resonance imaging

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory immune-mediated disease, probably of autoimmune origin. The diagnosis according to the International Panel on the Diagnosis of Multiple Sclerosis from 2005 revised the original McDonald's Diagnostic Criteria from 2001 [1]. The Criteria formally incorporated magnetic resonance imaging (MRI) into the well-established diagnostic workup that focuses on detailed neurological history and examination and a variety of paraclinical laboratory examinations. They laid particular emphasis on determining dissemination of lesions in time and space, incorporating different types of imaging criteria into the diagnostic scheme and assessing the value of cerebrospinal fluid analysis, particularly for the diagnosis of primary progressive multiple sclerosis. The incorporation of CSF (cerebrospinal fluid) findings into the McDonald's criteria has been supported by studies suggesting that CSF analysis increases diagnostic sensitivity, though at the cost of specificity. The Revised Criteria stated the belief that a positive CSF finding was preferably based on isoelectric focusing evidence of oligoclonal IgG bands with immunofixation demonstrating that bands that are different from those in the serum or an increased IgG index or both increase the "comfort level" for the diagnosis of MS in individuals with insidious progression of disease from the onset. However, such CSF findings are not specific and may be detected in patients with progressive myelopathies of other causes, especially those associated with infections [2, 3, 4]. Besides IgG-OB the MRZ reaction (intrathecal polyspecific immune response against neurotropic viruses: measles, rubella, varicella zoster) is a marker for differential diagnosis between MS and other demyelinating diseases of CNS because of its higher specificity for chronic autoimmune inflammation comparing oligoclonal IgG bands and the IgG index as mentioned above [5]. The specificity of MRZ to chronic inflammatory processes of autoimmune origin such as multiple sclerosis is in the range between 84 and 94 %. Combinations of M+R, M+Z or R+Z that are rarely seen in other diseases (e.g. acute infections) are clues to the presence of a chronic, especially autoimmune-type, disease. In other neurological diseases (neuroborreliosis, neurosyphilis, neurotuberculosis) the frequency of MRZ is below 1 % for the single species and far below 0.1 % for M+R+Z [6]. We previously published an evaluation of both the MRZ reaction and IgG-OB in a group of patients with OND (other neurological diseases) compared to the positivity of these parameters in a group of patients with MS [7].

The combined differential diagnostic value of both parameters for diagnosis between relapsing remitting (RR-MS) and primary progressive (PP-MS) courses has not been evaluated till now, in spite of supposed principal differences in the pathogenesis of both different MS courses. It is supposed that the RR-MS course is predominantly based on inflammation-demyelination and the PP-MS course on predominant neurodegeneration and less inflammation.

Our objectives were to assess the presence of MRZ reaction and IgG-OB in cohorts of patients with RR-MS and PP-MS and to evaluate their differential diagnostic potential between both courses.

MATERIALS AND METHODS

We evaluated the CSF and the serum for MRZ reaction and IgG-OB as the most specific and sensitive parameters of neuroinflammation in 29 patients (n–29) with RR-MS and 10 patients (n–10) with PP-MS.

The clinical diagnosis of multiple sclerosis was based on Diagnostic Criteria for Multiple Sclerosis from the International Panel published in Ann Neurol 2005; 58: 840–846 [1]. All patients fulfilled diagnostic criteria demonstrating dissemination of lesions in space and time including MRI (magnetic resonance imaging) positivity. None of these patients had a clinically isolated syndrome (CIS).

Serum and CSF samples were analysed in each patient.

ANALYTICAL PROCEDURES

Measles, rubella, and varicella zoster virus-specific IgG antibodies were detected both in the serum and CSF by sandwich enzyme immunoassay using commercial kits from Human, Germany (Measles-Virus Human ELISA IgG Antibody Test, Rubella-Virus Human ELISA IgG Antibody Test, Varicella-Zoster-Virus Human ELISA IgG Antibody Test). In this assay microtitre strip wells as a solid phase are coated with cell-culture derived Measles, Rubella, and VZV antigens. If the corresponding specific antibodies are present in a sample, they are bound to the antigens at the solid phase. After a washing step to remove unbound material, anti-human IgG peroxidase conjugate is added, which binds specifically to IgG class antibodies. After a second washing step to remove unbound conjugate, the enzyme-linked complexes are detected by incubation with a substrate solution. The subsequent development of a blue colour is changed into yellow by stopping the enzymatic reaction with sulphuric acid. Absorbances are measured at 450 nm using an ELISA microtitre plate reader.

Absorbances of serum and CSF samples were converted to arbitrary units (AU) in a log/log diagram based on a standard curve derived from seven serial dilutions of a positive standard serum. The highest standard concentration (approximately 2.0) was defined as 100 arbitrary units [5].

The specific antibody index (AI) was calculated according to the Reiber's formula: AI = Qspec /QIgG. Qspec is the ratio of

Table 1
Prevalence of MRZ reaction and IgG-OB in cohorts of patients with PP-MS and RR-MS

	MRZ reaction	IgG-OB
RR-MS (n–29)	11/29	20/29
PP-MS (n–10)	2/10	1/10

Table 2
Sensitivity of MRZ reaction and IgG-OB in cohorts of patients with PP-MS and RR-MS

	MRZ reaction (%)	IgG-OB
RR-MS (n–29)	37 %	69 %
PP-MS (n–10)	20 %	10 %

specific antiviral IgG antibodies in CSF and serum. QIgG is the ratio of total IgG antibodies in CSF and serum. The upper limit (Qlim) for IgG is Qlim = 0.93 *SQRT (Alb^2 + (6*10^{-6}) –1.7 *10^{-3}. If QIgG >Qlim, then AI = Qspec/Qlim. The values AI>1.4 are positive and indicate intrathecal synthesis of specific antibodies [8].

Oligoclonal IgG bands
Oligoclonal IgG bands in the serum and the CSF were detected by isoelectric focusing with subsequent immunoenzymatic staining using a commercial kit (SEBIA, France).

RESULTS

MRZ reaction was positive only in 2 of 10 (n–10) patients with primary progressive MS and IgG-OB were positive in 1 of 10 (n–10) patients with PP-MS.
MRZ reaction was positive in 11 of 29 patients (n–29) with RR-MS. IgG-OB were positive in 20 of 29 patients (n–29) with relapsing remitting course of MS.
The differences in positivity of MRZ reaction and IgG-OB between those cohorts of patients were statistically significant with a calculated sensitivity for MRZ reaction of 20 % in PP-MS and 37 % in RR-MS.
For IgG–OB the sensitivity was 10 % in PP-MS course and 69 % in RR–MS.

DISCUSSION

We found significantly less positivity in both parameters (MRZ and IgG-OB) in the primary progressive course of multiple sclerosis. It supports the widely accepted hypothesis about different pathogenesis and distinctive prognosis between both MS courses. The report exists about IgG-OB negative patients with relatively better prognosis [9]. True IgG-OB negative clinically definite multiple sclerosis occurs and, according to our results, is more common in the PP-MS course. From the short- and middle-time observational period this is in accord with the slower accumulating disability, i.e. temporarily and concomitantly better prognosis. MRI detected fewer lesions in patients with lower inflammatory disease activity, also reflected by the negativity of IgG-OB [11]. In the clinical setting the negativity of IgG-OB rather reflects the PP-MS course, but temporary evolution of IgG-OB positivity must be taken into account, especially in the case of clinically isolated syndrome (CIS), where the initial positivity is reported to range between 40 and 70 %, but when CSF examination is repeated after 1/2 to 1 year, the positivity of IgG-OB increases over 90 % [11]. One study described a significant delay in disability progression during the treatment with interferon-beta in the subgroup of MS patients with no IgG-OB detectable by IEF, compared to the patients with this CSF abnormality [12]. Whether IgG-OB negative MS patients have additional characteristic immune features remains to be settled. There is a need for concerted long-term follow-up studies of the subgroup of MS patients without CSF IgG OB regarding prognosis and immunological features.
As regards MRZ antibodies, 84–94 % of MS patients have intrathecal antibody synthesis against one, two or three of the measles, rubella, and varicella zoster viruses, and slightly more if herpes simplex is included. The frequencies and magnitudes of the antibody indices rise with increased total IgG synthesis [13]. Although much less frequent than the MRZ reaction, increased intrathecal synthesis of HSV antibodies (28% frequency in MS patients) is still higher than other antibody species in MS (e.g. intrathecal toxoplasma antibodies – 10 %, or intrathecal autoantibody synthesis against dsDNA – 20 %).
On the contrary, the MRZ reaction is strongly predictive of an autoimmune-type chronic inflammatory disease such as

multiple sclerosis even at the time of the first clinical symptoms. In the case of acute infections the specific antibody index has a higher diagnostic sensitivity than the oligoclonal IgG [14]. The rather lower sensitivity of MRZ reaction in our cohort of RR-MS patients could be explained by the smaller number of patients comparing numbers in relevant reports.
The relevance of the MRZ reaction is different from that of oligoclonal IgG for evaluating multiple sclerosis. The presence of IgG-OB in CSF has a lower specificity for MS and must be generally evaluated in the context of clinical setting and MRI findings [15]. The MRZ reaction has not been evaluated until now in the different courses of MS; we stated that the frequency is significantly lower in the PP-MS course, likely reflecting less pronounced inflammation in CNS as was similarly detected in IgG-OB.

CONCLUSION

If both parameters are taken together, we can conclude that the negativity of the MRZ reaction and IgG-OB does not exclude the possibility of diagnosing multiple sclerosis and rather has a better prognostic significance for both courses of MS and is much common in the PP-MS course. These significant differences in prevalence between the two courses can be used as an additional differential diagnostic marker.

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Electromyography laboratory

Stroke unit – nurse staff control centre

Inpatient clinic - nurse staff room

Outpatient clinic - nurse staff room

NON-JEWISH CHILD WITH CANAVAN DISEASE

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Received after revision February 2009

KEY WORDS

Canavan disease
Leukodystrophy
N-acetylaspartate
Developmental delay
Macrocephaly

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ABSTRACT

Canavan disease (CD) is a rare autosomal recessive inherited disorder caused by a deficiency of aspartoacylase, which leads to defective myelinisation and occurrence of leukodystrophy. Most frequently it occurs in the population of Ashkenazi Jews, while in the Central European non-Jewish population only about 5 % of outbreaks occur. The most common symptoms are progressive spasticity, serious developmental delay, macrocephaly, blindness, and seizures. The features crucial for diagnostics include clinical history, laboratory proof of N-acetyl-L-aspartate (NAA) accumulation in urine, magnetic resonance imaging (MRI) of the brain, and molecular analysis of the genetic mutation. Genetic therapy has already been known and used, while therapy with stem cells and glyceryl triacetate is still in the phase of research. We diagnosed a six-month non-Jewish girl with leukodystrophy suspect symptoms without EEG or clinically expressed epileptic activity. NAA elevation was proved in urine. Brain MRI showed diffused affection of the brain's white matter. The diagnosis was definitively confirmed by molecular analysis where the 914C>A mutation was found, which is most frequently expressed in the Central European non-Jewish population. The genetic confirmation explained the essence of this serious neurological disorder and allowed for a better determination of prognosis, which is unfavourable. In Central Europe, only symptomatic therapy of epileptic paroxysms has been used, along with rehabilitation. Negotiations leading to the establishment of gene therapy, which has already been used elsewhere, have not yet been initiated in this region, as it is extremely demanding and rare in Central Europe.

ABBREVIATIONS USED

ASPA – gene for enzyme aspartoacylase
BAEP – brainstem-evoked potentials
CD – Canavan disease

DNA – deoxyribonucleic acid
EEG – electroencephalography
EMG – electromyography
CSF – cerebrospinal fluid
MRI – magnetic resonance imaging
NAA – N-acetyl-L-aspartate
TORCH – intrauterine infections (toxoplasmosis, rubella, cytomegalovirus, herpetic viruses)
VEP – visual evoked potentials

Table 1
The severity of the disease is dependent on mutation and population

	Jewish population	Non-Jewish population
Mild disease	854 A>C (E285>A) 82.9 %	(G212>A)
Severe disease	693C>A (tyr231>ter) 14 %	914C>A (A305>E) 60 %

ASPA gene placed on the 17th chromosome has 6 exons and 5 introns. Dependence of the severity of the disease on location of the mutation and representation of the mutations in populations has been described. Up to date, there exist in the non-Jewish population over 14 mutations with different percentage substitution

INTRODUCTION

In Central Europe, CD is a very rare autosomal recessive inherited leukodystrophy with a known metabolic defect. It is caused by the mutation of the gene for N-acetylaspartate acid amidohydrolase (aspartoacylase), which catalyses the transformation of N-acetylaspartate acid to acetyl and aspartate, and actively participates in myelinisation. Due to the defective enzyme, the substrate accumulates in astrocytes with the result of leukodystrophy followed by spongiform degeneration of the brain's white matter [1, 4]. There are several types of mutation of the gene for aspartoacylase, occurring predominantly either in the population of Ashkenazi Jews (854A>C) or in the non-Jewish Central European population (914C>A) (Table 1) [3]. This means that the diagnosis of CD is, besides clinical symptoms, laboratory proof of NAA elevation in urine and MRI signs of leukodystrophy, based mostly on molecular analysis of the genetic mutation characteristic of a particular population. We have described a case of a six-month-old non-Jewish child suffering from CD, which is very rare in our country.

Case: The six-month-old girl, born to non-related non-Jewish parents, from uncomplicated gravidity and without perinatal risks, was brought to our department for the diagnostics of aetiology of severe developmental delay which was on the level of a pathological newborn infant with central hypotonic syndrome and beginning spasticity of the lower extremities. Epileptic seizures were not expressed yet in the infant. Brain ultrasound examination had led to the suspicion of an inherited developmental brain disorder. The objective neurological finding also noticed suspect amaurosis and macrocephaly. EEG, EMG from the legs and BAEP were normal, VEP examination confirmed lesion in the visual tract without the possibility of a closer topisation. The basic metabolic sampling was complemented by special tests for inherited metabolic disorders with the discovery of NAA elevation in the urine. After structural brain examinations, MRI was carried out, upon which diffused affection of the white matter was described along with a myelinisation disorder and reduction of the cortex, which are all signs of leukodystrophy (Figures 1, 2). Now we could express our suspicion of CD. To prove it we sent the patient's and her parents' DNA for genetic analysis. Heterozygote mutation 914C>A was proved in both parents, and upon the test of the ASPA gene (6th exon) of the patient, 914C>A (A305E) mutation was found in homozygote condition, which is characteristic of the Central European non-Jewish population (Table 1). In our conditions the only therapy available is symptomatic treatment of epileptic seizures and rehabilitation. The girl's prognosis is therefore catastrophic due to the very substance of the disorder, as well as to the therapeutic possibilities in our country. The parents were instructed about the 25% risk of the same disorder occurring in any of their children, boys or girls, and in the case of the mother's next gravidity amniocentesis would be carried out as part of the perinatal diagnostics and monitoring of this family.

Discussion: The main symptom of our little patient was a serious developmental delay and a central hypotonic syndrome with spasticity on the lower extremities. The same symptoms are present in children with infantile poliomyelitis, which includes positive perinatal risks in the

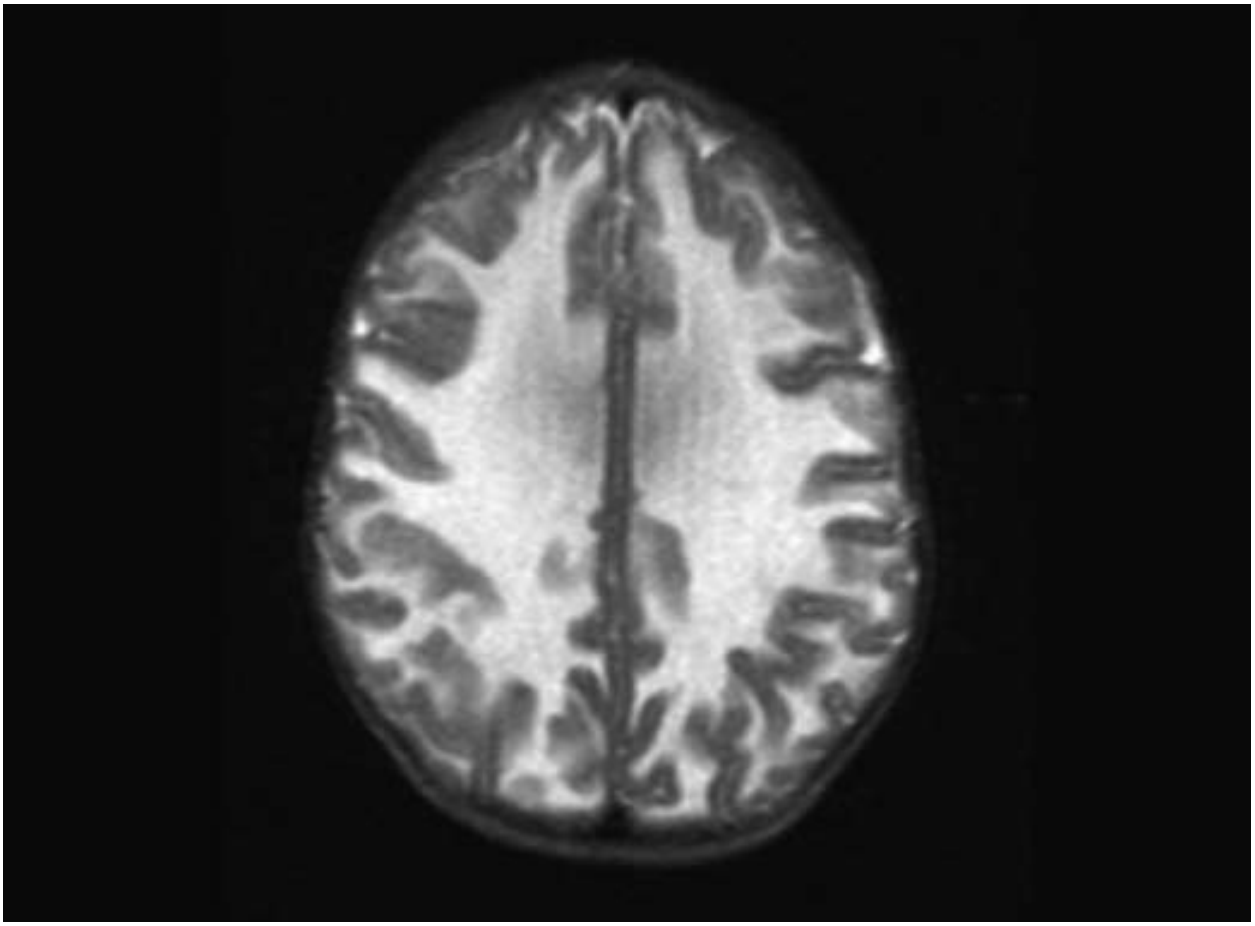

Figure 1
MRI T2W imaging – hyperintensity of white matter mainly localised subcortically, signs of grey matter reduction

patient's history that are negative in our case. Macrocephaly and developmental delay are also mentioned with the Pelizaeus-Merzbacher leukodystrophy, including a similar expression of the defective myelinisation in the T2-weighted brain MRI images, but in detailed biochemical examination and molecular analysis no concrete metabolic defect is found [1, 4]. In our case we found NAA elevation in the urine and, if lumbar puncture had been carried out, the increased NAA concentration would be present in the CSF as well [2]. Macrocephaly and developmental delay can also be diagnosed in hydrocephalus occurring after intrauterine infection. Laboratory proof of TORCH was negative in our patient. Clinical epileptic seizures have not yet occurred in our case, as these develop with the reduction of the brain's grey matter; EEG showed a mature curve of basic activity without any abnormities.

Unlike patients described in the literature, who suffer defective development of the cochlear apparatus and deafness, there was no proof of any hearing defect or lesion in the auditory pathway of our little patient [5].

For the purpose of the molecular analysis of the ASPA gene mutation, the non-Jewish origin of both parents was considered and the analysis was carried out directly for the A305E mutation, which was confirmed [3]. This mutation is present in approximately 60 % of the Central European non-Jewish population, and currently about 14 new mutations are available, whose detection would surely extend the time of the early diagnostics (Table 1). However, the prolongation of diagnostics unfortunately has no influence on the patient's prognosis. In Central Europe, no therapy other than symptomatic has been used at the moment. In 1996 gene therapy was launched in New Zealand followed by the USA; it is based on the introduction of the normal ASPA gene in the affected areas of the brain

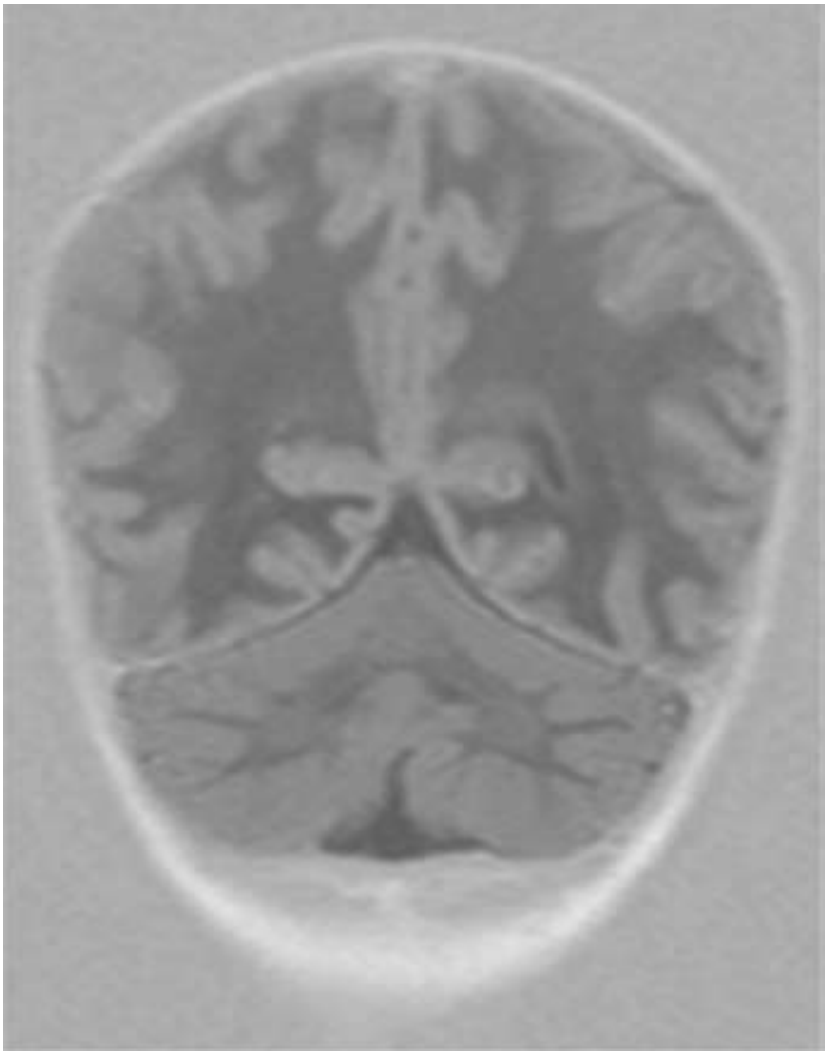

Figure 2
True inversion recovery – juxtacortical changes, reduction of grey matter

via recombinant virus particles [6]. The prognosis of patients treated in this way is much more positive, the quality of life improves, and they survive longer. The method of replacing the missing acetate to nerve cells for normal myelinisation is still in the research phase, with the chemical being glycerol triacetate [7]. Lithium citrate, on the other hand, drains the accumulating NAA from the glial cells – currently this is carried out in vitro only [8]. Studies with stem cells should be opened in the near future, which should offer even better prognoses to patients. The establishment of special treatment methods depends on the complications of the therapy, as well as on the incidence of the cases in individual states. With the increasing achievements of the research, specialised therapy should also be implemented in areas with low incidence of the disorder, thus improving the future prospects of patients and their families.

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