Critical illness polyneuropathy and myopathy; a neuromuscular disorder encountered in the intensive care unit

M.A.C.J. De Letter

De Letter, Marie-An Celeste Joseph
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Promotor

Prof. dr F.G.A. van der Meché

Overige leden

Prof. dr P.J. Koudstaal

Prof. dr H. F.J. Savelkoul

Prof. dr J.H.J.Wokke

Copromotor

Dr L.H. Visser

In memoriam copromotor

Dr A.A.W. Op de Coul

Voor mijn ouders

Co-authors

Neurology

P.A. van Doorn MD PhD[1]
F.G.A. van der Meché MD PhD[1]
A.A.W. Op de Coul MD PhD[2] †
R.L.L.A. Schellens MD[2]
G.A.M. Verheul MD[3]
L.H. Visser MD PhD[2]

Statistics

P.I.M. Schmitz PhD[4]

Immunology

J.D. Laman PhD[5]
H.F.J. Savelkoul PhD[5]

Neuropathology

J.M. Kros MD PhD[6]
J.L.J.M. Teepen MD PhD[7]

[1]Dept. of Neurology, University Hospital Rotterdam and Erasmus University Rotterdam, the Netherlands.
[2]Dept. of Neurology, St. Elisabeth Hospital Tilburg, The Netherlands.
[3]Dept. of Neurology, Groene Hart Hospital Gouda, the Netherlands.
[4]Dept. of Trials and Statistics, University Hospital Rotterdam and Erasmus University Rotterdam, the Netherlands.
[5]Dept. of Immunology, University Hospital and Erasmus University Rotterdam.
[6]Dept. of Neuropathology, University Hospital Rotterdam and Erasmus University Rotterdam, the Netherlands.
[7]Dept. of Neuropathology, St. Elisabeth Hospital Tilburg, The Netherlands.

CONTENTS

I	Polyneuropathies encountered in the intensive care unit		1
	Ia	General aspects	2
	Ib	Clinical and electrophysiological features of Critical Illness Polyneuropathy and Myopathy (CIPNM)	3
	Ic	Variants of CIPNM	5
	Id	Differential diagnosis of polyneuropathies encountered in the intensive care unit	5
	Ie	Aims of the prospective study of CIPNM	10
II	Risk factors for the development of polyneuropathy and myopathy in critically ill patients		11
III	A clinical and electrodiagnostic prospective study of patients with CIPNM: the natural history		25
IV	Immune activation in CIPNM		41
	IVa	How critical illness may trigger a powerful immune cascade in patients in the intensive care unit	42
	IVb	Immune activation in the muscle tissue of patients with CIPNM	50
V	Discussion: a pathogenetic model of CIPNM and future directions		67
VI	Summary/Samenvatting		73
References			83
List of abbreviations			93
Dankbetuigingen			97
Curriculum vitae			101
Publications			105

I

Polyneuropathies encountered in the intensive care unit

Ia General aspect

Ib Clinical and electrophysiological features of Critical Illness Polyneuropathy and Myopathy (CIPNM)

Ic Variants of CIPNM

Id Differential diagnosis of polyneuropathies encountered in the intensive care unit

Ie Aims of the prospective study of CIPNM

Ia GENERAL ASPECTS

Patients with neuromuscular disorders encountered on the ICU can be divided into two main categories. One category has been admitted to the intensive care unit (ICU) due to an underlying neuromuscular disorder, mainly the Guillain Barré Syndrome (GBS) and myasthenia gravis. The other category develops a so-called acquired neuromuscular disorder during their stay on the ICU after admission for other reasons (multi-trauma, severe infections, (multiple) organ dysfunction, etcetera). In these patients the neurological symptoms mainly consist of muscle weakness, wasting, and difficulties in weaning from the artificial respirator. The muscle weakness acquired in the ICU can be due to:

1) A polyneuropathy known as critical illness polyneuropathy, which is an axonal motor or sensomotor polyneuropathy. The myopathic changes in the histopathology of a notable part of the patients led to the use of the more descriptive term critical illness polyneuropathy and myopathy (CIPNM)[De Letter 2000 a].
2) A neuromuscular transmission defect due to a transient neuromuscular blockade related with the use of neuromuscular blocking agents.
3) A myopathy consisting of: a) Critical illness myopathy [Gutmann 1999, Hund 1999] in which both electrophysiological and histopathological features are consistent with a myopathy only. As this is mostly not the case we consider them as part of CIPNM. b) Myopathy with thick filament loss or acute quadriplegic myopathy (AQM) [Danon 1991, Douglas 1992, Hirano 1992, Lacomis 1996] and c) acute necrotizing myopathy of intensive care [Bolton 1994, Ramsay 1993, Zochodne 1994].

Ib CLINICAL AND ELECTROPHYSIOLOGICAL FEATURES OF CIPNM

The most frequent cause of the acquired neuromuscular disorders in the ICU is critical illness polyneuropathy and myopathy. The first reports on critical illness polyneuropathy were published in the early eighties by Bolton, Op de Coul, Roelofs and Couturier. These patients were admitted to the ICU, required artificial respiration and multiple drugs such as sedatives, neuromuscular blocking agents [Gooch 1991, Kupfer 1991, Op de Coul 1985/1991, Rossiter 1991, Subramony 1991], steroids [Lacomis 1996, Mac Farlane 1977] and antibiotics [Sokoll 1981].
The clinical features of CIPNM are muscle weakness and wasting, decreased or absent tendon reflexes and difficulties in weaning from the artificial respirator. Due to the variability of its clinical features CIPNM can easily be overlooked following a prolonged stay on the ICU [Coackley 1992]. The muscle weakness is most prominent in the lower extremities and located more distally than proximally. The exact onset of these symptoms can be difficult to determine due to the use of neuromuscular blocking agents or sedatives. About three days after discontinuation of neuromuscular blocking agents both clinical and electrophysiological testing of the motor functions is reliable and the motor symptoms become evident. In many cases difficulties in weaning from the artificial respirator is the presenting symptom of CIPNM and the reason to consult the neurologist. Muscle wasting is observed in one third of the patients [Zifko 1998]. Tendon reflexes are usually absent, but are not a prerequisite for the diagnosis [Hund 1996]. Sensory symptoms are often difficult to test in the sedated or intubated patients on the ICU. The presence of pain or paresthesias however often suggests a polyneuropathy secondary to the use of metronidazole, which primarily affects the sensory nerve fibers [Witt 1991]. Zifko et al [1998] found that in 17 of the 62 patients with CIPNM (27%) reliable information about sensory function could be obtained. Ten of them had distal, symmetric hypesthesia. Impaired consciousness, suggestive for an encephalopathy is often present, which makes clinical testing even more difficult. Leyten et al (1997) found that the sensitivity of the clinical judgment of CIPNM was 60% as compared to concurrent EMG diagnosed polyneuropathy.
Critical illness polyneuropathy is often preceded by critical illness encephalopathy, which is a poorly recognized entity. The clinical features of

the encephalopathy are impaired attention, concentration, orientation and writing [Jackson 1985, Young 1990], the presence of excessive theta activity on the EEG may be helpful in the diagnostic process [Young 1992].
Electrophysiological testing demonstrates a decrease of amplitudes of the compound muscle action potentials within one week of onset of CIPNM. Conduction velocities, distal motor latencies and responses to repetitive nerve stimulation are normal. The sensory conduction study can show decreased sensory nerve action potential amplitudes, which also confirms the presence of an axonal polyneuropathy. Such information is usually obtained by serial monitoring.
Needle electromyography reveals spontaneous activity of muscle fibers in rest and is present more abundant in distal than in proximal muscles. Spontaneous activity of the muscle fibers is usually present 3 weeks after start of artificial respiration [Bolton 1996], but can be found within the first two weeks [Op de Coul 1991]. Sometimes there are signs of myopathic changes of the motor unit potentials with a short duration and low amplitudes on voluntary activation.
Diaphragm and chest wall needle electromyography has been performed in patients who presented with failure to wean from the ventilator; this showed also spontaneous activity of the diaphragm [Bolton 1993a, Zifko1998].
Dysfunctioning of the neuromuscular junction in the ICU due to a preexisting disorder or the toxic effect of neuromuscular blocking agents or aminoglycosides can be excluded by repetitive nerve stimulation [Segredo 1992]. For suspected disturbances of the spinal dorsal columns the somato-sensory evoked potentials can be tested.

Ic VARIANTS OF CIPNM

As critical illness is not necessarily associated with artificial respiration, this should mean that CIPNM also occurs outside the ICU. Reports of polyneuropathy in patients suffering from severe burns and SIRS were described by De Saint Victor et al [1994]. Gorson and Ropper [1993] reported the development of critical illness polyneuropathy in five patients with severe respiratory problems, who later required artificial respiration. The severe sub-acute mainly motor axonal polyneuropathy that occurs in end-stage renal disease with trauma or sepsis may also be considered as a variant of CIPNM [Young 1990].

Id DIFFERENTIAL DIAGNOSIS OF POLYNEUROPATHIES ENCOUNTERED IN THE ICU

- **Critical illness myopathy**

There has been much debate as to the incidence of a myopathy, which may occur independently or in association with a polyneuropathy in critical illness patients [Hund 1999]. It is often difficult to distinguish between axonal motor polyneuropathy and myopathy in CIPNM. Clinical testing of the sensory function is often not reliable and therefore is not helpful either in the differential diagnosis. Theoretically predominant proximal weakness, neck flexor weakness and facial weakness occur more often in patients with myopathy. In the needle electromyography low amplitudes and short duration of the motor unit potentials suggest a myopathy. Normal sensory nerve action potentials (SNAP) amplitudes and decreased compound motor action potential (CMAP) amplitudes are usually present in conduction studies. However absence of or a decrease in SNAP amplitudes does not exclude a myopathy. Because of tissue edema, SNAP amplitudes may be spuriously low. Serial studies may reveal a significant fall in SNAP amplitudes in critical illness polyneuropathy. Reliable examination of the motor unit potentials (MUP) on voluntary activation is also difficult in cases of severe muscle weakness. Rich et al [1997] described that in neuropathy or endplate

dysfunction the excitability of the muscle remains normal, which appeared not to be the case in acute quadriplegic myopathy. Direct muscle stimulation may therefore be helpful in the distinction of polyneuropathy and myopathy. Determination of creatinine phosphokinase in serum is also helpful in revealing a myopathy. Highly elevated levels of creatinine phosphokinase suggest a necrotizing myopathy. Furthermore severe myopathy is associated with myoglobinurea. At present the best method to differentiate between underlying polyneuropathy and myopathy is by performing a muscle biopsy using standard light microsopic examination of the muscle tissue.

- Guillain Barré Syndrome

The Guillain Barré Syndrome (GBS) is a (sub-) acute immune-mediated polyneuropathy. Clinical features required for diagnosis are I) progressive, more or less symmetrical motor weakness of more than one limb. The degree ranges from minimal weakness to paralysis of the muscles of all four extremities, the trunk, bulbar and facial paralysis and external opthalmoplegia. II) Areflexia: universal areflexia is the rule, though distal areflexia with definite hyporeflexia of the biceps and knee jerks will suffice if other features are consistent. The diagnostic criteria are based on clinical, laboratory and electrodiagnostic criteria and are defined by Asbury and Cornblath (1990).

To distinguish CIPNM from the acute motor axonal variant of Guillain-Barré syndrome the following characteristics may be useful [De Letter 2000b]:

1) GBS is the primary neurological reason of admission to the intensive care unit (ICU). CIPNM on the other hand develops during a patient's stay on the ICU for another reason. Development of GBS on the ICU is considered to be very rare.
2) Infectious symptoms like fever and diarrhea have usually subsided before the clinical features of GBS appear.
3) The characteristic alterations in the cerebrospinal fluid of GBS patients with a raised protein and normal to slightly elevated cell count.
4) The possibility to detect IgG anti-bodies against GM1, GM1b, GD1a and N-acetylgalactosaminyl-GD1a in the serum of patients with acute motor neuropathy.
5) Electrodiagnostic changes in GBS occur in both sensory and motor nerves in about 80% of the patients in the Western world. In CIPNM there is clinically predominantly motor dysfunction. Both CIPNM and axonal type

GBS show sensomotor or pure motor axonal features. CIPNM can sometimes be distinguished from GBS by the presence of myopathic motor unit potentials on voluntary activation.

6) During the progression of GBS the demyelinating features of the nerve conduction study may change into a secondary axonal pattern. In the latter slow nerve conduction velocity remains in some patients and the initial needle electromyographic study lacks spontaneous activity [Chen 1998]. In CIPNM spontaneous activity of the muscle fibers is an early feature. Further phrenic nerve conduction studies usually show no significantly prolonged latencies in CIPNM [Bolton 1986].
7) Severe autonomic disturbances are more common in GBS than in CIPNM [Bolton 1986].
8) Septic encephalopathy may be present before onset of CIPNM. Patients with GBS do not exhibit disturbed consciousness.

- Porphyric Neuropathy

Porphyric neuropathy is an acute or subacute predominantly motor neuropathy. Disturbances of porphyrin metabolism are associated with acute attacks of neurologic disease in case of hepatic porphyrias. These porphyrias consisting of acute intermittent porphyria (AIP), hereditary coproporphyria (HCP) and variegate porphyria (VP) are caused by enzyme defects (uroporphyrinogen-1-synthetase, coproporphyrinogen oxidase or protoporphyrinogen oxidase). AIP [Kappas 1983, Stein 1970], HCP [Magnussen 1975] and VP [Eales 1980] all have a genetic origin. The attacks of acute hepatic porphyrias may be precipitated by drugs (most often barbiturates, estrogens, ethanol excess, griseofulvin, hydantoins, meprobamate, oral contraceptives and sulfonamides), hypoglycemia and hormonal influences. The latter results in the occurrence of AIP in the luteal phase of the menstrual cycle. Hormone-induced AIP does not occur before puberty in either sex. The excess of heme precursors that is being produced during the attacks is detectable in urine, feces and blood. Therefore they serve as diagnostic tools for the specific types of porphyria [Windebank 1993].

The change of color of the urine is due to the oxidation of the porphyrinogens. The conversion to the corresponding porphyrin causes the urine to turn red or purple. Porphobilinogen may polymerize to porphobilin, which has a black color.

The clinical features are: 1) Acute colicky abdominal pain. It is not known if the pain is caused by local effects of heme precursors or heme deficiency on muscles and nerves of the gastrointestinal tract or to acute autonomic neuropathy with sympathetic hyper reactivity. The latter is a prominent feature during porphyric attacks. 2) Psychiatric disturbances that vary from restlessness and agitation to psychosis, coma and seizures. 3) Acute neuropathy that develops within 2-3 days of onset of abdominal and psychiatric symptoms. The first symptoms may be back or limb pain. As in CIPNM motor symptoms are the earliest and most prominent clinical features. However in porphyric neuropathy the weakness is occasionally asymmetric or patchy, may begin in the upper limbs or cranial nerves (commonly facial weakness and swallowing difficulty), and proximal muscle are as likely to be affected as the distal ones. Atrophy is seen early as in CIPNM, may be severe, and is probably due to axonal degeneration and generalized weight loss. Sensory symptoms are less prominent but may involve patchy or migratory

painful paresthesias. Progression to maximal deficit, with possible respiratory insufficiency, usually occurs in a few days, but may occur gradually during a period of several weeks.

Electrophysiology of this acute neuropathy may show normal nerve conduction studies and needle electromyography during the first few days of the illness. As in CIPNM the primary process in porphyric neuropathy involves an axonal degeneration resulting in a decrease of the compound action potentials, which is usually proportional to the severity of muscle weakness. There is no prominent sensory involvement except when degeneration of the majority of motor axons occurs and during the regeneration phase slowing of the conduction velocity is present. Somato sensory evoked potentials and spinal motor-evoked potentials are useful as proximal and cranial innervated muscles are mostly involved. Needle electromyography of clinically involved muscles reveals denervation potentials in 5-10 days and features of reinnervation during recovery [Windebank 1993].

The following features may be used to disciminate the clinical pictures of porphyric neuropathy and CIPNM:

1) Porphyric neuropathy shows prodromal abdominal and psychiatric symptoms.
2) Porphyric neuropathy patients may have a family history of porphyric attacks.
3) CIPNM occurs during the patient's stay on the ICU for other reasons, the porphyric patient that develops neuropathy may become respiratory insufficient and therefore be admitted to the ICU.
4) Porphyric neuropathy patients have asymmetric or patchy weakness beginning in upper limbs or cranial nerves, CIPNM patients have symmetrical tetraparesis and occasionally involvement of cranial nerves.
5) Excretion of heme precursors causes the described color changes in urine.
6) Avoiding drugs that induce cytochrome p450 and situations leading to hypoglycemia can prevent porphyric attacks. Treatment is possible for the underlying defect using heme intravenously.

Ie AIMS OF THE PROSPECTIVE STUDY OF CIPNM

CIPNM has only a short history and interest arose to analyze the epidemiological, clinical, electrophysiological and pathological features of this disease. In a prospective study on the ICU we compared the clinical and electrodiagnostic characteristics of patients that developed CIPNM with patients that did not. After a pilot study on CIPNM in the St Elisabeth Hospital Tilburg performed by Freek Verheul [1994], the prospective study performed by Marie-An de Letter outlined in this thesis of 98 patients started in 1994. One of the aims of the study was to determine risk factors for developing CIPNM (Ch II). Therefore variables such as patient characteristics, medication and scores that express severity of illness were used. Defined risk factors were analyzed to identify patients that are at low, medium or high risk of developing CIPNM. Chapter III describes the natural history of CIPNM, its clinical and electrophysiological aspects. Further clinical variables, patient characteristics, medication and neuro-pathological features were compared with the severity of disease in an attempt to identify prognostic factors. In addition to an introduction on the process of immune activation in critically ill patients in general (Ch IV_a), chapter IV_b describes the structural changes in the muscle tissue of CIPNM patients compared with some ICU patients that did not develop CIPNM. This neuro-pathological analysis consisted of standard light microscopical examination of the muscle tissue and tested the presence of immune activation. The neuropathological findings were also analyzed statistically to exclude the influence of systemic (inflammatory) variables. In chapter V a general discussion is presented high-lighting the strong and weaker aspects of this prospective study with recommendations for future research.

II

Risk factors for the development of polyneuropathy and myopathy in critically ill patients

INTRODUCTION

Critical illness polyneuropathy and myopathy (CIPNM) is a neuromuscular disorder that has been recognized in critically ill patients [Bolton 1993c, Latronico 1996]. The clinical picture consists of difficulty in weaning from the artificial respirator, tetraparesis and muscle wasting of the limbs. The tendon reflexes are mostly decreased or absent. The neurophysiological examination shows an axonal polyneuropathy and sometimes myopathic altered motor unit potentials. The morphological features in the nerve point to a primarily distal axonal degeneration of motor and sensory fibers [Zochodne 1987]. Muscle biopsy shows scattered atrophic fibers in acute denervation and grouped atrophy in chronic denervation. Also necrotic muscle fibers can be found suggesting the contribution of a myopathy or a primary myopathy [Latronico 1996,Op de Coul 1991]. On clinical and electrodiagnostic grounds neuromuscular complications in the critically ill patients may be due to a polyneuropathy or myopathy. Since it is not always possible to differentiate between an axonal motor neuropathy and myopathy [Gutmann 1999], we prefer to use the descriptive term critical illness polyneuropathy and myopathy.

Hypotheses regarding the etiology of CIPNM include a role for (1) the presence of the systemic inflammatory response syndrome (SIRS) or sepsis and its influence on activation of the body defense system [Latronico 1996,Zochodne 1987, Leijten 1995, Wijdicks 1994b, Hund 1997, Witt 1991] or (2) a combination of (1) and neuromuscular blocking agents (NBA) with or without steroids [Op de Coul 1991, Bolton 1996]. Also other conditions as malnutrition, underlying disease, immobility and drugs used in the ICU have been postulated [Coackley 1993]. Because of this controversy, we prospectively followed critically ill patients. After inclusion into the study systematically serial clinical, laboratory and electrophysiological studies were performed. In that way we were able to diagnose CIPNM at an early stage and could compare the characteristics of these patients with patients who did not develop CIPNM.

METHODS

In a longitudinal prospective observational study at the intensive care unit of the St. Elisabeth Hospital Tilburg 98 patients were included in the period from May 1994 to July 1996. The medical ethical committee of the hospital approved the study.

Patients could be included from day 4 of the artificial respiration after a written informed consent was obtained from the family of the patient. The exclusion criteria for this study were the presence of a Guillain-Barré syndrome, acute or chronic spinal cord lesion, hypophosphataemia, myasthenia gravis and a history of a pre-existing disease, which might be responsible for the development of polyneuropathy or myopathy.

After the patients were enrolled, they underwent clinical and electrophysiological monitoring. The neurological examination was performed twice a week during the stay in the intensive care unit and once a week in the general ward until recovery. The examination assessed motor deficit, muscle wasting, sensory loss and tendon reflexes. Motor sum score of three upper and three lower limb muscles was used to evaluate muscle weakness. This involved the deltoid, biceps, wrist extensor, iliopsoas, quadriceps femoris and the foot extensor muscles on the most affected side if paresis was asymmetric (maximum score 30 in non-comatose patients). In the non-cooperative only tendon reflexes and motor sum score on pain stimuli were scored consisting of flexion in the elbow and flexion the hip, with a maximum sum score of 10.

For the clinical assessment of CIPNM only the motor sum score and tendon reflexes counted at least three days after the use of neuromuscular blocking agents was stopped (Table 1).

Electrophysiological monitoring was performed on day 4, 11 and 25 after start of the artificial respiration and consisted of conventional orthodromic motor (ulnar and peroneal) and antidromic sensory (ulnar and sural) nerve conduction studies. If edema was present the results of decreased CMAP or SNAP amplitudes were not used for analyses. The ulnar and peroneal nerve conduction studies were performed also along the elbow segment and at the fibula to exclude underlying entrapment neuropathies. Limb temperature was registered and warming was performed until limb temperature of at least 32° Celsius.

Table 1: Clinical and electrophysiological criteria of CIPNM.

Patients used no neuromuscular blocking agents for at least three days for both clinical and electrophysiological criteria. For diagnosis of CIPNM clinical and electrophysiological criteria should be met.

Clinical; present in at least two consecutive visits.

Co-operative patient
- Motor sum score <26.
- Decreased or absent tendon reflexes.

Non co-operative patient.
- Motor sum score < 8.
- Decreased or absent tendon reflexes.

Electrophysiological
Presence of axonal polyneuropathy defined as in I or II:

I distal CMAP ulnar nerve absent or below <4.2 mV and entrapment at the elbow and demyelination* were excluded.
and
distal CMAP peroneal nerve absent or below <2.6 mV and entrapment at the fibular head and demyelination* were excluded.

II Criteria for ulnar or peroneal nerve as mentioned above
and
Spontaneous activity of the muscle fibers in rest (fibrillations or positive waves) in at least two of the examined muscles.
* Ratio of the area of the proximal CMAP versus the distal CMAP >0.89, in the nerve with decreased CMAP amplitude.

Furthermore repetitive nerve stimulation at 3 Hz of the ulnar nerve and monopolar needle electromyography of the biceps, abductor digiti quinti, quadriceps femoris and tibialis anterior muscle were performed. When patients suffered from disability due to CIPNM neurological and

electrophysiological examinations were repeated every 6 weeks. Both the clinical and electrophysiological criteria had to be met for the diagnosis of CIPNM (Table 1).
The use and dosage of midazolam, vecuronium and steroids were registered at every visit. The use of aminoglycosides was noted at entry. The creatinine phosphokinase, erythrocyte sedimentation rate and liverfunctions were tested weekly. If a patient developed clinical or electrophysiological signs of a neuromuscular disorder the myoglobin in the urine was analyzed.
At entry the Apache-III score, sepsis severity score and presence of SIRS were determined. Developing SIRS during the stay on the ICU was also noted. The Apache-III score, the Acute Physiology, Age, Chronic Health Evaluation, is a methodology that is known to predict hospital mortality risk for critically ill hospitalized adults [Friedland 1996, Knaus 1991]. The score results from addition of three groups of variables concerning physiology, age and chronic health. The physiology is represented in weighted laboratory abnormalities and vital signs: pulse, mean bloodpressure, temperature, respiratory rate, PaO_2 , hematocrit, white blood cell count, creatinine, urine output, blood urea nitrogen, sodium, serum albumin, bilirubin, glucose [Knaus 1991].
The sepsis severity score consists of scoring five organ systems (lungs, heart, liver, kidneys, intestine) and the blood coagulation on a scale of 0 to 5 of which 5 reflects the worst situation. Eventually the three highest of these six scores are put to a square and summated resulting in the sepsis severity score (minimum 0 and maximum 75) [Stevens 1983]. The presence of SIRS includes two or more of the following clinical manifestations: (1) body temperature greater than 38°C or less than 36°C; (2) heart rate greater than 90 beats per minute; (3) tachypnea, manifested by respiratory rate greater than 20 breaths per minute, or hyperventilation as indicated by $PaCO_2$ of less than 32 mm Hg; and (4) alteration in the white blood cell count, such as a count greater than 12,000/cu mm, a count less than 4,000/cu mm, or the presence of more than 10 percent immature neutrophils [Bone 1992].

Statistical analysis

The Apache-III score, the presence of SIRS, the reason of admission, the sepsis severity score and its components were related at entry with the fraction of patients that developed CIPNM from entry (day 4 of the artificial respiration) using the Kaplan-Meier method and the log rank test. This was also done for the use of aminoglycosides at entry and the dosage of

midazolam, vecuronium, and steroids at entry and until day 7 of the artificial respiration when still no CIPNM was diagnosed. Day 7 was chosen because at that time only one case of CIPNM had been diagnosed. Analyzing earlier in time would be to close to the moment of entry in the study. Finally the total dose of vecuronium used until day 7 of the artificial respiration combined with the kidney function were related with the fraction that developed CIPNM. P-values <0.05 were considered significant.
The factors studied in the univariate analysis were used in a stepwise Cox's proportional hazards model. With this approach a predictive index was calculated using the two eventual significant risk factors. Three groups (low, medium and high risk) were determined from the distribution of this index. Kaplan-Meier curves are presented, showing the risk of developing CIPNM at certain time from start of the artificial respiration for each of the three risk groups.

RESULTS

From May 1994 to July 1996 117 patients were evaluated of which 98 were eligible. Nineteen patients were not eligible of which 11 refused to give permission for this study and 8 patients had exclusion criteria. CIPNM occurred in 32 (33%) of the 98 prospectively monitored critically ill patients. The 95% binominal exact confidence interval was 24%-43%.
Analysis could be performed on the data of 97 of the 98 patients, including 31 CIPNM patients.
The clinical characteristics showed 55 (57%) male and 42 (43%) female patients. Their median age was 70 years, ranging from 15-85 years. The reason of patient admission was a multi-trauma in 13 (13%), elective surgery in 38 (39%), infectious disease in 27 (28%) and a category others of 19 (20%). This last category consisted mainly of patients with severe cardiac insufficiency and two patients with exacerbation of their chronic obstructive pulmonary disease.
Univariate analysis revealed the Apache-III score (Fig. Ia), the presence of SIRS (Fig. Ib) and the use of aminoglycosides as being significant factors for the development of CIPNM at entry of the study (Table 2). The sepsis

Table 2: Patients characteristics and results of univariate analysis.

4 days after start of artificial respiration		Number of patients	Percentages with CIPNM 15 days[1]	30 days[2]	P-value Log rank test
Overall		97	15	35	---
Apache-III score	≤70	24	4	17	0.02
	>70- ≤ 85	30	14	28	
	>85	43	22	50	
Sepsis Severity score	10-57	97	-	-	0.91
SIRS	Yes	47	20	46	0.04
	No	50	10	26	
vecuronium	0 mg	66	14	31	0.38
	>0 mg	31	16	43	
midazolam	≤500 mg	51	20	32	0.86
	>500 mg	46	9	38	
steroids	0 mg	70	15	35	0.67
	>0 mg	27	15	34	
aminoglycosides	Yes	37	22	51	0.049
	No	60	10	26	
7 days after start artificial respiration. (n=96)[3]					
vecuronium	0 mg	62	17	33	0.28
	>0 mg	34	21	48	
midazolam	≤1780mg	48	22	35	0.95
	>1780mg	48	16	44	
Steroids	0 mg	67	16	34	0.31
	>0 mg	29	25	48	

[1] After 15 days of artificial respiration.

[2] After 30 days of artificial respiration.

[3] One patient deleted because the patient developed CIPNM before day 7 of the artificial respiration (n=96).

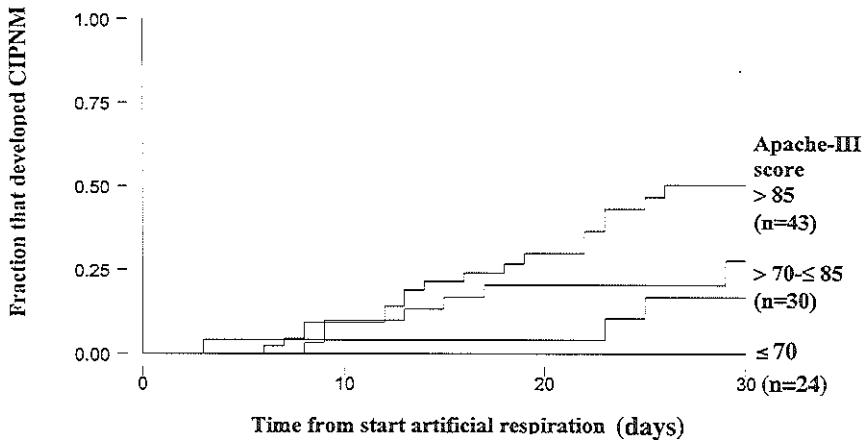

Figure Ia.

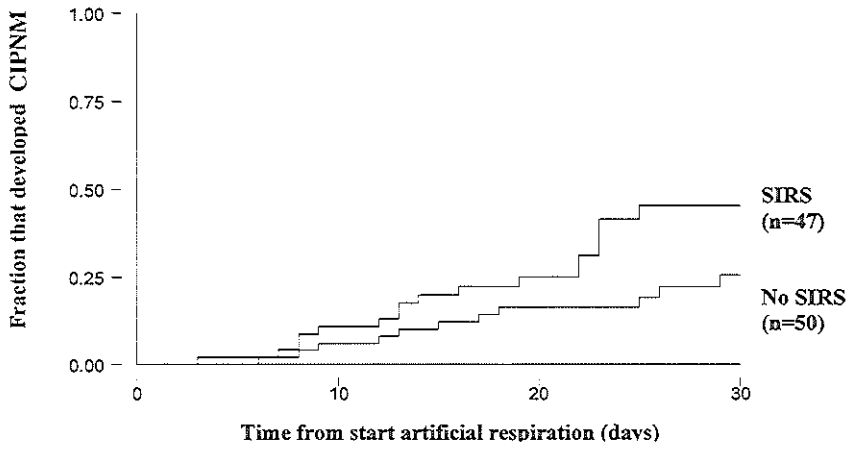

Figure Ib.

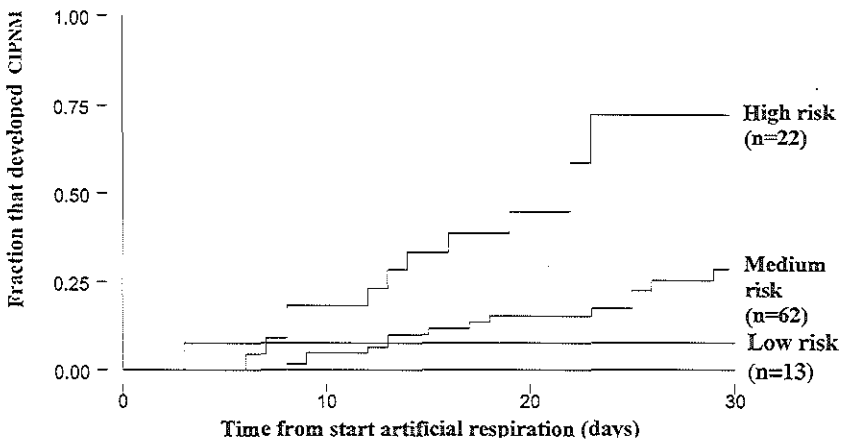

Figure II.

Figure Ia, Ib and II;
Kaplan Meier curves showing the fraction of patients developing CIPNM during 30 days after start of the artificial respiration. Ia: the Apache-III score ≤ 70, > 70- ≤ 85 and > 85 (p=0.02). Ib: presence or absence of SIRS or no SIRS (p=0.04). II: Low-risk group with an Apache-III score ≤ 70 and no SIRS, the high-risk group with an Apache-III score >85 and presence of SIRS and the medium-risk group containing all other patients.

severity score (also its individual components representing the organ functions and the coagulability), the reason of admission, the amount of vecuronium, midazolam or steroids used (Table 2), did not show a relationship with the occurrence of CIPNM. Nor did the total dose of vecuronium used until day 7of the artificial respiration combined with the kidney function relate with the development of CIPNM.
SIRS was present in 47 of 97 cases (48%). There was a clear relationship between the presence of SIRS and the use of aminoglycosides (P-value 0.03). Using a stepwise proportional hazards model with Apache-III score, presence of SIRS, amount of vecuronium, midazolam and steroids and the use of aminoglycosides as potential risk factors, only the Apache-III score and the presence of SIRS remained significant. Based on the distribution of the predictive index derived from these two variables three risk groups were

identified. The low-risk group with an Apache-III score ≤70 and no SIRS (n=13), the high-risk group with an Apache-III score >85 and presence of SIRS (n=22) and the medium-risk group containing the other patients (n=62). The Kaplan-Meier curves show that the probability of developing CIPNM within 30 days of artificial respiration is 8% for the low-risk group, 28% for the medium-risk and 72% for the high-risk group (Fig. II). Although the variables SIRS and Apache-III score are correlated, they both appear to contribute to the multivariate Cox regression.

DISCUSSION

The incidence of CIPNM in these 98 prospectively studied critically ill patients was 33%. In previous studies using similar inclusion criteria this varied from 33 to 44% [Leijten 1995,Coakley 1993]. If patients suffer from sepsis the incidence increases up to 70% [Hund 1997,Witt 1991,Coakley 1993]

Risk factors for the development of CIPNM

The Apache-III score, presence of SIRS and use of aminoglycosides were all significant in the univariate analysis of the exposed risk factors for the development of CIPNM. Multivariate analysis showed that the presence of a high Apache-III score and SIRS both were independent factors contributing to the development of CIPNM. When both factors are present the risk was found to be 72%.

With regard to a high Apache-III score this appeared to be an important predictor for the development of CIPNM. The Apache-III scoring system is used to predict the mortality risk in ICU patients and is a quantitative index of disease severity based on clinical and laboratory physiological data [Friedland 1996, Knaus 1991]. A high Apache-III score has been associated with the development of MODS in patients with perforated viscus [Barie 1996]. The group of Bolton et al was the first to relate critical illness polyneuropathy with multiple organ failure and sepsis and Bolton hypothesized about the pathogenic role of SIRS [Bolton 1993c, Zochodne 1987, Witt 1991, Bolton 1996]. In this study organ dysfunction is reflected

by the APACHE III score giving a quantitive index of disease activity, and the results more or less agree with the studies using MODS score [Leijten 1995]. Although in the univariate analysis the use of aminoglycosides at entry was related significantly with the development of CIPNM, this was no longer so after multivariate analysis. This may be due to the correlation with the presence of SIRS. Since in our ICU in case of (threatening) sepsis aminoglycosides are standard treatment, it cannot be ruled out that either SIRS or aminoglycosides may have a pathogenic effect. However, no effect was found on the neuromuscular junction using repetitive stimulation in the electrophysiological monitoring of the patients, which would be expected in case of a neurotoxic effect of aminoglycosides [Sokoll 1981]. Furthermore we note that the effect of the use of aminoglycosides in the muscle tissue was not visible at the light microscopical level in our neuropathological study performed on the same population that was studied for the presence of risk factors [De Letter 2000a]. More detailed cellular study was not performed. Therefore, we consider SIRS more likely as risk factor.

As no relationships were found for the total amount of midazolam, vecuronium or steroids used with regard to developing CIPNM, it seems that this medication does not play a role in the pathogenesis. Our previous results indicated an important role for neuromuscular blocking agents [Op de Coul 1985]. However, results were obtained retrospectively in that study. A prospective study of Douglass et al assessing the occurrence of myopathy as a complication of therapy of severe asthma in patients requiring mechanical ventilation showed that myopathy was associated with a higher total dose of vecuronium. It is likely that these patients had an acute quadriplegic myopathy with selective loss of thick filaments, which is a different entity than CIPNM. In our study we did not include patients with an acute quadriplegic myopathy [Douglass 1992]. Our current results agree with other prospective studies, which report that drugs do not play a part in development of CIPNM on the ICU [Latronico 1996, Leijten 1995, Witt 1991, Coakley 1993, Berek 1996]. The causative descriptions of mainly retrospective case series of prolonged neuromuscular blockade can be divided into pharmacokinetically based [Segredo 1990,Shearer 1991, Hoyt 1994, Fahey 1981, Lynam 1988, Smith 1987] and neuromuscular function based [Shanks 1985, Darrah 1989, Wokke 1988, Vanderheyden 1992, Patridge 1990]. The pharmacokinetically based blockade is attributed to decreased renal clearance of active metabolites of vecuronium. The combination of the

amount of vecuronium used during the first week of the artificial respiration and disturbed kidney functions were not found to be a risk factor for the development of CIPNM in our study. Concomitant use of several drugs alters the duration of action of NBA's. Drugs, which we investigated, that can potentiate blockade are aminoglycosides and midazolam. Combinations of such medication in our study did not reveal any differences between the patients that developed CIPNM and those who did not. However the influence of the medication as prognostic factors on the recovery of CIPNM patients should also be considered. This might support the hypothesis of previous publications that such medication especially neuromuscular blocking agents are of importance in CIPNM.

Pathogenetic model

In the critically ill patients which is reflected in a high Apache-III score, the host response seems to lead to dysfunction of the neuromuscular system. On the other hand the pro-inflammatory cytokines TNF-α and IL-1 are known to play a pivotal role in SIRS. Tissue damage, infection or severe organ dysfunction may serve as a trigger inducing antigen presenting cells to produce IL-1, IL-6, IL-8, IL-10, IL-12 and TNF-α, resulting in activation and influx of mainly CD4+ Th-cells, monocytes, macrophages and neutrophils. As a result of this immune activation adhesion molecules are also being produced, leading to an increased vascular permeability [Nieuwenhuijzen 1997]. Extravasation of (inflammatory) cells, edema and therefore tissue damage are the result in various organs; one of them being the neuromuscular system. We recently found evidence for immune activation in the muscle tissue of our CIPNM patients [De Letter 2000a]. A disturbed microcirculation with resulting endoneurial edema and hypoxia may be responsible for primary axonal degeneration [Bolton 1996].

Our study indicates that critically ill patients with both a high Apache-III score and SIRS are most prone to the development of CIPNM (Fig. II), which agrees with the pathophysiological model discussed. The high incidence of CIPNM of about 70% when patients suffer from sepsis or SIRS as found in previous studies supports this hypothesis [Hund 1997,Witt 1991, Segredo 1990]. In conclusion, the risk of developing CIPNM is higher in patients with a high Apache-III score and the presence of SIRS. So, the more sick the patient on initial evaluation, the greater the risk of developing CIPNM.

Practical implications

The APACHE III scoring system and the presence of SIRS can be useful tools in assessing who will be at risk of developing CIPNM on the ICU. Our data however need to be validated in a second study.

Many intensive care doctors use these scores, which are relatively easy to obtain. Patients with an initial high APACHE-III score and development of SIRS during follow-up should have regular neurological assessments of muscle strength. To obtain this one should be careful with the use of sedation and NBA's in these patients. As soon as CIPNM is suspected an EMG should confirm these findings and supportive therapy can be started. Although NBA's or steroids are not causing CIPNM it is possible that after the onset of CIPNM NBA's or steroids can be detrimental to muscles. Moreover, because of the weakness in limb and respiratory muscles due to CIPNM NBA's are no longer necessary and should be withheld in these patients. This could become an early diagnosis of CIPNM. Most CIPNM patients are diagnosed within 2 to 3 weeks after the start of artificial respiration, therefore we advise that an EMG be performed at day 10-14 after start of ventilation and if necessary 1 week later if neurological assessments are not possible. Difficulty in weaning from the artificial respirator was a common reason for asking neurologists to assess whether the patient has a CIPNM. In our opinion this is a late sign and with the advice mentioned above can be anticipated. Multi-organ failure and sepsis, risk factors for CIPNM, probably result in an exaggerated immune response with a local (muscle tissue) low-level immune activation as a consequence [De Letter 2000a]. It is likely that effective therapy of multi-organ failure and sepsis may result in a lower incidence of CIPNM. Immune-modulating therapy, like the use of intravenous immunoglobulins, for patients at risk for CIPNM could be a theoretical option. A retrospective study gave some indication of this [Mohr 1997], but should be tested in a prospective randomized placebo controlled trial.

III

A clinical and electrodiagnostic prospective study of patients with critical illness polyneuropathy and myopathy: the natural history

INTRODUCTION

Critical illness polyneuropathy and myopathy (CIPNM) is a neuromuscular disorder frequently encountered in the intensive care unit. The incidence of CIPNM varies from 33-40% [Leijten 1995, Coakley 1993] and reaches 70% in septic patients [Hund 1997, Witt 1991, Coakley 1993]. The clinical characteristics are difficulty in weaning from the artificial respirator, muscle weakness and wasting, and decreased or loss of tendon reflexes. We prefer to use the descriptive term critical illness polyneuropathy and myopathy since it is not always possible to differentiate (clinically and electrophysiologically) between an axonal motor neuropathy and myopathy [Gutmann 1999]. Furthermore, muscle biopsies of patients with the clinical and electrodiagnostic features of critical illness polyneuropathy often show myopathic changes [Latronico 1996, De Lettter 2000a].
Until recently, it has been stated, that the patients with CIPNM generally recover well [Hund1996], but long term follow-up studies of patients with CIPNM are scarce. Moreover, death in critical ill patients may overestimate this general accepted good prognosis. Therefore, in a longitudinal prospective observational study we followed 98 patients who needed at least 4 days of artifical respiration. All patients were monitored clinically and neurophysiologically for the development of CIPNM. Development of clinical and electrophysiological features, the outcome during follow-up, assessment of prognostic factors for bad outcome and the correlation of clinical characteristics with structural changes in the muscle tissue of 30 of the 32 patients who developed CIPNM are described.

METHODS

In the intensive care unit of the St. Elisabeth Hospital Tilburg we monitored 98 patients of which 32 met the clinical and electrophysiological criteria of CIPNM as defined earlier [De Letter 2001].
The exclusion criteria for this study were the presence of a Guillain-Barré syndrome, acute quadriplegic myopathy, acute or chronic spinal cord lesion, hypophosphatemia, myasthenia gravis and a history of a pre-existing polyneuropathy or myopathy. The medical ethical committee of the hospital approved the study.
Monitoring of these patients for the development of CIPNM started four days after the onset of the artificial respiration. Written informed consent was obtained from the family of the patient.

Clinical assessment, medication and laboratory monitoring (n=98):
Clinical data were recorded twice a week during the stay on the intensive care unit and once a week in the general ward until discharge from the hospital. Clinical monitoring consisted of:

1) The motor sum score reflected by the MRC sum score of three upper and three lower limb muscles. This involved the deltoid, biceps, wrist extensor, iliopsoas, quadriceps femoris and the foot extensor muscles on the most affected side if paresis was asymmetric (maximum score 30).
2) Touch and pain sensation were tested in all extremities and classified as normal, disturbed (in upper, lower or all extremities) or unreliable.
3) The presence of muscle wasting defined as absent, present or unreliable to establish.
4) The tendon reflexes were classified as normal, decreased, slight contraction visible and no contraction (the worst score was used).

The use of aminoglycosides was noted at entry. The use and dosage of midazolam, vecuronium and steroids was registered on every neurological examination. The creatinine phosphokinase, erythrocyte sedimentation rate and liver functions were tested weekly. If a patient developed clinical or electrophysiological signs of a neuromuscular disorder the presence of myoglobin in the urine was analyzed.

Monitoring severity of underlying disease and infections (n=98):
At entry of the study the Apache-III score, sepsis severity score and presence of SIRS were determined. Developing SIRS during the stay on the ICU was also noted. The Apache-III score, the Acute Physiology, Age, Chronic Health Evaluation, is a methodology that is known to predict hospital mortality risk for critically ill hospitalized adults [Friedland 1996, Knaus 1991]. The score results from addition of three groups of variables concerning physiology, age and chronic health. The physiology is represented in weighted laboratory abnormalities and vital signs: pulse, mean bloodpressure, temperature, respiratory rate, PaO2 , hematocrit, white blood cell count, creatinine, urine output, blood urea nitrogen, sodium, serum albumin, bilirubin, glucose [Knaus 1991].
The sepsis severity score consists of scoring the function of five organ systems (lungs, heart, liver, kidneys, intestine) and of the blood coagulation on a scale of 0 to 5 of which 5 reflects the worst situation. Eventually the three highest of these six scores are squared and summated resulting in the sepsis severity score (minimum 0 and maximum 75) [Stevens 1983]. The presence of SIRS includes two or more of the following clinical manifestations: (1) body temperature greater than 38°C or less than 36°C; (2) heart rate greater than 90 beats per minute; (3) tachypnea, manifested by respiratory rate greater than 20 breaths per minute, or hyperventilation as indicated by PaCO2 of less than 32 mm Hg; and (4) alteration in the white blood cell count, such as a count greater than 12,000/cu mm, a count less than 4,000/cu mm, or the presence of more than 10 percent immature neutrophils [Bone 1992].

Electrodiagnostic studies before, at time of diagnosis and during follow-up (n=98):
Electrophysiological investigations were performed at day 4, 11 and 25 after start of the artificial respiration. The examination consisted of conventional orthodromic motor (ulnar and peroneal) and antidromic sensory (ulnar and sural) nerve conduction studies. The compound muscle action potential (CMAP) was recorded using a surface tendon-belly montage over the abductor digiti minimi for the ulnar nerve and over the extensor digitorum brevis in case of the peroneal study. The ulnar nerve conduction study was performed at the wrist, below and above the elbow and for the peroneal nerve at the ankle, fibular head and at the knee. Using this method pressure

palsies of the ulnar or peroneal nerve could be excluded. Furthermore repetitive nerve stimulation at 3 Hz with train counts of six of the ulnar nerve and concentric needle electromyography (EMG) of the biceps, abductor digiti quinti, quadriceps femoris and tibialis anterior muscle were performed. Spontaneous muscle fiber activity scored in rest and; motor unit potential on voluntary activation was described as normal, neuropathic, myopathic, or unclassifiable for testing.
Once patients fulfilled the clinical and electrophysiological criteria of CIPNM a somato-sensory evoked potential were performed of the median nerve stimulating at the wrist and tibial nerve stimulating at the knee. Data from the conduction studies, repetitive stimulation, motorscore and tendon reflexes were used for analysis only if no vecuronium (neuromuscular blocking agent) was administered.

Muscle biopsy:
In 30 patients an open biopsy from the quadriceps muscle was taken from the leg opposite to the site on which the needle EMG was performed. Standard light microscopic analysis was used to classify the muscle tissue as myopathic, neuropathic or showing both characteristics (mixed).

Statistical analysis;

Part I: Clinical features
The analysis was performed to describe the clinical features of CIPNM. Therefore the motor sum score, sensory symptoms, muscle wasting and tendon reflexes were judged over time until discharge from the hospital or death. The statistical analysis of the motor sum score and tendon reflexes was performed only if patients did not use vecuronium for at least three days prior to analysis.

Part II: Electrophysiological features
From the conduction studies the compound muscle action potentials (CMAP) and conduction velocities of the ulnar and peroneal nerve, the sensory nerve action potentials (SNAP) and conduction velocities of the ulnar (sensory) and sural nerve measured for all patients were interpolated to predefined days. Therefore the scale t= -10, 0, 10,20,30 and 40 days was used of which t=0 stood for time of diagnosis of CIPNM. Besides for the total group of CIPNM

patients, analysis of the conduction study was also performed for each motor sum score group separately. For the patients without CIPNM the values of the distal CMAP amplitudes and the SNAP amplitudes of the ulnar and sural nerve were determined on day 4, 11 and 25 of the artificial respiration. For the EMG the presence of spontaneous muscle fiber activity and changes in the motor unit potentials were analyzed on t=-1 (time before diagnosis CIPNM), t=0 and t>1 (time after diagnosis CIPNM) using chi-square test. The CMAP amplitude of the ulnar nerve recorded after wrist stimulation was correlated with the presence of spontaneous muscle fiber activity using the random effects logit model. This was also performed for the sum score of the CMAP amplitude of the ulnar nerve (wrist) and the peroneal nerve (ankle).
The statistical analysis data of the CMAP amplitudes were performed only if patients had stopped using vecuronium for at least three days.

Part III: assessment of prognostic factors

For the planned analysis of the prognostic factors of CIPNM the motor sum score was used for further correlation. After fifty days of follow-up the CIPNM group showed different subgroups of patients defined as:

1) Recovered CIPNM with no functional disability and motor sum score ≥ 26. The score of 26 was chosen because a lower score meets the clinical criteria of CIPNM [De Letter 2001].
2) The motor disability group with motor sum score between 10 and 26 and
3) A group with early mortality and therefore period of follow-up that was too short to perform statistical analysis.

The motor sum score groups 1 and 2 were used for further correlation with the presence of sensory symptoms, muscle wasting and improvement of tendon reflexes using Pearsons chi-square test. This was also performed for age, sex, duration of artificial respiration, Apache-III score, sepsis severity score, SIRS, the standard light microscopical changes of the muscle biopsies and the total amounts of vecuronium and steroids used on day seven of the artificial respiration.
A comparison was made of the creatinine phosphokinase in the serum of the CIPNM patients with the 66 patients without CIPNM.
The results of the standard light microscopy (neuropathic, myopathic or mixed changes) of the muscle biopsies were correlated with the presence of spontaneous activity in the muscle fibers using the random effects logit model.

RESULTS

Part I: Clinical features

The general characteristics of 21 (group 1 and 2) of the 31 patients with CIPNM are listed in table 1. Seventeen (55%) of the 31 CIPNM patients died in comparison to 19 (29%) of the 66 patients that did not develop CIPNM (P=0.01). There was full recovery in 6 (20%) and persistent disability in 15 (48%) of the 31 CIPNM patients after 50 days of follow-up. All patients showed flaccid quadriplegia, the minimum motor sum score was zero. Follow-up of the motor sum score during the first month of the stay on the ICU is shown in the figures 1a-b. For all 31 patients the mean duration of the artificial respiration was 21 days. The mean duration in the recovered CIPNM group and the motor disability group was 14 and 23 days respectively (Table 1). In group 1 all patients were alive after one month on the ICU, in groups 2 mortality was 7/15 (47%) (P =0.06).

Patients did not mention sensory complaints during the monitoring. The presence of muscle wasting and recovery of tendon reflexes showed no significant differences between the motor sum score subgroups 1 and 2.

In the CIPNM patients the mean serum creatinine phosphokinase was 23 and in the group of patients that did not develop CIPNM 27 mmol/l. (not significanty different).

Part II: Electrophysiologic features

Follow-up of the CMAP (Fig 2a-b) and SNAP amplitude after stimulation of the ulnar nerve showed a decrease towards t=0 (time of diagnosis CIPNM) and a gradual increase afterwards for the motor sum score subgroups 1 and 2. The minimum CMAP amplitude of the abductor digit quinti muscle after stimulation at the wrist for the MRC sum score groups 1 and 2 were 3.52 mV and 2.69 mV respectively. In the patients that did not develop CIPNM (n=66) on day 4, 11 and 25 of the artificial respiration this was 5mV, 5 mV and 8 mV respectively. For the CMAP amplitude after stimulation of the peroneal nerve and the SNAP amplitude after stimulation of the sural nerve on every interpolated moment in time decreased values were found (Table 2). The mean CMAP amplitude of the extensor digitorum brevis muscle after ankle stimulation in the patients that did not develop CIPNM (n=66) on day 4, 11 and 25 of the artificial respiration was 1mV, 2 mV and 2 mV

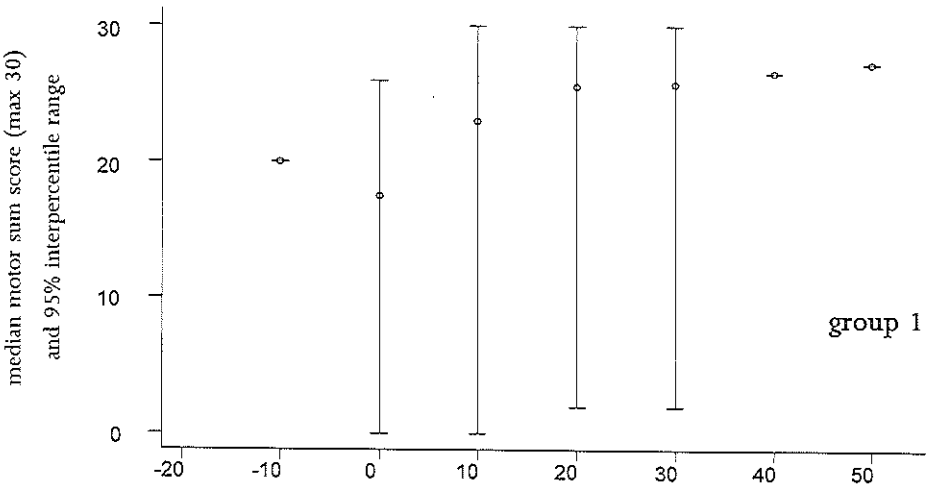

Figure 1a. T= 0; time of diagnosing CIPNM (days)

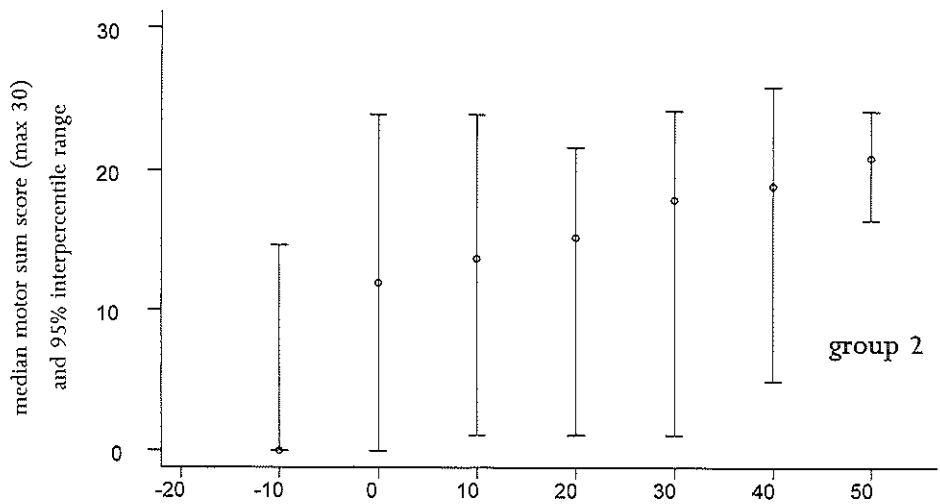

Figure 1b. T= 0; time of diagnosing CIPNM (days)

Table1: Clinical features of patients suffering from CIPNM (n = 31).

	MRC-score ≥ 26	MRC-score ≥ 10 - < 26	Overall P-value
	Group 1	Group 2	
Motorscore in time	6 (20%)	15 (48%)	--
Numbers of deaths	0	7 (47%)	0.06
Muscle wasting	5/6 (83%)	14/15 (93%)	0.50
Recovery tendon reflexes	4/6 (67%)	8/15 (53%)	0.66
Age (years)	60	68	0.48
Sex (M/F)	4/2	10/5	1.00
Duration artificial respiration (days)	14	22	0.17
Apache-III score	79	8	0.26
Sepsis severity score	25	19	0.56
Presence SIRS	4 (67%)	7 (47%)	0.41
Use of vecuronium until day 7 (x patients)	2	7	0.66
Use of steroids until day 7 (x patients)	0	5	0.26
Number muscle biopsies	5	15	
Standard light microscopy:			0.21
- myopathy	4	5	11
- neuropathy	1	6	10
- mixed	0	4	8

respectively. Moreover these 66 patients showed a decreased median SNAP amplitude after stimulation of the ulnar and the sural nerves. On day 4, 11 and 25 of the artificial respiration the values for the ulnar nerve after wrist stimulation were 10.63 μV, 10.07 μV and 9.33 μV. For the sural nerve these values were 5.57 μV, 4.10 μV and 6.60 μV respectively.

Conduction velocities of the ulnar nerve, peroneal nerve (until t= 20 days in the distal part of the lower leg) and sural nerve were normal during the period of monitoring on the intensive care unit. In one patient evidence was found for a superimposed pressure palsy of the peroneal nerve across the

fibular head showing a significant decrease of the conduction velocity. Repetitive stimulation of the ulnar nerve showed no abnormal decrement. The EMG of the biceps muscle showed no spontaneous muscle fiber activity, however for the abductor digiti quinti, quadriceps femoral and anterior tibial muscle spontaneous muscle fiber activity increased significantly over time. At the time of the first needle EMG the abductor digiti quinti and anterior tibial muscle already showed spontaneous activity of the muscle fibers. In the quadriceps femoral muscle such early spontaneous activity was found in 2 of 25 patients. From the second needle EMG on spontaneous activity of the muscle fibers was present in the abductor digiti quinti and anterior tibial muscle in 14 of 28 patients (P= 0.003) and for the quadriceps femoral muscle in 9 of 28 patients (P=0.031). During EMG monitoring this activity disappeared in a proximal to distal gradient. The analysis showed no relationship between the CMAP amplitude of the ulnar nerve and the presence of spontaneous activity of the muscle fibers. Polyphasic motor unit potentials were present in all muscles examined without signs of a proximal-distal gradient in time.
Neuropathic or a combination of neuropathic and myopathic changes in the standard light microscopy of the muscle biopsies was related with the presence of spontaneous activity of the muscle fibers (P=0.029) and polyphasic motor unit potentials with increased duration (P=0.037).
Somato-sensory evoked potentials were normal in all 22 CIPNM patients that were tested.

Part III: Assessment of prognostic factors using subgroups 1 and 2.
In both motor sum score groups muscle wasting and recovery of tendon reflexes were present and did not correlate with the clinical severity of CIPNM. The outcome did not relate to age, sex, duration of artificial respiration, Apache-III score, sepsis severity score, SIRS, the use of vecuronium or steroids until day seven of the artificial respiration and with the results of the standard light microscopical examination of the muscle biopsies. Although no significant relationship was found, age and Apache-III score were higher in group 1 compared to group 2.

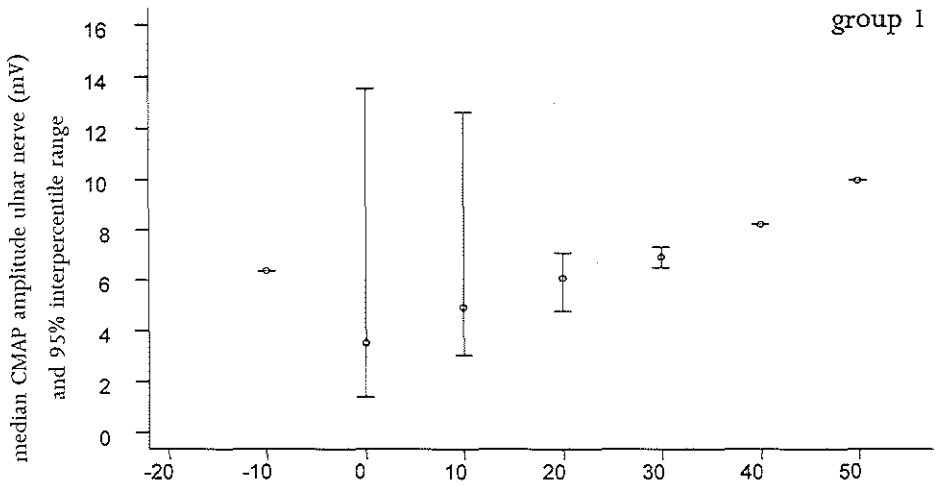

Figure 2a. T= 0; time of diagnosing CIPNM (days)

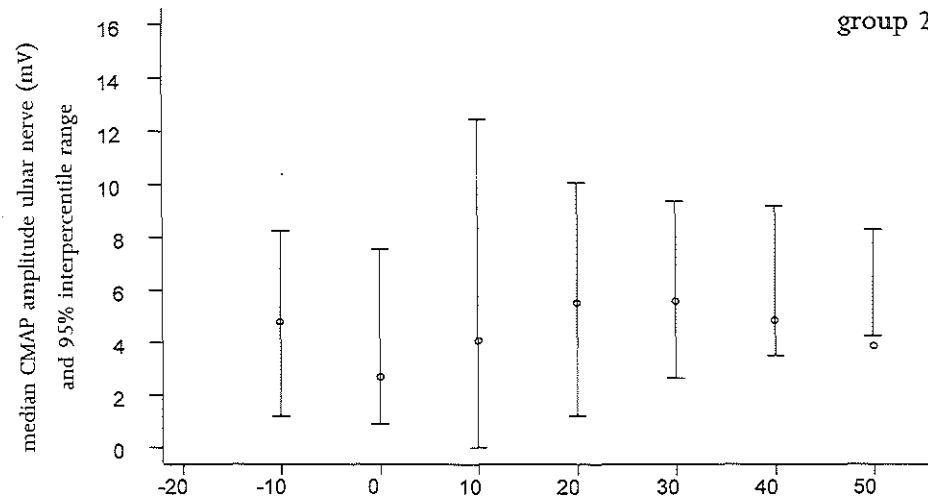

Figure 2b. T= 0; time of diagnosing CIPNM (days)

Table2: Interpolated data of the conduction studies of 31 patients suffering from CIPNM. t= 0, is time diagnosis CIPNM.

	t=-10 (days)	t=0 (days)	t=10 (days)	t=20 (days)
CMAP amplitude abd dig v muscle (mV)				
-wrist	4.03	2.69	4.08	5.81
-below elbow	3.26	2.44	3.25	4.70
-above elbow	2.77	1.94	2.70	4.79
CMAP amplitude abd dig br muscle (mV)				
-ankle	0.22	0.40	0.57	0.33
-fibular head	0.15	0.27	0.42	0.29
-above knee	0.18	0.19	0.49	0.42
Sum CMAP amplitude abd dig v muscle (wrist) & abd dig br muscle (ankle) nerve (mV)	4.87	3.34	4.19	5.81
Conduction Velocity ulnar nerve (m/s)				
-wrist-below elbow	50.19	47.84	49.98	52.69
-across ulnar sulcus	42.58	46.80	50.00	50.00
Conduction Velocity peroneal nerve (m/s)				
-ankle-fibular head	39.98	37.70	36.94	27.90
-across fibular head	36.22	37.34	44.85	34.16
SNAP amplitude ulnar nerve (microV)				
-wrist	7.26	6.64	9.01	11.64
SNAP amplitude sural nerve (microV)				
-lower leg	1.85	2.74	5.89	5.06
Conduction Velocity sural nerve (m/s)				
-ankle-lower leg	38.93	35.43	33.66	35.28

DISCUSSION

Part I: Clinical features

Development of CIPNM

This study shows that most patients were detected early in the course of the disease and some died already within the first two weeks of follow-up. These patients may be missed in earlier studies, since EMG was usually performed at a later stage. During the monitoring testing of motor function was influenced by severe underlying illness, the use of sedatives and neuromuscular blocking agents. This underlines the role of the electrophysiological study regarding the early detection of CIPNM.

Recovery of the tendon reflexes and the presence of muscle wasting are important features of CIPNM without significant differences in the motor sum score groups. Zifko et al observed muscle wasting in 1/3 of the 62 patients suffering from CIPNM [Zifko 1998]. We found that testing of tendon reflexes is not a useful tool in the early diagnosis of CIPNM because of the use of neuromuscular blocking agents in the acute phase of critical illness. Patients did not mention sensory complaints during clinical monitoring, however prior to diagnosis of CIPNM sedation or the severity of the underlying illness could cause these symptoms to remain unnoticed. Particularly if the presence of such disturbances occurs in only a short period of time or is of minor severity compared with motor symptoms. Zifko et al [1998] also found that in 17 (27%) of the 62 patients with CIPNM reliable information on sensation could be obtained. Ten of them had distal, symmetric hypesthesia. Berek et al also described the difficulties to clinically confirm sensory symptoms [Berek 1996].

As expected, determination of the serum creatinine phosphokinase did not differ significantly with the patient group that did not develop CIPNM. Therefore the serum creatinine phosphokinase appears not useful in diagnosing CIPNM and should be tested only when an acute myopathy is suspected.

Prognosis and recovery of CIPNM

The outcome of the CIPNM patients is related directly to the prognosis of the underlying critical illness. Since the prognosis of patients with multiple organ failure is poor; a significant number of the patients (about 50-60%) will die

from the underlying critical illness. Previous reports on the prognosis of CIPNM are rather optimistic [Hund 1996, Leijten 1995], the overall mortality rates vary in prospective studies from 36% [Leijten 1995] to 55% in our study. The patients who survive usually recover from critical illness polyneuropathy and it has been stated that the long-term outcome is good. In five patients from the motor disability group who were willing to inform us about their functional status disability had recovered one year after discharge from the hospital however clinical and electrophysiological details are lacking. Leijten et al [1995] found that 5 of 8 (63%) patients with delayed recovery beyond 4 weeks after discharge from the ICU still had a persistent motor handicap after one year. Zifko [2000] studied eleven of 13 surviving patients with CIP 1-2 years after the onset of the disease. Five of them had signs of mononeuropathy and quality of life was seriously impaired. Their electrophysiological features revealed abnormalities in the motor, sensory and phrenic conduction studies. De Seze et al [2000] analyzed the critical care conditions and 2-year clinical follow-up of 19 patients who suffered from severe forms (quadriplegia or quadriparesis) of CIP. Characteristics of patients who recovered clinically were compared with those of patients who did not. Two patients died within 2 months, 11 recovered completely, 4 remained quadriplegic and 2 remained quadriparetic. All patients suffered from sepsis, MODS and a catabolic state before the onset of CIP. Outcome appeared difficult to predict with clinical or electrophysiological data. Three variables were significantly correlated to poor recovery: longer length of stay on the critical care unit, duration of sepsis and loss of body weight.

Part II: Electrophysiological features

The electrophysiological data show both motor and sensory axonal dysfunction in the upper and lower extremities. Clinically the motor dysfunction is much more evident. Previous prospective studies also found such electrophysiological features [Latronico 1996, Witt 1991, Zochodne 1987, Coakley 1993], however the findings of Hund et al [1997] revealed normal SNAP amplitudes. As the SNAP amplitudes after stimulation of the ulnar and sural nerve in the 66 patients without CIPNM were also decreased, the sensory findings in the CIPNM and the non-CIPNM patients should be considered as unreliable in the diagnosis of CIPNM. Furthermore we note that electrophysiological follow-up of the lower extremities was often technically difficult and unreliable due to edema. This explains the lack of the

pattern, which was found during follow-up of the CMAP amplitude after stimulation of the peroneal nerve or the sum CMAP amplitude of the abductor digiti quinti and abductor digitorum brevis muscle. Therefore the motor conduction study of the upper extremities should be primarily used in diagnosing patients that are at risk for developing CIPNM. The pattern of the CMAP amplitudes in time (until t= 50 days) as mentioned above showed complete recovery in the recovered CIPNM group and decreased values in the motorscore disability group. The EMG of CIPNM patients contained the most pronounced spontaneous muscle fiber activity in the distal muscles with a proximal to distal gradient during recovery of the clinical and electrophysiological dysfunctions. Together with the results of the conduction study these were mainly features of an axonal (senso) motor polyneuropathy. The latter was sustained by the correlation of spontaneous muscle fiber activity and the presence of neuropathic changes or both neuropathic and myopathic changes in the standard light microscopical examination of the muscle biopsies. The EMG signs of acute spontaneous muscle activity are one of the features of CIPNM [Hund 1996]. The problem of testing myopathic changes of the motor unit potential is mainly due to the inability of voluntary contraction in these patients. Mostly the myopathic changes in CIPNM are found in the muscle biopsies [Latronico 1996, De Letter 2000a, Op de Coul 1991]. To discriminate neuropathy from myopathy by means of the EMG Rich et al [1997] already described the use of direct muscle stimulation. In case of myopathy the muscle remains electrically inexcitable. At the time of our study this method was not yet available.

During CIPNM no dysfunction of the neuromuscular junction was found, reflected in the normal response after repetitive nerve stimulation. Nor are there significant disturbances of the spinal dorsal columns in CIPNM as the somato-sensory evoked potentials were normal.

Part III: Assessment of prognostic factors using subgroups 1 and 2.

The clinical variables age, sex and duration of the artificial respiration showed no relation with the motor sum score groups. Furthermore the severity of critical illness reflected in the Apache-III score, the sepsis severity score or the presence of SIRS was not related with these groups either. We however note the gradual increase of the Apache-III score in the relatively small subgroups from the recovered CIPNM group (1) towards the motor

disability group (2). Thusfar these parameters only play a role as risk factors [Bolton 1996, De Letter 2001]. The use of vecuronium or steroids did not correlate with the severity of CIPNM.
Nor did the specific structural changes in the muscle biopsies of the patients correlate with the motor sum score groups. Earlier we mentioned the lack of a relation between the use of medication as vecuronium, steroids and midazolam with the standard light microscopical findings [De Letter 2000a].
Only few data from prospective studies provide suggestions that might influence the natural history of CIPNM. Therapeutically the importance of an early start of rehabilitation therapy in CIPNM patients has been described [Leyten 1995]. De Seze et al [2000] described the apparent relationship between the severity of CIP and that of sepsis and its associated hypercatabolism. The authors recommended aggressive measures against sepsis to limit CIP and its sequelae. We also emphasize the possibility of therapeutic strategies in CIPNM. As local (muscle tissue) low-level immune activation was demonstrated to play a role in CIPNM, immune therapy should be considered. Previous results from treating patients in the early phase of sepsis with immunoglobulins intravenously were promising, the incidence of CIPNM decreased dramatically [Mohr 1997]. This study was however retrospective and could have suffered from a selection bias. We suggest that patients at risk for developing CIPNM may be better off with immunoglobulin treatment, whereas treatment after CIPNM has developed appeared to be unsuccessful [Wijdicks 1994]. The early treatment option should however be tested in a proper trial.

IV

Immune activation in CIPNM

IVa How critical illness may trigger a powerful immune cascade in patients in the intensive care unit

IVb Immune activation in the muscle tissue of patients with CIPNM

IVa HOW CRITICAL ILLNESS MAY TRIGGER A POWERFUL IMMUNE CASCADE IN PATIENTS IN THE INTENSIVE CARE UNIT

Muscle biopsies from patients in the intensive care unit are yielding important new clues to the basic mechanisms involved in their critical illness polyneuropathy and myopathy. By discovering what drives the disease process, new investigations could have a sharp impact on therapies of the future.

Recently, progress has been made in the understanding of the basic mechanisms involved in critical illness polyneuropathy and myopathy (CIPNM). This is of great importance as polyneuropathy and myopathy are frequently encountered in patients in the intensive care unit. Despite this progress, however, the pathophysiological basis of this entity is largely speculative and involves histological and serologic analysis of these disorders. Although many explanations for the polyneuropathy and myopathy associated with critical illness initially looked promising, investigators have failed to link such neuromuscular problems with the extensive use of neuromuscular blocking agents, steroids, aminoglycoside antibiotics, and specific nutritional deficiencies. New studies include critical illness as the likely cause of a cascade of inflammatory events culminating in nerve and muscle disease. Biochemical changes are well-recognized manifestations of the sepsis and multiple-organ failure syndrome [Bolton 1993d]. Such disturbances and the accompanying immunological activity in the microcirculation of various organs in sepsis could contribute to a growing appreciation of mechanisms that could underlie a primary axonal degeneration. These recent studies have focused on the fact that blood vessels supplying peripheral nerve lack auto-regulation, rendering them particularly susceptible to such disturbances [Bolton 1996]. As this recognition has gained favour, researchers have directed their attention to the series of events leading to the influx of potentially inflammatory cells, including monocytes/macrophages, neutrophils, and CD4+ (helper T) cells. As part of this hypothesis, renewed attention has also been given to cytokines because cytokines secreted in sepsis have histamine-like properties that may increase microvascular permeability. Cytokines have important functions in inflammatory processes, as both up-regulating and down-regulating factors and are likely to participate in the inflammatory process of myopathies [Lundberg1997]. The inflammatory

cascade in inflammatory myopathies has also been described in a number of recent reports, notably by Dalakas [1995] who suggests that when activated, the CD8+ cells (cytotoxic T cells) recognize antigens presented in the context of MHC class 1 molecules whereas the CD4+ cells recognize antigen presented by the MHC class II-bearing cells. Some of the CD4+ cells can interact with the B-lymphocytes via various lymphokines to produce specific antibodies.

NEUROLOGICAL AUTOIMMUNE DISEASE: HOW THE CASCADE BEGINS

Generally triggering of an immune response occurs in the circulation and lymphoid tissues but outside the nervous system. T cells play a key role in the regulation of these immune responses but also as effector cells generated during the response. Dysregulation occurs in T cells that normally are self-tolerant. Once they no longer become self-tolerant, they are activated and capable of recognizing various auto-antigens within the nervous system [Dalakas 1995]. Although the nature of these molecules is largely unknown and consequently, the antigenic specificity of these auto-reactive T cells has yet to be determined, the following key processes are proposed to be involved (Fig 1):

- *Cytokines.* During a viral or bacterial infection cytokines are released as a consequence of the induced immune response and play an important role in changing T cell tolerance to self-antigens. When an auto-antigen in the circulation is presented to a T cell by the antigen-presenting cells such as macrophages in the lymphoid organs, these macrophages release certain cytokines (interleukin IL-6, IL-1) that are able to stimulate T cells. Generally, quiescent auto-reactive T cells lose their tolerance to the self-antigens and become activated by the combined presence of the antigen and the cytokines. Even if the infectious agent is missing from the T cell, the released cytokines provide enough stimulation to activate these T cells to respond to auto-antigens present on the target cells, e.g. muscle fibers, neurons.

- *Infection triggers molecular mimicry.* The concept of molecular mimicry may explain what occurs when an infectious agent shares epitopes with host antigens. Viral or bacterial antigens sharing epitopes with the central or peripheral nervous system induce an immune response consisting of antibodies and sensitized T cells specific for the infectious agent but also cross reacting with auto-antigens. In dermatomyositis, for example, it has been postulated that an auto-antibody or immune-complex-mediated response against vascular endothelium might be the primary pathogenic mechanism. Also essential to understanding the pathophysiology of this disease is the deposition of membrane attack complex (MAC) due to the activated complement cascade on the vascular endothelium. Secondary to microvascular damage is the influx of potentially inflammatory cells, like monocytes/macrophages, neutrophils, and CD4+ helper T cells.

- *T helper cells.* Naive CD4+ / helper T lymphocytes (Th) develop into functionally mature effector cells upon stimulation with relevant antigenic peptides presented on the major histocompatibility complex (MHC) class II molecules by antigen-presenting cells (APC). Based on the characteristic set of cytokines produced, Th cells are commonly segregated into at least two different subpopulations: Th1 cells and Th2 cells. These Th1 and Th2 subsets appear to be extremes in cytokine production profiles and within these polarized subsets, individual Th cells exhibit differential rather than coordinated cytokine gene expression. These subsets develop from common Th precursor cells (Thp) after triggering with relevant peptides into Th0 cells producing an array of cytokines, including IL-2, IL-4, IL-5 and IFN-γ. These activated Th0 cells subsequently polarize into the Th1 or Th2 direction based on the cellular and cytokine composition of their microenvironment. Antigen-presenting cells like the various subsets of dendritic cells besides subsets of macrophages largely determine this polarization into Th1 or Th2 subset development. The Th1-Th2 subsets appear to cross regulate each other's cytokine production profiles, mainly through IFN-γ and IL-10, and from this concept it was rationalized that disturbances in the balance between these two subsets may result in different clinical manifestations. IL-12 is a dominant factor promoting Th1 subset polarization and dendritic cells and macrophages produce IL-12. Moreover, IL-12 induces IFN-γ production by T cells and natural killer (NK) cells. Recently, it was reported that IL-18 acts synergistically with IL-12 to

induce Th1 development [Tews1996]. Polarization of Th2 cells is critically dependent on the presence of IL4 produced by T cells or basophils and mast cells. APC-derived IL-6 has also been shown to induce small amounts of IL-4 in developing Th cells. IL-10 and APCderived prostaglandin E_2 (PGE_2) inhibit IL-12 production and Th1 priming [Nakamura 1997]. The Th1-Th2 paradigm has been useful in correlating the function of Th1 cells with cell-mediated immunity (inflammatory responses, delayed type hypersensitivity, and cytotoxicity) and Th2 cells with humoral immunity. In general, among infectious diseases, resistance to intracellular bacteria, fungi, and protozoa is linked to mounting a successful Th1 response. Th1 responses can also be linked to pathology, like arthritis, colitis and other inflammatory states. Effective protection

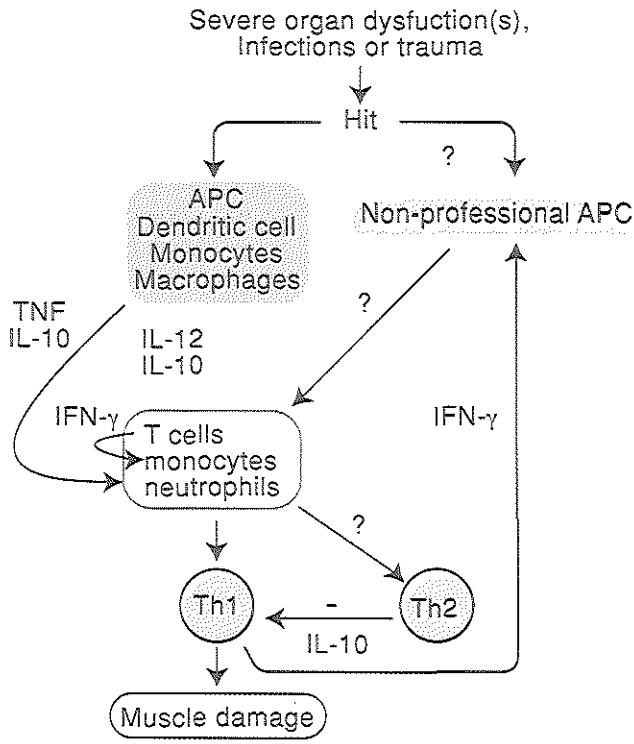

Fig 1. Hypothetical representation of possible relevant interactions in the immunopathology of CIPNM. An as yet unidentified "hit" influences the activity of antigen-presenting cells (APC), including presentation of (auto) antigens to specific T cells and cytokine production. As a result, the Th1-Th2 balance will be disturbed, leading to activity of the Th1 cells and possible stimulation of muscle cells to act as (non-professional) APC. – = inhibiting effect; ?= as yet unresolved issue.

THE IMPACT OF NEW FINDINGS: CLUES TO THE PATHOPHYSIOLOGY OF CIPNM

The new results have added clues for an immune-mediated process in critical illness polyneuropathy and myopathy. Inflammatory infiltrates found in the biopsies of these patients presented as either small clustered infiltrates or isolated inflammatory cells and were comprised mainly of macrophages and CD4+ lymphocytes, not CD8+ cells or B cells. The evidence for IL-1, TNFαR75 and IFN-γ in muscle fibers lends support to previous work by Tews et al [1996] who suggested that the expression of these cytokines by muscle fibers in inflammatory myopathies may induce and mediate the process of autoimmunization and antigen expression without the primary presence of inflammatory cells. Our finding of IL-12 is also important

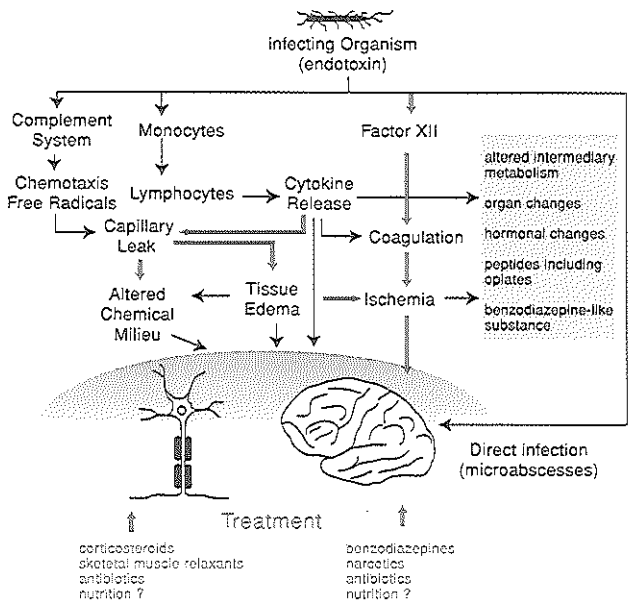

Fig 3.- Possible mechanisms for septic encephalopathy and critical illness polyneuropathy. Arrows pointing to the curved line indicate mechanisms that may apply to both central and peripheral nervous system. Lower arrows designate treatments that may affect these systems independently. The heavy arrows highlight the most likely mechanisms. Release of cytokines from macrophage and from T lymphocytes may directly affect the brain or act directly on the blood-brain barrier and microcirculation. Encephalopathy may also be due to the failure of other organs or to direct infection of the brain with formation of microabscesses. Critical illness polyneuropathy may be due to disturbance of microcirculation of peripheral nerve through effects similar to those in brain. Various treatment may play a role as well in triggering pathophysiology.

because this cytokine could be a powerful activator of the immune cascade and induces the proliferation of T cells. Overall, the immunological findings support the hypothesis that local low-level immune activation seems to be important in patients with CIPM. These critically ill patients suffer from severe organ dysfunction, tissue damage or severe infections resulting into sepsis or multiple organ dysfunction syndrome (MODS). All these factors could trigger immune activation eventually leading to damage of the nervous system. Hopefully new data will confirm and expand upon the results obtained so far. Armed with this information, investigators will be able to tackle the issue of tailoring therapeutic strategies with more confidence because they understand the basic mechanisms involved (Fig3).

IVb IMMUNE ACTIVATION IN THE MUSCLE TISSUE OF PATIENTS WITH CIPNM

INTRODUCTION

Critical illness polyneuropathy and myopathy (CIPNM) can be encountered in critically ill patients during or after (prolonged) artificial respiration. The clinical features are difficulty in weaning from the ventilator, generalized wasting of the limbs with severe tetraparesis, hypo- or areflexia and prolonged rehabilitation. Electromyographical examination reveals abundant denervation potentials compatible with an axonal polyneuropathy and often low and short amplitudes compatible with a myopathy [Bolton 1984, Op de Coul 1985, Zochodne 1987]. On clinical and electrodiagnostic grounds neuromuscular complications in the critically ill patients may be due to a polyneuropathy or myopathy. Since it is not always possible to differentiate between an axonal motor neuropathy and myopathy, we prefer to use the term critical illness polyneuropathy or myopathy (CIPNM).

Severe cases of CIPNM should be distinguished from the acute motor axonal variant of Guillain-Barré syndrome. Other diagnostic considerations include damage to anterior horn cells resulting from acute or chronic spinal cord ischemia and acute motor neuropathy associated with administration of nondepolarizing neuromuscular blocking agents [Hund 1997]. The following conditions have been associated previously with CIPNM: prolonged artificial respiration with the usage of neuromuscular blocking agents, systemic inflammatory response syndrome (SIRS), multi organ failure (MOF), and treatment with high dose steroids or with aminoglycosides [Bolton 1993d, Bolton1996]. But the etiology of CIPNM has not been elucidated thusfar.

We have postulated that cytokines such as TNF-α and IL-6 may be involved in the pathophysiology of CIPNM, but no convincing evidence was found after analyzing their serum [Verheul 1994]. However, there may well be a local process of immune activation. Therefore in this study the presence of immune activation was investigated in the muscle tissue. Bazzi et al studied muscle biopsies of two patients with an electrophysiological diagnosed as

critical illness myopathy. Immunohistochemical analysis revealed a positive reaction for HLA-I and staining for membrane attack complex (MAC). In one muscle biopsy single lymphocytes were present but no cellular infiltrates. They concluded that their patients could be affected by an autoimmune-inflammatory myopathy [Bazzi 1996].
In a large prospective study we analyzed the incidence of this neuromuscular disorder and investigated the hypothesis whether local immune activation in muscle tissue could play a role in the development of CIPNM.

METHODS

Patients

From May 1994 to July 1996 98 patients were included in a longitudinal prospective observational study held at the intensive care unit of the St. Elisabeth Hospital Tilburg. The medical ethical committee (MEC) of the hospital approved the study and a written informed consent was obtained from the family of the patient.
Patients could be included if they needed at least three days of artificial respiration. All patients included in the study were monitored clinically and neurophysiologically for the development of CIPNM. Exclusion criteria for follow up in this study were the presence of the Guillain-Barré syndrome, acute or chronic spinal cord lesion, hypophosphatemia, myasthenia gravis and a history of polyneuropathy or myopathy. In addition patients younger than 15 years of age were not admitted.
This neurological examination was performed twice weekly and neurophysiological monitoring on day 4, 11 and 25 after starting artificial respiration. Clinically CIPNM was defined as severe tetraparesis with wasting of the limbs and hypo- or areflexia at least three days after the use of neuromuscular blocking agents. The severity of the paresis consisted of a MRC sum score < 26 (maximum 30) of the three upper and three lower limb muscles that were tested. The neurophysiological study consisted of conventional motor (ulnar and peroneal nerve) and sensory nerve (ulnar and sural nerve) conduction studies. Further repetitive nerve stimulation at 3 Hz of the ulnar nerve and monopolar needle electromyography of two muscles

of the upper and lower limb respectively (EMG). In cases of CIPNM this study shows an axonal polyneuropathy with or without signs of a myopathy. This results in a reduction of the compound motor action potential (CMAP) and the sensory nerve action potential (SNAP) and only a mild reduction of the conduction velocities. The needle electrode study shows signs of acute denervation with fibrillation potentials and positive waves in the resting muscle. In those cases of critical illness myopathy the motor unit potentials have low amplitude and a short duration. To define a patient as having CIPNM the clinical and the neurophysiological descriptions as mentioned above had to be met.

In 30 of the 32 patients with CIPNM and 2 of the 66 patients without CIPNM controls an informed consent was obtained to perform an open biopsy from the quadriceps femoral muscle. This was taken at least three days after stopping treatment with neuromuscular blocking agents and in cases of CIPNM within 3 days after the diagnosis. No permission was given by the MEC to perform a nerve biopsy. The two control patients also needed artificial respiration, they were severely ill, like the CIPNM group of patients, and were therefore matched controls. They did not show clinical or neurophysiological signs of CIPNM. One of the control patients suffered from Goodpasture's disease, the other from severe hyperglycemia. To avoid artefacts the biopsy was always taken from the leg opposite to the site at which the EMG was performed. The biopsies were frozen and stored at -70° C until analysis. The immunohistochemical analysis was performed at the Erasmus University Rotterdam.

Standard light microscopy and immunohistochemical analysis

Standard light microscopic and immunohistochemical analyses were performed. For the latter, a streptavidin-biotin method was used with a broad panel of antibodies [Hoefakker 1995]. Briefly, cryostat sections (6μ) were fixed for 10 minutes in buffered formaline 10% pH 6.9. After blocking using human serum the slides were incubated with a monoclonal antibody. All reagents were titrated to achieve optimal results. Subsequently the slides were incubated with biotinylated goat anti-multilink (Biogenex) containing normal human serum 2% and normal goat serum 2%. Enzyme detection was carried out after incubation with streptavidin biotin alkaline phosphatase. First napthol as-mx (Sigma) 18 mg and TRIS-HCL 60 ml pH8.0 (Sigma) and secondly fuchsine 200 μl and sodium nitrate 200 μl were mixed. Finally

levamisole 15 mg (Sigma) was added. After staining the reaction was stopped and counterstaining with haematoxylin was performed.
The mouse monoclonal antibodies (MoAb IgG) as listed in table 1 were used. For immunohistochemical analysis of cytokines we used the MoAb VHP20 recognizing human IL-1β, a rat MoAb 2-4A1 recognizing human IL-12 p40 and a rat MoAb recognizing human IFN-γ, all of the IgG class. Additionally a rabbit polyclonal antibody recognizing human TNFαR75 was used.
Two different controls for our staining method were performed. First, control staining of the sections with an isotype-matched control antibody without the primary antibody. In these controls no staining was seen. Secondly, positive and negative tissue controls were used to verify the specificity of the staining in every staining procedure. The negative control was a muscle biopsy of the quadriceps femoral muscle from a healthy subject. A muscle biopsy from a patient with dermatomyositis served as a positive control for the analysis of CD4, CD8, CD20, CD68, E-selectin, ICAM, VCAM, HLA-I, HLA-DR and TNFαR75. Muscle tissue from a patient with polymyositis was used as a positive control for MAC and IL-12. For IL-1β and IFN-γ a skin biopsies from patients with psoriasis and for E-selectin a human tonsil served as a positive control.

Interpretation of the biopsies

Scoring of the standard light microscopic and the immunohistochemical analysis was performed separately by a neuropathologist and two experienced neurologists. The classification was carried out without knowledge of the clinical or laboratory data of the patients or the controls. Thereafter the results were discussed. The biopsies were classified as primary neuropathic, myopathic or mixed as defined by Dubowitz [1985]. For the immunohistochemical analysis the following classification was used; stainings were scored as negative (-)/ not present or positive (+)/ present.

Clinical findings

The neuropathological findings were compared with clinical and laboratory parameters using the erythrocyte sedimentation rate (ESR), creatine phosphokinase (CK) and the sepsis severity score in the CIPNM group at entry.
The sepsis severity score consists of scoring five organ systems (lungs, heart, liver, kidney, intestine) and the coagulation on a scale of 0 to 5 of which 5

Table 1: Listing of the antibodies used, including the dilution and their source.

	CD-marker	Dilution	Source
Inflammatory cells			
Th-cells	CD4	1:100	Becton Dickinson, Mountain View, CA
Tc-cells	CD8	1:75	Becton Dickinson, Mountain View, CA
B-cells	CD20	1:400	Biogenex, San Ramon, USA
Macrophages	CD68	1:300	DAKO, Glostrup, Denmark
Adhesion molecules			
Eselektin	CD62E	1:50	DAKO, Glostrup, Denmark
ICAM-1	CD54	1:25	Neomarkers, Fremont, USA
VCAM	CD106	1:25	DAKO, Glostrup, Denmark
Complement molecule			
MAC (C5b-9)		1:50	DAKO, Glostrup, Denmark
Proinflammatory cytokines			
IL-1 β		1:20	E. Claassen, Erasmus University Rotterdam,NL
IL-12		1:10	E. Claassen, Erasmus University Rotterdam,NL
IFN γ		1:40	W.A. Buurman, University Hospital Maastricht,NL
TNFαR75		1:25	W.A. Buurman, University Hospital Maastricht,NL
Anti-inflammatory cytokine			
IL-10		1:250	J. Laman, Erasmus University Rotterdam,NL
Antigen presentation			
HLA-I		1:2000	E. Claassen, Erasmus University Roterdam, NL
HLA-DR		1:100	Becton Dickinson, Mountain View, CA

reflects the worst situation. Eventually the three highest of these six scores are put to a square and summated resulting in the sepsis severity score (minimum 0 and maximum 75) [Stevens 1983].

Statistical analysis

The distribution of the standard light microscopic findings related to the results of the immunohistochemistry was compared using the Kruskal Wallis test. Percentages of the immunohistochemical findings in the CIPNM group (n=30) and the two control biopsies were compared with Fisher's exact test. Differences in ESR and sepsis severity score between the CIPNM patients and the 66 patients without CIPNM were tested with the Mann-Whitney test. This test was also used to analyze a possible pattern of the ESR and sepsis severity score in the neuropathological changes in the CIPNM group. P-values <0.05 were considered significant.

RESULTS

Incidence

CIPNM was diagnosed in 32 of the 98 (33%) patients who underwent prolonged artificial respiration, leaving 66 patients without CIPNM

Histology

Standard light microscopic examination showed neuropathic changes in 37% (11/30), myopathic changes in 40% (12/30) of the patients and both neuropathic and myopathic changes in 23% (7/30) of the patients, without overlap between the groups. Muscle fiber necrosis was only present in 30% (9/30) of the muscle biopsies, showing a sparse and scattered pattern. There was no relation between the presence of muscle fiber necrosis and the level of CK.

The effect of high dose steroids inducing pure myopathy with loss of type-II muscle fibers and fast myosine was present in one patient with an exacerbation of her COPD and was excluded from the CIPNM group.

Immunohistochemical analysis

Immunohistochemical analysis showed small infiltrates containing 10 to 20 inflammatory cells in 27% (8/30) of the biopsies. The perivascular infiltrates consisted of different celltypes all expressing CD68 and CD4 and less frequently CD8 (3% (1/30)) and were located within the perimysium. Additionally, isolated macrophages were seen scattered in the perimysium and near necrotic muscle fibers when present. Overall, macrophages (CD68) were found in 40% (12/30) of the biopsies (Fig. 1a). CD4 positive cells were present in 60% (18/30) in the perimysium, usually in the perivascular region and sometimes in the endomysium. CD20 positive B-cells could not be detected.

In all biopsies there was an upregulation of HLA-I and HLA-DR. HLA-I was strongly present on the surface of all nucleated cells compared with muscle biopsies of inflammatory myopathies (positive controls) and a normal muscle biopsy (negative control). HLA-DR was positive on the surface of the nucleus of the muscle fibers and on the vascular endothelium. In general infiltrates contained HLA-DR positive cells (Fig. 1b).

ICAM-1 and VCAM staining were positive in 58% (15/26) and 53% (16/30) of the biopsies respectively on the peri- and endomysial vascular endothelium (Fig. 1c). On the other hand E-selectin staining was negative in all CIPNM biopsies. It however was positive in the tonsil.

MAC was present in 79% (21/28) of the biopsies being found mainly on the vascular endothelium and on necrotic muscle fibers when they were present (Fig. 1d). Positive MAC staining was not associated with perivascular infiltrates (data not shown).

TNFαR75 cytoplasmic staining was found in 90% (27/30) of the biopsies on the vascular endothelium and diffuse juxtanuclearly in the muscle fibers (Fig 1e). IL-1β staining was present in 71% (20/28) of the biopsies and showed diffuse juxtanuclear cytoplasmic staining in the muscle fibers (data not shown). IFN-γ was positive in 40% (10/25) of the biopsies. Morphological analysis revealed these IFN-γ positive cells to be lymphocytes and not other cell types since these were not detected in any of the biopsies analyzed. Surprisingly IFN-γ also showed staining in the juxtanuclear cytoplasm in the muscle fibers (Fig. 1f). IL-10 was present in 96% (27/28) of the biopsies both on the vascular endothelium (Fig 1g) and the cytoplasm of cells that were located mainly perivascularly in small clusters.

IL-12 staining was positive in 73% (19/26) of the biopsies in the cytoplasm of cells with a horseshoe-like nucleus containing a spot, which were shown histo-morphologically to be monocytes located in the perimysium (Fig. 1h). The two control biopsies showed the presence of HLA-I on nucleated cells. In the muscle biopsy from the patient with Goodpasture's disease the endothelium of some capillaries stained with ICAM-1 and VCAM.

Only very minor interobserver differences were found. After discussing without knowing the patients data agreement was achieved in all cases. Not all 30 muscle biopsies could be tested for the complete panel of antibodies due to lack of sufficient muscle tissue, these are marked as unknown in table 2. The results of antibody staining in the CIPNM biopsies were not significantly correlated with the outcome of the standard light microscopy. But the presence of HLA-DR (100%), TNFαR75 (90%) and IL-10 (96%) in the muscle biopsies of the CIPNM patients was significantly higher compared with the two control biopsies (0%). This resulted in P-values of 0.002, 0.02 and 0.007 respectively.

The ESR and the sepsis severity score showed no significant differences between the CIPNM group and the patients that didn't develop CIPNM or a pattern within the CIPNM group.

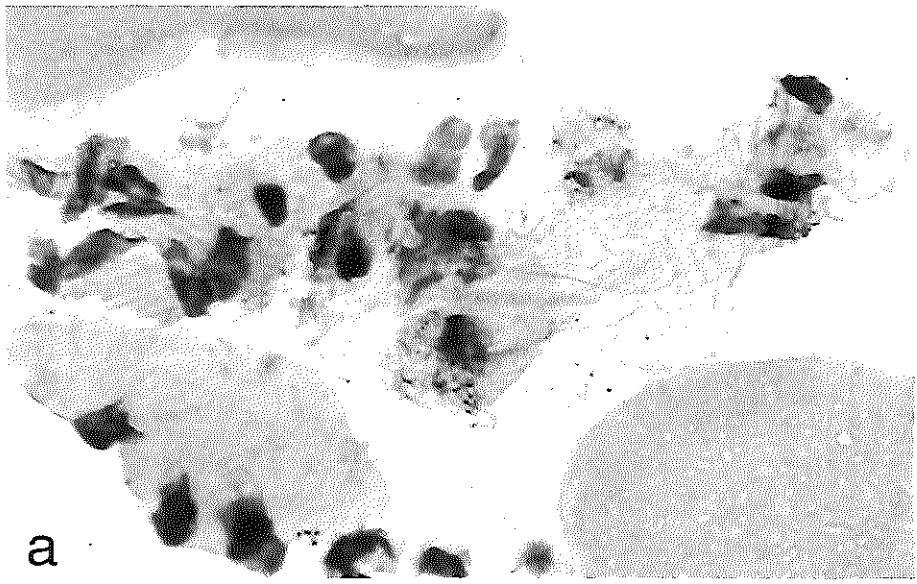

Fig. 1a:
Macrophages (CD68) near a necrotic muscle fiber (red staining).

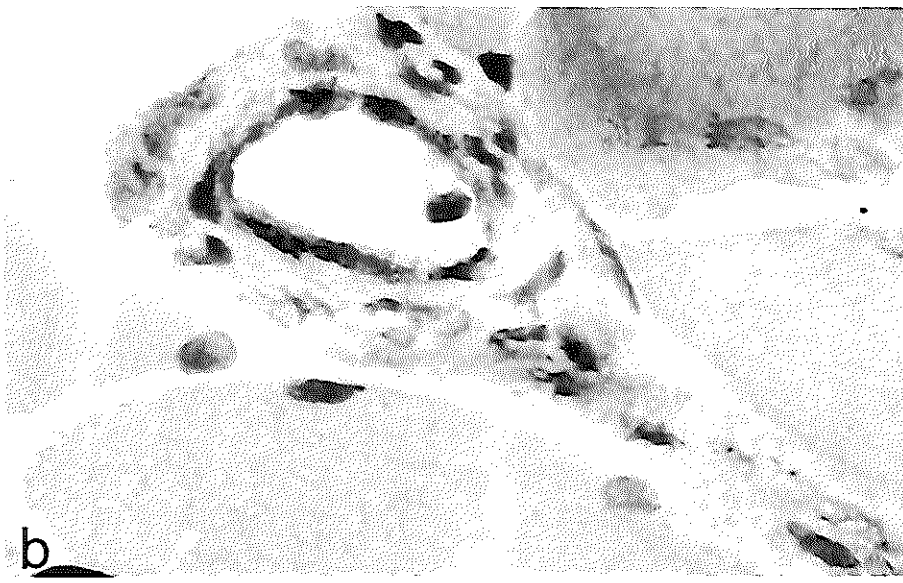

Fig. 1b:
HLA-DR staining on the vascular endothelium (red staining).

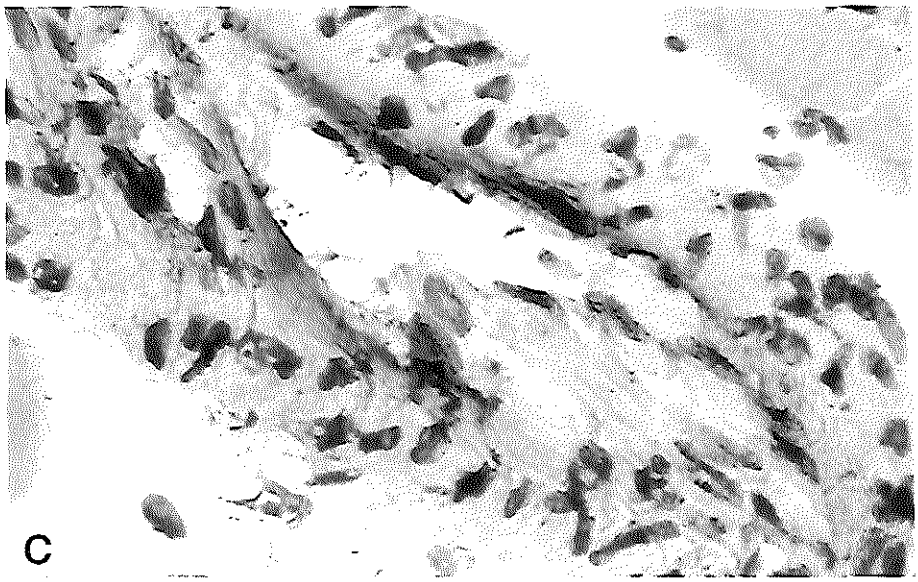

Fig. 1c:
VCAM is present on the endothelium of a blood vessel (red staining).

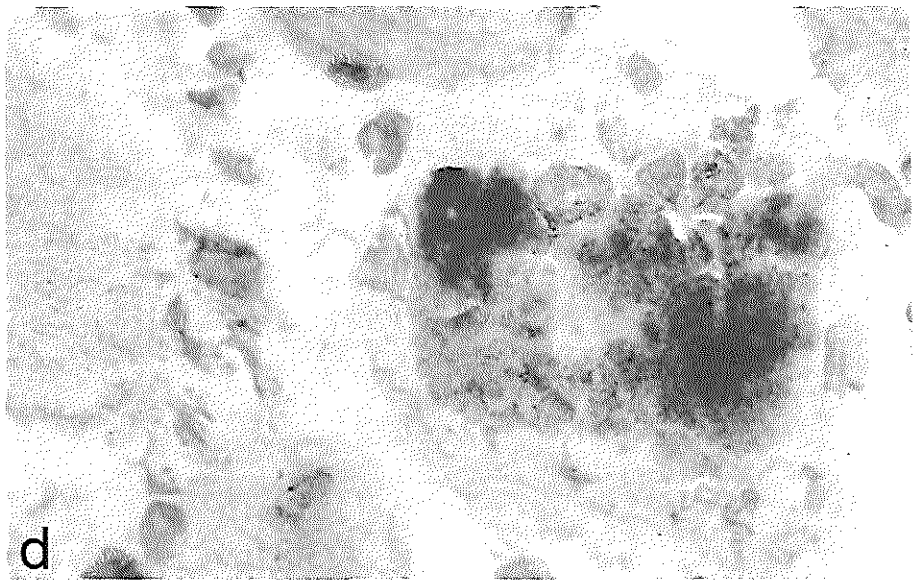

Fig. 1d:
Membrane Attack Complex (C5b-9) staining in a necrotic muscle fiber (red staining).

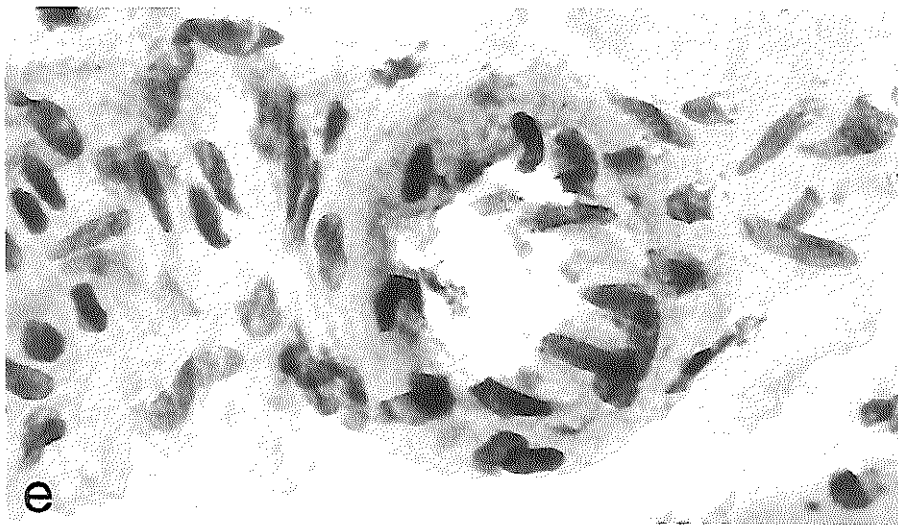

Fig. 1e:
TNFαR75 is present on the endothelium of a blood vessel in the perimysium (red staining).

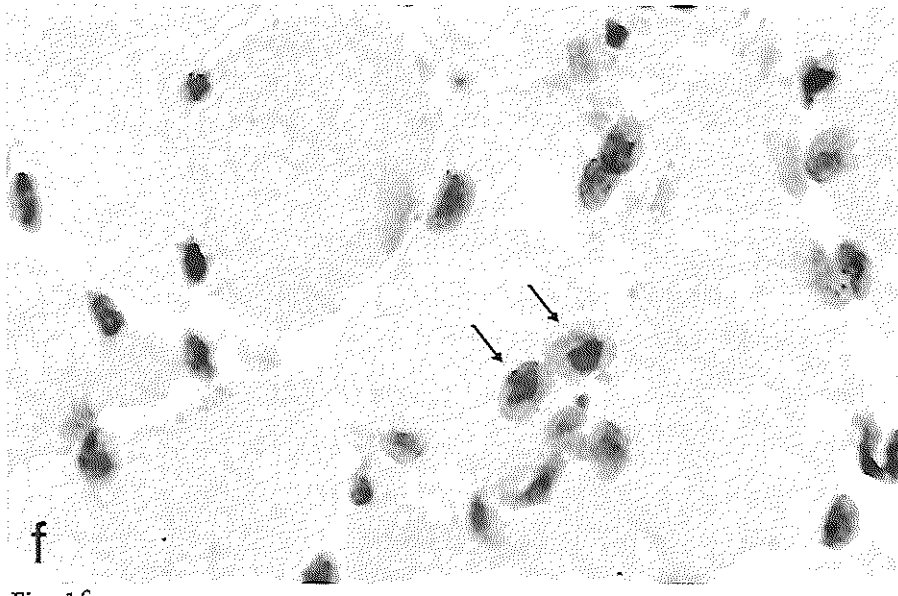

Fig. 1f:
The arrows point at IFN-γ staining juxtanuclear in the cytoplasm of a muscle fiber (red staining). The juxtanuclear production of this cytokine makes it look like a nuclear staining.

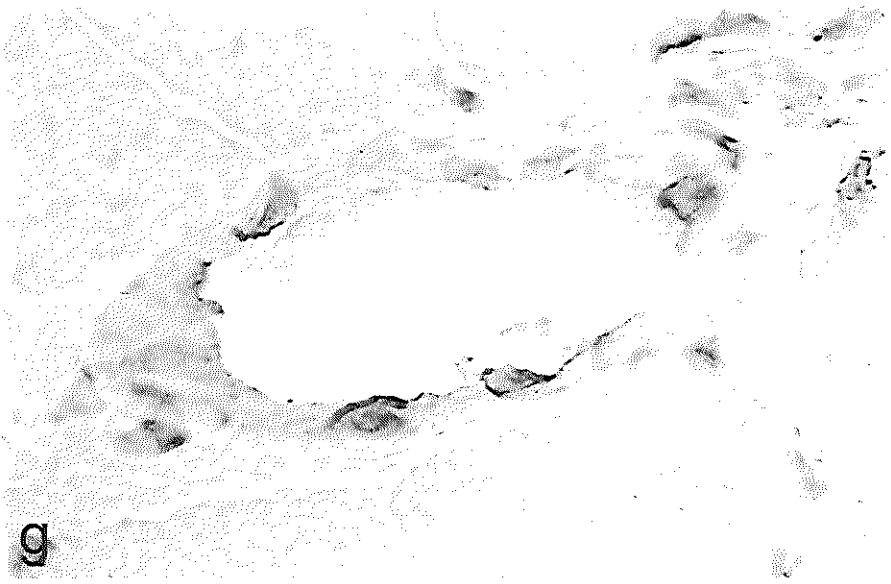

Fig. 1g:
IL-10 is present on the vascular endothelium (orange-brown staining).

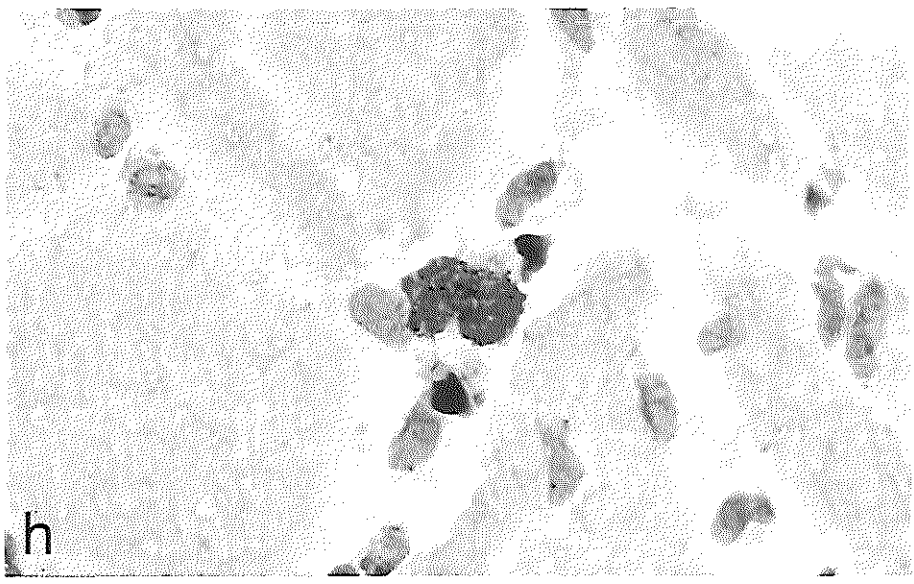

Fig. 1h:
IL-12 staining is positive in the cytoplasm of a cell with a horseshoe-like nucleus containing a basophilic spot (red staining), histomorphologic characteristics of a monocyte.

Table 2: Results of the immunohistochemical analysis of muscle biopsies in CIPNM.

		CIPNM patients																														total	% pos	control	
Inflammatory cells																																			
Th-cells	CD4	-	+	+	-	+	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	+	-	+	-	+	+	-	+	+	+	+	18/30	60	-	-
Tc-cells	CD8	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	+	-	+	-	8/30	27	-	-
B-cells	CD20	-	-	-	-	-	-	-	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/29	0	-	-
Macrophages	CD68	-	+	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	+	-	-	+	+	-	12/30	40	-	-
Adhesion molecules																																			
Eselectin	CD62e	-	-	-	-	-	-	-	-	-	-	-	/	-	-	/	-	-	/	-	-	-	-	/	-	-	-	-	-	-	-	-	0/26	0	-	-
ICAM-1	CD54	+	+	-	-	+	/	-	-	/	+	+	+	+	+	-	-	-	+	+	-	+	+	+	-	+	-	+	/	/	-	15/26	58	+	-
VCAM	CD106	+	-	+	+	-	-	-	+	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	16/30	53	+	-
Complement molecule																																			
MAC (C5b-9)		+	-	+	-	+	+	-	+	+	/	+	+	-	+	-	-	+	+	+	+	+	+	/	+	+	+	+	+	+	+	21/28	79	-	-
Proinflammatory Cytokines																																			
IL-1β		-	+	-	+	+	+	+	+	+	/	-	+	+	+	+	-	+	+	+	+	-	-	-	+	+	+	+	-	+	/	20/28	71	-	-
IL-12		+	+	+	+	/	+	-	+	-	/	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	/	+	-	-	/	19/26	73	-	-
IFN-γ		-	-	-	+	-	/	-	+	-	/	+	-	-	+	/	+	+	-	+	-	-	+	/	-	+	+	-	/	-	-	10/25	40	-	-
TNFαR75		+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	27/30	90	-	-
Anti-inflammatory Cytokine																																			
IL-10		+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	/	+	+	+	+	+	+	+	+	+	+	/	27/28	96	-	-
Antigen presentation																																			
HLA-I		+	+	+	+	+	+	+	+	/	+	+	+	+	+	+	/	+	+	+	+	+	+	+	+	+	+	+	+	+	+	28/28	100	+	+
HLA-DR		+	+	+	+	+	/	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	29/29	100	-	-
Light microscopy		n	n	n	m	M	m	n	M	M	n	n	M	n	n	n	m	M	n	M	n	m	m	m	m	m	m	M	m	m	m				

n= neuropathic, m= myopathic, M= mixed, - = negative, + = positive, Control= control biopsies, / = not done.

DISCUSSION

In this study we found evidence for local immune activation in patients suffering from CIPNM. This disease is clinically characterized by muscle weakness, which may be related to inflammatory factors. This infiltration presents itself either as small clustered infiltrates or by the presence of isolated inflammatory cells. These cells are comprised mainly of macrophages and CD4+ lymphocytes, not of CD8+ cells or B-cells. The macrophages and CD4+ cells showed an activated phenotype (HLA-DR-positive) as other cell types were not present. Equally, these inflammatory cells are more likely to be responsible for the observed production of proinflammatory and anti-inflammatory mediators.

In the same muscle biopsies we found evidence for expression of several adhesion molecules on the vascular endothelium. This is consistent with a model explaining the infiltration of the muscle tissue. Lundberg et al. already postulated the importance of the endothelial cells in inflammatory myopathies (IM). The presence of IL-1α on the vascular endothelium may lead to the induction of endothelial cell adhesion molecules. In IM this can result in the extravasation of inflammatory cells [Lundberg 1997].

Moreover, MAC-positive staining was seen on the endothelium and on necrotic muscle fibers, when present. The expression of MAC initiates capillary destruction and increases vascular permeability [Hohlfeld 1994].

The site and the size of the inflammation do not reflect a strong pathological stimulus leading to the heterogeneity in the standard light microscopy as found here and which was described previously [Zochodne 1987, Al Lozi 1994, Coakley 1993, Subramony 1991, Danon 1991, Gutmann 1996, Wokke 1988, Op de Coul 1991, Latronico 1996]. The weak, but persistent inflammatory response is accompanied by the release of proinflammatory and anti-inflammatory mediators. In our study IL-1β, TNFαR75 and surprisingly also IFN-γ were expressed by muscle fibers. Tews et al. postulated that the expression of cytokines (IL-1α and -β, IL-2 and TNFα) by muscles fibers in inflammatory myopathies may induce and mediate the process of autoimmunization and antigen-expression without the primary presence of inflammatory cells [Tews 1996]. We found the presence of tissue damage in the biopsies of the CIPNM patients. This is possibly due to MAC-induced capillary destruction, and occasionally by necrotic muscle fibers which may explain the abundant expression of HLA-DR positive cells. We speculate that these endothelial cells and scattered macrophages could

present these potential auto-antigens to the migrating CD4+ T-cells. Bazzi et al studied the expression of HLA-I, CD4, CD8, CD19 and MAC in two patients with CIPNM and could not find cellular infiltrates. In one patient CD4, CD8 and CD19 positive staining was present. Both cases however also showed HLA-I upregulation and scattered microvascular deposits of MAC suggesting that at least some patients with CIPNM could be affected with an autoimmune-inflammatory myopathy [Bazzi 1996].

The production of IL-12 by monocytes as recognized histomorphologically could be considered a powerful activator of the immune cascade and induces the proliferation of T-cells. A possible effect of IFN-γ could be the expression of HLA-DR on the surface of the vascular endothelium and macrophages as we found in our study. IFN-γ also sustains the activation of macrophages, and the production of proinflammatory cytokines, which leads to an upregulation of adhesion molecules on the vascular endothelium [Debets 1996]. We demonstrated the expression of ICAM-1 and VCAM, the adhesion molecules that induce increased vascular permeability. E-selectin is synthesized rapidly after cell stimulation by cytokines and is present in severe inflammation. The absence of severe inflammation in the current study may explain why we did not find positive staining on the vascular endothelium for E-selectin.

The immune activation seen in the muscle tissue was associated with minor infiltrates with some inflammatory cells observed in only 27% of the biopsies. The size and relatively low percentage of inflammatory cells were unlikely to be due to a sampling error as there were no biopsies that showed larger infiltrates or infiltrates with celltypes other than CD68, CD4 and less frequently CD8 positive cells. On the other hand there seems to be a role for anti-inflammatory cytokines, as demonstrated by the abundant expression of IL-10. In our opinion the presence of IL-10 could prevent severe inflammation in the muscle tissue, eventually leading to reconvalescence.

To support the hypothesis that the presence of immune activation in the muscle tissue was due to CIPNM and not to an inflammatory process elsewhere in the body, it was important to analyze matched controls. We were fortunate to get muscle biopsies from two such patients. They were critically ill, on the artificial respirator and died shortly after the biopsies were taken. One patient suffered from Goodpastures disease, an immune mediated illness in which immune activation could be expected. The other had severe hyperglycemia and multi organ failure (MOF), which means that

systemic immune activation was present in this patient. Therefore these patients were relevant controls. The importance of local immune activation and the presence of both a pro- and anti-inflammatory stimulus in muscle tissue of CIPNM patients is shown in the role of HLA-DR, TNFαR75 and IL-10 that differ statistical significantly from the control biopsies. The small control group of two patients implies a wide 95% confidence interval of the percentages of HLA-DR positive, TNFαR75 positive and IL-10 positive patients, namely 0%-84%. These intervals for the CIPNM patients are 88%-100% (HLA-DR positive), 73%-98% (TNFαR75 positive) and 82%-100% (IL-10 positive). Especially the results for HLA-DR, representing immune activation in the muscle tissue of CIPNM patients, with non-overlapping confidence intervals are an indication of a statistically significant outcome. Other arguments against non-specific changes in the muscle tissue of CIPNM patients, resulted from the fact that the distribution of the erythrocyte sedimentation rate and the sepsis severity score did not differ between the CIPNM patients and the patients without CIPNM (n=66). And the level of the ESR or the sepsis severity score did not correlate with specific structural changes in the muscle biopsies of the CIPNM patients. We postulate that systemic or local (as yet unresolved) factors are required to cause the local immunohistopathological changes as described in the muscle tissue of our CIPNM patients.

In conclusion, our findings support the hypothesis that local low level immune activation seems to be of importance in patients with CIPNM. The release of cytokines may have contributed to the clinical and electrophysiological findings of a neuropathy and/or myopathy. Factors causing tissue injury may trigger macrophages, leading to the production of cytokines and temporary T-cell-activation. The expression of ICAM-1, VCAM and MAC suggests the possibility that increased vascular permeability could play a role. We propose that together these processes lead to damage of the muscle fibers in which the immunopathology is involved.

V

Discussion: a pathogenetic model of CIPNM and future directions

DIAGNOSIS OF CIPNM

The results of our study indicate that critically ill patients with a high Apache-III score and presence of SIRS are most prone to the development of CIPNM. Clinical and electrophysiological criteria are used for diagnosing CIPNM. In previous studies general definitions of axonal polyneuropathy were used but never defined specific towards CIPNM. Therefore this study provided such criteria (Chapter II) using variables of the clinical and electrophysiological examination of ICU patients that are technically reliable. Also factors as the use of neuromuscular blocking agents and the level of consciousness of the patient were taken into account.
In the near future it is important to reach an international consensus for the clinical and electrophysiological criteria of CIPNM. The following criteria are proposed, which are open for discussion.

DIAGNOSTIC CRITERIA OF CIPNM

Assessment of clinical findings

Clinical assessment is reliable when patients have not been exposed to any neuromuscular blocking agents for at least three days. For diagnosis of CIPNM both clinical and electrophysiologcal criteria should be met.
Clinical; present in at least two consecutive visits.
Co-operative patient
- Motor sum score <26.
- Decreased or absent tendon reflexes.

Non co-operative patient.
- No reaction to pain stimulus or discrepancy between facial movements and movement of the extremities with motor sum score < 8
- Decreased or absent tendon reflexes.

Electrophysiological;
Presence of axonal polyneuropathy:

I distal CMAP of the ulnar nerve is absent or below <4.2 mV and entrapment at the elbow and demyelination* is excluded.

and

distal CMAP of the peroneal nerve is absent or below <2.6 mV and entrapment at the fibular head and demyelination* is excluded.

II Criteria for ulnar or peroneal nerve as mentioned above

and

Spontaneous activity in rest (fibrillations or positive waves) in at least two of the examined muscles using needle electromyography.

*) Ratio of the area of the proximal CMAP versus the distal CMAP >0.89, in the nerve with decreased CMAP amplitude.

There is need for further evaluation of the exact incidence of critical illness polyneuropathy, myopathy or the combination of the two in the ICU population. The findings distal muscle weakness (with or without the presence of facial muscle weakness) and sensory loss differentiate critical illness polyneuropathy from critical illness myopathy clinically with mainly proximal musle weakness. Other tools are electrophysiological testing using direct muscle stimulation and muscle biopsy for more detailed discrimination. At this stage it is my opinion that most of the patients have a combination of peripheral nerve and muscle involvement resulting in CIPNM.

RISK FACTORS

The highest incidence of CIPNM of about 70%, if patients suffer from sepsis or SIRS as found in earlier studies, is supportive for the type of risk factors (SIRS and Apache-III score) found in this study that attribute to the

development of CIPNM. However, there are drawbacks to these two scoring systems. The criteria of SIRS are met rather easily and the Apache scoring system is very complex, consisting of general characteristics and physiological features. To minimize these disadvantages of the scoring methods each organ system was also analyzed separately using the components of the sepsis severity score. This however did not reveal a relation with the development of CIPNM. Most important the statistical analyses showed that SIRS and the Apache III score contributed both independently to the multivariate Cox regression.

IMMUNE MECHANISMS

It is clear that immune mechanisms are involved in MODS and SIRS and our findings indicate that immune activation is present in CIPNM, although immunohistochemistry was only assessed in muscle tissue and not in peripheral nerve.

At present investigators are still far from defining the specific pathogenetic factors of CIPNM. Thus far in the immunohistochemical analysis of this study a model was described for patients suffering from severe trauma, burns or sepsis, patients that underwent major surgery or combinations as possible triggers of the immune system.

In this thesis the immunohistochemical study of muscle biopsies of CIPNM patients a comparison could only be performed with two biopsies of patients that did not develop CIPNM. Ethical aspects limit the performance of biopsies in patients without CIPNM. Therefore in future studies serum analysis of soluble immunological parameters (cytokines, adhesion molecules etcetera) may be another method to investigate the role of immune activation in CIPNM patients. If future studies confirm the presence of (local) immune response therapeutic strategies using immune-modulating therapy could be developed. These may then influence the course of CIPNM or prevent it from occurring, which needs to be tested in a prospective randomized placebo controlled trial. Electron microscopical examination of the muscle tissue could also add important information in describing specific structural changes as for example to the thick filaments, which enables the discrimination of CIPNM from acute quadriplegic myopathy.

FOLLOW-UP

This study had a relatively short follow up of the natural history of CIPNM patients with a relatively small number of patients that survived. High mortality is due to the fact that the severity of critical illness was high. The occurrence and prognosis of the polyneuropathy is considered to be related to the prognosis of the underlying critical illness. Since the prognosis of patients with multiple organ failure is fatal in about 50-60% of the patients, a significant number of patients will die from the underlying critical illness. This study supports the hypothesis that in some patients CIPNM is an additional factor in the organ failure and contributing to the persistent severe illness, resulting in death. The patients who survive usually recover from critical illness polyneuropathy and in general the long-term outcome is considered to be good. However, the residual findings in CIPNM are underestimated. As for long term follow-up very few data are available which are obligatory to supply more insight about the course of CIPNM.

VI

Summary/Samenvatting

Critical illness polyneuropathy and myopathy (CIPNM) is a neuromuscular disorder encountered in the ICU. During critical illness patients develop muscular weakness of the limbs with atrophy. Respiratory muscles and occasionally cranial nerves are involved and tendon reflexes are decreased or absent. The electrophysiological features include an axonal polyneuropathy and possible myopathic changes in the needle electromyographic examination. Neuropathological findings in nerve and muscle tissue show either neuropathic, myopathic changes or both. The entity CIPNM has been recognized first in the early eighties. Chapter I describes the characteristics mentioned above more elaborately, furthermore the variants and differential diagnosis of CIPNM is discussed. A large prospective study on CIPNM also studying patients on the ICU under the same conditions that did not develop CIPNM had not been performed thus far. This was the goal of this thesis including the following subjects: the risk of development of CIPNM (chapter II), its natural history (chapter III), the neuropathological features including variables involved in immune activation (chapter IV_b) and constructing a model on the pathogenesis and possible therapeutic strategies (chapter V).
Chapter II describes 98 patients that were on the artificial respirator were studied of whom 32 developed CPNM. To diagnose CIPNM clinical and electrophysiological criteria were defined, which both had to be met. From several patients characteristics multivariate analysis showed that a high Apache-III score ($p=0.02$) and the presence of SIRS ($p=0.04$) in the early phase of the stay at the ICU differed significantly between the group of patients that developed CIPNM (32 patients, 33%) and those that did not (66 patients, 67%). Medication as midazolam, vecuronium, steroids and aminoglycosides did not play a role as risk factor for the development of CIPNM. Based on the distribution of the predictive index derived from the variables Apache-III score and SIRS three risk groups were identified. The low-risk group with an Apache-III score ≤ 70 and no SIRS, the high-risk group with an Apache-III score >85 and presence of SIRS and the medium-risk group containing the other patients. The Kaplan-Meier curves show that the probability of developing CIPNM within 30 days of artificial respiration is 8% for the low-risk group, 28% for the medium-risk and 72% for the high-risk group.
In chapter III both clinical and electrophysiological features of 31 of the 32 patients with CIPNM were analyzed. After 50 days follow-up of 6 of the 31 CIPNM patients (19%) had a good functional recovery and 15 of the 31

patients (48%) showed persistent motor disability defined as poor outcome. The ten other CIPNM patients died early after inclusion. The clinical and electrophysiological features were compared with the study population that did not develop CIPNM (66 patients). Overall mortality in the CIPNM group was 55% and 29% for the patients that did not develop CIPNM (p=0.01). After six months 10 patients of the poor outcome group still had motor disability.

The main tools for the clinical diagnosis of CIPNM are assessment of muscle weakness using the MRC sum score and assessment of tendon reflexes as mentioned in the diagnostic criteria for both co-operative and non co-operative patient (Ch II). The electrophysiological criteria can be met earlier in time than the clinical criteria of CIPNM. Assessment of muscle weakness was often not reliable because of the severe underlying illness and use of neuromuscular blocking agents. These factors may also explain why we did not find sensory complaints clinically. Using EMG conduction studies, CMAP amplitudes of the nerves of the upper extremities together with needle EMG are the most reliable measurements to diagnose CIPNM as edema of the lower limbs is often a problem in ICU patients. In patients without edema the SNAP amplitude of the sural nerve was decreased in most cases and therefore supportive for the diagnosis of CIPNM (Ch II). Needle EMG showed spontaneous activity of the muscle fibers mainly in the distal muscles of the limbs (p=0.003) comparing the first recording (day 4 of the artificial examination) with the second examination (day 11 of the artificial respiration). Spontaneous activity of the muscle fibers was related with neuropathic or both neuropathic and myopathic changes in the muscle biopsies of the CIPNM patients (p=0.029). Conduction velocities were normal during monitoring on the ICU. One patient had superimposed pressure palsy of the peroneal nerve across the fibular head. SSEP of the median and tibial nerve was tested in 22 patients with CIPNM and were all normal.

No prognostic factors were present after statistical analysis of the patients' characteristics, their medication, the Apache-III score, SIRS, severity of sepsis or light microscopical findings when comparing two groups with good or poor outcome during follow-up. Age and Apache-III score were higher in the poor outcome group, but the differences were not significant.

After an introduction on the mechanism of immune activation during critical illness, chapter IV describes a neuropathological study of the muscle tissue of

30 of the 32 patients with CIPNM and 2 patients that did not develop CIPNM during prolonged artificial respiration. In the muscle biopsies of the CIPNM patients neuropathic changes were found in 37%, myopathic in 40%, and a combination in 23%. The immunohistopathology showed macrophages and Th1-cells in 40% and 60% of the muscle biopsies respectively. Small mainly perivascular infiltrates were present containing macrophages and Th1-cells. ICAM-1, VCAM and MAC were found on the vascular endothelium in 58%, 53% and 79% of the muscle biopsies respectively. In all biopsies of the CIPNM patients there was an upregulation of both HLA-I and HLA-DR. Proinflammatory cytokines and TNFαR75 were also produced locally (IL-1β in 71%, IFN-γ in 40%, IL-12 in 73%, TNFαR75 in 90%). The anti-inflammatory cytokine IL-10 was expressed simultaneously in 96% of the biopsies. HLA-DR, TNFαR75 and IL-10 differed significantly when compared with the muscle biopsies from the patients that did not develop CIPNM. These data show that small numbers of activated leukocytes producing both pro- and anti-inflammatory cytokines infiltrate skeletal muscle of CIPNM patients.

Samenvatting

Critical illness polyneuropathie en myopathie (CIPNM) is dè neuromusculaire aandoening die patiënten ontwikkelen, die ernstige orgaanfunctie stoornissen hebben en daarmee de benaming "critically ill" verdienen. Deze patiënten zijn veelal afhankelijk van kunstmatige beademing en onder deze conditie ontwikkelen zij een aandoening van het perifere zenuwstelsel. Een dergelijke aandoening vertoont kenmerken van algehele zwakte aan de extremiteiten en van de ademhalingsmusculatuur , de spieren zijn atrofisch en spierrekkingsreflexen zijn verlaagd of afwezig. Soms kunnen ook de hersenzenuwen meedoen, hetgeen zich veelal uit in aangezichtszwakte en oogbewegingsstoornissen. Het gaat dus om een klinisch beeld van met name het motore deel van het perifere zenuwstelsel, maar ook sensibele functiestoornissen bestaande uit hypaesthesie/gevoelsverlies of paraesthesieën/tintelingen kunnen aanwezig zijn. In dit laatste geval pleit de kliniek voor een polyneuropathie. Het aanvullend onderzoek in de vorm van electromyografie/EMG bevestigt een dergelijke polyneuropathie wanneer de amplituden na stimulatie van de motore en eventueel ook de sensibele zenuwen verlaagd zijn en er bij naald-electromyografie in rust sprake blijkt te zijn van spontane spiervezelactiviteit. Er is dan sprake van een axonale (senso)motore polyneuropathie. Mocht er desondanks onvoldoende diagnostische duidelijkheid zijn, dan is ook de betrokkenheid van de intercostaalmusculatuur of de nervus phrenicus te testen. De myopathische component is veelal terug te vinden in het spierweefsel, waarbij standaard lichtmicroscopisch onderzoek van spierbiopten veranderingen toont bij sommige patiënten die passen bij een myopathie. De spiervezels zijn daarbij veelal atrofisch met een variërende diameter en rond aspect. Wanneer de structurele kenmerken voornamelijk neuropathisch zijn dan zijn de spiervezels hoekig en atrofisch. Verder kunnen in een gevorderd stadium de type-I en type-II spiervezels zich gaan hergroeperen door reïnnervatie (type-grouping). Ook mengbeelden in het microscopisch onderzoek met myopathische en neurogene kenmerken zijn mogelijk.

CIPNM werd begin jaren tachtig onderkend en voor het eerst beschreven door de pioniers Dolf Op de Coul en Charles Bolton. Sedertdien volgden voornamelijk casuïstische en retrospective publicaties. Deze postuleerden beschreven en retrospectieve analyses postuleerden verschillende pathogenetische factoren voor CIPNM. In dit proefschrift wordt een prospectieve studie beschreven waarin patiënten gemonitord worden op het ontwikkelen

van CIPNM (hoofdstuk II) en het beschrijven van verscheidene aspecten van de aandoening (hoofdstukken III, IV en V).
In hoofdstuk I wordt ingegaan op de klinische en electrofysiologische kenmerken van CIPNM. Daarnaast worden de varianten en differentiaal diagnose van CIPNM beschreven.
Hoofdstuk II beschrijft de analyse van de risico factoren voor het ontwikkelen van CIPNM. Van de 98 patiënten die kunstmatig beademd werden voldeden er 32 gedurende de follow-up aan zowel de klinische als electrofysiologische criteria van CIPNM. In de speurtocht naar risico factoren voor het ontwikkelen van CIPNM werd uitgegaan van een drietal categorieën: 1) medicatie in de vorm van sedativa, spierverslapping, steroiden en aminoglycosiden, 2) aanwezigheid van SIRS (systemic inflammatory response syndrome) en de sepsis severity score en 3) Apache-III score (Acute Physiology Age and Chronic health Evaluation score), een gewogen score van algemene patiënt karakteristieken en fysiologische parameters. De statistische analyse toonde dat de genoemde medicatie niet als risico factor aan te merken was voor het ontwikkelen van CIPNM. Na multivariate cox-regressie analyse bleken de aanwezigheid van SIRS ($p=0.04$) en een hoge Apache-III score ($p=0.02$) wel significante risicofactoren. Al in de vroege fase van de beademingsperiode bleek er een significant verschil voor deze twee parameters voor patiënten die CIPNM ontwikkelden (32 patiënten, 33%) en patiënten waarbij CIPNM uitbleef (66 patiënten, 67%). De sepsis severity score opgebouwd uit 5 orgaansysteemscores en een score voor de stolling bleek geen risico factor. Evenmin was dat het geval voor de 6 componenten van deze score, die ook ieder apart werden geanalyseerd.
Vervolgens werd een predicatieve index verkregen middels de hoogte van de Apache-III score en de aanwezigheid van SIRS voor het ontwikkelen van CIPNM. De lage risico groep heeft een Apache-III score ≤ 70 en geen SIRS, de hoge risico groep heeft een Apache-III score >85 en SIRS is aanwezig. In de medium risk groep zitten de overige patiënten. De Kaplan-Meier curves tonen een kans voor het ontwikkelen van CIPNM binnen 30 dagen in de lage risico groep van 8%, voor de medium risk groep van 28% en een kans van 72% voor de high risk groep.
In hoofdstuk III wordt het natuurlijk beloop van 31 van de 32 patiënten met CIPNM beschreven. Voor het stellen van de klinische diagnose bleken de motore somscore en de spierrekkingsreflexen de belangrijkste onderdelen van het neurologische onderzoek. Het feit dat sensibele klachten veelal niet vast

te leggen zijn, wordt veelal veroorzaakt door de mate critical illness en het gebruik van sedativa. Het electrofysiologische onderzoek wordt vaak beïnvloed door het gebruik van spierverslappers en de mate van "critical illness" waarbij oedeem aan de extremiteiten het geleidingsonderzoek bemoeilijkt. In het geleidingsonderzoek zijn het meten van de CMAP amplituden aan de bovenste extremiteiten en het naald-EMG het meest betrouwbaar voor het stellen van de electrofysiologische diagnose CIPNM.
Na een follow-up van 50 dagen was er bij 6 van de 31 patiënten (19%) een goed functioneel herstel, 15 van de 31 patiënten (48%) toonde nog steeds een motore handicap gedefinieerd als slechte outcome. De overige 10 patiënten overleden vroeg na de inclusie, waardoor zij niet mee konden worden genomen in de langere termijn statistische analyse. De klinische en electrofysiologische parameters van de twee groepen CIPNM patiënten werden vergeleken met die van de 66 patiënten zonder CIPNM. Overall was de mortaliteit voor de CIPNM groep 55% en de patiënten zonder CIPNM 29% ($p=0.01$). Na 6 maanden hadden patiënten van de slechte outcome groep nog steeds een motore handicap van betekenis. Bij navraag bleek er na 1 jaar van vijf ondervraagde patiënten bij allen geen sprake meer van een functionele beperking in het dagelijks leven. Er bleken geen prognostische factoren voor het verloop van CIPNM na statische analyse van de Apache-III score, de aanwezigheid van SIRS, sepsis severity score, medicatie in de vorm van sedativa, spierverslapping, steroïden en aminoglycosiden.
Hoofdstuk IV geeft een introductie omtrent immuun activatie tijdens critical illness. Vervolgens worden de resultaten besproken van de weefsel studie in de vorm van standaard lichtmicroscopisch en immuunhistochemisch onderzoek van de spierbiopten van 30 van de 32 patiënten met CIPNM en 2 spierbiopten uit de groep van 66 patiënten die geen CIPNM ontwikkelden. In het standaard lichtmicroscopische onderzoek bleek de verdeling myopatische, neurogene afwijkingen en mengbeelden met kenmerken van beide verdeeld als 40%, 37% en 23%. De immuunhistopathologie van de patiënten met CIPNM toonde de aanwezigheid van macrofagen en Th1-cellen in respectievelijk 40% en 60%. Kleine perivasculair gelegen infiltraten bevatten macrofagen en Th1-cellen. Adhesie moleculen, die leiden tot een verhoogde permeabiliteit van de endotheel wand van de bloedvaten in de spiebiopten waren aantoonbaar in de vorm van ICAM-I in 58% en VCAM in 53%. Het membrane attack complex (MAC) was aanwezig in 79% van de spierbiopten en leidt eveneens tot een permeabeler endotheliale vaatwand. Alle biopten

van de CIPNM patiënten toonden een verhoogde expressie van HLA-I en HLA-DR, de human leucocyte antigens die tonen dat er sprake is van immuunactivatie in het onderzochte weefsel. Tevens werden cytokinen kleuringen verricht van zowel het anti- als pro-inflammatoire type, die de immuunactivatie respectievelijk remmen en stimuleren. Bij statistische analyse van de spierbiopten van CIPNM patiënten en patiënten die geen CIPNM ontwikkelden, bleek er een significant verschil voor de aanwezigheid van HLA-DR, het pro-inflammatoire cytokine TNF-alfa R75 en anti-inflammatoire cytokine IL-10. Deze verschillen en de homogeniteit van de bevindingen binnen de CIPNM groep leidden tot de conclusie dat een klein aantal geactiveerde leucocyten zowel pro- als anti-inflammatoire cytokinen produceren die het skeletspierweefsel infiltreren bij CIPNM patiënten. De balans van anti- en pro-inflammatoire activiteit bepaalt behalve het ontstaan van de aandoening ook het eventuele herstel. Wat de uiteindelijke trigger is die leidt tot de beschreven immuun activatie is tot dusver speculatief en is mogelijk in de vorm de aanwezigheid van sepsis, multi-trauma, uitgebreide brandwonden, uitgebreide chirurgische ingrepen of combinaties ervan. Meer ondersteuning voor het pathogenetisch model zou verkregen kunnen worden indien bij herhaling de beschreven afwijkingen worden aangetoond. Verder suggereert de impact van de immuunactivatie, dat dit een ingang zou kunnen betekenen voor therapeutisch interveniëren. Een immuun-modulerende therapie dient echter in de vorm van een prospectief gerandomiseerde placebo gecontroleerde trial te worden onderzocht.

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List of abbreviations

APACHE	Acute Physiology Age and Chronic Health Evaluation
AQM	Acute quadriplegic myopathy
CD4	Marker Thelper cells
CD8	Marker Tcytotoxic cells
CD20	Marker B cells
CD54	Marker ICAM-1
CD62E	Marker E-selektin
CD68	Marker macrophages
CD106	Marker VCAM
CIPNM	Critical illness polyneuropathy and myopathy
CMAP	Compound muscle action potential
CK	Creatine phosphokinease
EMG	Electromyography
ESR	Erythrocyte sedimentation rate
GBS	Guillain-Barré Syndrome
HLA	Human leucocyte antigen
ICAM	Intercellular adhesion molecule
s-ICAM	soluble-Intercellular adhesion molecule
ICU	Intensive care unit
IFN	Interferon
IL	Interleukine
IM	Inflammatory myopathies
MAC	Membrane Attack Complex or Complement 5b-9
MEC	Medical ethical committee
MFS	Miller Fisher syndrome
MODS	Multiple organ dysfunction syndrome
MOF	Multi organ failure
MUP	Motor unit potential
NBA	Neuromuscular blocking agents
SIP	Sickness Impact Profile
SIRS	Systemic inflammatory response syndrome
SNAP	Sensory nerve action potential
SSEP	Somato-sensory evoked potential
TNF	Tumor necrosis factor
VCAM	Vascular cell adhesion molecule
s-VCAM	soluble-Vascular cell adhesion molecule

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Curriculum Vitae

The author of this thesis was born on December 6th 1967 inTerneuzen, the Netherlands. In 1986 she graduated from the Hoogveld College (Athenaeum B) in Hintham ('s-Hertogenbosch).
After completing this study, she started studying medicine at the University Medical Centre in Utrecht and obtained her medical degree in March 1993.
In April 1993 she started working at the department of Neurology at the St.Elisabeth Hospital in Tilburg, followed by a residency in Neurology from January 1994 until December 1999 (former head, Op de Coul AAW, MD, PhD, head Tijssen CC, MD, PhD), this training included a qualification in Clinical Neurophysiology (head Schellens RLLA, MD, PhD).
During her residency she performed the prospective study of critical illness polyneuropathy and myopathy as described in this thesis.
Since June 2000 she is working as a neurologist at the St. Antonius Hospital in Nieuwegein, the Netherlands.

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