Acquired Neuromuscular Disorders

Pathogenesis, Diagnosis and Treatment

Corrado Angelini
Editor

Second Edition

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Introduction

Acquired Neuromuscular Disorders and Covid-19 Pandemia

This second edition of the book was finalised during the Covid-19 pandemia, and I thank all contributors that prepared their chapters in such difficult times.

In Europe. the first severe acute respiratory syndrome coronavirus (SARS-CoV-2) cases were documented in January 2020, and the first affected case was found in Codogno Hospital, Milan, and the first deceased case occurred in February 2020 in Schiavonia Hospital, Padova, Italy.

There were almost two years of ongoing cycles of imposing and easing restrictions to suppress the epidemia since most European countries experienced its first large wave in March 2020–July 2020, which stretched to the second wave in autumn 2020.

With the appearance of alpha, beta, delta, omicron variants of coronavirus, there were several diffuse outbreaks that further stretched the fragmented resources of the National Health Systems.

The category of patients afflicted by neuromuscular disease covers a wide range of different diagnoses with widely varying levels of disability, even in patients with the same diagnosis. It is difficult therefore to make specific recommendations for Covid-19 that apply generally.

The following are recommendations that apply to acquired neuromuscular disorders. The recommendations are designed primarily for patients, carers, and non-specialist medical providers. They are also intended to inform neuromuscular specialists particularly regarding basic service requirements.

Patients with Neuromuscular Disease (NMD) at Higher Risk

National neurological associations and neuromuscular networks, such as EURO-NMD and the World Muscle Society (WMS), have produced guidance on the impact of Covid-19 pandemia on neurological and neuromuscular disorders and their management. The concepts expressed here are derived from NMD expert discussion and appear in websites (see References).

These documents define the risk of a severe course of Covid-19 as high or moderately high in all, except for the mildest forms of acquired NMD.

Features conferring a high risk of severe disease include the NMD patients with:

- muscular weakness of the chest or diaphragm, resulting in respiratory volumes less than 60% predicted (FVC < 60%), especially in patients with kyphoscoliosis
- use of ventilation via mask or tracheotomy
- weak cough and weak airway clearance due to oropharyngeal weakness
- presence of tracheostoma
- cardiac involvement (and/or on medication for heart involvement)
- risk of deterioration with fever, fasting, or infection
- risk of rhabdomyolysis with fever, fasting, or infection
- concomitant diabetes and obesity
- patients taking steroids and undergoing immunosuppressive treatments

Advice for Patients with Acquired NMD to Avoid Covid-19 Infection

Covid-19 spreads through droplet infection when an infected person coughs, sneezes or talks, or potentially via touching a surface carrying infectious droplets.

Patients with acquired NMD at high risk of a severe course of Covid-19 infection should undertake the following precautions:

- Social distancing of at least 1–2 m is a minimum requirement.
- For high-risk individuals, self-isolation is advised.
- Patients are encouraged to do homework or reduce their working times.
- Avoid large gatherings and public transport.
- People are urged to limit visits to vulnerable persons.
- Frequent hand-washing (20 s with soap and warm water), use of 60% alcohol-based hand sanitisers, and surface disinfection are crucial.
- Caregivers should be in-house, if possible.
- Essential visiting caregivers (for instance, providers of backup support for ventilatory assistance) should wear face masks and adequate personal protection, according to up-to-date guidance, to prevent passing on the virus.
- Visiting a physiotherapy service is discouraged; however, physiotherapists should provide advice on maintaining physical activity remotely, via phone or video link through telemedicine.
- It is important to be prepared for all eventualities including when assistants are absent for illness or quarantine.

  Plans should be made for how to best meet the needs of the individual NMD patient without possibly resorting to hospitalisation.

Consequences of the Risk of Covid-19 Infection During Treatments Used in NMD Patients

- Patients must ensure that they have an adequate supply of medication and of ventilatory support equipment for a period of prolonged isolation (1-month supply).
- Patients and carers should make use of online and telephone-based pharmacy and equipment.
- Patients and carers need to be comfortable with emergency procedures, specific to their condition and their equipment.
- Patients on steroid regimens should continue their medication. Steroids should not be stopped suddenly, there might be a need to increase the steroid dose when an NMD patient is sick.
- Immunosuppression in inflammatory muscle disease, myasthenia gravis, and peripheral nerve disease patients should not be discontinued except under specific circumstances and in consultation with the neuromuscular specialist.
- Isolation requirements may impact treatment regimens requiring day-hospital procedures (i.e. IVIg and rituximab infusions or treatments related to clinical trials). These treatments should not be stopped, but if possible moved to a non-hospital setting (home-visiting nurses). IVIg can be changed to subcutaneous immunoglobulin.

Importance to Assure Ventilatory Services (Home Ventilators, etc.)

- Backup and advice hotlines should be offered by the patients' neuromuscular centres.
- Patients should have and alert card or a medical bracelet providing the neuromuscular centre contact.
- Neuromuscular centres should actively contact patients on ventilatory support to ensure they have relevant information and adequate equipment.

Acquired NMD Admission to Hospital for Symptoms of Covid-19 Infection

Inpatient admission should be avoided if possible, but should not be delayed when necessary. This can be a difficult decision. Patients with NMD need to be aware that:

- Emergency services may be under severe pressure.
- Individual countries may have triaging procedures in place. These may affect the potential for intensive care admission for patients with NMD, who require ventilation.

- Specifically, the terms “incurable” and “untreatable” may be confused by medical staff in Hospital.
- Neuromuscular disorders may be difficult to treat, but patients relatively untreatable such as ALS, paraneoplastic syndrome, or other motor neuron diseases have different implications for treatment decisions.
- Use of patients’ home equipment (i.e. ventilators) may be forbidden in hospitals if ICU are available.

Effects on Neuromuscular Disease Treatments by Covid-19 Common Drugs

- Numerous specific drugs for the treatment of Covid-19 are under investigation. Some of these can affect neuromuscular function significantly, for example, chloroquine and azithromycin are unsafe in myasthenia gravis, except when ventilatory support is available.
- Other treatments may have effects on specific neuromuscular diseases (in particular, nutritional disorders, motor neuron diseases, and neuromuscular junction disorders) and anatomical peculiarities of patient may influence options for treatment (e.g. prolonged prone ventilation).
- Experimental treatments for Covid-19 may be offered “compassionately”. They should only be taken after consultation with the patient’s neuromuscular specialist.

Neuromuscular Specialists Advice for Emergency Medical and Intensive Care Decisions on Admission to Units, and Care in Acquired Neuromuscular Patients

Decisions on patient admission to Intensive Care Units may be affected by anticipated or existing capacity problems. Triaging may have been instituted. This can have practical and ethical consequences.

- There should be close collaboration between neuromuscular and respiratory physicians.
- The neuromuscular specialist must be available to play a role in ensuring fair provision of intensive care to acquired NMD patients.
- Ideally, neuromuscular specialists will have to be involved in formulating hospital policies, decision-making algorithms, and documentation forms.
- Neuromuscular specialists must develop guidelines for treatment that ensure that patients remain at home as long as possible.

Patient Support by Neuromuscular Centres

Neuromuscular centres and specialist services should aim to provide the following:

- Patient hotlines with advice by neuromuscular, physiotherapists, and other specialist personnel, with specialist physician backup (paediatric and adult).
- The possibility to continue routine clinics by structured telemedical phone and video links.
- Outreach ventilatory support strategies should be provided.
- Strategies to maintain hospital-based treatments with minimal disruption.
- Neuromuscular specialists should be in discussion with their hospitals' Emergency, Medical, and Intensive Care Units on restriction for use of home NIV equipment.
- Neuromuscular specialists should support their hospital to define approved devices and ensure their availability (i.e. ICU mask systems with viral particle filters).
- Liaison and shared care with Intensive Care Unit services might be useful.

Vaccination

The spread of coronavirus disease-2019 (COVID-19), that was caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), can be mitigated through safe and effective vaccines. Different vaccine types are currently being used to protect individuals from SARS-CoV-2 infection and disease. The four main types of COVID-19 vaccine include whole virus, protein subunit, Adeno Viral Vector (AAV), and messenger RNA or mRNA vaccines (i.e. produced by Pfizer or Moderna). Limitations in vaccine supply, however, can affect the outcomes of the global vaccination campaign.

There are different types of vaccines available: the AAV-linked vaccines (i.e. produced by AstraZeneca or Janssen Johnson & Johnson) and their type of subministration varies in different countries as well as the subministration of the ones with mRNA vaccines.

So far, there are no live virus vaccines, where there could be a risk for immunosuppressed patients.

For more detailed advice on COVID-19 vaccines, see the WMS vaccination advice website.

The choice and availability of vaccines is different in various countries and are regulated in Europe by EMA and in the United States by FDA regulations. National vaccines are in use in China or Russia.

In other countries, i.e. Canada, South America, Australia, or India and developing African countries AAV-linked vaccines are more in use than mRNA vaccines, for economic reasons.

Individual country regulations are important in time and subministration of vaccinations.

There have been complications from individual vaccines, that are collected by the National and International Agencies, including some neuromuscular syndromes such as Guillain-Barrè syndrome (after getting the Janssen Johnson & Johnson COVID-19 vaccine) and Miller-Fisher syndrome. These are rare disorders that are described in Chaps. 14 and 15 and cause muscle weakness or tingling sensation, especially in legs and arms and spread to other districts. Such complications are rare, but patients with such syndromes are distressed.

It is advisable to be vaccinated and individual choice depends on a series of individual factors to be discussed with the neuromuscular specialist.

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Contents

Part I

Diagnostic Tools

Autoantibodies in Neuromuscular Disorders

1

Luis Querol, Eduard Gallardo, and Isabel Illa

1.1 Antibodies in Autoimmune Neuromuscular Disorders

1.1.1 General Principles

The search for autoantibodies is an important topic of research in autoimmune diseases. Describing disease-specific antibodies is usually the first step leading to the description of the pathogenic mechanisms involved in the development of an autoimmune disease although not all autoantibodies detected in specific diseases have pathogenic or diagnostic implications. The definition of the clinical utility and the technical caveats of an autoantibody test and the pathogenic implications of a detected autoantibody are key to understand the relevance of autoantibodies and their role in a specific disease or subset of patients. The field of neuromuscular diseases was the first one describing a disease-specific, relevant, pathogenic autoantibody, when antibodies against the nicotinic acetylcholine receptor (AChR) were described four decades ago [1]. Since then, many other autoantibodies, in the myasthenia field, inflammatory neuropathies and myositis, have been described.

Only a fraction of autoimmune neuromuscular disorders has been associated with specific autoantibodies; in those diseases that have specific autoantibodies described, their detection has provided a three-fold utility. First, they inform about the target antigen and are the first step to describe the upstream and downstream mechanisms leading to loss of tolerance or to tissue damage in an autoimmune disease. On the other hand, autoantibodies, when they are specific enough, have diagnostic implications and may uncover disease subtypes with different pathogenesis,

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prognosis, or response to treatment. The discovery of anti-MusK antibodies in myasthenia [2], for example, led to the description of a disease subtype in which clinical features and response to conventional therapies, significantly differs from the typical myasthenia with antibodies against the acetylcholine receptor [3]. Finally, specific autoantibodies are frequently used to monitor the response to treatment. This is not useful for all antibody-mediated diseases, but in some, the correlation is clear. In a context in which detection of an autoantibody will lead to substantially different therapeutic protocols it is crucial to place the focus in those autoantibodies that are highly specific and ensure that false positives are minimized. Moreover, some autoantibodies are tested with techniques that show significant technical challenges and that may be associated with diagnostic inaccuracy when performed with non-standard techniques. This is the case in anti-ganglioside antibodies (ELISA and thin-layer chromatography) [4] or cell-based assays (nodal/paranodal antibodies and, in some cases, anti-MusK) for example [5]. This means that physicians, pathologists, and researchers should be aware to the techniques that are considered standard to interpret a result that does not match the clinical suspicion or when titers are low.

The quest for specific autoantibodies in neuromuscular disorders has not been easy, and, still today, some diseases in which the involvement of humoral factors was suspected since their description, do not have identifiable autoantigens that could serve, at the same time, for the study of their pathogenesis and as diagnostic biomarkers [6]. There are several clinical, technical, and interpretation caveats when studying the importance of autoantibodies in neuromuscular diseases. From the clinical perspective, neuromuscular disorders are often diagnosed with clinical, electrophysiological, or pathological criteria that don't necessarily reflect common pathogenic mechanisms. This, in fact, leads, to high degree of clinical heterogeneity within the classical syndromes. Defining clinically homogeneous subgroups, no matter how infrequent they are, is the necessary step to avoid noise when searching for specific antigenic reactivities, although, frequently, it is only possible to clarify the underlying heterogeneity when a specific biomarker allows the detection of the features that make that autoantibody-associated disease unique. The technical problems that have frequently misguided autoantibody search are, first, the use of biased approaches (candidate-antigen approach, animal models, etc.); second, the bias towards the identification of single-chain protein antigens (and negligence, with the exception of inflammatory neuropathies of glycolipid, lipid, and glucosidic antigens and of protein complexes); and third, the use of techniques that detect non-conformational antigens, such as western-blot. Western-blot-detected autoantibodies can have some clinical utility but they do not always recognize true pathogenic antigens. Finally, when an autoantibody is detected, interpretation of the findings is not always easy. Researchers tend to look for sensitive antibodies that are present in a high proportion of patients of the studied syndrome, when the description of very specific antibodies (regardless of their frequency) might be more relevant. These are common problems to other antibody-mediated autoimmune diseases that now try to be overcome using multimodal autoantibody search approaches.

High disease-specificity, homogeneous clinical features associated to it, relevance of the antigen in disease pathogenesis, membrane location, and existence of knock-out animal models of the antigen resembling the autoimmune disease phenotype are the features that strongly suggest the importance of the antigen found.

1.2 Autoantibodies to Neuromuscular Junction Components

Autoimmune disorders of the neuromuscular junction (NMJ) include Myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS). In these pathologies, a series of autoantibodies to different pre- or postsynaptic NMJ antigens have been identified, and some of them have been proven to unequivocally contribute to their pathogenesis. In clinical practice, to test for the autoantibodies to NMJ proteins is useful for their precise diagnosis, prognosis, and in some cases to select a specific treatment.

1.2.1 Myasthenia Gravis

Myasthenia gravis (MG) is an autoimmune disease characterized by muscle weakness, fatigability, and autoantibodies against proteins of the skeletal muscle membrane at the NMJ. MG is a "classical antibody-mediated autoimmune disease" caused, in most cases, by autoantibodies directed against the acetylcholine receptor (anti-AChR antibodies). Significant progress was made in the knowledge of MG immunopathology when the pathogenic role of the anti-AChR antibodies was demonstrated, long ago [7, 8].

The search for novel antigenic reactivities in seronegative myasthenia (SNMG) patients has been a hot topic in MG research that has led to the description of autoantibodies to the Muscle-Specific Kinase (MuSK) in up to 50% of patients without autoantibodies to AChR [8], autoantibodies to Lipoprotein-Related Protein 4 (LRP4) [9–11], autoantibodies to "low-affinity" acetylcholine receptor (AChR) antibodies [12], and autoantibodies to cortactin [13–15]. According to these findings, the percentage of seronegative MG has been dramatically reduced.

A number of autoantibodies to proteins of the skeletal muscle—anti-striated muscle antibodies—such as titin, myosin, actin, and anti-ryanodine receptor antibodies may be present in MG patients [16].

Therefore, MG is not only a clinically but an immunologically heterogeneous disease, as determined by its various subgroups, AChR+, MusK+, LRP4+, low-affinity AChR+, cortactin, and seronegative MG [17, 18].

1.2.1.1 Autoantibodies to Acetylcholine Receptor

The acetylcholine receptor autoantibody response is T cell mediated. The pivotal role of the acetylcholine receptor autoantibodies in the pathogenesis of MG was demonstrated long ago. The anti-AChR antibodies were shown to act basically through three main mechanisms, including (a) the destruction of the postsynaptic

membrane by complement pathway activation, leading to a reduced expression of AChR at the muscle endplate, (b) the blockade of the acetylcholine binding to the receptor by antibodies attached to the acetylcholine binding site, or (c) reducing the number of functional AChRs by increasing their degradation and turnover due to the cross-linkage of AChRs by divalent antibodies [19–21].

1.2.1.2 Autoantibodies to MuSK

In MuSK-MG, the autoantibodies are against the receptor tyrosine kinase MuSK at the neuromuscular junction. A characteristic of these antibodies is that they are of the IgG4 isotype. IgG4 is a peculiar antibody isotype, formed by divalent heavy-light chain pairs [22, 23]. Several studies demonstrate the pathogenic properties of MusK antibodies [24–28]. It has been shown that IgG4 MuSK antibodies prevent MuSK to interact with LRP4 in a complement-independent manner [24, 29]. In MusK, unlike AChR+-MG, there is a clear correlation between severity and antibody levels [30–33] and none of the Musk-MG patients have thymoma.

1.2.1.3 Autoantibodies to LRP4

Antibodies against LRP4 were described in a subgroup of SNMG patients [30] and, in additional studies, it was confirmed that a subset of seronegative MG have autoantibodies to this NMJ protein [9, 11]. Prevalence ranges from 12 to 50% of AChR and MusK double negative patients, probably due to ethnic and/or technical differences in the published studies. Recent study indicates that the incidence of this antibody is likely to be very low [34]. The role of these antibodies in causing myasthenic symptoms in vivo has not been completely elucidated [35]. It is proposed that they can inhibit the interaction of agrin-LRP4-MuSK and disrupt AChR aggregation. No specific MG phenotype has been associated to antiLRP4 antibodies although it was described that these patients have a more severe clinical phenotype [9]. Furthermore, the prevalence of antiLRP4 antibodies in seropositive MG cohorts and in other autoimmune diseases is still to be clarified as well to determine their specificity.

1.2.1.4 Low-Affinity Antibodies to AchR

It was reported that up to 66% of patients with SNMG have low-affinity IgG1 antibodies against the AChR, that are only detectable in a cellular assay using HEK cells co-transfected with human AChR subunits and rapsyn to induce AChR clustering on the cell surface [36]. Later on, the same authors reported that up to 50% of patients with SNMG, generalized or ocular, have antibodies that recognize clustered AChR and their levels correlate with the electrophysiologic features [37]. These antibodies fix complement and decrease miniature motor endplate potentials to the same extent as anti-AChR antibodies, suggesting a pathogenic role comparable to that of classical anti-AChR antibodies [38, 39]. Low-affinity anti-AChR antibody test is not currently commercially available. Patients with this type of autoantibodies generally have an ocular MG or a mild generalized disease.

1.2.1.5 Autoantibodies to Cortactin

We reported the presence of autoantibodies to cortactin, an NMJ subsarcolemmal membrane protein, in 20% of SNMG patients [13]. Testing for these antibodies, in

addition to clinical and electrophysiological studies can be used as a potential marker of an underlying immune mechanism related to a suspected MG specially for patients with ocular symptoms. We found that patients with cortactin antibodies and SNMG had an ocular or mild generalized phenotype of MG [39, 40]. Cortactin is needed for the formation of AChR clusters. Cortactin signaling downstream from agrin/MuSK promotes actin polymerization and AChR/rapsyn clustering [40]. These functions of cortactin indicated that it could be a new antigen playing a role in the development of MG. Research should be done to determine the pathogenic role of these autoantibodies.

1.2.1.6 Striational Antibodies

Some patients with MG have autoantibodies to proteins expressed in the cytoplasm of the striated muscle. These striational antibodies do not participate in the pathogenesis of the disease, but they may have some value as predictors of disease severity or thymoma [41]. For instance, they are detected in 75–85% of patients with MG and thymoma, particularly in young patients. The presence of titin and RyR antibodies in early-onset MG (<50 years) has a 90% positive predictive value and a 95% predictive negative value for thymoma. However, these antibodies are not so clearly associated to disease severity [42].

1.2.2 Lambert-Eaton Myasthenic Syndrome (LEMS)

LEMS is a rare autoimmune disease of the neuromuscular junction in which predominant symptoms are lower limb weakness and dysautonomia, due to acetylcholine release failure in the pre-synaptic terminal. Almost 60% of patients present as a paraneoplastic syndrome, in which small-cell lung carcinoma is the most frequently associated tumor. Ninety percent of LEMS patients have antibodies against voltage-gated calcium channels (VGCC) of the P/Q type. Although it has not been formally demonstrated, these antibodies are thought to have pathogenic potential. They target a membrane channel that is relevant in disease pathogenesis, disease can be transmitted from mother to child during pregnancy, mutations in the VGCC result in an LEMS-like phenotype and active immunization in rats determines a mild LEMS-like disease in the animals.

An important issue in LEMS care is ruling-out a hidden neoplasm. Anti-SOX1 antibodies are present in a high proportion of small-cell lung carcinoma-associated LEMS patients while are absent in non-paraneoplastic LEMS. This antibodies are, thus, a very good biomarker with immediate clinical implications in the search for a hidden neoplasm in LEMS [42].

1.2.3 Autoantibody Testing

1.2.3.1 Diagnosis

An international consensus guidance for MG diagnosis and care has been published. This guideline task force, formed by a large group of experts in MG, agreed that the

study of autoantibodies to the NMJ proteins should be done, for diagnostic purposes, in all patients with suspected MG [43, 44]. Antibodies against AChR are found in around 85% of patients with MG while anti-MusK antibodies are detected in 5–10% of patients previously classified as seronegative [43]. The finding of these autoantibodies confirms unequivocally the diagnosis of MG and distinguishes subgroups of autoimmune MG. Although patients may have similar clinical characteristics, it is now established that MG-MuSK+ patients are predominantly young females and have a preferential involvement of facial, bulbar, and axial muscles. Furthermore, the diagnosis of MG-MuSK+ may have therapeutic implications. We reported the clinical, immunologic, and long-term response to rituximab of patients with MuSK+MG and AChR+MG resistant to other therapies. A benefit of the therapy was observed in both groups of patients, however, in view of the long-lasting benefit observed in MuSK+MG patients, we recommend to use rituximab as an early therapeutic option in this group of patients with MG if they do not respond to prednisone [44, 45].

The study of LRP4 in selected SNMG patients will help to define the clinical characteristics of this subgroup of MG and the presence of cortactin Abs in patients with suspected SNMG may help to support the diagnosis of immune MG and consequently the use of immune therapy.

1.2.3.2 Correlation with Disease Severity

Studies addressing the diagnostic value and the correlation of anti-AChR antibody titers and disease severity were done long ago [46] and are reviewed periodically [47]. It is well known that anti-AChR antibody titers vary among patients. However, patients with similar degrees of weakness may have quite dissimilar titers of anti-AchR antibodies and, consequently, cannot reliably predict the severity of disease in individual patients. The anti-AchR antibody test is widely used by clinicians to diagnose and evaluate clinical status and is especially useful to diagnose elderly patients with bulbar symptoms [48, 49]. A retrospective study showed that antibodies against the main immunogenic region of AChR differentiate between ocular and generalized MG [6, 48].

Autoantibodies to MuSK correlate with disease severity [49, 50] and have been demonstrated useful as markers of response to immune therapy. For instance, rituximab has shown effectiveness in both AChR+ and MusK-MG. However, while AChR antibody titers varied along the follow-up and did not correlate with disease improvement, MuSK antibodies decreased dramatically during the follow-up after a single rituximab cycle, the response was long-lasting and it was associated with a dramatic clinical improvement [3]. No studies are available for LRP4, low-affinity AChR, or cortactin antibodies.

1.3 Antibodies in Inflammatory Neuropathies

The field of inflammatory neuropathies is one of the fields in which the discovery of autoantibodies has yielded more relevant results, especially in Guillain-Barre syndrome (GBS) and its variants [51–55] but also in chronic autoimmune neuropathies,

such as multifocal motor neuropathy or anti-MAG-associated polyneuropathy [56]. In the last years, the discovery of autoantibodies directed against structures of the node of Ranvier has placed the focus on chronic inflammatory demyelinating polyneuropathy (CIDP) variants harboring these antibodies and has boosted research on the topic [57].

1.3.1 General Considerations

The inflammatory neuropathies are a heterogeneous group of peripheral nerve disorders that can be acute (GBS and its variants) or chronic (CIDP, multifocal motor neuropathy, polyneuropathy associated to monoclonal gammopathy of unknown significance, etc.) that share an immune-mediated pathogenesis [48, 58, 59]. The search of autoantibodies has been an important topic of research in all the diseases of this group since the description of the first animal models of experimental autoimmune neuritis [60]. The autoantibodies described in these diseases share several common features that are specific of these diseases and are not common in other organ-specific autoimmune diseases. First, the antigens described are usually glycosylated structures, mainly glycosphingolipids (gangliosides) and glycoproteins [61], second, the detection of specific autoantigens has led to the discovery of specific microbial strains associated to the development of the disease in the GBS-spectrum of diseases [61] (indeed, GBS is the paradigmatic disease of autoimmune post-infectious pathophysiology) and, third, despite being all of them very rare disorders, the discovery of autoantibodies has helped defining even smaller subgroups of patients with homogeneous clinical features.

1.3.2 Guillain-Barre Syndrome and Variants

1.3.2.1 Anti-ganglioside Antibodies

GBS is the most frequent inflammatory neuropathy. In GBS, it is widely accepted that the peripheral sensitization to a microbial antigen results in an immune response that includes antibodies against microbial structures [62]. These antibodies, by a molecular mimicry mechanism, cross-react with nerve molecules that share some structural similarities with microbial antigens and fix complement, determining nerve pathology. Diverse infectious agents have been described to associate with the development of GBS, including *Campylobacter jejuni*, cytomegalovirus, or influenza viruses [63]. In the recent years, the ZIKV pandemic was followed by a surge of GBS cases [60] and the SARS-CoV2 pandemic has been associated to the development of GBS in specific cases although there has not been an increase in GBS incidence [64–66]. In these two last cases, specific autoantibodies have not been found. The development of the axonal motor variant of GBS after a *Campylobacter jejuni* infection is the first and best characterized model of molecular mimicry in a human autoimmune disease [67, 68]. In this model, a patient with a *C. jejuni* gastrointestinal infection develops antibodies of the IgG subclass against the lipooligosaccharides (LOS) of the *C. jejuni* bacterial wall. The structural similarity of the LOS

with the nerve ganglioside GM1 determines a cross-reaction of the antibodies directed against the bacterial structures with nerve structures and, finally, complement fixation leading to nerve damage [69]. Although a less frequent variant, this same model, explains the axonal sensory and motor variant of GBS, in which IgG anti-GM1 antibodies are also present. This model has led to the exploration of complement inhibitors as GBS treatments. In vitro and in vivo studies support this disease model that reinforces the importance of molecular mimicry in the development of acute post-infectious autoimmune diseases.

Miller-Fisher syndrome (MFS) is an uncommon variant of GBS in which, again, the peripheral sensitization to a microbial antigen precedes the development of ophthalmoparesis, ataxia, and areflexia [70]. In this case, the microorganisms involved and the mechanisms leading to a crossed reactivity are not characterized in detail. However, up to 95% of patients develop antibodies against the GQ1b ganglioside with very high specificity. The GQ1b ganglioside is abundant in the muscle spindles, oculomotor nerves, and nerve roots and, thus, the presence of complement-fixing autoantibodies directed against these structures ultimately leads to the development of the typical features of the disease. Passive transfer studies in animal models demonstrate the pathogenic potential of the anti-GQ1b autoantibodies in MFS [71, 72]. Not surprisingly, anti-GQ1b antibodies have a diagnostic accuracy for MFS that exceeds that of lumbar puncture and represent one of the best examples of the clinical utility of defining homogeneous clinical subgroups to detect highly specific and clinically relevant autoantibodies. The detection of anti-GQ1b autoantibodies can also help detecting patients that do not present with the typical clinical features, such as those few including brainstem signs like Bickerstaff encephalitis variants of the MFS or those with *forme fruste* variants such as ataxic GBS or pure isolated ophthalmoparesis, in which anti-GQ1b antibodies are also positive [53]. On the contrary, the absence of these antibodies should raise doubts regarding a diagnosis of MFS and other diagnostic mimics (Wernicke's encephalopathy, brainstem encephalitis of any origin, botulism, etc.) should be ruled out.

Anti-ganglioside antibodies are also useful in the clinical definition of other GBS variants. For example, anti-GT1a antibodies are frequently found in the rare pharyngeal-cervico-brachial palsy variant of GBS and antibodies directed against gangliosides bearing disialosyl epitopes (GD1b, GD3, GT1a, GQ1b) can be detected in a significant proportion of patients with acute ataxic neuropathy [55, 73].

1.3.2.2 Other Autoantibodies in GBS

Despite the description of anti-ganglioside reactivities has led to a better understanding of the disease pathogenesis and to clinical utility in some GBS variants, in the acute inflammatory demyelinating polyradiculoneuropathy (AIDP) variant, the most frequent one in the western world, the discovery of specific autoantibodies remains elusive. Disease onset and progression of the AIDP variant shares many features with the other GBS variants and the response to intravenous immunoglobulin and plasma exchange supports a role of humoral factors in its pathogenesis. Several studies describe antibodies against gangliosides, myelin and axonal proteins, node of Ranvier structures, and intracellular antigens [50–54]. So far, none of

these autoantibody reactivities has been replicated or demonstrated useful for clinical practice. Some patients with aggressive-onset autoimmune neuropathies, including patients classified as classical GBS, have been associated to nodal-paranodal autoantibodies. The autoimmune nodopathies, described with the chronic autoimmune neuropathies in the following subheading, may present with aggressive phenotypes that may be misdiagnosed as GBS [74, 75]. The antibodies in these patients play a crucial role since their detection implies a completely different therapeutic approach.

1.3.3 Chronic Inflammatory Neuropathies

The group of chronic autoimmune neuropathies includes CIDP, multifocal motor neuropathy (MMN), and polyneuropathies associated with monoclonal gammopathy of unknown significance (MGUS-P). Currently, the appearance of the autoimmune nodopathies, generally included within the spectrum of CIDP but clearly differentiated from the typical CIDP, has expanded the field of chronic autoimmune neuropathies to incorporate chronic aggressive autoimmune neuropathies associated with nodo-paranodal antibodies that may be sometimes misdiagnosed as GBS. Despite their clinical heterogeneity, these diseases share their response to immune therapies and, as in acute inflammatory neuropathies, the study of autoantibodies has been the main topic of research in their immune-pathogenesis [51–55].

1.3.4 Chronic Inflammatory Demyelinating Polyradiculoneuropathy

CIDP is the most frequent disease of the group. Experimental evidence and the response to intravenous immunoglobulin and plasma exchange suggest an immune-mediated pathogenesis and a role for humoral factors in its pathogenesis [51, 76–78]. As it happens with the AIDP variant of GBS, clinically useful antibodies have been elusive despite extensive research efforts. This is particularly true for the typical variant of CIDP, in which there is no autoantibody serving as a useful biomarker in the everyday practice.

1.3.4.1 Nodo-Paranodal Autoantibodies

The field of autoantibodies in CIDP has recently regained interest after the description of small subsets of patients harboring antibodies against node of Ranvier structures with clinical utility [52]. Our group and others described the presence of a subset of patients fulfilling CIDP diagnostic criteria but frequently presenting with more aggressive and subacute phenotypes that harbored antibodies of the IgG4 isotype targeting node of Ranvier structures (nodes and paranodes). One of the newly identified antigens is contactin-1 (CNTN1), an axonal protein of the paranode of Ranvier that forms a dimer with contactin-associated protein-1 (CASPR1), essential for maintaining nodal integrity and saltatory conduction. Patients harboring

anti-CNTN1 antibodies present with an aggressive neuropathy with poor response to intravenous immunoglobulin and frequently associated with nephrotic syndrome. These antibodies were initially described in older-age patients and associated with motor predominance but younger patients, including children, and other phenotypes (ataxia, tremor) have also been associated to these autoantibodies [79].

Neurofascin 155 (NF155) is the glial ligand of the CNTN1-CASPR1 complex and is also essential for the maintenance of the paranodal loops at the axo-glial junction that enable node of Ranvier integrity and salutatory conduction. A German group detected antibodies against NF155 in a few patients with CIDP and GBS [80, 81] and our group described that patients harboring anti-NF155 antibodies present with typical features that include distal predominance of the motor involvement, a prominent intention tremor and lack of response to intravenous immunoglobulins [82, 83]. These clinical associations with the anti-NF155 have been confirmed in other cohorts [84]. The description of these autoantibodies has enabled the identification of a specific pathological phenotype in which inflammation and segmental demyelination are rare but in which loss of transverse bands and detachment of the paranodal loops and axons appear instead [55]. These patients are also very strongly associated to the DRB1*15 human-leukocyte antigen class II alleles (in contrast to typical CIDP that do not associate to an specific HLA haplotype) [54].

After these two antigens antibodies against CASPR1, a protein complexing with CNTN1 at the paranodes, have been associated with specific subsets of patients with subacute inflammatory neuropathies fulfilling CIDP diagnostic criteria [85]. Anecdotal case-reports, including patients with CIDP and GBS had been described and prompted the search of patients with these autoantibodies. In a very recent study, Pascual-Goñi et al. describe the clinical and immunological features of anti-CASPR1 antibodies. These antibodies associate, again, with an aggressive-onset neuropathy, associated with cranial nerve involvement and, sometimes, with respiratory failure, poor response to intravenous immunoglobulins and, frequently, with pain.

Finally, antibodies against pan-neurofascin (these antibodies target all neurofascin isoforms but, primarily the nodal isoforms: neurofascin-140 and 186), have also been described in patients with aggressive neuropathies, cranial nerve involvement, poor response to intravenous immunoglobulins, and nephrotic syndrome [58, 86].

Interestingly, the majority of patients with autoimmune nodopathies harbor antibodies of the IgG4 isotype [87]. IgG4 is an anti-inflammatory autoantibody that is unable to activate complement or bind Fc gamma receptors in inflammatory cells. This means that the autoantibody itself must be pathogenic interfering with the function of the target antigens. Anti-CNTN1 antibodies disrupt the union between CNTN1/CASPR1 and NF155 and determine paranodal disruption in in vitro models and passive transfer of human IgG from patients harboring anti-CNTN1 antibodies disrupt paranodes and cause a demyelinating neuropathy resembling the one found in patients in experimental animal models [56, 88]. In a similar fashion anti-NF155 antibodies from patients prevent the incorporation of NF155 to the NF155/CNTN1/CASPR1 complex and cause a demyelinating neuropathy with features resembling those found in patients with anti-NF155 antibodies when injected

intrathecally into animal models [89]. Formal demonstration of the pathogenicity of anti-pan-neurofascin and anti-CASPR1 antibodies is still pending. The appearance of the autoimmune nodopathies have boosted the interest in describing new antigenic reactivities in immune neuropathies and questioned the homogeneity of CIDP as a single entity [90, 91].

1.3.4.2 Antibodies Against Myelin Proteins

Considering the demyelinating nature of CIDP, most initial studies, using the candidate-antigen approach, focused in the description of antibodies against myelin proteins in CIDP. Some studies found antibodies against myelin protein zero, myelin protein 2, connexin 32, or myelin protein 22 [92]. These autoantibodies were found in significant proportions in control populations or in hereditary neuropathies and CIDP specificity was not confirmed in other cohorts.

Two recent studies have described the presence of anti-myelin-associated glycoprotein antibodies in patients fulfilling CIDP diagnostic criteria in which there was no evidence of monoclonal gammopathy [93, 94]. Some of these patients, clinically similar to the typical patients with monoclonal gammopathy-associated polyneuropathies, developed the monoclonal gammopathy years after identifying the anti-MAG antibodies. Although preliminary, these studies could have important implications for diagnosis of anti-MAG neuropathies since current guidelines only recommend anti-MAG tests whenever a monoclonal gammopathy is found [95].

1.3.4.3 Anti-ganglioside Antibodies

A few reports also link CIDP to antibodies against glycosphingolipids. LM1 ganglioside is the predominant ganglioside in peripheral myelin. Some reports describe antibodies against the LM1 ganglioside in a small subset of CIDP patients that presented more frequently with ataxia than anti-LM1-negative CIDP patients [49]. Also, anti-GM1 antibodies of the IgM isotype, identical to those found in multifocal motor neuropathy, have also been described in small subsets of patients with CIDP although confirmation in larger cohorts is needed [96].

1.3.5 Multifocal Motor Neuropathy

Multifocal motor neuropathy (MMN) is a chronic, focal, exclusively motor neuropathy that responds to intravenous immunoglobulin. As most diseases of the group, it is diagnosed based on clinical and electrophysiological criteria that, in this case, include the presence of nerve conduction blocks. The immune pathogenesis of the disease, again, is inferred from the fact that it responds to intravenous immunoglobulin [97].

Approximately half of the patients with MMN have IgM antibodies against the GM1 ganglioside. These autoantibodies bind to node of Ranvier structures of motor axons (where GM1 is enriched) and fix complement, disrupting nodal and paranodal molecular organization. These autoantibodies cause motor dysfunction in an analogous way IgG anti-GM1 antibodies do in axonal GBS. Nevertheless,

anti-GM1 testing leaves half MMN without identifiable antigens. Some authors propose that testing antibodies against combinations of gangliosides could increase the frequency of patients positive and, thus, improve the diagnostic yield of these autoantibodies. For example, testing IgM antibodies against the galactocerebroside/GM1 complex increased the sensitivity of the test to 75% (from a 48% when testing anti-GM1 antibodies only) [98]. Some series also describe IgM antibodies against GM2 gangliosides in a subset of MMN patients but their frequency is much lower than GM1 [99].

Although their clinical relevance is unknown, it has been described that some patients with multifocal motor neuropathy in which anti-GM1 antibodies are not identified, react against human motor neurons derived from induced pluripotent stem cells with reactivity patterns identical to those of patients in which anti-GM1 antibodies are present [100].

One important clinical utility of anti-GM1 IgM antibodies appears in the context of a lower motor neuron syndrome in which clear motor nerve conduction blocks are not identified in the electrophysiological studies. The detection of high-titer anti-GM1 IgM antibodies in this context may identify those patients that differentiate from amyotrophic lateral sclerosis patients and that are eligible for therapy with intravenous immunoglobulins [59].

1.3.6 Polyneuropathy Associated to Monoclonal Gammopathy of Unknown Significance (MGUS-P)

Monoclonal gammopathy frequently associates with immune neuropathies. In CIDP, for example, up to 30% of patients can present with a monoclonal gammopathy of unknown significance. Whether this association is key in the disease pathogenesis is unknown. Up to 50% of patients with an IgM monoclonal gammopathy have a polyneuropathy, regardless of the origin of the gammopathy (Waldenstrom's macroglobulinemia, multiple myeloma, MGUS, etc.) [101]. Approximately 50% of these patients have a specific demyelinating neuropathy characterized by distal phenotype, large-fiber sensory predominance, distal tremor, slow progression, and poor response to conventional immune therapies that associates to antibodies against myelin-associated glycoprotein (MAG) [102]. Anti-MAG antibodies define a specific subgroup within all IgM monoclonal gammopathy-associated polyneuropathies with diagnostic and prognostic implications. Deposition of IgM and complement fixation in myelinated fibers of these patients and the development of a demyelinating neuropathy when animals are immunized with MAG suggest that, although studies are scarce, the anti-MAG antibodies can be pathogenic [103–105].

Some ataxic neuropathies (sometimes associated with ophthalmoparesis) and IgM monoclonal gammopathy that not necessarily display demyelinating features in the electrophysiological studies and, thus, may be difficult to identify as treatable neuropathies, display antibodies against disyalosil-epitope bearing gangliosides (mainly GD1b, but also GQ1b, GD2, GD3) of the IgM isotype. These anti-GD1b-associated neuropathies respond to intravenous immunoglobulins and, thus, the

detection of the anti-GD1b antibodies may differentiate them from other monoclonal IgM-associated neuropathies that may not respond to therapy [106].

1.3.7 Other Diseases of the Peripheral Nerve

1.3.7.1 Neuromyotonia

Although very infrequent, autoimmune neuromyotonia is another disease in which recent discovery of disease-specific associated autoantibodies has changed its diagnosis and knowledge on its pathogenesis. Contactin-associated protein 2 (CASPR2) is a protein of the juxtaparanode that is necessary for potassium channel function and normal saltatory nerve conduction. One of the antigens targeted by antibodies initially attributed to voltage-gated potassium channel complex antibodies in Morvan's syndrome and peripheral nerve disorders with peripheral nerve hyperexcitability was identified as CASPR2. These antibodies also associate with specific HLA haplotypes and have been described to be pathogenic in animal models [107].

1.4 Myositis-Specific Autoantibodies

1.4.1 General Considerations

A number of antibodies against skeletal muscle antigens have been described in inflammatory myopathies [108]. Myositis-specific autoantibodies (MSA) are mainly intracellular; therefore, its pathological significance is still under debate. In this section, we will describe briefly each autoantibody discovered so far in the different inflammatory myopathies that can be used for the diagnosis/prognosis of these diseases. We will focus on MSA that are present in 40–50% of patients with inflammatory myopathies. Most patients with myositis present a single MSA. Table 1.1 summarizes the most relevant autoantibodies associated to the different types of myositis.

1.4.1.1 Anti-synthetase Antibodies

Multiple antibodies to different aminoacyl t-RNA synthetases have been described. These are enzymes expressed in the cytoplasm that bind the aminoacids that have affinity for each transference RNA (t-RNA). Anti-Jo-1 (anti-histidyl-t-RNA synthetase) are the most frequently found associated to myositis and can be found in 25–30% of patients with anti-synthetase syndrome (AS) dermatomyositis (DM) and polymyositis (PM). The prevalence of the other anti-synthetase antibodies falls down to 1–5% and includes anti-threonyl-t-RNA synthetase (PL7), anti-alanine-t-RNA synthetase (PL12), and anti-glycyl-t-RNA synthetase (EJ) among others. After Jo-1, anti-PL7, and anti-PL12 are the most frequently detected and are associated with a less severe myositis but a more severe interstitial lung disease (ILD) than Jo-1.

Table 1.1 Myositis-specific autoantibodies

Antibody	Target antigen	Frequency	Diagnosis	Useful clinical features
Anti-Jo1	Histidyl-t-RNA synthetase	15–30%	PM, DM, IMNM, ASS	Mechanic hands ILD, heart involvement
Anti-PL7	Threonyl-t-RNA synthetase	5–10%	PM, DM, IMNM, ASS	Mechanic hands ILD
Other anti-synthetases	Other t-RNA synthetases	<5%	PM, DM, IMNM, ASS	Mechanic hands ILD
Anti-TIFIγ	Transcriptional intermediary factor γ	10–20%	DM adult	Cancer
Anti-Mi2	Nucleosome remodeling-deacetylase	6%	DM	More severe muscle involvement
Anti-MDA5	Melanoma differentiation associated gene 5	19%	DM amyopathic	ILD
Anti-SAE	Small ubiquitin-like modifier activating enzyme	8%	DM adult	Dysphagia, skin disease
Anti-SRP	Signal recognition particle	5–10%	IMNM	Heart involvement
Anti-NXP2	Nuclear matrix protein 2	23% JDM 11% DM	DM juvenile > adult	Cancer, calcinosis
Anti-HMGCR	3-hydroxy-3-methyl glutaryl coenzyme A reductase	6%	IMNM	–
Anti-cN1A	Cytosolic 5'nucleosidase 1A	30%	IBM	Dysphagia, distal weakness UULL

ILD interstitial lung disease, *PM* polymyositis, *DM* dermatomyositis, *IBM* inclusion body myositis, *IMNM* immune-mediated necrotizing myopathy, *ASS* anti-synthetase syndrome, *UULL* upper limbs

(Modified from Allenbach and Benveniste J Neuromusc Dis 2: 13-25, 2015)

Patients with reactivities against these antigens share a wide variety of clinical features such as ILD, arthritis, fever, and skin lesions known as "mechanic hands." All these different manifestations are known as AS although not all patients with these antibodies show all these clinical features. Some patients with acute or subacute myalgias and weakness associated to anti-synthetase antibodies can present necrotizing myopathy features in the muscle biopsy [109].

1.4.1.2 Anti-Mi2 Antibodies

Anti-Mi-2 targets the Mi-2/NuRD complex that participates in chromatin remodeling. This complex participates in development and differentiation of T and B cells and the formation of the basal lamina of the epidermis. They are found almost exclusively in patients with DM (10–30%). They have been related to skin changes, better response to corticosteroids and a lower risk of developing cancer. It has been

recently reported that DM patients with anti-Mi2 antibodies display a more severe muscle involvement than those negative for these antibodies. Also, the titers correlate with the disease and may normalize in patients who enter remission [110].

1.4.1.3 Anti-TIF-1γ Antibodies

Initially called anti-p155/140, transcriptional intermediary factor γ (TIF-1γ) antibodies are detected in patients with DM, especially in those associated to neoplasia. Therefore, this biomarker is relevant to the early detection of one of the major complications that represent a serious risk for the patient. 50–100% of patients, depending on the series, with DM and neoplasia have these antibodies in Japan. In contrast, in Europe and America between 15 and 20% of patients with DM without neoplasia are also positive for these antibodies. This discrepancy could be explained not only by racial differences but also different criteria to select patients. The sensitivity and specificity together with the negative and positive predictive values obtained by ELISA and Western-blot are similar to those obtained using radiolabeling-based immunoprecipitation; therefore, this last technique could be substituted by the former ones [74].The presence of anti-TIF1-γ antibodies may be found before symptoms of cancer and antibody titers are reduced or disappear after successful treatment of cancer with remission of DM [111]. It has been reported that genetic analysis of tumors from DM patients with anti-TIF1 antibodies show an increased number of mutations and loss of heterozygosity in TIF1 genes. The authors suggest that these changes may generate new antigens that would trigger an autoimmune response by a phenomenon of molecular mimicry [111, 112].

1.4.1.4 Anti-SRP Antibodies

Signal recognition particle (SRP) is a ubiquitously expressed protein involved in the transport of newly synthesized proteins to the endoplasmic reticulum. Although formerly associated to classical myositis, it is now accepted that they are associated to immune-mediated necrotizing myopathies (IMNM) that can also present with lung or heart involvement. In patients with IIM, the prevalence of anti-SRP positivity ranges from 5 to 15%. The antibody titers correlate with muscle strength and creatine kinase levels. Anti-SRP-positive IMNM affects predominantly women and girls [113].

1.4.1.5 Anti-HMGCoAR Antibodies

The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) is a key molecule in the biosynthesis of cholesterol. The presence of antibodies against HMGCR is associated with patients treated with statins who develop a myopathy sometime after initiating the treatment and that persists after discontinuation of the treatment. Anti-HMGCR antibodies recognize the intracellular C-terminal fragment of the enzyme. However, these antibodies have been also found in patients that have not taken statins. These patients are generally younger and several pediatric cases have been reported. This is important because these patients often are incorrectly diagnosed of an untreatable limb girdle muscular dystrophy and can be treated successfully if the antibodies are detected. Anti-HMGCR antibodies have not been

detected in genetically confirmed muscle diseases. Typically, these antibodies have been found in patients with clinical features of a necrotizing myopathy. As in anti-SRP antibodies, anti-HMGCR antibody titers correlate with muscle strength and creatine kinase levels. Between 6 and 10% of patients with IMNM present anti-HMGCR antibodies. These antibodies are more frequently detected in women >40 years of age [114].

1.4.1.6 Anti-MDA5 Antibodies

Melanoma differentiation-associated gene 5 (MDA5) is a RNA helicase that belongs to the family of intracellular receptors of innate immunity as a defense against viral infections. Sato et al. discovered in 2005 the presence of these antibodies in Japanese population. Anti-MDA5 antibodies have been found exclusively in patients with amyopathic dermatomyositis, and most of them develop a rapidly progressive interstitial lung disease that often has fatal consequences. Therefore, detection of these antibodies is of high prognostic value for these patients [115]. It has been recently reported that three different subgroups can be observed among MDA5+ patients: (1) Rapidly progressive interstitial lung disease with severe lung involvement and poor prognosis; (2) Anti-MDA5+ rheumatic DM with good prognosis; and (3) Anti-MDA5+ vasculopathic DM with an intermediate prognosis [116].

1.4.1.7 Anti-SAE Antibodies

Anti-small ubiquitin-like modifier activating enzyme (SAE) identify a subgroup of patients with adult DM. Most of them present skin lesions and systemic features that can include dysphagia. These antibodies have been associated to the haplotype HLA DRB1*04-DQA1*03-DQB1*03 [117].

1.4.1.8 Anti-NXP2 Antibodies

The target antigen of these antibodies is the nuclear matrix protein 2. DM patients presenting these antibodies normally present with proximal and distal muscle weakness, edema, and/or dysphagia [118] and are more prone to develop calcinosis. These antibodies are very infrequent, are associated to develop cancer within 3 years of the diagnosis, and have a higher incidence in adult DM.

1.4.1.9 Anti-cN1A Antibodies

The presence of antibodies against different nuclear and cytoplasmic antigens in skeletal muscle in patients with IBM had been reported years ago, especially in those patients with a monoclonal gammopathy [119, 120]. Using mass spectrometry analysis, it was reported that antibodies to a 44 KDa protein that had been called Mup44, recognize the cytosolic 5′ nucleosidase 1A [121]. These antibodies are found in 30–60% of IBM patients, 5–10% PM, 15–20% DM and in patients with Sjogren syndrome and lupus [122]. Anti-NT5C1a autoantibodies have also been associated with increased clinical severity and a higher risk of mortality [121]. Finally, passive immunization of mice with anti-NT5C1a IgGs leads to the formation of p62 aggregates and macrophage infiltration [122].

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Electromyography

2

Lucio Santoro and Fiore Manganelli

2.1 Introduction

Myopathies are a heterogeneous group of diseases induced by numerous pathogenic mechanisms that include many different phenotypes and show a variable muscle pathology. Diagnostic approach can be simple in some instances, but it can also be ambiguous when symptoms are very light or when the hyposthenia involves distal muscles. In the majority of cases, a careful clinical examination, the personal and family history, and the biochemical data are sufficient to formulate a differential diagnosis with a neurogenic process. When this is not possible, the most useful investigations are the electrodiagnostic studies and more specifically the muscle examination by means of needle EMG. This technique is also very useful for guiding the choice of the muscle to be eventually biopsied, to characterize the distribution of the involved muscles, and to set the severity of the myopathy. Nerve conduction studies and tests for neuromuscular transmission (NMT), which includes the repetitive nerve stimulation (RNS), and single-fiber electromyography (SFEMG), are needed in special cases with the aims to confirm the clinical suspect of an NMT disorder and to establish whether it is presynaptic or postsynaptic.

In this chapter, we will illustrate the neurophysiologic findings most useful for the differential diagnosis of a myopathy with respect to a neurogenic disease, and moreover we will try to correlate EMG findings with muscle pathology with the aim to give useful data for the identification of a specific form of myopathy.

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2.2 Anatomo-Physiological Basis

A motor neuron in the anterior horn of the spinal cord, its axon, and all the muscle fibers it innervates constitute a motor unit (MU), according to the definition of Sherrington [1]. The MU is the smallest part of a muscle that can be activated voluntarily. The number of muscle fibers belonging to the same MU (innervation ratio) varies considerably among the muscles. Normally, the muscles which perform fine movements have few fibers for MU (i.e., 5–10 in the external muscles of the eye), while muscles whose task is to generate as much strength as possible can have thousands of fibers in their MU (i.e., almost 2000 in the gastrocnemius muscle). Each MU occupies a circular territory in the muscle of about 5–10 mm in diameter, and in this area, fibers of several MU (from 5 to 30) are intermingled. The distribution of muscle fibers in the cross-sectional area of a muscle shows that fibers sharing the same innervation (belonging to the same MU) are generally isolated, less often in pairs and very rarely they are associated in number of three or more. This peculiar distribution avoids the interference between twitches of neighboring MUs and limits the interaction between action potentials of muscle fibers of the same MU. According to their biochemical and physiological characteristics, the MUs of human muscle can be classified into three main groups, namely, the slow-twitch, oxidative type which have the smallest axons, slow firing frequency, high content of oxidative enzymes, low content of glycogen and phosphorylase, and high resistance to fatigue and express low tension. The second type of MUs is called fast-twitch, oxidative, and glycolytic type, and they have high content of both oxidative enzymes and glycogen and phosphorylase; they also have high resistance to fatigue but can express a medium tension. The last type are the fast-twitch, glycolytic type; these MUs have the largest axons, high content of glycogen and phosphorylase, low content of oxidative enzymes, and low resistance to fatigue but can express a high tension during bursts of high-frequency discharge [2]. The electrical activity produced by voluntary contraction of muscles or in response to motor nerve stimulation can be recorded by intramuscular electrodes (needle EMG) or by cutaneous electrodes (motor nerve conduction) and is a very important tool for the investigation of muscle and peripheral nerve diseases. Cutaneous electrodes can record the electrical potential generated by the whole muscle (compound muscle action potential, CMAP), while concentric needle intramuscular electrode can record the electrical potential generated by a single MU (motor unit potential, MUP); both potentials are in volume since they are recorded in the extracellular space; therefore, their peak-to-peak voltage declines steeply with radial distance from muscle fibers that originate the corresponding transmembrane potential. Needle electrode can also capture the single-fiber potential when they originate spontaneously from a denervated muscle fiber and are called fibrillation or denervation potentials. The MUPs recorded with a concentric needle electrode have three parameters that need to be considered for a reliable evaluation of the investigated muscles: duration, amplitude, and morphology. All the parameters largely depend on the number of muscle fibers, belonging to the same MU, included in the recording area of the electrode, on their caliber, and on their degree of synchronization. In other words, the clustering of muscle

fibers of the same MU close to the leading-off surface of the electrode will increase the peak-to-peak amplitude of the MUP; vice versa, a reduced number of muscle fibers will reduce the amplitude. However, if the caliber of few surviving fibers is clearly increased (i.e., hypertrophic fibers), it is still possible to record a high-amplitude MUP. The MUP duration is a more stable and repetitive parameter than the amplitude and largely depends on the number of the MU fibers present in a large recording area (almost 2.5 mm). Therefore, the pathological processes which induce primary loss of muscle fibers habitually also determine a reduced MUP duration, while the diseases which imply axon sprouting or regeneration with clustering of muscle fibers belonging to the same MU increase MUP duration. The MUP morphology can vary from the classical biphasic or triphasic shape to a polyphasic shape (more than four phases crossing the baseline) when muscle fibers do not discharge synchronously. This can happen when the neuromuscular transmission is compromised or when noncontractile tissues (lipids or connective) have modified the MU spatial distribution. All the MUP parameters vary according to the patient age and the examined muscle; therefore, every evaluation must be performed with respect to the normative data that should be produced by the laboratory which has performed the neurophysiological exam. The CMAP amplitude is the most important parameter for evaluating the integrity of MU number in an examined muscle. However, some variability due to technical and anatomical aspects is always present, and a variation from normal values of at least 40% should be considered. In addition, it is true that the loss of motor units is the most frequent cause of reduction of CMAP amplitude; however, if there is a severe loss of muscle fibers in a long-standing dystrophic process, the same finding can also be recorded. The order of MU recruitment is task related and can also vary according to the preexisting experience. However, the size principle of Henneman is the rule when a gentle movement is required; therefore, the small MU will be recruited first [3]. Thereafter, if a greater tension is needed, the large MU will intervene. The electromyography system can analyze the single MUPs of small MU, but cannot see individual MUP of large MU, which can be analyzed only by automatic system. It is possible to record the MU recruitment, and this is normally progressively increasing until a maximum where the single MUP cannot be recognized from each other (interference pattern). This technique needs the cooperation of the patient and is anyway difficult in some muscles (e.g., gastrocnemius). With these limitations, it is however possible to observe a reduced recruitment pattern (single oscillations or mixed pattern) with high amplitude in neurogenic diseases, while a fast interference pattern with low amplitude is frequent in myopathic diseases, at least if this is not very long lasting. Overall, a normal MUP requires that in the recording area of the needle electrode, muscle fibers of a single MU are present with normal density and have a homogenous caliber and an efficient neuromuscular transmission. When a disease modifies the anatomical setting with a new pattern that occupies a large part of the muscle, the MUP parameters will change accordingly. In these cases, the analytical evaluation of at least 20 MUPs will show an increase or a decrease of duration and of amplitude and a high or normal percentage of polyphasic shape. All myopathic processes change the anatomical picture of the muscle, and many induce a prevalent

pattern. However, some muscular diseases are characterized by a variegated anatomical picture in the muscle, and the EMG findings will change and will depend on the characteristic of the area where the needle has been collocated. Therefore, one of the most striking EMG findings in muscle diseases is the high variability of MUP parameters in the same muscle.

The neuromuscular junction (NMJ) consists of the motor axon terminal, the synaptic cleft, and the highly organized postjunctional folds on the muscle membrane. The chemical transmitter at the NM junction is acetylcholine (ACh). The nerve terminal is the site of synthesis and storage of ACh, which is released in the discrete quanta. The quanta are located in three separate stores: primary (immediately available), secondary (mobilization store), and tertiary (reserve store). The number of ACh molecules in each quantum was estimated to be fewer than 10,000.

When a nerve action potential depolarizes the presynaptic terminal, voltage-dependent calcium channels are activated, allowing an influx of calcium that results in a release of ACh from the presynaptic terminal through the proteins of SNARE complex. A nerve impulse results in a release of 50–100 quanta.

The ACh diffuses across the synaptic cleft and binds to ACh receptors (AChR) on the postsynaptic membrane, resulting in an end-plate potential (EPP).

In the healthy condition, the EPP always reaches the threshold for the opening of voltage-gated sodium channel on muscle membrane, and hence EPP triggers a muscle fiber action potential (MAP) that, propagating along sarcolemma down T tubules, results in muscle contraction. The amplitude of the EPP above the threshold value needed to generate an MAP is called the safety factor (SF).

The SF is reduced in patients with a disorder of NMT. The failure of the EPP to reach MAP threshold represents the basis of the electrodiagnostic abnormalities in patients with disorders of NMT [4]. The resulting impulse blocking accounts for the decremental responses seen on repetitive nerve stimulation (RNS) studies and the impulse blocking seen with single-fiber electromyography (SFEMG). In addition, the time variability of when the EPP reaches MAP threshold accounts for the neuromuscular jitter seen in the latter technique [5, 6].

SFEMG is an electromyography (EMG) technique that allows to record action potentials from individual muscle fibers (i.e., single MAPs). The selectivity of this technique relies on the small recording surface of needle electrodes. This can be obtained by using either dedicated SFEMG needle electrodes that have a small recording area (0.0005 mm^2) or conventional EMG needle electrodes after proper filter setting since these have larger recording area (0.07 mm^2).

SFEMG recordings can be performed during electrical stimulation of the nerve (S-SFEMG) or during voluntary activation (V-SFEMG) of the tested muscle [7–10].

When MAPs are elicited by nerve stimulation, the latency from stimulus to response (i.e., MAP) varies. This variation is due to physiologic fluctuation in the time for EPP to trigger MAP, and it represents the neuromuscular jitter.

When SFEMG is performed during voluntary activation, the needle electrode, inserted into the tested muscle, records from two or more MAPs that belong to the same motor unit (MU) and that hence depolarize synchronously. In this case, the neuromuscular jitter is the variations in the time intervals between pairs of MAPs.

This variation is related to physiologic fluctuation in the time that EPP takes to trigger each MAP in the examined pair of potentials.

Jitter is the most sensitive electrophysiological measure for the safety factor of NMT. In disorders of NMJ, the reduction of EPP may cause a delay in triggering MAPs, or if the EPP falls below the threshold, MAP is not generated. In the former, jitter will be increased; in the latter, SFEMG recording will demonstrate neuromuscular blocking. For *V-SFEMG* the subject is asked to maintain a steady contraction, and recordings of two or more MAPs belonging to the same MU can be performed by dedicated SFEMG or conventional EMG needle electrode. Around 20 different potential pairs are collected from the tested muscle. Theoretically, all muscles can be investigated with SFEMG. However, OO and EDC muscles are commonly investigated in clinical practice even though in some patients (e.g., MuSK positive), the examination of the most severely involved muscles to demonstrate abnormal jitter may be necessary. The following parameters should be recorded: the mean jitter (MCD) of all ($n = 20$) potential pairs or stimulated MAPs, the percentage of potential pairs or stimulated MAPs in which jitter is abnormal, and the percentage of NM blocking.

The SFEMG examination is considered abnormal if at least one of the following criteria is satisfied:

1. The mean jitter (MCD) of all potential pairs or stimulated MAPs recorded exceeds the upper limit of mean jitter for that muscle.
2. Ten percent or more of potential pairs or stimulated MAPs have jitter that exceeds the upper limit of normality in that muscle or if more than 10% of potential pairs/stimulated MAPs exhibits NM blocking.
   (a) Generally, NM blocking is observed when also jitter value is markedly increased.

Repetitive nerve stimulation (RNS) is a variant of the nerve conduction study since electrical stimulation is delivered to a motor nerve repeatedly several times per second [6].

The function of NMT is assessed by measuring after a train of stimuli the change in amplitude/area of the compound muscle action potential (CMAP) that represents the sum of the individual MAPs generated in a muscle. The train of stimuli may be carried out at low- (3 Hz) or high-frequency stimulation (20–50 Hz).

Low-frequency stimulation (e.g., train of 10 stimuli at 3 Hz) causes a depletion of ACh level in synaptic space since after the releasing of primary (immediately available) storage, it will be needing a time of 1–2 s for the mobilization of quanta from secondary storage. In this meantime, the amplitude of the EPP reduces (this phenomenon is known as synaptic fatigue). However, in normal subjects for the safety factor, EPP never falls below the threshold needed to generate a MAP. Vice versa for patients with NMJ disorders, the EPP of some muscle fibers may fall below the threshold level, and MAPs will not be generated. This reduction of MAPs is responsible for decremental response of CMAP when performing RNS studies. The size of CMAP may be assessed by measuring either the amplitude or the area of the negative peak of the CMAP. In disorders of NMT, there is a progressive

decrement of the second through the fourth or fifth response, with some return toward the initial size during the subsequent responses, the so-called U-shaped pattern. Decrement is defined as the percent change comparing the negative peak amplitude or area between the fifth (or fourth or lowest potential) and the first CMAP. The decrement is generally considered abnormal when greater than 10%.

High-frequency stimulation (20–50 Hz for 5–10 s) may be used to investigate presynaptic level of NMJ. The high-frequency stimulation induces (rate faster than time needed [100–200 ms] for exit of calcium from terminal nerve) the accumulation of calcium ions in the preterminal space producing a transient increase in the amount of ACh released from the motor nerve. This greater ACh release increases the EPP and may improve synaptic transmission briefly (this phenomenon is known as facilitation). In healthy subjects, the EPP is already (safety factor) above the threshold for eliciting MAP, and high-frequency stimulation does not induce any change in CMAP size. A phenomenon of "pseudofacilitation" (increase of amplitude associated with reduction of duration of CMAP; area remains unmodified) can be observed, and it is attributed to increased synchronization of MAPs or to hyperpolarization of the muscle fiber membrane from increased sodium-potassium pumping. In normal muscle, pseudofacilitation may increase the CMAP amplitude to 50% during stimulation at rates up to 50 Hz.

In a patient with a disorder that affects NMJ at presynaptic level (e.g., Lambert-Eaton myasthenic syndrome), after single electrical stimulus, few quanta of ACh are released, and the EPP frequently fall under the threshold. Therefore, the CMAP size may be reduced after single electrical stimulus, while during (or immediately after) high-frequency stimulation, the calcium accumulation in terminal nerve causes a massive releasing of ACh, and the EPP raises above MAP threshold resulting in an incremental response of CMAP. An increment greater of 60–100% is considered of significance.

High-frequency stimulation is painful and requires patient tolerance, and thus in clinical practice, maximal voluntary muscular contraction (protracted for 10–60 s) is used to obtain the same effect of high-frequency nerve stimulation.

After the phase of facilitation, NMJ develops a phase of postactivation exhaustion, in which less ACh is released by each nerve impulse. The exhaustion lasts 2–5 min (maximum at 3 min) after the end of activation. In this period, low-frequency stimulation worsens the decrement of CMAP or may unmask a decrement not evident at the basal stimulation performed before the activation. Generally, after 5 min the change of CMAP size observed during low-frequency stimulation comes back to basal condition. RNS is more likely to be abnormal in proximal and facial muscles, rather than in limb distal muscles. To have the maximum diagnostic sensitivity, examination of several muscles, including those that are involved clinically, may be necessary. Hand muscles are easy to test but scarcely sensitive. Recording can be made from thenar or hypothenar muscles by stimulating median or ulnar nerves at wrist. Such stimulation is suitable if prolonged high-frequency stimulation is required. Proximal muscles have greater sensitivity than distal muscles. The trapezius is the easiest shoulder muscle to test. The spinal accessory nerve is stimulated at the neck where it is superficial so that it can be maximally

stimulated with low-intensity pulses, minimizing discomfort, and stimulation of other muscles. Recordings can also be made from biceps brachii or deltoid muscles by stimulating musculocutaneous nerve in the axilla or axillary nerve at the Erb's point. However, such stimulations are often disturbed by movement artifacts and stimulation/activation of near muscles. Facial muscles have the greatest sensitivity. Recordings are made from orbicularis oculi or nasalis muscles by stimulating facial nerve at tragus or stylomastoid foramen. This study may be performed with the patient either sitting or lying. Temperature influences the CMAP size and decremental response is less evident when the muscle is cool. Low temperatures reduce enzymatic activity of acetylcholinesterase in synaptic cleft, increasing the availability of ACh and increasing the EPP. Hand or foot muscles should be warmed to a surface temperature of at least 34 °C to avoid false-negative results in patients with a disorder of NMT. In a patient with suspected disorder of NMT, the standard procedure consists of:

1. Low-frequency stimulation (10 stimuli at 3 Hz) to detect the decrement of CMAP amplitude/area.
2. If the test is positive (decrement of CMAP >10%), the patient should undergo:
   (a) High-frequency stimulation (20 Hz for 5 s) or preferentially maximal voluntary muscular contraction (protracted for 30 s) to evaluate facilitation.
   (b) Low-frequency stimulation every minute up to 5 min to evaluate postactivation exhaustion.
3. If the test is negative (decrement of CMAP ≤10%), the patient should undergo:
   (a) High-frequency stimulation (20–50 Hz for 5–10 s) or preferentially maximal voluntary muscular contraction (protracted for 60 s).
   (b) Low-frequency stimulation every minute up to 5 min in order to unmask a decremental response of CMAP during the phase of postactivation exhaustion.

The high-frequency stimulation or maximal voluntary muscular contraction is essential in detecting presynaptic neuromuscular diseases by showing a significant increment of CMAP size (>60%). If a small CMAP amplitude is observed at basal examination after single electrical stimulus, a presynaptic disorder should be strongly suspected. In this case, also a brief maximal voluntary muscular contraction (10 s) may be sufficient to disclose an incremental response of CMAP size.

In the next paragraphs, we will describe how to plan the EDX examination and the interpretation of most frequent findings according to the anatomical picture [11, 12].

2.3 Plan of the Electrodiagnostic Examination

Nerve conduction studies (NCS) in patients with suspected myopathy should include at least one motor and one sensory recording, in at least one upper and lower limb. Both sensory and motor nerve conduction studies are generally

normal in myopathies. However, in some distal phenotypes, it is possible that the loss of muscle fibers is enough to decrease the CMAP amplitude, but in this case, the distal latency and motor conduction velocity should be normal. Needle EMG is required to differentiate a motor neuron disease. A reduced CMAP amplitude is also present in case of presynaptic disorder (e.g., Lambert-Eaton disease), and a differential diagnosis is required by means of RNS. Sensory nerve conduction could be distally reduced in some myopathic disorders as the myotonic dystrophy type 1 or the critical illness myopathy, in which sensory endings can be involved in the context of a coexistent neuropathy. Proximal and distal muscles should be investigated, and upper and lower limb of one side must be considered. The easiest muscle to explore are deltoid, biceps, abductor digiti minimi, quadriceps (rectus), and tibialis anterior; however, the choice should be guided by clinical observation or by the diagnostic hypothesis. If an inclusion body myositis (IBM) is supposed, the flexor digitorum muscle should be considered; when a glycogen storage disease (e.g., Pompe disease) is a possibility, then paraspinal muscles must be investigated. In this case, cervical and lumbosacral levels should be avoided for the frequent coexistent radiculopathy, and a thoracic level is the best choice. EMG analyses can help for choosing the muscle to be biopsied; it should be a weak but not severely affected muscle. The EMG examination is more sensitive than clinical observation and can reveal an involved muscle that has escaped clinical evaluation. However, the biopsy cannot be performed in a muscle recently investigated by needle EMG. Needle EMG should give data about the presence of muscle irritability on insertion of the needle, the state of the muscle at rest, and the analyses of MUPs during slight effort. Finally, it is important to record the recruitment behavior at moderate and maximum effort [13].

2.4 General Findings

2.4.1 Resting Activity

In a normal resting muscle, the only electrical activity that is possible to record is derived by the miniature end-plate potentials when the needle electrode is very close to the end plates. However, the needle introduction in the muscle can induce discharges of MFs that are called insertional activity and is generally very short (less than 250 ms). This activity is produced by the mechanical irritation of the needle electrode on the nearby end plate. The insertional activity can be abnormally prolonged when the muscle is denervated or in case of myotonias, polymyositis, or some muscular dystrophies suggesting an aspecific hyperexcitability of MFs. However, the normal insertional activity can also be decreased, for example, in long-lasting or end-stage myopathies, when most of the muscle fibers are replaced by fat or connective tissue. In this case, it is also possible to feel an increased resistance to needle insertion due to the advanced fibrotic substitution of the muscle. Other types of abnormal electrical activity can originate in the muscle itself [14] as fibrillations, complex repetitive discharges (CRD), and myotonia.

Fibrillation potentials are muscle fiber action potentials recorded outside the endplate zone; are spontaneous, biphasic, or triphasic in shape; and are of very short duration (1–5 ms) [15]. They are generally seen in neurogenic diseases but can be present in several primary muscle disorders, as Duchenne muscular dystrophy, dermatomyositis and polymyositis, Pompe disease, sporadic inclusion body myositis (sIBM), and centronuclear myopathy (CNM). The origin of fibrillation potentials in these diseases can be produced by the denervation of muscle fibers secondary to focal fiber necrosis, as in Duchenne dystrophy, or to the inability of a regenerated but isolated fiber to be innervated for the excessive distance from the innervation zone. An additional explanation could be a modification of muscle membrane properties with an increased excitability. In polymyositis, it is also possible that there is an inflammatory direct damage of intramuscular axon branches [16]. Less consistently fibrillation can be observed in facioscapulohumeral (FSHD), limb girdle (LGMD), and oculopharyngeal dystrophies. Overall fibrillation potentials are the most frequently pathological spontaneous activity that can be observed in muscle diseases [17].

CRD are complex potentials showing multiple spike components, with a total duration ranging from 50 to 100 ms. They discharge repetitively at a low (5 Hz) or high (100) frequency, generally with a stable waveform that is typically polyphasic, from one discharge to another, and they have an abrupt onset and cessation. However, the waveform can change suddenly at the higher firing frequency due to the intermittent block of some spike components. The origin of CRD is the spontaneous firing of a pacemaker muscle fiber which emphatically drives few or several adjoining muscle fibers [18]. The CRD have been described both in muscle and peripheral nerve diseases. Since CRD are very frequent in adult onset form of Pompe disease and especially in paraspinal muscles, they have been included as a diagnostic feature in the diagnostic guidelines of the American Association of Neuromuscular and Electrodiagnostic Medicine [19]. CRD are rarely observed in LGMD and FSHD, while they seem to be more frequent in Duchenne than in Becker muscular dystrophies [20]. CRD are also present in almost half of patients affected by sIBM, with a higher frequency in paravertebral muscles. According to a recent analysis, CRD seem to occur more frequently in myopathies with protein accumulations, vacuoles, and nuclear protein defects, for example, Pompe disease, sIBM, and centronuclear myopathy, rather than in myopathies with a sarcolemmal protein defect, for example, Becker and LGMD [17].

Myotonic rhythmic discharges can be induced by a voluntary movement or by an electrical or mechanical stimulation of a muscle and can be seen in congenital myotonias (induced by both Na and Cl channel alterations), dystrophic myotonia (DM1 and DM2), congenital paramyotonia, Pompe disease, and sodium channel myotonias. Myotonic discharges may be present with or without clinical myotonia. They are characterized by a burst of potentials, with positive or negative spikes, of short duration (less than 5 ms), which progressively increase and then decrease their amplitude and frequency of firing. The pathophysiology of myotonic discharges is not completely known in human diseases, but it probably relates on Na and Cl channel abnormalities. In fact, a reduced conductance for chloride can reduce the leak of this ion in the transverse tubular extracellular space after the depolarization with a consequent relative increase of extracellular potassium

concentration (K ions are released with chloride ions). This K concentration could raise until a level which determines depolarization of transverse tubular membrane with repetitive responses to a single presynaptic impulse. The reduced chloride conductance theory can be applied to congenital myotonia, but it has not been shown in myotonic dystrophies or congenital paramyotonia. In these diseases, mutations of Na channels can cause cellular membrane instability and sensibility to temperature. In fact, myotonic discharges are increased with cooling in DM1 and paramyotonia and with warming in DM2. Several observations describe a longer myotonic discharge in myotonic dystrophies than in congenital myotonias. The electrophysiologic differential diagnosis among myotonias can be approached using the short-exercise protocol. With this technique, the variation of amplitude of the CMAPs, habitually recorded in the abductor digiti minimi muscle, at baseline and after 10 s of maximum effort every 2 s for 1 min can show three different patterns. These patterns have a good sensitivity and specificity in distinguishing among the chloride and sodium myotonias [21].

2.4.2 MUP Analysis

In myopathic disorders, the number of functional muscle fibers per MU is reduced. This has the consequence of a contraction of the area of the MUs since some of the most distant fibers are lost. The MUP duration is largely dependent on the number of muscle fibers active in the relatively large (2.5 mm) recording area of the needle electrode; therefore, if the fiber loss is consistent, the MUP duration can be shortened. Sometimes, the duration and the shape of the MUP suggest that it is composed by one fiber only. In this case, it is likely that the surviving fibers belonging to the same MU are too thin to evoke a recordable potential or too isolated by the recording electrode for the presence of no contractile tissue. This finding can be more evident in weak muscles and in patients with chronic myopathy [22, 23]. However, in some instances, the short and often polyphasic main component of MU potentials is linked to late or less often preceding small potentials that discharge several ms (at least 5 ms) far from the main component of the MUP. These satellite components, if included in the measure of the MUP duration, make it very long, sometimes more than 30 ms. This finding is recordable in dystrophic diseases when there is some fiber regeneration from satellite muscle fibers or from split fibers. Overall, MUP duration in myopathies is shorter than normal when MUPs with satellite components are not considered. The presence of MUPs with late component is frequent also in neurogenic diseases, but in this case short MUPs are not or exceptionally recorded. The amplitude of the main component of the MUPs depends on the number and diameter of muscle fibers very close (within 1 mm) to the recording area of the needle electrode. This makes the amplitude parameter variable also in normal subjects when single MUPs are analyzed. In muscle diseases, the fiber loss and their reduced diameter induce habitually a reduction of MUP amplitude, but the occasional presence of hypertrophic fibers close to the electrode can determine an increased amplitude of a MUP spike, otherwise short in duration. Therefore, a

highly increased variability of MUP amplitude can be a finding suggestive of a primary muscle disease.

The biphasic or triphasic shape of the majority of normal MUPs is due to the homogeneity of fiber diameter and to the regular distribution of end-plate zone within the MU area, with consequent synchronous contraction of the recordable component of the MU. When there is an increased variability of muscle fiber diameter, the reinnervation of split or regenerated fibers and the presence of fat or connective tissue, the synchrony of MU components can be lost, and MUPs can appear polyphasic and sometimes with late components. This finding is very frequent in many muscle disorders.

2.4.3 MU Recruitment

In normal subjects, the number and the discharge frequency of MUs recruited are proportional to the tension required by the voluntary movement. If the MUs have a reduced number of functional muscle fibers or there is uncoupling between electrical and mechanical events, it is necessary to increase the number and the rate of discharge of recruited MUs. This is what happens in myopathic patients when they are requested to develop a certain muscle tension. In other words, in muscle disorders, it is possible to see in weak muscles a recruitment pattern very similar to that of normal muscles (interference pattern) with regard to the richness of MUs, but with a reduced amplitude. The increased number of recruited MUs, their high rate of discharge, and the complex shape of MUPs all determine the interference patterns seen in myopathic patients even in weak contraction, while the reduced number of functional muscle fibers in each MU is the explanation of the reduced amplitude of the potentials. This finding can be no more present when the myopathic process is long lasting and has induced a severe reduction of muscle fibers with the possibility that the electrical activity of some MUs is no more recordable.

2.5 Specific Findings

Electromyographic findings obtained with needle electrode do not permit a differential diagnosis among the several muscle diseases. However, some peculiar aspects can orientate toward a specific myopathy and can likely anticipate the histological aspects. In the following paragraphs, these findings will be shortly described for some muscular dystrophies; inflammatory, endocrine, metabolic, and congenital myopathies; and myotonias.

2.5.1 Muscular Dystrophies

The most frequent muscular dystrophies are the dystrophinopathies, Duchenne muscular dystrophy (DMD), and Becker muscular dystrophy (BMD). The diagnostic procedure, after the clinical evaluation, can be addressed with genetic testing, particularly when there is a positive family history or in some cases on muscle

biopsy. Therefore, the EMG examination may be helpful in sporadic cases when clinical and biochemical data are equivocal. In these cases, needle EMG can reveal increased insertional activity, some sparse fibrillation potentials, and short, small, polyphasic MUPs with early recruitment. In late stages, EMG findings can be somewhat different according to the supervening morphological changes (muscle tissue necrosis, muscle fiber splitting, reinnervation, and muscle replacement by connective and fatty tissue). In these stages, the insertional activity is reduced and alongside with short MUP; long duration MUPs with satellite components can be appreciated [23]. At the MU recruitment, the interference pattern may be incomplete.

In other dystrophies, as LGMD, FSHD, and oculopharyngeal needle EMG can be necessary when disease onset is in adult age, CK levels are mildly elevated and clinical data are not clearly expressed. In these forms of dystrophies, fibrillation potentials and CRD are rare, while MUPs can be short, small, and polyphasic [17]. The EMG can also be useful to evaluate the distribution of weak muscles.

2.5.2 Inflammatory Myopathies

In classical poly-dermatomyositis (PM and DM), the majority of patients show classical myopathic EMG abnormalities with short, small, and polyphasic MUPs, but the presence of spontaneous activity in the form of fibrillation potentials is a constant finding. In addition, the fiber irritability is revealed also by the occasional presence of CRD or myotonic discharges (only electrical). Fibrillation and CRD can decrease with the improvement of the disease [24]. On the other end, in long-lasting forms, it is possible that long duration and complex MUPs will appear, making the differential diagnosis with a neurogenic lesion more difficult. For this, the MU recruitment and the distribution of involved muscles should be useful. In polymyositis, the EMG sampling of several muscles is also very useful for the choice of the muscle to be biopsied since sometimes the biopsy can miss the morphological abnormalities.

The sporadic inclusion body myositis (sIBM) is likely a degenerative disorder rather than an inflammatory muscle disease; however, it is traditionally included in the inflammatory myopathy chapter. The electrodiagnostic findings are similar to those in DM and PM, but the incidence of the irritative aspects and the presence of a double population of MUPs, with myopathic and neurogenic aspects, is higher in IBM than in PM and DM [25, 26]. The differential diagnosis with a neurogenic disease can be further complicated by nerve conduction studies that can reveal a mild sensory axonal polyneuropathy in up to 30% of patients with IBM [27]. A confirmatory muscle biopsy is mandatory.

2.5.3 Endocrine Myopathies

The presence of a thyrotoxicosis can be complicated by a concomitant autoimmune myasthenia gravis. This possibility must be excluded by adequate neurophysiological techniques. Otherwise, EMG analysis does not show fibrillation; rarely, it is possible to record some fasciculations, and MUPs are generally normal but short,

small MUP can be present. A steroid myopathy is more commonly induced by a prolonged prescription for the treatment of inflammatory disorders. This can happen during the treatment of a PM and can induce some diagnostic error. The EMG findings are habitually normal since there is the selective atrophy of type 2 muscle fibers and a biopsy is necessary.

2.5.4 Metabolic Myopathies

Glycogen and lipid are both important source of energy for muscle fibers. Therefore, disorders of their metabolism may have significant muscle involvement. There are several glycogen storage diseases and two well-known diseases (carnitine deficiency and carnitine palmitoyltransferase deficiency) of lipid metabolism that induce weakness, hypotonia, and sometimes respiratory insufficiency in patients of different age. Unfortunately, EMG examination can be normal or not specific for most of these diseases, with the exception of two glycogen storage forms (acid maltase deficiency-glycogenosis II or Pompe disease and myophosphorylase deficiency-glycogenosis V or McArdle disease). In all Pompe disease forms, needle EMG shows a prominent spontaneous activity with fibrillation potentials, CRD, and myotonia discharges without clinical myotonia. Myotonia discharges origin very often from a single muscle fiber [28]. Spontaneous activity is widespread in infantile and childhood onset, while in adult form it must be investigated in proximal and paraspinal muscles [19]. McArdle disease is characterized by painful muscle contracture after a vigorous exercise that shows electrical silence at needle examination. This finding is different from all other diseases with painful cramp, where an intense electrical activity can be recorded. The contracture can also be induced by a high-frequency (50 Hz) repetitive stimulation of a motor nerve, but this painful procedure is not recommended.

2.5.5 Congenital Myopathies

Congenital myopathies are a group of clinically and genetically heterogeneous muscle disorders, which cannot be distinguished from each other by means of the neurophysiological examination. Some recent observations have shown that among the centronuclear myopathy in the adult onset form mutations were identified in DNM2 gene [29], and some of patients showed CRD at needle examination (personal observation).

2.5.6 Myopathies with Myotonic Discharges

Myotonic discharges with or without clinical myotonia can be observed in dystrophic (DM1 and DM2) and in congenital myotonias which include some channelopathies. Myotonia on needle EMG is the electrophysiologic hallmark of myotonic dystrophies. The discharges are more easily obtainable in distal and proximal muscles in DM1 than DM2 and tend to be classically waxing and waning in DM1 and waning only in DM2.

Moreover, myotonic discharges are longer in DM than in congenital myotonias. However, both in dominant (Thomsen disease) and recessive (Becker disease) congenital forms, the discharges are very frequent and at times it is impossible in the analysis of single MUPs. In congenital paramyotonia, cold temperature and exercise exacerbate myotonia. This paradoxic myotonia is more easily seen during hand grip or eye closure [30, 31]. Sodium channel myotonias could be distinguished from other congenital myotonias using the short-exercise protocol [21].

2.5.7 Myasthenia Gravis

MG is an autoimmune disease caused by the presence of antibodies against components of the muscle membrane localized at the NMJ. In most cases, the autoantibodies are against the acetylcholine receptor (AChR). Recently, other targets have been described such as the MuSK protein (muscle-specific kinase) or the LRP4 (lipoprotein-related protein 4) [32–34].

RNS demonstrates an abnormal decrement in a facial or shoulder muscle in 70–80% of patients with generalized MG and in only 50–60% of patients with ocular MG. However, in patients with MuSK-positive MG, RNS studies are frequently normal in commonly examined muscles, and testing the most severely involved muscles to detect a decremental response may be useful. SFEMG demonstrates abnormal jitter in at least one muscle in 99% of patients with generalized MG and in 97% of those with ocular MG. Increased jitter and blocking frequently are found in muscles in which no decrement is detected on RNS. Unlike RNS, SFEMG cannot differentiate presynaptic from postsynaptic disorders. However, in MG characterized by a postsynaptic defect, the rapid firing rate of MU increases the jitter, while in presynaptic disorders (LEMS, botulism), jitter increases at slow firing rates and decreases at fast rates.

2.5.8 Congenital Myasthenic Syndromes (CMS)

CMS are a heterogeneous group of genetically determined structural disorders of the presynaptic, synaptic, and postsynaptic element of the NMJ. Decremental response is absent in asymptomatic CM with episodic apnea and is absent at rest but elicited by 10 Hz stimulation (protracted for 5 min) in congenital choline acetyltransferase (ChAT) deficiency. Decreased amplitude of CMAP after 10 Hz RNS for 5 min may persist up to 30 min.

Lastly, in slow-channel syndrome, there is a peculiar repetitive CMAP response to single stimulus. This response is typically observed in the small muscles of the hand and foot and is abolished by RNS at 10 Hz [6, 35].

2.5.9 Lambert-Eaton Myasthenic Syndrome

Electrodiagnostic studies in LEMS are exemplificative of presynaptic disorder of NMT. LEMS is a rare autoimmune disorder due to antibodies against presynaptic

voltage-gated calcium channels; most of patients with LEMS have an underlying malignancy. The most striking electrophysiological features are a reduction in the basal CMAP amplitude after single stimulus and a marked postactivation facilitation following a brief period (generally are sufficient 10 s) of maximal voluntary contraction or high-frequency stimulation (20–50 Hz for 5–10 s). Generally, CMAP amplitude increases greatly (over 150–200%).

Decremental response to slow stimulation rates before and after the phase of facilitation can be observed as well [5, 36].

2.5.10 Botulism

Botulism is a rare and potentially fatal disease caused by toxin produced by the bacteria *Clostridium botulinum*. Botulin toxin cleaves some of the SNARE proteins inhibiting or reducing the release of ACh into the synaptic cleft. The electrodiagnostic abnormalities are quite similar to those observed in LEMS. However, the degree of facilitation is usually less marked than that observed in LEMS (>60%). Moreover, facilitation may be initially absent in adult in whom facilitation may require more prolonged high-frequency stimulation (up to 20 s). Postactivation exhaustion is not seen in botulism [6].

Highlights

- Motor unit potentials in myopathies are generally shortened and of polyphasic shape. However, the most frequent finding of needle EMG in myopathies is the increased variability of MUPs.
- Some fibrillation potentials can be found in most myopathies, but they are more frequent in sIBM, acute necrotizing myopathy, and polymyositis.
- CRD are present when some hyperexcitable muscle fibers act as pacemaker with ephaptic transmission to near fibers. This happens more frequently in Pompe disease and sIBM.
- Cramps are associated to a florid electrical activity, while muscle contractures (McArdle disease) are electrically silent.
- A reduced CMAP amplitude in a patient with fatigability must induce the suspicion of a presynaptic disorder.

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Imaging of the Muscle in Idiopathic Inflammatory Myopathies

3

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3.1 Introduction

In the last years, muscle imaging has progressively become fundamental in the diagnostic workflow in neuromuscular disorders. Despite ultrasonography being readily available and inexpensive to assess muscular echogenicity and trophicity, magnetic resonance imaging (MRI) has progressively emerged as the main agent in neuromuscular imaging given the elevated number of features that can be assessed, the independence from the operator, and the possibility to explore even deeper muscles and larger body regions [1–4].

Muscular MRI proven to be not only a valuable assistant to the correct location to perform a muscle biopsy, but it increasingly plays a crucial role in identifying the localization and extension of muscle diseases and in helping the diagnostic process, especially when a phenotypic overlap is present, by describing specific patterns of distribution of muscle damage and also staging its extent [4, 5]. Last but not least,

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MRI is also helpful in monitoring the disease evolution and, if available, in assessing the efficacy of therapy [6].

One of the principal applications of muscle MRI is certainly in the management of **idiopathic inflammatory myopathies** (IIM), mainly but not limited to the role of guiding the biopsy and assessing response to therapy [7, 8].

In this chapter, we aim to summarize current knowledge on muscle imaging techniques and their usefulness, with an eye on technical advances, also briefly discussing the radiological correlates of the principal acquired myopathies, as dermatomyositis (DM), polymyositis (PM), and inclusion body myositis (IBM).

3.2 MRI Imaging of Muscular Diseases

The cardinal radiological abnormalities to be searched in a muscle MRI of a subject with a suspected neuromuscular disorder are both volume abnormalities (atrophy, hypertrophy, and pseudo-hypertrophy) and structural changes (fat replacement and edema). For such comprehensive assessment, a conventional MR protocol must include at least a T1-weighted (T1w) and a fluid-sensitive sequence, such as T2-weighted fat-suppressed sequences (T2 FS) or short-tau inversion recovery (STIR).

Contrast administration is generally not required; also in the field of inflammatory muscular diseases, it is used only in selected cases as infectious complications [9, 10].

3.2.1 Volume

Volume abnormalities consist in atrophy, hypertrophy, or pseudo-hypertrophy and can be assessed on T1w sequences as well as in T2w sequences (though the first are preferable).

Atrophy is a reduction of the volume of the muscular bulks and can be easily detected by MRI; by comparison to adjacent ones, atrophy of a single muscle is easily detected visually (i.e., rectus femoris as the only affected muscle among the quadriceps components at thigh level). MRI also quite easily detects real **hypertrophy**, defined as an increase in the muscular volume with maintenance of its normal structure, and distinguishes it from **pseudo-hypertrophy** in which muscular volume increases due to the presence of adipose and/or connective tissues substituting muscular fibers; in the latter case, MRI shows an increase in volume associated with structural disruption of muscle tissue (Fig. 3.1a–c).

To go beyond visual assessment, CSA (cross-sectional area) of muscles has been implemented and used for quantitative purposes, mainly in the setting of dystrophies, yet practice has suggested the use of more reliable trophicity measures, obtained by removing the "noise" given by the fat replacement occurred in the muscle (e.g., contractile mass index)[11, 12].

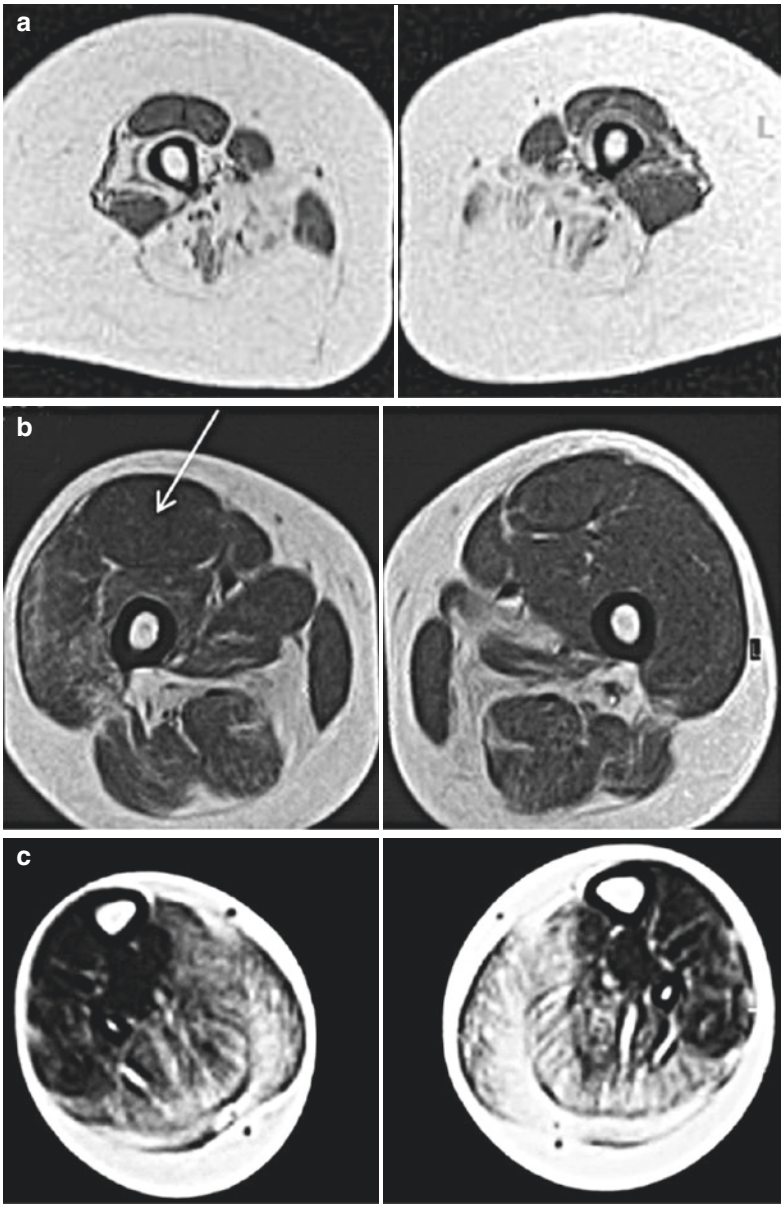

Fig. 3.1 Volume alterations. Axial T1-weighted images at the level of the thigh (**a**, **b**) and leg (**c**) showing examples of muscle atrophy (**a**), hypertrophy (**b**), and pseudohypertrophy (**c**). (**a**) Shows an increase of the subcutaneous adipose tissue and a diffuse atrophy of all muscle bulks, associated with adipose substitution mainly in the posterior compartment, bilaterally, and at the level of the right vastus intermedius muscle. (**b**) Shows right quadriceps atrophy and adipose substitution, together with selective ipsilateral hypertrophic rectus muscle (arrow) which maintains its normal structure. (**c**) Shows volume muscular increase of soleus, medial and lateral head of gastrocnemius muscle bilaterally due to the presence of adipose and connective tissues substituting muscular fibers, detectable as diffuse hyperintensity

3.2.2 Fat Replacement

Intramuscular **adipose replacement** (or commonly **fat replacement**) is usually detected on T1-weighted (T1w) sequences (Fig. 3.2) in which adipose tissue has a high signal because of its short relaxation time, differently from normal muscle tissue which has a much lower signal intensity [13, 14]. As fatty degeneration does not affect all muscle groups with the same extension and severity (i.e., specific muscles or muscle groups can be electively spared or specifically involved in different clinical conditions), MRI has proven to be very useful in identifying specific patterns of distribution of the pathological process.

In clinical practice, several **MR rating scales** (usually, 4 or 5 grades ranging from normal to severe changes depending on the percentage of the involved muscle volume) are applied to evaluate the extension of fat replacement and semi-quantitatively assess the degree of muscle damage (Tables 3.1 and 3.2) [15, 16].

The degree of fat replacement correlates with severity and duration of the disease [17]. In the early stages or in mild cases, MRI scans may show normal or mildly affected muscles, possibly with a selective involvement of a few muscles or muscular groups, whereas in more advanced stages, such pattern of sparing/involvement may become scarcely recognizable because of the extensive muscular damage and fatty degeneration [14]. Therefore, an early diagnostic suspect and an early MRI examination may sometimes be fundamental to guide the diagnosis, especially in those cases where the phenotype is overlapping with other diseases.

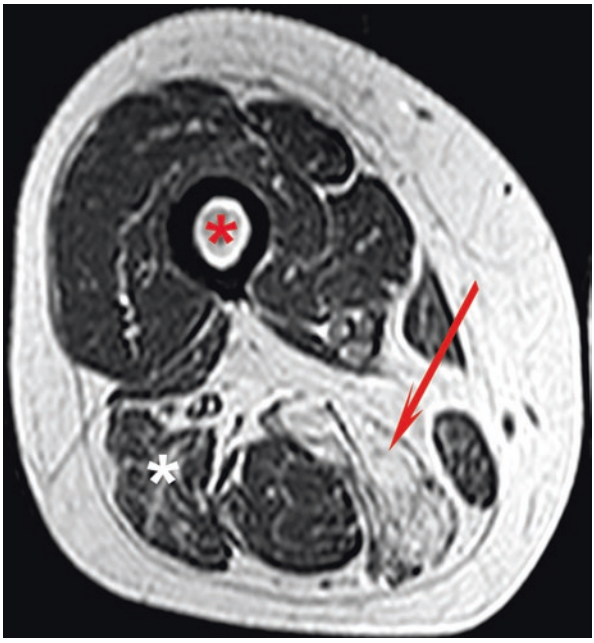

Fig. 3.2 Structural alterations-adipose tissue. Axial T1-weighted image at the level of the thigh showing adipose intramuscular infiltration characterized by high signal intensity at the level of the adductor magnus and semimembranosus (*red arrow*) and in a lesser extent of the long head of the biceps (*white* *). No other tissue apart from yellow marrow bone—which can be easily distinguishable for its site in the bone (*red* *)—has the same signal intensity

Table 3.1 Modified Lamminen rating scale

Score	Description
0	Normal
1	Mild with only traces of increased signal intensity
2	Moderate with increased signal intensity in less than 50% of affected muscle
3	Severe with increased signal intensity in more than 50% of affected muscle
4	Entire muscle replaced by abnormal signal

Table 3.2 Mercuri Score, MRI, T1 sequences

Score	Description
0	Normal appearance
1	Early moth-eaten appearance, with scattered small areas of increased signal
2a	Late moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising less than 30% of the volume of the individual muscle
2b	Late moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising 30–60% of the volume of the individual muscle
3	Washed-out appearance, fuzzy appearance due to confluent areas of increased signal
4	End-stage appearance, muscle replaced, increased density connective tissue and fat with only a rim of fascia and neurovascular structures distinguishable

Visual assessment of T1w images provides a rapid visualization of fat replacement and, as explained before, potentially guides the diagnostic process; yet it must be underlined that it is extremely difficult to quantify the degree of fatty degeneration and fat content in a robust way on these sequences, especially where multiple serials scans are acquired and there is need to compare the degree of involvement between time-points.

Quantitative MR techniques, such 3-point Dixon sequences or parametric T2 measures, are progressively inserted into the clinical routine of neuromuscular imaging and allow a precise quantification of the amount of intramuscular fat tissue, separating it from water (the so-called **fat fraction, FF**, i.e., the percent of fat within a certain muscular volume of interest) [9, 18]. Fat fraction quantification allows a more precise evaluation of the fat replacement in the muscle and strongly facilitates longitudinal evaluation even when only subtle changes are present. Although post-processing of sequences necessary for FF quantification needs specific expertise, commercial software that allow a quite fast quantification of fat replacement are continuously implemented and generally easily available. In addition to their more robust precision in detecting the real fat replacement degree, the application of quantitative MR techniques as Dixon is of particular utility in longitudinal follow-up.

3.2.3 Edema

T2-weighted sequences without fat signal suppression may be used in detecting **intramuscular edema** (Fig. 3.3) and fat infiltration. Both water and fat have longer T2 relaxation times than healthy muscle, and this explains the elevated signal of

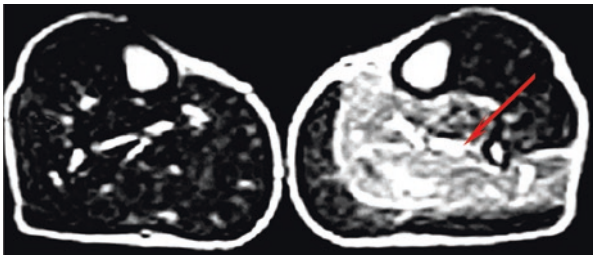

Fig. 3.3 Structural alterations-edema. Axial T2-weighted image of the leg showing a hyperintense edematous left soleus muscle (*red arrow*). Note that both water and fat have long T2 relaxation times, as can be seen by the signal of the subcutaneous adipose tissue

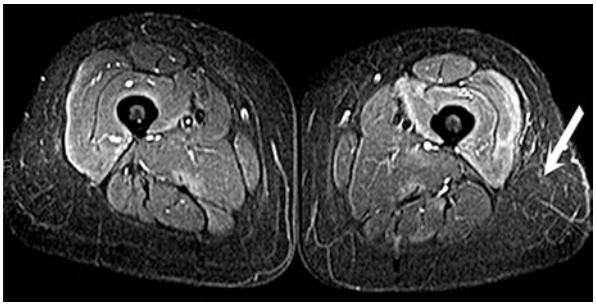

Fig. 3.4 Structural alterations-edema. Axial short-tau inversion recovery (STIR) image of the thigh, where fat signal is suppressed, evidencing pathologic scattered edematous muscle changes of both anterior and posterior muscular compartments, mainly involving the peripheral portions of the quadriceps bilaterally. Note subcutaneous edema apparent as crisscrossing linear pattern in the subcutaneous fat (*white arrow*)

muscle tissue on T2 sequences when it is affected by either adipose infiltration and/or edema [13, 14].

The presence of the two components of the image (i.e., water and fat degeneration) can thus confuse the reader of MRI scans: the high signal common to water and fat, in some conditions where both components are present at the same time, may thus mask the detection of edema by an increase of intramuscular fat, and vice versa (fat replacement, in fact, is commonly assessed on T1w sequences). This is the reason why **STIR (short-tau inversion recovery)** sequence, a T2w sequence where the signal of adipose tissue is suppressed, is preferred to allow visualization of increased water in tissues (Fig. 3.4) [13].

When finding muscular hyperintensity on fat-suppressed T2-weighted sequences as STIR, clinicians are prone to attribute such finding to muscle edema; the exact correlates of this hyperintensity at tissue level, however, are still poorly understood. Increased T2 times have also been documented in healthy muscle after exercise and are thought to be due to a number of physiological mechanisms including water shift from intra- to extracellular space, increase in vascular fluid volumes, and/or in the proportion of “free” water to macromolecular “bound” water. It has also been

described in cases of pathological abnormal cellular infiltrates (lymphoma, bacterial myositis), rhabdomyolysis (sport-related injuries, trauma, diabetic infarction, metabolic myopathies stress-exercise related, drug and alcohol abuse), subacute or chronic denervation, and inherited myopathies/muscular dystrophies [10, 19].

Similarly to the evaluation of fatty degeneration, several rating scales have been introduced to classify and quantify muscle edema, also taking into account intra/interfascicular edema, global/segmental myoedema, percentage of muscles involved, or entity of the signal alteration [10, 20, 21]. The spectrum of imaging findings in the context of muscular edema range from focal edema involving certain parts of a single muscle to diffuse edema involving several muscle groups, as in rhabdomyolysis [22]. Edema can have a myofascial distribution (perifascicular edema) around individual muscles or muscles groups or a subcutaneous localization (soft-tissue edema) with subsequent abnormal reticulation of the subcutaneous adipose tissue.

The quantitative evolution of visual assessment as per STIR sequence has led to the development of T2 mapping to measure tissue T2 values through multiple echos [23]. It is known that free water content in inflamed muscles can increase their T2 relaxation time; some studies, in fact, found a positive correlation between T2 value is and the severity of the inflammatory lesions [24]. Quantification of T2 values in the muscular tissue (depurated from the component derived from fat), i.e., **water T2** of muscle appears as promising to detect even subtle changes in ongoing edematous phenomena in the tissue, to go beyond the operator dependency of STIR evaluation [17, 25]. Some technical difficulties and relatively long acquisition times have so far limited its implementation in clinical practice. Yet a few experiences have already been attempted, even with accelerated sequences that greatly limit the total scan duration [26].

3.2.4 The Role of Whole-Body MRI (WB-MRI)

In clinical practice, a certain region of interest (pelvic girdle, thighs, etc.) has generally to be chosen to reduce the overall length of scan acquisitions and also costs. In the last decades, whole-body MR imaging (WB-MRI) has been developed to investigate the entire body in a reasonable time (1 h or less); in the field of muscular diseases such approach has the potential to unveil at a glance the presence of distribution patterns of muscular involvement in the entire body, also indicating abnormalities in clinically silent muscles or regions [27–29]. It is quite clear how this can potentially help the diagnostic process, also even guiding muscle biopsy, indicating the most involved muscles in the whole body [30]. So far muscular WB-MRI protocols generally include T1w and STIR sequences, yet diffusion imaging is also increasingly applied [31].

3.3 Conventional MRI of Dermatomyositis and Polymyositis

Inflammatory myopathies can be classified as idiopathic or secondary [1]. Adult subacute-onset idiopathic inflammatory myopathies (IIM) are classified into polymyositis (PM) including immune mediated necrotizing myopathy (NAM), dermatomyositis (DM) and overlap syndrome with myositis [32–35]. Diagnosis is usually

made on the basis of clinical signs, such as symmetrical progressive proximal muscle weakness and worsening muscle fatigue, electromyographic findings, serum CK elevation, muscle biopsy, and assessment of myositis-specific antibodies [1, 34].

Biopsy-proven inflammation is usually present in muscle tissue of subjects with PM and DM but it is absent in subjects with NAM [33]. Muscle biopsy, however, may show only nonspecific abnormalities because of the scattered distribution of inflammatory infiltrates in the affected muscles (of note the importance of targeting the biopsy) [36]. In this view, MRI can be helpful in distinguishing affected muscles from non-affected ones as the use of STIR allows the precise detection of the inflammation-related muscle edema and its extension and severity [36–38]. Muscle biopsy performed on affected muscles as indicated by MRI showed significantly more inflammatory changes than blinded biopsies [34, 39, 40]. By contrast, since inflammatory cells have been found also in muscles appearing unaffected on MRI, a certain degree of inflammation is probably necessary to make edema visible on MRI.

In the acute phase of PM/DM, the signal intensity on STIR sequences is associated with disease activity and, as expected, it decreases after immunosuppressant treatment (Figs. 3.5 and 3.6). By contrast, the histological picture does not always substantially change after therapy [33, 38, 40]; the lack of a clear-cut correlation

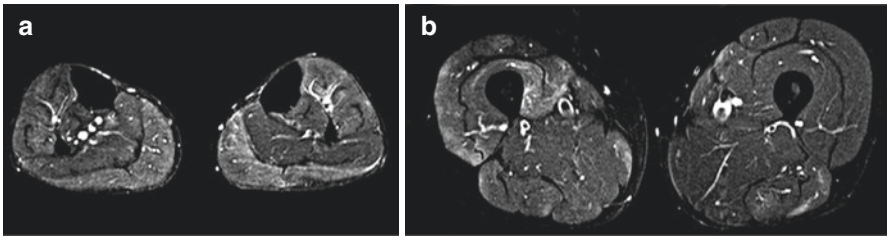

Fig. 3.5 Polymyositis in the acute phase. Axial STIR images at the level of the leg (**a**) and thigh (**b**) of a patient with polymyositis in the acute phase. Diffuse scattered hyperintensities indicative of muscle edema are evident in both districts, predominantly involving medial and lateral head of gastrocnemius muscle and anterior compartment at the level of the legs (**a**) and the right quadriceps in the thighs (**b**)

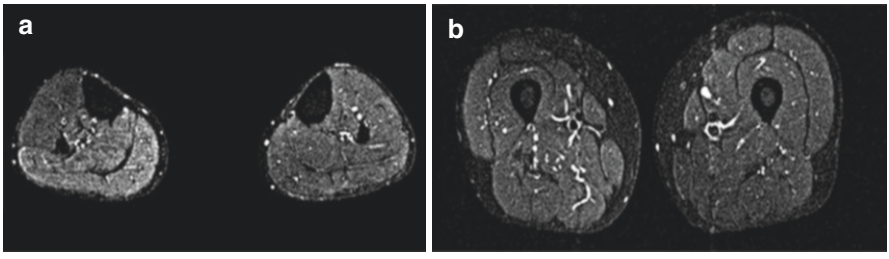

Fig. 3.6 Polymyositis after treatment. Axial STIR images at the level of the leg (**a**) and thigh (**b**) of the same patient of Fig. 3.5 with polymyositis, after immunosuppressant treatment. Muscular edema is diffusely and markedly reduced in both districts, still slightly evident on the posterior compartment of the right leg (**a**), while in the thigh is not detectable anymore (**b**)

between muscle biopsy findings and clinical and MRI improvement supports the notion that different mechanisms, other than the presence of inflammatory infiltrates, are involved in the pathogenesis of this group of diseases [40].

The role of imaging in the IIM workup, however, is not limited to a triage test before performing muscle biopsy or in the evaluation of the efficacy of treatment, but MRI is currently considered extremely valuable in those subjects presenting with clinical history and clinical examination that are consistent with IIM. Interestingly, an STIR hyperintensity in such patients has a very high positive predictive value (>80%) when the a priori chance of having myositis is more than 60%, even if muscle biopsy was not diriment (normal appearance or nonspecific findings) [33]. Although MRI can be negative whether disease activity is quite low, it nonetheless demonstrated to reduce the false-negative diagnosis when used as an add-on diagnostic test. This is a very important target for treatable diseases, even if it is at the expense of a higher false-positive rate [33, 39, 40].

Although DM and PM share many clinical features, they have to be considered distinct diseases with different pathophysiological and histological features [1, 7]. Some studies conducted to assess whether this distinction can reflect also in muscle changes observed in MRI, showed that muscle edema is significantly found in the proximal regions of thigh muscles in DM, whereas PM is predominantly characterized by fat replacement occurring mostly in the in the distal muscles, in their medial and distal regions [41].

The usefulness of muscle MRI has also been tested in the juvenile form of dermatomyositis (JDM), whose course and evolution is often hardly predictable [42, 43]. A reliable **scoring system** that defines several markers of active JDM has been proposed [42]: the first marker of disease activity is the degree of muscle inflammation in selected four muscle groups (gluteal, hamstrings, quadriceps, and adductor), and it is based on a 4-point score (none = 0, mild = 1, moderate = 2, and severe = 3) and on the overall impression about the entire muscle group. The presence of soft-tissue and perifascicular edema is also scored. Unfortunately, muscle or fascia MRI findings do not seem to predict clinical outcome in newly diagnosed JDM children, even if abnormal subcutaneous fat signal appears to be significantly associated to an aggressive chronic disease course [42].

WB-MRI may provide additional information to clinical evaluation of IIMs and represents a promising tool to estimate total inflammatory burden, tailor treatment, and monitor its efficacy. In 2014, Malattia et al. evaluated disease activity with STIR sequences by comparing WB-MRI with clinical examination in 41 patients affected with JDM and 41 matched controls. Muscle, subcutaneous tissue, and myofascial signal abnormalities were scored in 36 muscular groups and on proximal and distal extremities. WB-MRI revealed distal leg and forearm muscle inflammation undetected during clinical examination and allowed a precise assessment of subcutaneous and myofascial involvement. WB-MRI score was higher in JDM active patients than in inactive patients and control group and the correlation between WB-MRI muscle score and disease activity measures resulted good [41]

3.4 MRI Advanced Techniques in Inflammatory Idiopathic Myopathies

A number of different advanced MRI techniques have been progressively implemented and applied in the field of neuromuscular diseases, with the possibility to evaluate and quantify several features including tissue composition and architecture, metabolic properties and function.

In parallel to T2w sequences, **diffusion-weighted imaging (DWI)** has been applied to characterize inflamed muscles. In DWI, the signal is built on the random movement of water in tissue; abnormalities in such movement, as those provoked by inflammatory infiltrates, fiber degeneration, necrosis, and fiber hyperplasia alter the free movement of water. In this sense, the inflamed muscles have been shown to present with increased water content and increased transmembrane water movement. Already in 2008, Qi et al. characterized total fluid motion within the muscle using the apparent diffusion coefficient (ADC), diffusion in the extra- and intracellular muscle compartments (D), perfusion in capillaries (pseudodiffusion), and volume fraction of capillary perfusion (f). Unaffected patient muscles had DWI coefficients equivalent to those of normal muscles; inflamed muscles showed elevated ADC and D values whereas fat infiltrated muscles had lower values than control muscles. Inflamed muscles had also lower f values, thus suggesting decreased fractional volume of capillary perfusion [44].

Despite the lack of a pathologic correlation, Faruch et al. recently suggested that DWI (applied with a WB-MRI approach) was superior to STIR in detecting muscular edema, possibly related to its higher signal-to-background ratio. The authors also suggest that DWI is more sensitive than STIR at detecting low-grade edema, presumably as STIR could not be sensitive enough to see the pathophysiological changes associated with low-grade edema [31].

Interestingly, ADC has also been also correlated to electromyographic studies: Meyer et al. found that ADC_{mean} and ADC_{min} had a positive correlation with the duration of motor unit action potential in patients with myositis [45]. Another study from the same group searched for correlation between histogram analysis of ADC and seric markers in myositic subjects and, found, among others, that percentiles of ADC as ADC_{10} and ADC_{90} correlated with seric creatine kinase and kurtosis (an advanced diffusivity imaging technique) inversely correlated with seric c-reactive protein [46].

As an evolution of DWI, **diffusion tensor imaging (DTI)** also proved to be useful for characterizing normal and pathological muscle tissue [47, 48] . Muscle proteins and membranous structures represent a barrier to diffusion and cause low self-diffusion coefficient of water in muscle and diffusion anisotropy. Since some changes observed in chronic myopathies (i.e., Z-line abnormalities, increased membrane permeability to water and inflammation) affect the spacing of physical barriers to free diffusion, DTI-MRI is potentially a very sensitive method to investigate such modifications and follow-up the disease evolution. Wang et al. found that in DM/OM fractional anisotropy (FA) values of edematous muscles were lower than

unaffected muscles and of normal muscles of controls; conversely the ADC and eigenvalues (λ1, λ2, and λ3) of edematous muscles were higher than both unaffected muscles and of healthy controls [49]. Finally, Sigmund et al. applied a novel dynamic DTI technique to DM subjects and found, among others, significantly larger mean (MD) and radial diffusion (RD) exercise response in patients compared to controls, suggesting an interesting role for dynamic imaging to increase our insight into muscular abnormalities in myopathies [50]

Quantitative magnetization transfer (qMT) MRI is used in characterizing the spatial distribution of the relative contents of the macromolecular and free water proton pools of biological tissues, deriving a ratio of the sizes of these two pools (pool size ratio, PSR) [51]. It has been demonstrated that PSR may be used as a biomarker of inflammation [51, 52]. However, quantitative MRI studies in skeletal muscle are challenged by low signal-to-noise ratio (SNR), motion artifacts .and intramuscular adipose component [51, 52]. Li et al. developed an approach for performing qMT imaging in thigh muscles using a pulsed saturation method; their data support the use of a two-parameter modeling approach in qMT imaging of skeletal muscle in order to reduce total imaging time and to permit additional signal averaging [51]

Interesting data come from preclinical experiments, somehow indicating the pathway for future clinical studies. Bryant et al. used a **multi-parametric MRI technique** to investigate muscle inflammation by calculating proton relaxation, DTI, qMT-MRI, and dynamic contrast-enhanced (DCE-MRI) parameters [52]. Data were acquired in a single imaging session conducted 6–8 h following the injection of λ-carrageenan, a local inflammatory agent, in eight healthy male C57BL/j6 12- to 14-week-old mice. T2 relaxation was elevated in inflamed skeletal muscle, and this parameter is highly sensitive to inflammation; the global increases in T2 of inflamed muscle in this model are largely due to the expansion of the extracellular compartment. A significant increase in ADC also occurred in the inflamed muscle, having been brought about by a general increase in diffusivity in all directions, which is probably a direct effect of the expanded extracellular space. Analysis of the qMT data revealed that the T1 of the free pool and the observed T1 both increased in the inflamed tissue, while the PSR significantly decreased as the free water pool increased. DCE-MRI data also supported observations of an increase in extracellular volume.

A **7-Tesla MRI** provided a method for noninvasively assessing inflammation and remodeling of the skeletal muscle and seems to represent an informative tool for studying in vivo immune-mediated muscle damage [53]. In a mouse model with anti-synthetase syndrome, 7-Tesla MRI allowed a precise identification of the events occurring in muscle tissue and showed that they were temporally associated with establishing autoimmunity linked to the development of anti-HisRS antibodies. Muscle changes detected by MRI paralleled edematous and inflamed areas at the histopathological studies. MRI reflected muscle damage and remodeling even if the disruption of myofibers, as appreciated by serum creatinine phosphokinase concentration, was scarce.

3.5 Imaging of Inclusion Body Myositis

Inclusion Body Myositis is the most common form of myopathy in subjects older than 50 years of age, has a progressive course, and predominantly affects the quadriceps and the gastrocnemius muscles in the lower limbs and the digitorum flexors in the upper limbs [1, 54–56]. In particular, MRI abnormalities (STIR hyperintensities) seem to more extensively affect the lower extremities, generally in an asymmetric way, and with greater involvement of the more distal muscles [56, 57].

It is not infrequent that subjects with incomplete clinical presentations and/or lacking the classical pathological features of the disease may be frequently misdiagnosed as a different inflammatory myopathy, especially PM, or degenerative diseases, i.e., myofibrillar myopathies, GNE myopathy, and other rimmed vacuolar myopathies. In this sense, additional tools are needed to confirm the diagnosis and muscle MRI has proven to be useful for the diagnostic workup, especially of patients with early disease or who lack the classical IBM pathology [57].

Recently, a typical lower limb MRI muscle involvement has been described in IBM patients, which was not found in any no-IBM patients. The major criterion seems to be the presence of slightly granular fatty-fibrous infiltration and/or atrophy of both quadriceps muscles in the distal portion (above the knee), particularly involving the vastus intermedius and medialis muscles, thus giving a "melted" appearance (Fig. 3.7). Involvement of the sartorius is also a useful additional hint, as it is usually spared in other adult-onset myopathies. The presence of these criteria is considered enough to define a typical case.

As aforementioned, IBM shows involvement of flexor digitorum profundus in the forearm (Fig. 3.8), with extensive degree of fat replacement. Upper limb imaging, however, is not routinely performed in the diagnostic workup of muscle diseases, yet could help in the differential diagnosis.

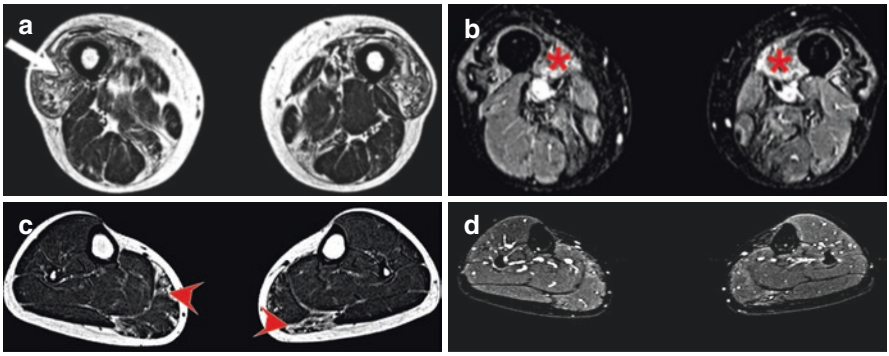

Fig. 3.7 Inclusion body myositis. Axial T1 and STIR images at the level of the distal thigh above the knee (**a**, **b**) and at the level of the leg (**c**, **d**) in a patient with inclusion body myositis. "Melted" appearance with slightly granular fatty infiltration and atrophy of the distal quadriceps *(white arrow),* together with abnormalities on STIR sequences (*red **) mainly in the medial vasti. Note the predominant involvement of the medial head of gastrocnemius muscle (*arrowhead*) at lower leg level

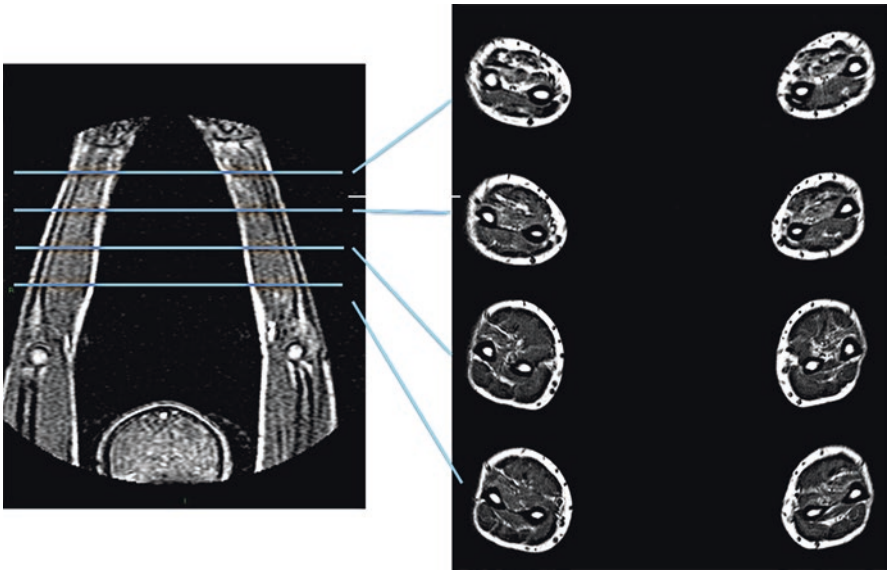

Fig. 3.8 Inclusion body myositis. Axial T1 images at the level of the forearm (see corresponding level in the scout coronal view) in a patient with inclusion body myositis showing fat replacement at the level of the **flexor digitorum profundus** bilaterally

In uncertain cases, supporting criteria, neither necessary nor sufficient per se to define a typical case, were considered, in order of importance: (1) in the legs, the most involved muscle is the medial gastrocnemius, even if the legs can also be normal; (2) pelvic muscles should not be heavily involved, or at least less involved than thigh muscles. In their series, Tasca et al. found a typical IBM pattern in 10 out of 17 definite IBM patients, 0 out of 2 possible IBM, and 0 out of 118 non-IBM patients. A consistent pattern was found in 6 out of 17 definite IBM patients, 2 out of 2 possible IBM, and 3 of 118 non-IBM patients [57].

3.6 Imaging of Other Acquired Neuromuscular Diseases

Only few studies on the role of imaging in other acquired neuromuscular diseases are so far available in the literature, yet this topic will certainly be a challenge for the future.

Myasthenia Gravis (MG) related to antibodies to muscle-specific tyrosine kinase (MuSK) is often associated with bulbar involvement, fixed facial weakness, and tongue muscle atrophy. In MuSK-MG patients compared with healthy controls and AChR-MG cases, MRI demonstrates thinning of the buccinator, orbicularis oris, and orbicularis oculi muscles as well as tongue areas having increased T1w signal; an ocular-bulbar-facial-respiratory (OBFR) score was also proposed to correlate MRI changes with clinical and treatment histories [58]. MRI revealed fat

replacement and marked atrophy of temporalis, masseters, and lingual muscles also in MuSK-MG patients having short duration of symptoms and still not treated with immunosuppressive therapy, thus suggesting that MuSK antibodies "per se" may be related to muscle atrophy development [59]. Otherwise, significant muscle atrophy and fatty replacement were only rarely found in the AChR-MG patients [58, 60].

Muscle MRI has been studied in a limited number of cases of **lipid-lowering agent-associated myopathy**. Although a tendency to preferential involvement of dorsal muscle groups of both thighs and legs with evidence of muscle edema, fatty replacement, or both has been observed, a distinctive MRI pattern of abnormalities was not identified [61], even if attempts in such direction are on the go [62].

In **alcoholic myopathy** high muscle signal intensities especially on T2-weighted images were observed and interpreted as indicating "pre-rhabdomyolysis" related to alcohol abuse [63] or type II fiber atrophy [64]. In **diabetic myopathy**, the anterior compartment of the thigh was involved in all the studied patients and, interestingly, muscle infarction and necrosis was observed in 38% of the patients [65].

3.7 Other Imaging Options

Computed tomography (CT) was widely used in the past to evaluate muscle changes, in particular morphology, volume and fatty degeneration, the latter detectable as muscular hypodensity (fat has lower density than muscular tissue)[4, 14, 66]. The contrast of soft tissues contrast, however, is poor and it is not adequate to identify inflammatory changes. Importantly, CT also implies elevated doses of ionizing radiation; in the context of muscular diseases, ultrasound (US) and MRI have globally taken the place of CT [14, 67].

Ultrasound (US) is a valid low-cost and widely available imaging technique in the suspect of a muscular disease. By evaluating echogenicity changes, it makes possible to identify atrophic changes and fatty degeneration, is able to guide muscle biopsy and to follow-up patients [68, 69]. Ultrasound scans have good resolutions, up to 1 mm, and allow dynamic studies of muscle contraction by short videos in order to examine muscle contraction and pathological muscle movements (i.e., fasciculations, myokymia) [68, 70]. However, ultrasound application in clinical practice is limited as it is operator-dependent and can usually efficaciously study only superficial muscle groups.

Last but not least, the application of US in the field of muscular diseases may only play a role in those centers where operators have a specific experience in the field.

Contrast-enhanced ultrasound (CEUS) blood flow demonstrated to be a good measure in sensitivity and specificity of perfusion in clinically affected DM and PM skeletal muscles [71, 72]. By applying a modified model that analyzed the replenishment kinetics of microbubbles, the perfusion-related parameters (blood flow, local blood volume, and blood flow velocity) were measured and findings were compared with muscle biopsy appearances and with the results of a 1.5-T MRI. Patients with histologically confirmed DM or PM showed significantly higher blood flow velocity, blood flow, and blood volume than those with no inflammatory

myopathy signs. An increase in signal intensity on T2-weighted MR images was found in all patients with myositis [71].

Last but not least, **[^{18}F]-Fluorodeoxyglucose positron emission tomography (FDG PET),** a standard tool for evaluating malignancies, can also detect inflammatory changes in the muscle [73]. Tanaka et al. demonstrated that [^{18}F]FDG uptake can discriminate PM/DM from non-muscular diseases and is highly sensitive in detecting muscle inflammation in proximal muscles, providing useful information in the management of treatment-naıve PM/DM patients [74]. The regional FDG uptake reflects muscle weakness and correlates with the infiltration of inflammatory cells at muscle biopsy.

The visual assessment of FDG uptake (vFDG) as well as the calculation of standardized uptake value (SUVmax) can improve clinical practice and provide insights into patho-mechanisms of PM/DM [75]. vFDG was observed in multiple muscle lesions with different distribution in two-thirds of the patients with PM/DM, with most lesions being symmetrical. Histological findings correlated with both the mean SUVmax and number of vFDG-positive regions. Serum creatine kinase levels were higher in patients with more than two vFDG-positive regions than in those with two or less regions.

Attempts to even propose cut-off values for SUVmax in the field of inflammatory myopathies are promising yet still need larger cohorts [76].

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4 Ultrasound Evaluation of Peripheral Nerves: Evidence, Clinical Application, and Recent Developments

Luca Padua and Daniele Coraci

4.1 Basics of Ultrasound

Nerve ultrasound (US) is a medical tool based on mechanical waves able to mainly evaluate the morphology of a body part. Its use for the assessment of peripheral nerves is now well known and supported by a large number of literature evidence.

US technique requires a proper machine equipped with specific probes containing the piezoelectric crystals. These ones are able to produce sound waves higher than 20 kHz (the upper limit of the acoustic waves) when stimulated by electric energy [1]. When the waves are transmitted towards the tissues, some are reflected as echoes (Fig. 4.1). Hence, the crystals translate in electric signals the mechanical stimulation due to the echoes received. This property allows the visual reconstruction of the studied organs, similarly to the sonar functioning. Finally, US performer's skill is fundamental for a proficient US assessment and for a correct interpretation of the findings. For nerve US study, some physical properties should be kept in mind. The nerves are relatively small particles and, for this reason, we need high-resolution level of US. Considering the inverse relationship between wavelength and probe frequency and considering that the low wavelengths increase the resolution power, we should use high-frequency US probes [2]. This explains the usefulness of 15–18 MHz probes to assess the nerves. On the other hand, the higher frequencies imply a lower capability of tissue penetration. This induces to privilege

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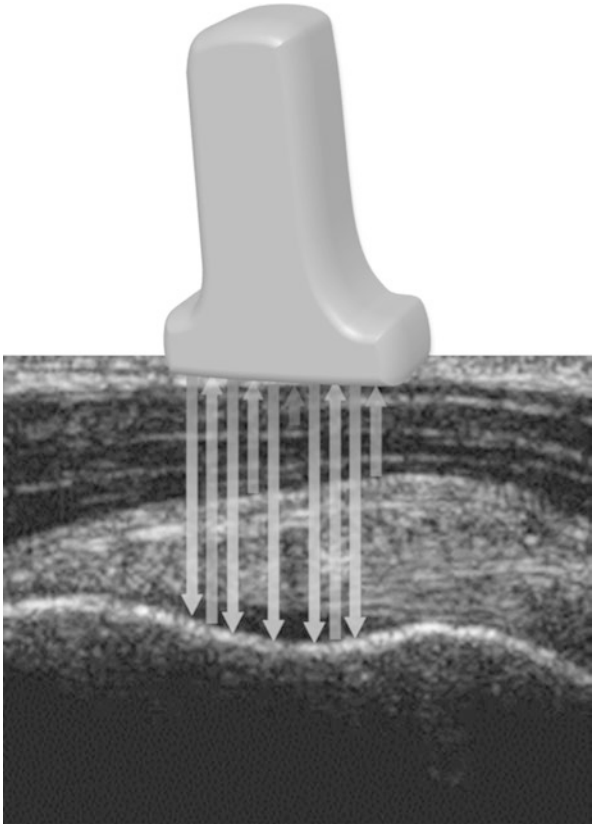

Fig. 4.1 Schematic representation of the ultrasound beam. The mechanical waves produced by the probe are transmitted in the tissues and soma of them are reflected. The probe receives the echoes and allows the translation of the signals into images

the use of lower frequency probes (around 8–10 MHz) to examine the deep structures, like the sciatic nerve, even if this reduces the resolution. In a few words, a good nerve US is the result of the interactions of the US machine, the US probe, the US performer, and the anatomical conditions.

The application of US in peripheral nerve evaluation is in constant development and, when associated with the clinical and neurophysiological examinations, provides several data about the nerves, the muscles, and the surrounding elements. In particular, US allows the differentiation between the axonotmesis (interruption of the sole axons) and the neurotmesis (discontinuation of the nerve). Indeed, the electrophysiological (EDX) exam shows comparable findings in both these conditions, but the prognosis and the management of the two situations are completely different

[3]. Obviously, this is allowed by the possibility to directly and safely see the nerve through US. This ability additionally reveals the localization of the nerve abnormalities (anatomic variants included) and the characterization of the space close to the nerve. For example, conflicts with vessels are visible. For these reasons, US is applicable for several diseases: entrapments, traumas, even immune-mediated, inherited neuropathies, nerve tumors, and other less common diseases.

4.2 Literature History of Nerve Ultrasound

The literature about nerve US has continuously increased since the first publications about this topic. The search on PubMed of "nerve US," shows a small number of papers in the late 1940s. In particular, the most ancient paper on PubMed is dated 1946. In this year, and until 1951, just one paper per year was published, with the exception of 1948, when no result is found. From the 1950s and especially the 1960s, the increase of publications has never stopped. In order to reveal the high impact of nerve US in literature, we can evaluate the relative number of papers, calculating the percentage in comparison with the total number of papers on PubMed for each year. Considering the last decade, since 2016 the search reveals an increment, meaning an unceasing interest in this matter (Fig. 4.2). Among the diseases which can be usually studied with this technique, the traumatic conditions appear the most present in association with nerve US. The literature shows a plateau of literature production during the first 5 years of the considered time, followed by a relative growth. This is expectable for the high frequency of traumas and the important data provided by US, even in the hyper-acute phase [4]. With a similar behavior, the entrapments represent the second most considered sub-topic with a relative number of papers about ten times higher than other less common diseases. Interestingly, an important rapid increase of the results is visible for the Charcot-Marie-Tooth disease (the most common inherited neuropathy) in 2013. The same sub-topic shows another increase in 2017–2018, when its relative number of publications overcame the production about chronic inflammatory demyelinating polyneuropathy (CIDP) and Guillain-Barré syndrome (GBS). For these two immune-mediated polyneuropathies, the relative increase of publications has occurred in the last decade, until a peak in 2016. After this year, the literature interest about CIDP has continued, while the scientific production about GBS has apparently weakened.

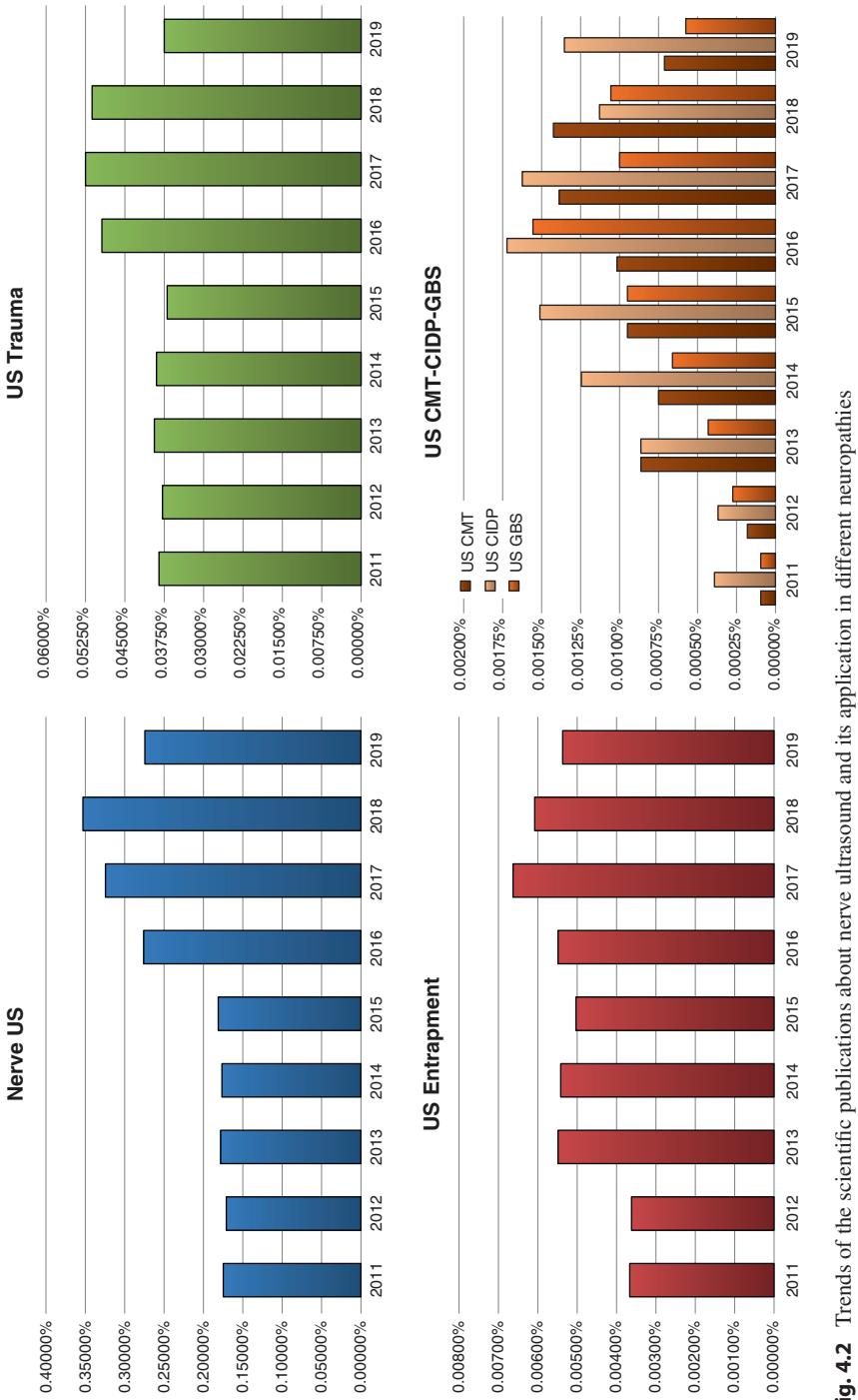

Fig. 4.2 Trends of the scientific publications about nerve ultrasound and its application in different neuropathies

4.3 Nerve Ultrasound in Clinical Practice

4.3.1 Fundamentals

Nerve US requires enough knowledge about the anatomy and the physiology of the peripheral nerves. The usual course of the nerves and the main anatomical landmarks have to be acknowledged [5]. Particular attention should be paid to the anatomical variants. During the examination, abnormalities in nerve localization or irregular relationships with other structures (muscles, tendons, bones, vessels) can be found [6]. These conditions can be misinterpreted and, for this reason, the examiner should have enough experience to recognize them. The nerves are mainly studies in axial view, meaning the US beam is perpendicular to the long axis of the nerve. With this transverse scan, the usual US pattern of a nerve is characterized by a hyperechoic rim surrounding an ellipsoid area containing hypoechoic fascicles (Fig. 4.3) [7]. This pattern shows some variations in normal nerves, for example, scarcely visible fascicles in the sites of common entrapments, even if no disease is present. The transverse scan should be performed along the whole nerve course (when possible), in order to find changes in morphology. In some cases, a comparison with the same nerve of the other side may be suggested to help the identification of unilateral abnormalities. The main finding indicating a focal nerve suffering is the increased cross-sectional area (CSA, area of the nerve section assessed in the transverse scan), possibly associated with the loss of the fascicular pattern and changes in echogenicity [7]. Each nerve presents its normal values of areas in the different points of its course. Additionally, other abnormalities may be found, like the increased dimension of single fascicles or an increased echogenicity of the entire nerve. This last situation is visible in long-duration diseases and can be associated to area reduction. Besides the axial view, a longitudinal scan is possible. This

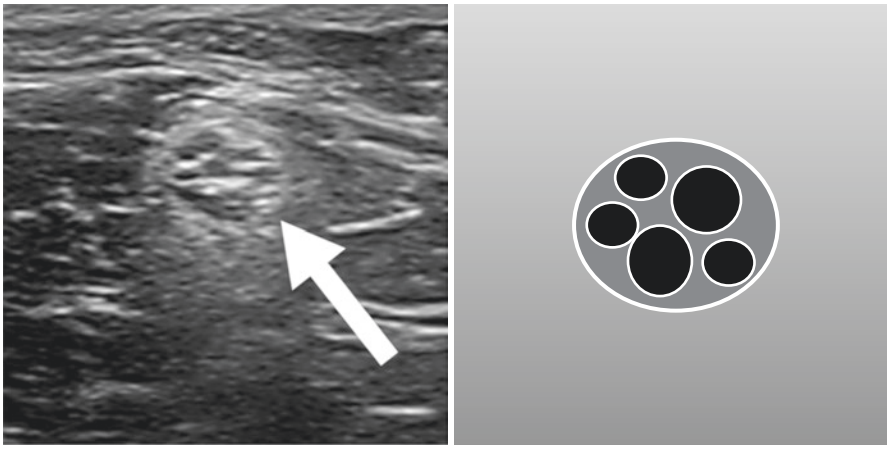

Fig. 4.3 Typical ultrasound image of a normal nerve (arrow) with the hyperechoic rim and the fascicles. The panel on the right shows the graphical representation of the nerve picture

approach should be only considered as the second step because it can provide a less precise visualization and can be used to show, in a single picture, the extension of a lesion. Additionally, the use of power Doppler, able to evaluate the slow flows, can be added to study the intraneural vascularization and to localize the vessels close to the nerve [8].

The measurement of the nerve dimension delivers a quantification of the morphological alteration. This makes the nerve US only partially operator-dependent. Obviously, the identification of the real US sign of nerve suffering is related to the operator's ability, but the demonstration is based on the measurement of the nerve area. On the contrary, the quantification of the echogenicity is not usually applied, especially because it can vary with the changes of US beam angle and, furthermore, because it is not directly measured. For this reason, semi-quantitative scales, based on the operator's perception, can be used, with risks of high variability [9].

The application of US is very useful for diagnosis because it shows specific elements indicating the nerve suffering. However, the utility of US is extended to: prognosis, because some findings can be linked to different levels of recovery; follow-up, because its safety and speed execution, allows serial exams; rehabilitation, because the morphological information can support the therapeutic choices.

4.3.2 Association with Electrophysiology and Magnetic Resonance

Nerve US can be seen as the third eye of the physician. Indeed, it represents an extension of the examiner's senses and allows directly visualizing most of the nerves. This exam can be correctly performed only after proper anamnesis and physical examination, in order to collect data about the patient's history, symptoms and signs, and formulate the correct diagnostic hypotheses. When possible, nerve US should be associated with an extensive EDX evaluation, which indicates and quantifies the nerve dysfunction. In this way, US represents a completion of the other assessment and adds valuable information about the morphology, difficultly obtainable with the EDX [3]. The two approaches can be considered as Castor and Pollux that together support the diagnosis and the management of the neuropathies [10]. The most important added values of US are the ability to identify the neurotmesis and the precise localization of the nerve damage. In these cases, nerve sheath and the axons are interrupted, while, in cases of axonotmesis, the sheath is preserved. The two conditions are very different in prognosis, in fact, the first one requires a surgical approach to restore the nerve continuity. Unfortunately, EDX cannot distinguish between them because the elements conducting the electrical signals are similarly interrupted. Nerve US sees the nerve and when it reveals its continuity, it allows the exclusion of neurotmesis. For the same reason, US localizes the nerve damage and discloses the extension of a morphological alteration, like in cases of neuromas due to closed trauma. Additionally, the application of US is fundamental to rapidly explore nerves not easily assessable with EDX (e.g., lateral

femoral cutaneous nerve) [11]. The contributions of US in an EDX lab also include: localization of compressive structures, explaining focal nerve damage; characterization of organs surrounding the nerve; identification of anomalous courses, facilitating the nerve conduction study. Recently, an additional practice of US in aiding EDX. Through US evaluation of the muscles innervated by the injured nerve, minimal voluntary contraction can be found in muscles (if they are present). This can guide the electromyographic needle examination, selecting the muscle parts presenting the activity and increasing the specificity of EDX examination [12]. In conclusion, a contemporary presence and integration of EDX and US services is desirable in the medical labs where patients with peripheral neuropathies are managed [13].

The main limitation of US is the bone, representing an almost absolute obstacle for the US beam. The nerves located under the bone (e.g., the lumbar plexus) are not visible. For this reason, a completion with magnetic resonance is necessary in particular cases. The two imaging techniques may be seen as two techniques balancing the respective limitations [14]. Obviously, US is fast and without any contraindications, but is not able to access every body part. Finally, some clinical conditions, like severe traumas with significant tissue alterations, can require an evaluation based on magnetic resonance.

4.3.3 Scores

In the last years, some US scores have been developed in order to increase the value of information related to the nerve dimensions. The absolute nerve area may be misinterpreted or not indicative of a real alteration. In cases of normal small nerves, an apparent mild increase (within the upper limit of normal values) may represent a sign of nerve suffering because the focal dimensional change becomes more important when compared with the dimension in other sites. For example, if the CSA of the median nerve at the wrist is 11 mm^2 (upper normal value 12 mm^2), while at the forearm is 5 mm^2, the result may indicate a nerve involvement at the carpal tunnel, especially if clinical and EDX findings suggest it. For this reason, besides the comparison with the contralateral side, a comparison between two different sites of the nerve is useful. Values of the wrist-forearm ratio higher than 1.4 indicate carpal tunnel syndrome. This ratio is calculated by dividing the nerve area (in the transverse scan) at the wrist for the area at the forearm [15].

Other scores have been built for the characterization of polyneuropathies. These scores can be applied when more nerves are assessed in the same subjects. Usually, in this situation the major nerves of the four limbs are studied: median, ulnar, radial, fibular, tibial, and sural in defined sites. Additionally, the assessment of cervical roots and brachial plexus can be added. The aim of the scores is the definition of the disease severity or the differentiation between different forms (e.g., axonal and demyelinating). Intra-nerve CSA variability is a simple calculation of the ratio between the maximum and the minimum area of the assessed nerves. This measurement can be associated with the inter-nerve CSA variability that is the ratio between

the maximum and the minimum values of these ratios. This calculation does not exclude the sites of the common nerve entrapments and is useful in cases of immune-mediated polyneuropathies, indicating a general degree of inhomogeneity of nerve enlargements [16]. Differently, Bochum US score is calculated on the basis of the number of sites with increased area. The nerves explored are ulnar (distal and proximal), radial, and sural. This score can distinguish between CIDP and multifocal demyelinating polyneuropathies [17]. A more complex score is the ultrasound pattern sum score. This latter assigns points on the basis of the enlargement severity of several nerves in different sites. The final score summarizes the general level of the nerve abnormalities and can help in distinguishing the different types of polyneuropathies [18]. In a similar way, nerve abnormality index can differentiate between inherited and acquired neuropathies and between axonal and demyelinating. This score combines the number of abnormal sites and the incremental ratio of enlargements of median and ulnar nerves, excluding the sites of common entrapments. The scores are based on the usual US differences of the nerves in the various neuropathies [19]. Generally, in the abovementioned diseases, the nerve appears larger in the demyelinating forms and the enlargement is less homogeneous in the acquired neuropathies.

4.4 Main Diseases

4.4.1 Entrapment Neuropathies

The entrapment neuropathies are focal nerve suffering usually occurring in specific anatomic sites [20, 21]. The ability of US to directly see the anatomy allows the evaluation of the nerve and the other local structures. In these neuropathies, the main US finding is the focal enlargement of CSA, possibly associate with hypoechogenicity. By US, the depiction of the causes of the entrapment is possible. In particular, the visualization of all local structures allows the distinction between idiopathic conditions and secondary nerve suffering [22]. Cases with clinical and EDX findings indicating nerve entrapment can be related to the presence of a cyst or an anatomical variant compressing the nerve [6]. These causes can be shown by US, changing the therapeutic approach. In fact, when similar abnormalities are found, the surgery becomes the most appropriate strategy to remove the cause. In idiopathic conditions, like carpal tunnel syndrome, external masses are absent and US shows an increase of nerve dimension that can be associated with the thickness of the transverse ligament. Considering the second most common entrapment, ulnar neuropathy at the elbow, the usefulness of US is especially related to the capability to localize the site of nerve suffering [23]. Indeed, in this disease, the compression can occur in at least four different sites and EDX cannot discriminate them without a long and challenging exam. The evaluation by US can show the focal enlargement with high precision and rapidity. In the lower limb, fibular neuropathy at the fibular head is assessable, recognizing the changes in nerve CSA (in terms of dimensions and shape) and showing the relationship with the bone. This approach may be useful

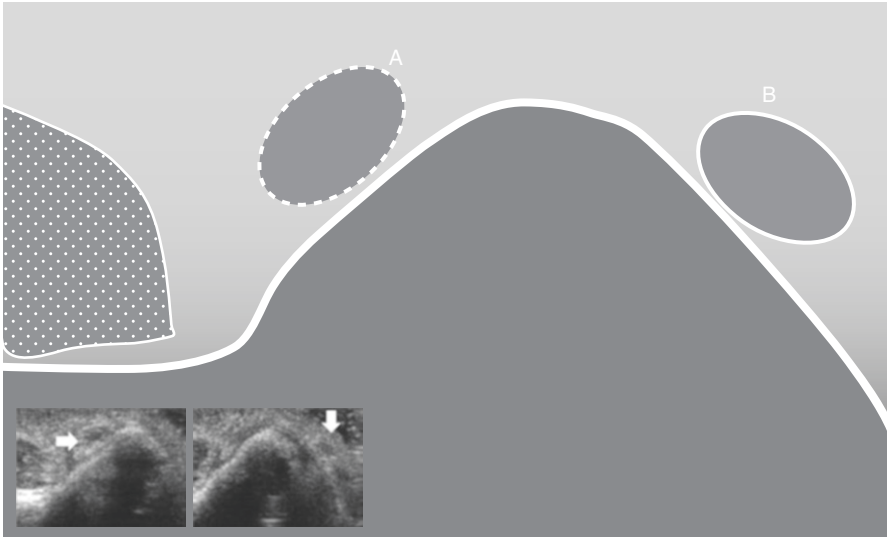

Fig. 4.4 Scheme of an ulnar nerve luxation. The ulnar nerve moves from position A (inside the epitrocleo-olecranic groove), when the elbow is extended, to position B (outside the groove) when the joint is flexed. The picture-in-picture shows the ultrasound images of ulnar nerve (arrows)

to confirm the diagnosis and characterize the origin of the nerve involvement, for example, revealing a cyst or an exostosis [24]. Similar to other tunnels, tarsal tunnel syndrome can be easily accessible to US for the entire anatomical tract. Because of the pathogenesis of the tarsal tunnel syndrome, the increased CSA is not always present, but US is a proficient completion even in this case, for its power in depicting the causes of compression external to the nerve [25]. Additionally, being the tarsal tunnel syndrome hardly valuable by EDX, the US should be a valid allied of the clinician. In entrapment neuropathies, another important strength of US is the dynamic evaluation. During the exam, the patient can move the examined anatomical segment and the operator can visualize the changes in the movement of the assessed structure (nerves, muscles, etc.). This evaluation, not usually possible with other imaging approaches, allows assessing the interactions of the different local anatomical elements, revealing a nerve luxation (for example of the ulnar nerve at the elbow) or an anomalous non-constant contact of two organs (Fig. 4.4) [26].

4.4.2 Traumatic Nerve Injuries

In cases of traumas, the nerves can be extremely compromised, causing significant motor and sensory deficits. EDX in these conditions can be very useful, especially to reveal the motor unit during voluntary contractions, but the other important information is not obtainable in the days after the injury. US, instead, can allow the visualization of the damaged nerve and can show if the nerve presents discontinuity (Fig. 4.5)

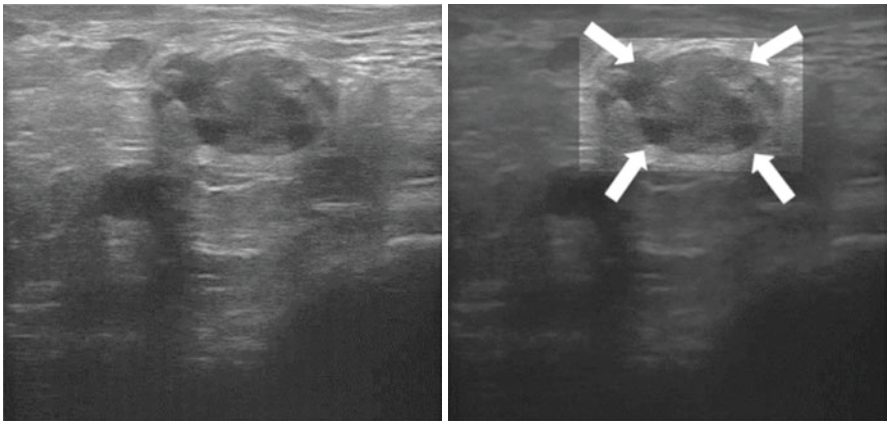

Fig. 4.5 A neuroma in a case of neurotmesis. In the right panel, the arrows delineate the neuroma. The nerve shows increased size, reduction of echogenicity, and loss of fascicular pattern

[3]. The diagnosis or the exclusion of neurotmesis determines the treatment decision. When the disruption of the nerve is complete, the surgical approach is necessary. A fast choice is fundamental for the patient's prognosis and US provides, before EDX, enough data for the decision. US can completely change the management avoiding a long follow-up to record the absence of recovery. Furthermore, in cases of neurotmesis, US shows the distance between the two nerve stumps, indicating the best surgical approach (raffia or graft), and displays the anatomical peculiarities of the single patients, thus guiding the intervention limiting the complications [27]. Besides these relevant abilities, US can show the nerve enlargement in the injured site, or in sites far from the main damage, and its extension [28]. In cases of traumas, the neuroma (nerve enlargement) is linked to general nerve damage (demyelinating and axonal) and its dimension can be directly related to the severity of nerve involvement and the poor recovery [29]. This potential prognostic value is interesting and needs extensive longitudinal studies [30]. The follow-up evaluation can take advantage from US because the evolution of nerve CSA can be quantified and associated with clinical and EDX changes. In a similar way, the morphological outcome after the surgery can be assessed, revealing if the nerve reconstruction has been effective or if the intervention caused complications (like a conflict between the nerve and a bone or a foreign body). Even the rehabilitation can be helped by US. The design of a proper recovery project can be more efficient when the severity and the localization of the damage is well known. This allows deciding the most appropriate maneuvers and the outcome definition of the rehabilitation [31].

4.4.3 Immune-Mediated Neuropathies

The immune-mediated neuropathies are a heterogeneous group of acquired diseases characterized by the abnormal action of the immune system against antigens expressed by the nerve structures. They include acute and chronic types and

demyelinating and axonal forms [32, 33]. The US assessment of these diseases should consider a wide assessment of the four limbs and the cervical roots. A correct evaluation of the main nerve trunks is desirable, paying attention, not only to the CSAs, but even to the nerve echogenicity and the fascicular patter. Inhomogeneous CSA enlargements, meaning increased nerve size in different sites interrupted by normal nerve dimensions, are expectable [34]. This justifies the utility of the examination of the whole nerves, where assessable. Among these neuropathies, GBS represents an acute type and comprises demyelinating and axonal neuropathies. It usually presents nerve enlargements in US, in the first month from the onset, while a normalization trend is visible in the following months [35]. Some differences are visible between the demyelinating and the axonal form, showing the first more enlargements in the proximal nerve sites [36]. The evolution of nerve size is different in CIDP, a chronic demyelinating form. In CIDP, the nerves and the fascicles are enlarged. In follow-up, only a small number of patients show CSA reduction, while most of them are characterized by a stability of US pattern when the clinical improvement occurs and further CSA increases if the disease worsens [37]. When the disease progression is long, peculiar changes in nerve dimension and echogenicity. In particular, the involved nerves become hyperechoic and the fascicles are less visible. In very late diseases, the nerves appear small and very hyperechoic and, usually, this stage implies a low response to the treatment [38]. In this way, US has further prognostic values, useful for the therapeutic decision. The US differentiation between the types of immune-mediated polyneuropathies is a matter of debate, but the use of the scores discussed above represents a recommended approach.

4.4.4 Charcot-Marie-Tooth Disease

CMT is a group of inherited neuropathies presenting demyelinating or axonal forms, severity, genetic transmission, nerve involvement. US has shown peculiarities of the morphological alterations in the different subtypes [19]. In the subtypes 1 (demyelinating), nerve CSA is generally increased and this enlargement is diffuse. This means that the nerves usually show CSA values higher the normal values in their entire course and the dimension increase uniformly proceeds in distal-proximal direction. The size increment is especially present in CMT1A, the most common form. The axonal subtypes (CMT2) usually have nerves with CSA within the normal values, but with a relative hyperechogenicity. Additionally, in these forms, US shows hyperechoic pattern of the muscles, indicating a substitution due to the damaged caused by the axonal neuropathy (Fig. 4.6). In the CMTX, increased nerve dimensions are visible but with levels lower than the CMT1. Finally, the hereditary neuropathy with liability to pressure palsy (HNPP), a particular neuropathy distinguished from CMT but classifiable in the same neuropathy family, shows clear enlargements in the sites of common entrapments and normal CSA values in the other locations [19]. Many studies about CMT are focused on the characterization of the nerves, but the real meanings of dimension differences among the CMT subtypes and the evolution of the nerve morphological abnormalities are not completely

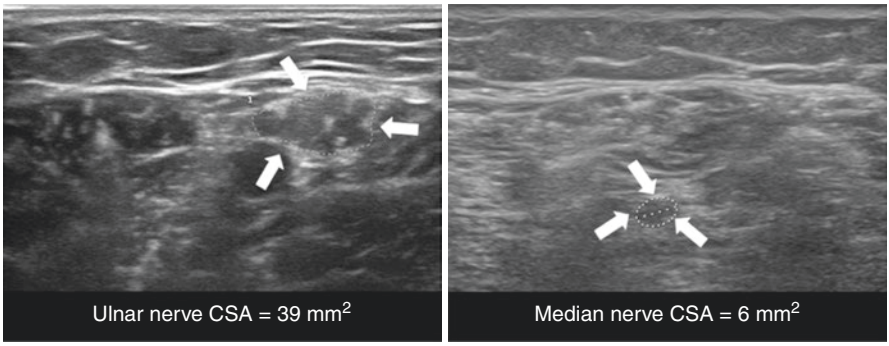

Fig. 4.6 Two examples of nerve ultrasound in Charcot-Marie-Tooth disease. In the demyelinating subtype (1A), the nerve is usually enlarged (left panel). The axonal subtypes can show small nerves and hyperechoic pattern of the muscles (right panel). The arrows indicate the nerves

clear [39]. Furthermore, correlation with clinical parameters has been studied. If no linear correlations should exist with age, a peculiar association was found for the CMT1A [40]. In particular, the disease seems to present increased nerve CSA in the first part of life, with a peak at 50 years and the following CSA reduction [41]. This datum requires further detailed studies but can be associated with the demonstration of a clinical worsening after 50 years [42]. Many studies have considered the possibility to employ US findings to guide the differential diagnosis of the subtypes and to direct the genetic examination, avoiding the long and expensive research for the disease confirmation. However, at the moment, this US application seems not realistic. The usefulness of nerve US in CMT is, at the moment, related to the characterization of the nerve involvement and the evaluation of possible associated abnormalities.

4.5 Other Diseases

4.5.1 Amyotrophic Lateral Sclerosis

The diagnosis of amyotrophic lateral sclerosis (ALS) is based on clinical and EDX examinations. However, US may support the assessment of the patients, through the valuation of nerves and muscles [43]. Considering the nerves, some studies found size reduction. The real diagnostic usefulness of US for the disease is not clear. When associated with clinical examination and EDX, US can support in differentiating ALS from other neuropathies (like multifocal motor neuropathy, which usually shows enlarged nerves). Considering the muscles, US is sensitive for revealing fasciculations, superior to EMG. They are not pathognomonic of ALS but represent an important diagnostic sign [43]. Furthermore, US can show the severity of muscle degeneration, giving information about muscle dimension and echogenicity. In particular, size reduction, hyperechoic pattern, and reduction of variability in the

echogenicity are usual findings. These findings are related to the disease progression. Finally, diaphragmatic muscle can be assessed by US, obtaining morpho-functional information [43]. In fact, besides muscle thickness, the variation between inhalation and exhalation is measurable. These US data can be useful for monitoring disease progression but extensive studies are needed.

4.5.2 Morton's Neuroma

The focal enlargement of the interdigital branches of the tibial nerve, in the metatarsal space, is the condition defined Morton's neuroma (MN). This is a painful condition that requires appropriate treatment. US can be an elective approach for the depiction of the enlargement of these little nerves. MN is visible by US, usually, as hypoechoic mass between two metatarsal heads [44]. With proper operator's maneuver, this neuroma changes its position on an ideal plane perpendicular to the long axis of the metatarsal bones. When a force is applied on the two sides of the foot, similar to the procedure used to evoke Mulder's sign, and the probe is positioned on the foot sole, a movement of the hypoechoic nerve mass towards the probe is visible. This sign, associated with the clinical evaluation, should confirm the MN. Neuroma dimensions can have a large variability, not necessarily associated to the severity of the symptoms. In addition, a patient can present neuromas in more than one metatarsal space. The information obtained by US can help the planning of the surgical intervention, exactly indicating the involved spaces and can be advantageous for follow-up, showing the morphological status after the surgery or the dimension evolution if conservative treatment is decided [45].

4.5.3 Neuralgic Amyotrophy

Neuralgic amyotrophy (NA) is a peripheral nerve disorder characterized by pain, in the first phase, and muscle atrophy after some week. The diagnosis is based on clinical examination, while the EDX can demonstrate the axonal involvement but does not have specificity for NA. Recently, US has shown some peculiar findings in cases of NA [46]. Obviously, this does not mean that the diagnosis can be performed by the exclusive application of US, but supporting findings can be obtained. In particular, the involved nerves in NA, besides the possibility to show focal enlargement, can present partial or total constriction. Additionally, in some cases, nerve torsions have been documented. This is a particular behavior consisting of a total (360°) or sub-total rotation of the nerve around its longitudinal axis. In some circumstances, the torsion can involve the fascicles inside the nerve [46]. These phenomena are visible in the transverse scan, but a longitudinal view can aid the identification and characterization. Furthermore, the scan along the whole nerve course is suggested in order to increase the nerve study efficiency. The presence of these morphological alterations is time-dependent and, hence, in the acute phase may be not visible.

4.5.4 Nerve Tumors

US, generally, can find a clear morphological change in nerve tumors. In particular, a focal enlargement of very variable 3D dimensions is visible where the accessibility of the nerve is guaranteed. The enlargement can be very high in comparison with the native dimensions of the nerve. For this reason, some small nerves, usually almost invisible with US, can get giant CSAs and become easily visible. Exploring by US the body segment of the suspected lesion, even where normally a nerve is not observable for its tininess, can be useful [47]. In these cases, when a large structure is found, a diagnostic suspicion can arise, especially when the contralateral side does not show the same features. The main issue related to the nerve tumors is the differentiation between schwannoma and neurofibroma. This is fundamental, especially for surgical management and prognostic value. In some elected cases, US can distinguish the two forms, revealing a hypoechoic eccentric mass in schwannoma and a general nerve enlargement with non-homogeneous echogenicity in neurofibroma [48]. Unfortunately, in many cases, the morphological patterns are less clear and a certain diagnosis is difficult. However, US utility in nerve tumors is linked to the localization and the dimensional characterization of the mass. These data are fundamental for surgical decision and strategy. Finally, in genetic diseases (e.g., neurofibromatosis), the evaluation of the number of lesions is possible with a rapid assessment of the four limbs, in symptomatic and asymptomatic patients.

4.5.5 Vasculitic Neuropathies

The diagnostic path of the vasculitic neuropathies (VN) consists of clinical, EDX and laboratory exams, with a final confirmation based on nerve and skin biopsy [49]. In non-traumatic axonal neuropathy, usually the nerves show normal CSA values. VN, a particular class of axonal neuropathies related to the vessel damage, should represent an exception among these axonal forms. Some authors showed focal enlargements in more nerve of patients with VN [50]. These enlargements appear less severe than the demyelinating neuropathies but seem to be able to distinguish VN from other axonal diseases [49]. This may be potentially useful to support the assessment of patients with axonal polyneuropathies and may guide the diagnosis. Finally, US can fast find the most involved nerves and, hence, can define which nerves are adequate for a biopsy.

4.6 Ultrasound-Guided Nerve Intervention

The treatment of peripheral nerve dysfunctions includes the injection of drugs or other preparations close to the nerves [51]. This approach can be especially useful in cases of neuropathic pain and in different conditions, like entrapment, phantom-limb syndrome, post-traumatic neuropathy. The side effects related to the injection can be due to mechanical and chemical causes. In fact, the intraneural injection and

the excessive diffusion of the drug have to be avoided in most cases [51]. Furthermore, the vessels represent a target to absolutely avoid because of the risks linked to the accidental spread of the drug in the cardiovascular system. US is able to guide the injections, increasing safety and efficacy for patients [52]. With US ability to show in real-time the anatomy of the site where the intervention is performed, the operator can know the position of the structures and even the needle location. Additionally, the physician can directly see the moment of the inoculation and can check the diffusion of the injected solution. In order to guarantee an effective US-guided procedure, the anatomy knowledge and the manual ability to manage the probe and the needle have to be high [51]. Certainly, specific cautions aimed to obtain a sterile condition are required. Two main approaches of US-guiding are possible: out-of-plane and in-plane. In the first case, the US beam is perpendicular to the long axis of the needle. In this way, just the needle tip is visible as a single hyperechoic point. With the in-plane approach, the needle *lies* on the US beam plane and it is entirely visible [51]. This technique can initially present more difficulties, but it provides a clearer visualization of the relationships between the needle and anatomical structures. In comparison with the blind injection, the exact localization of the nerve to treat, and the surrounding structures to avoid, increases the efficacy of the procedure reducing the risks.

4.7 New Developments and Future Directions

Recently, the research about US in peripheral nervous system assessment has shown further development by new approaches potentially able to evaluate the nerves from different points of view.

Elastography (E-US) is a US-based technique able to assess the stiffness of an organ [53]. Two main types of E-US are available: strain and shear-wave. The first one is based on external compressions, mainly obtained by the probe movements; the second one is based on the speed of the shear-wave. Strain E-US provides qualitative indications about the stiffness, providing the possibility to calculate the ratio between two points (Fig. 4.7). Shear-wave E-US is able to quantify the elasticity of tissue. Both techniques have been studied for the assessment of peripheral nerves but, at the moment, a few results are present in literature and referred mainly to CTS [53]. In this disease, the affected median nerve shows higher stiffness than normal nerves. Unfortunately, important variability in normative data has been found [54]. For this reason, E-US adds information different from the simple nerve morphology, but further studies are required.

Although the direct quantification of echogenicity is not usually possible, a post-processing analysis of the grey-scale levels of the muscles and the nerves is achievable [55]. Data about maximum, minimum, and most represented grey value of the US image are obtainable. Furthermore, the quantification of echogenicity variation is possible. Finally, other calculations based on grey relationships inside the image have been proposed [56]. All these approaches are becoming more and more

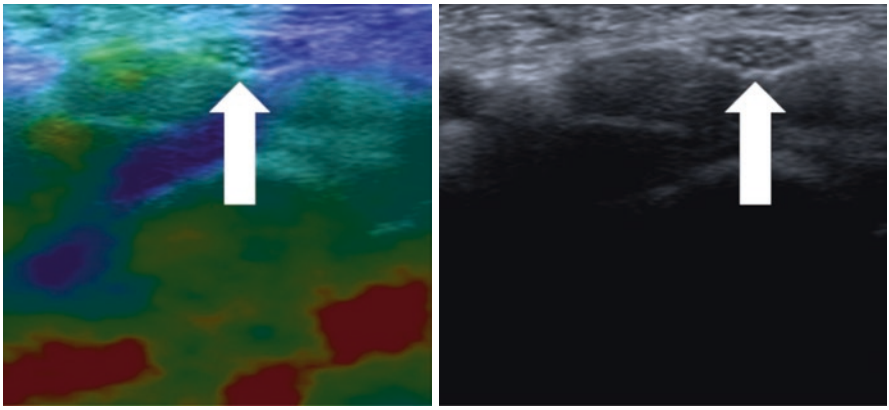

Fig. 4.7 Median nerve at wrist assessed by strain elastography. In the left part of the figure, the window with the level of stiffness is visible. These levels are represented by different colors (in this case, blue indicates the highest stiffness, red the lowest). The arrows indicate the nerve

common in research papers about nerve US, but their diffusion in clinical practice will require robust evidence and instrument developments.

Large interest has been shown about the use of Ultra-High Frequency US (UHFUS). This technique, already available, is based on US probes with very high frequency (70 MHz). These machines can notably increase the US resolution power but decreasing the accessibility to deeper structures [57]. UHFUS has been used for the evaluation of median nerve at the wrist, showing the possibility to discriminate different nerve fascicles in a way never seen before. This assessment may be helpful to better characterize the ultra-structural variations of a nerve, possibly increase the diagnostic accuracy. Furthermore, the very small superficial nerves can be better studied with UHFUS, visualizing minimal alterations [57].

Nerve US is a medical field in continuous expansion and many questions still remain. The interpretation of some US findings is unclear: differentiation between edema and hypertrophy, differentiation of the subtypes of diseases, relationships with the neuropathology, prognostic usefulness in some diseases [39].

The research seems to have a long and stimulating way to go.

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Magnetic Resonance Imaging of the Peripheral Nerve

5

Roberto Gasparotti and Massimiliano Filosto

5.1 Introduction

The diagnostic workup of peripheral neuropathies has traditionally relied on the patient's clinical history, physical examination, and electrophysiological studies [1].

Clinical and instrumental data usually provide enough information about the location, severity, as well as the etiology of the underlying nerve injury in the majority of patients. However, electrodiagnostic studies do not display the anatomic detail needed for precise localization and treatment planning, therefore imaging techniques, especially Magnetic Resonance Imaging (MRI) and nerve ultrasound (US), are gaining an increasing role in the evaluation of peripheral neuropathies [2, 3].

In conventional MRI studies, peripheral nerves are poorly visualized due to low contrast resolution between nerves, muscles and vessels, signal intensity variability, pulsatility artifacts, and small size. These disadvantages have been overcome with the development of Magnetic Resonance Neurography (MRN) in the 1990s [4, 5].

DTI is a functional MRI technique that represents a new source of information about the microstructural integrity of peripheral nerves, allowing quantitative measurements which have been proved useful for further characterization of neuropathies with different etiologies [6].

Ultrasound (US) is very suitable for dynamic assessment of the abnormalities of superficial peripheral nerves, such as changes in nerve caliber, continuity, and echogenicity and represents a useful complement of clinical and

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electrophysiological evaluation [7], although it is more operator-dependent than MRI, and less effective in cases of deeply situated nerves, especially of the pelvis and lumbosacral plexus.

5.2 Magnetic Resonance Neurography

MRN is a tissue-selective imaging technique, based on T2-weighted sequences with fat suppression, acquired with dedicated surface coils and small field of view, which is directed at identifying and evaluating specific characteristics of nerve morphology, such as internal fascicular pattern, longitudinal variations in signal intensity and caliber, and connections and relations to other nerves or plexuses [8].

The most efficient method of fat suppression is with T2-weighted Short Time Inversion Recovery (STIR) sequences, which provide a selective suppression of the fat signal using an inversion recovery pulse of 150 ms. These sequences, however, have some disadvantages, mainly represented by poor signal-to-noise ratio and pulsatility artifacts caused by vessels. Alternative methods for fat suppression are represented by T2 spectral adiabatic inversion recovery imaging (SPAIR) or DIXON-type fat suppression, which are both characterized by a better signal-to-noise ratio, although the contrast resolution is lower [9].

In order to obtain the best compromise between spatial resolution, field of view (FOV) and acquisition time, MRN sequences should be adapted to the anatomical region and the best echo time should be carefully selected in order to obtain a satisfactory differentiation between nerves and muscles, as the signal intensity of the nerve is very sensitive to small changes (Fig. 5.1).

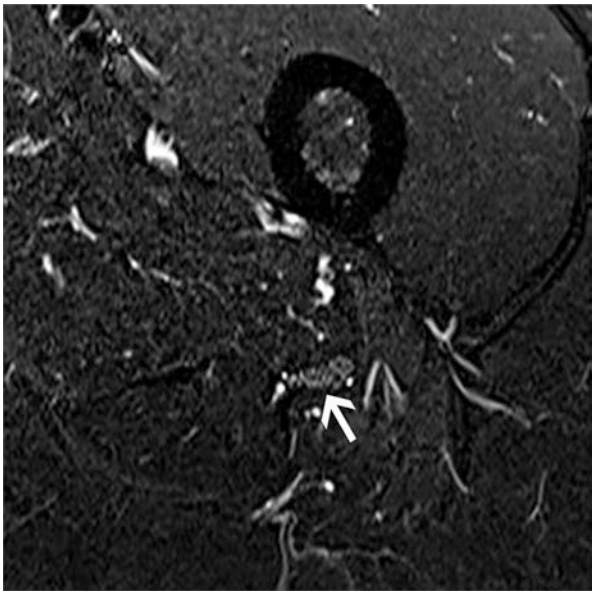

Fig. 5.1 MRN (1.5T), axial T2-STIR section at mid-thigh. Normal subject. The right sciatic nerve (arrow) is moderately hyperintense compared to the adjacent muscles and its transverse fascicular pattern is clearly identifiable

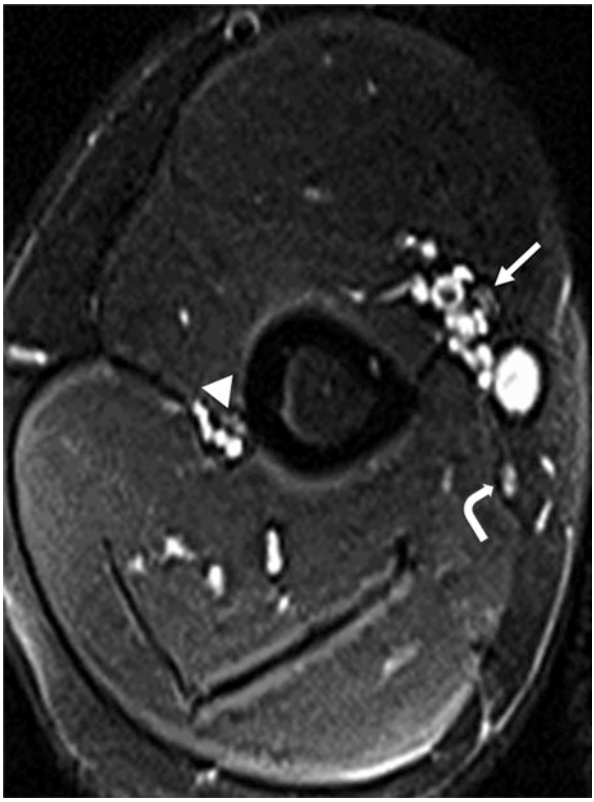

Fig. 5.2 MRN (3T), axial T2-STIR section at mid-humerus. Normal subject. The median (arrow), ulnar (curved arrow) and radial (short arrow) nerves, which are moderately hyperintense, can be differentiated from adjacent muscles and vessels

Recent technological advances, such as parallel imaging, new coil design and new sequences, together with an increasing use of 3T MR scanners, have led to the development of high-resolution MR peripheral nerve imaging, which provides significantly better depiction of peripheral nerve structures [10] (Fig. 5.2).

Three-dimensional (3D) MRN represents a further refinement of conventional MRN. 3D sequences provide enhanced contrast between nerves and muscles and are typically acquired with isotropic voxels, therefore conferring the advantage of generating oblique and curved-planar reformations of nerve roots, peripheral nerves, and plexuses [11]. Excellent contrast of the nerves can be obtained by suppressing muscle, blood and fat signal with fat-suppressed diffusion-prepared 3D MR Neurography sequences [12]. These features are particularly useful for imaging the anatomical complexity of brachial and lumbosacral plexuses which cannot be fully displayed by 2D imaging [13] (Fig. 5.3).

In MRN studies, normal nerves are identifiable as rounded or ovoid structures on axial images, are typically isointense to slightly hyperintense on T2-weighted images, depending on the size of the nerve, on the amount of endoneurial fluid and degree of fat suppression, whereas they are isointense to the adjacent muscles on conventional T1-weighted images [14].

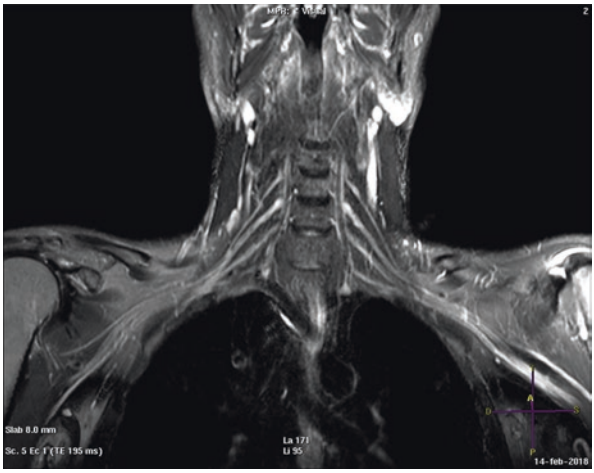

Fig. 5.3 3D MRN (3T) of the brachial plexus. Oblique coronal reformat. The supra and infraclavicular brachial plexus is simultaneously displayed in a single image

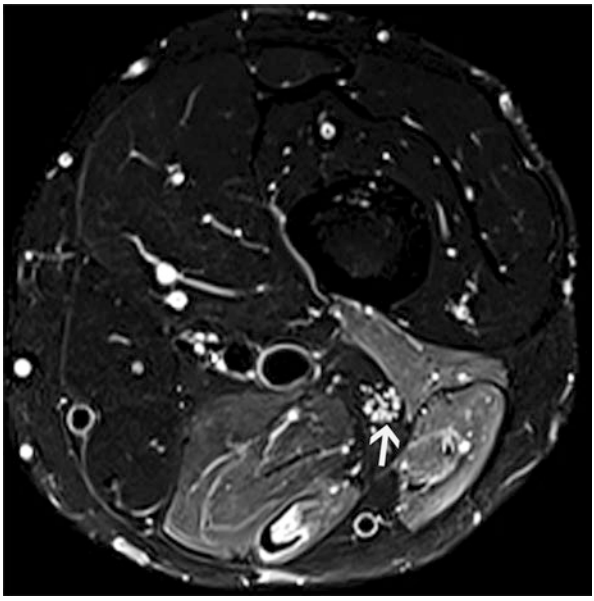

Fig. 5.4 MRN (1.5T), axial T2-STIR section of the left thigh. A 38-year-old male with left sciatic nerve injury. Enlargement and hyperintensity of the left sciatic nerve, which is characterized by fascicular hypertrophy (arrow). Increased signal intensity of semimembranosus, semitendinosus, and long head of the biceps femoris, due to acute denervation

The epineurium appears as a thin hypointense rim and the transverse and longitudinal fascicular pattern may be identified in larger nerves such as the sciatic nerve or the median nerve at the carpal tunnel.

The signal intensity of normal nerves is strongly influenced by the amount of collagen fibers contained in the perineurium and endoneurium and their magnetic properties, which depend on the angle with the principal vector of the magnetic field, and this concept should be taken into account in the image interpretation [15].

Diseased nerves become hyperintense to muscle on MRN images and are focally or globally enlarged [16] (Fig. 5.4).

The signal intensity change is due to increased water content in the epineurial space caused by blood–nerve barrier damage, blockade of axoplasmic flow, inflammation, distal Wallerian degeneration and is relatively independent from the etiology of the neuropathy [17].

Neuropathies with different etiologies cannot be distinguished only on the basis of signal intensity changes, as no reliable quantitative methods for evaluating the signal intensity of normal versus abnormal nerves have been developed up to now.

A common method to recognize variations of the size of the nerves is the measurement of the cross- sectional area (CSA) with manually drawn ROIs in the axial sections perpendicular to the course of the nerves. From the same ROI, a relative quantitative assessment of the signal intensity of the nerve can be obtained normalizing its value with the signal intensity of a reference adjacent muscle or with the calculation of nerve-to-muscle contrast-to-noise ratio (CNR) [18].

MRN has the advantage of a simultaneous exploration of nerves and muscles, therefore muscle denervation represents a useful MR sign of peripheral nerve disease (Fig. 5.4).

In the acute phase of muscle denervation increased signal intensity can be observed in T2-weighted sequences as early as 24 h after nerve injury and lasting for more than 2 months [19]. These denervation-related signal abnormalities are reversible and represent enlargement of the capillary bed and shift of fluid to the extracellular space. In the subacute phase, a progressive decrease of signal intensity is associated with an initial fat replacement, and in chronic phase muscles show atrophy and sever fat replacement, which is better displayed by T1-weighted images.

The observed MR changes precede the earliest EMG findings of denervation, which are not detectable until the second week, thus MR imaging may be useful in narrowing this diagnostic gap.

Diffusion Tensor Imaging (DTI) is a novel technique which has been recently applied to the investigation of peripheral nerve disorders. Nerves are characterized by greater water diffusion anisotropy compared to the surrounding tissues. These techniques are sensitive to subtle changes in tissue at the microstructural level and allow measurement of nerve microstructural integrity based on quantitative parameters such as Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD) [20].

Peripheral nerve tractography is increasingly used for selective visualization of peripheral nerves. With the same approach used in deterministic tractography of the brain white matter, seed points placed on the nerves with the aid of a reference anatomical image, allow successful tracking of major peripheral nerves (Fig. 5.5).

DTI has been extensively applied to the investigation of the median, ulnar and radial nerves at the arm [21–23] and more recently to the brachial and lumbosacral plexus with the aim to obtain quantitative measurements potentially useful for more precise imaging characterization of immune-mediated neuropathies.

A comprehensive MRI protocol for the investigation of peripheral nerves should include MRN, which provide both structural and functional information on the nerves and muscle denervation, T1-weighted sequences which are helpful for a precise anatomical identification of nerves and for the identification of muscular

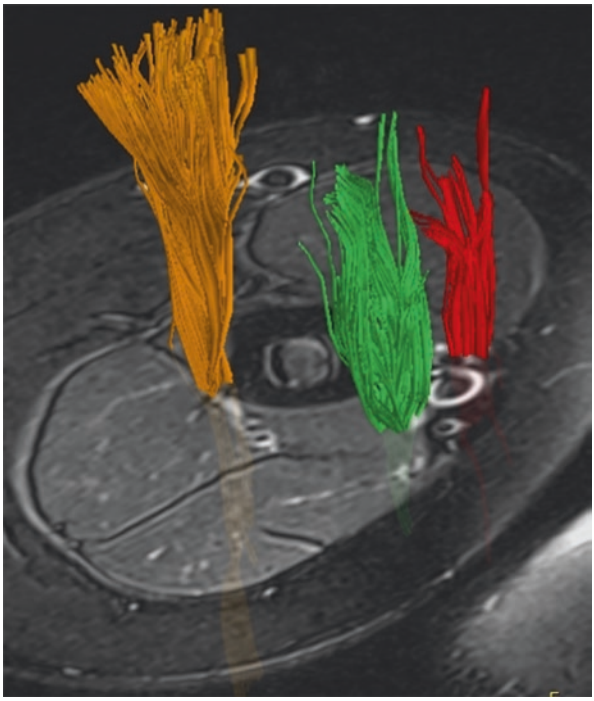

Fig. 5.5 DTI tractography (3T) of median (red), ulnar (green), and radial (orange) nerves at the arm in a normal subject

atrophy and T1-weighted sequences after contrast media administration for the evaluation of the blood–nerve barrier integrity.

MRN has been reported to be effective on the diagnostic workup of traumatic nerve injuries [24], nerve entrapment syndromes [25, 26], and nerve tumors [27]. More recently, MRN has been proposed for the evaluation of hereditary and immune-mediated disorders of peripheral nerves [28], diabetic polyneuropathy [29], and paraproteinemic neuropathies [30].

5.3 Guillain–Barre Syndrome

Guillain–Barre syndrome (GBS) is a well-known inflammatory disease of peripheral nerves, including the spinal and cranial nerves, characterized by albuminocytologic dissociation and demyelinating and/or axonal involvement at electrophysiologic testing.

GBS is divided into different subtypes: acute inflammatory demyelinating polyneuropathy (AIDP), which accounts for 90% of all GBS cases in Western countries and the axonal subtypes, acute motor axonal neuropathy (AMAN), and acute motor and sensory axonal neuropathy (AMSAN), most frequent in Asia and Japan and the Miller-Fisher syndrome (MFS) [31].

MRI studies are usually not necessary for diagnosis although a thorough medical assessment of patients may be needed to exclude "mimic disorders" [32].

Nerve conduction studies (NCS) and CSF analysis are important investigations that help confirming the clinical diagnosis of GBS although NCS may be unrevealing when studying patients within days of symptom onset and CSF may be normal in the first week of the illness [33].

In the initial phase of GBS, breakdown of the blood–nerve barrier is the characteristic pathologic change, which may lead to enhancement of nerve roots in MRI studies.

Although the enhancement of the intrathecal spinal nerve roots is not specific and can be seen in neoplasia and other inflammatory processes, the enhancement of only the anterior spinal nerve roots is strongly suggestive of GBS [34] (Fig. 5.6).

About 5% of patients initially diagnosed with GBS turn out to have chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) with acute onset (A-CIDP) [35].

Differentiating A-CIDP from GBS prior to relapse is challenging at the onset of the disease and has implications for treatment as well as prognosis. Electrodiagnostic

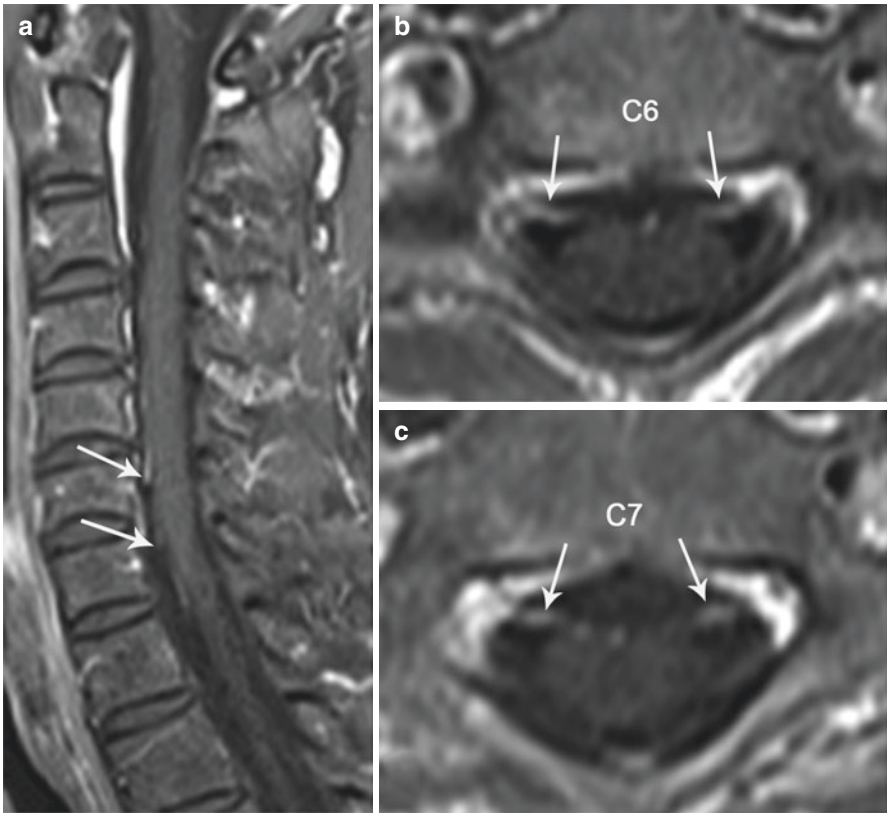

Fig. 5.6 GBS, MR (1.5T), sagittal (**a**), and axial T1-W sections (**b**, **c**) after gadolinium administration. Enhancement of ventral C6 and C7 nerve roots (arrows)

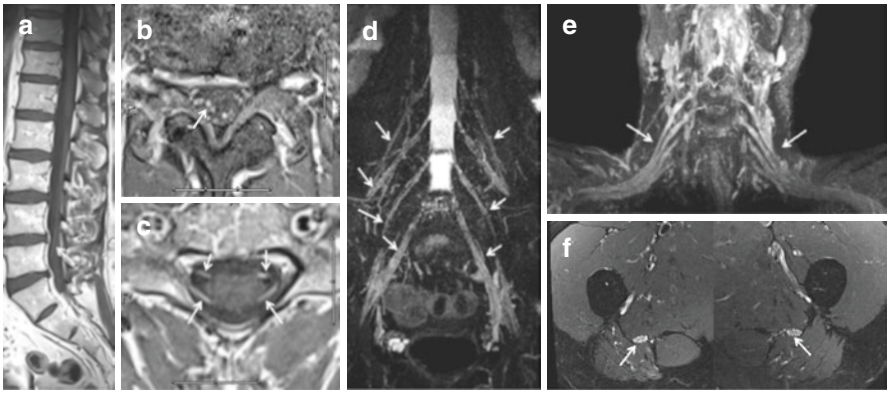

Fig. 5.7 Acute onset CIDP, MR (3T). Sagittal (**a**), axial L3–L4 (**b**), axial C5–C6 (**c**), T1-W sections after gadolinium administration, 3D MRN coronal view, lumbosacral plexus (**d**) and brachial plexus (**e**), axial 2D MRN (**f**), comparative evaluation of sciatic nerves at proximal thigh. Enhancement of cauda equina (arrows in **a** and **b**) and ventral and dorsal C6 nerve roots (arrows in **c**). Symmetric bilateral hypertrophy and increased signal intensity of the lumbosacral and brachial plexus (arrows in **d** and **e**). Increased signal intensity and diffuse fascicular hypertrophy of both sciatic nerves (arrows in **f**)

studies may distinguish patients with A-CIDP from GBS patients; however, the demonstration of brachial and/or lumbosacral plexus hypertrophy at MR imaging may be useful for the differential diagnosis (Fig. 5.7).

5.4 Chronic Immune-Mediated Neuropathies

5.4.1 CIDP

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an immune-mediated neuropathy characterized by symmetrical proximal and distal weakness, with sensory loss, impaired balance, and areflexia.

CIDP includes a broad spectrum of clinical phenotypes, including atypical forms with pure motor or sensory impairment or distal, multifocal, or focal distributions [36].

The diagnosis of CIDP is based on a combination of clinical, electrodiagnostic, and laboratory features, primarily directed at detecting signs of demyelination; however, in clinical practice CIDP may be difficult to diagnose, especially in atypical cases. Despite the good overall sensitivity and specificity of the current electrophysiological criteria, almost 20% of patients in CIDP cohorts do not match these criteria [37].

MRI showing gadolinium enhancement or hypertrophy of the cauda equina, nerve roots, or plexuses has been recommended as an additional supportive exam in a recent revision of the European Federation of Neurological Societies/Peripheral

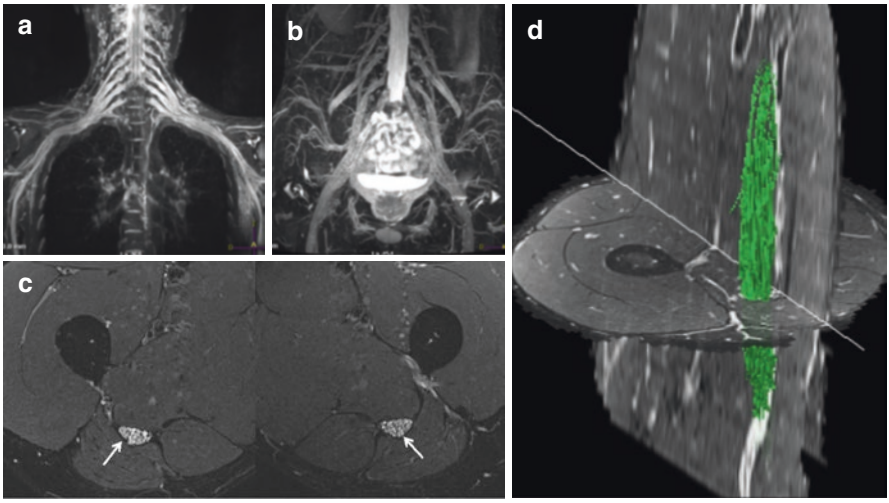

Fig. 5.8 CIDP, 3D MRN (3T), showing diffuse symmetric hyperintensity and enlargement of the brachial (**a**) and lumbosacral plexus (**b**). 2D MRN (**c**) of the proximal thigh, showing symmetric enlargement of sciatic nerves, with diffuse fascicular hypertrophy. DTI tractography of the right sciatic nerve (**d**) with quantitative evaluation of the microstructure of the nerve demonstrated decreased fractional anisotropy (FA, 0.34 vs 0.5) and increased radial diffusivity (RD, 1.5×10^{-3} mm^2/s vs 1.0×10^{-3} mm^2/s) compared to the reference values

Nerve Society guideline on management of CIDP [38] although contrast enhancement is rarely seen in clinical practice.

The most frequent MRI finding in patients affected by CIDP is represented by bilateral and symmetric hypertrophy of both brachial and lumbosacral plexus, which is invariably associated to increased signal intensity, better displayed by MRN (Fig. 5.8).

Hypertrophy and increased signal intensity of the cervical roots and brachial plexus at MRI have been reported in 57% of patients affected by CIDP, 75% of whom also had hypertrophy of the lumbar plexus [39].

Patients with nerve root hypertrophy usually have a relapsing-remitting course and a significantly longer disease duration, which may be related, according to some authors, with the process of demyelination and remyelination [39]. Similar findings of enlargement and increased MR signal intensity have been observed in the median and ulnar nerves of patients with CIDP, correlating with the site of conduction block and contrast-enhancement during relapses or active progression, possibly reflecting increased water content within the nerve fascicles and disruption of the blood–nerve barrier due to the inflammatory process [40].

3D MR Neurography has become a valuable tool for a thorough assessment of the symmetry and longitudinal extent of the disease.

Using 3D MRN techniques Shibuya et al. showed longitudinal morphological changes from the cervical roots to the nerve trunks in the proximal arm in 88% of patients affected by CIDP [41].

Phenotypic features can be noninvasively characterized in patients with atypical variants of CIDP using 3D MRN for a detailed evaluation of brachial and lumbosacral plexus hypertrophy and signal intensity abnormalities, which typically involve long segments with a different distribution, symmetric or asymmetric, diffuse, or multifocal.

Lewis-Sumner syndrome (LSS) or multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) is characterized by asymmetry, presenting as a multifocal multiple mononeuropathy most commonly in the upper limbs, accounting for 6–15% of CIDP patients [37].

The distribution of hypertrophy in typical CIDP is symmetric and predominant in the nerve roots, with gradual normalization toward the proximal arm segments distally, whereas in MADSAM nerve hypertrophy is usually asymmetric and multifocal in the peripheral nerve trunks [42] (Fig. 5.9).

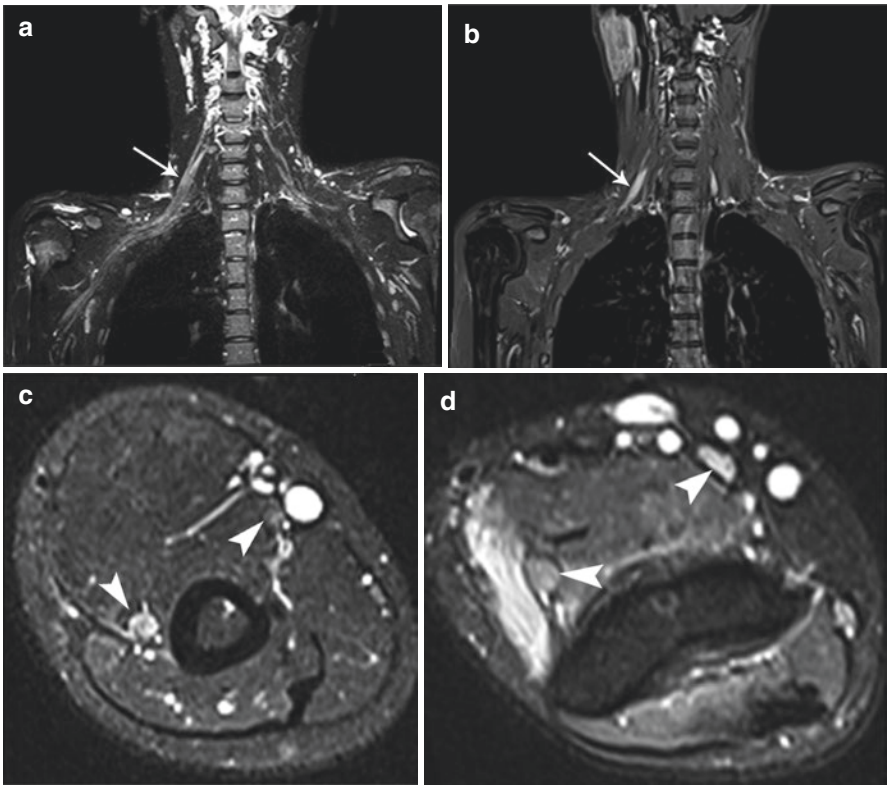

Fig. 5.9 MADSAM, 3D MRN (1.5T) of the brachial plexus (**a**), coronal T1-W section after gadolinium administration (**b**), 2D MRN, axial T2-STIR section of the right arm (**c**, **d**). Asymmetric hypertrophy of the right brachial plexus (arrow in **a**), with enhancement of the right C6 nerve root and superior primary trunk (arrow in **b**). Hypertrophy and increased signal intensity of the right radial and median nerves (arrowheads in **c** and **d**)

Sensory predominant CIDP occurs in 5–35% of patients, often starting with lower limb numbness [43]. The diagnosis is typically made on the basis of demyelinating electrodiagnostic features in motor nerves, which may occur without motor signs, although patients may develop weakness at a later date [44]. This entity may be under-diagnosed at the onset of symptoms which manifest at a young age and 3D MRN may represent a useful diagnostic tool when demonstrating symmetric hypertrophy of the brachial and lumbosacral plexus, which is comparable to the typical form of CIDP [45].

Recent comparative studies showed that both MRI and high-resolution sonography (HRUS) of the brachial plexus can be used to support the clinical suspicion of an inflammatory neuropathy [46]. HRUS appears to be more sensitive for nerve (root) thickening, whereas MRI complements this with an abnormal T2 signal. An increased T2 signal has been reported to be more frequently found in patients with CIDP than Multifocal Motor Neuropathy (MMN).

Brachial and lumbosacral plexus hypertrophy on MRI is also well documented in patients with demyelinating Charcot–Marie–Tooth disease (CMT) [47] and the differential diagnosis with CIDP, besides genetic abnormalities, can also rely on the measurement of the sciatic nerve cross-sectional area (CSA) at mid-thigh by means of MRN [48] (Fig. 5.10).

New MR techniques such as diffusion-weighted (DWI) and diffusion tensor (DTI) imaging have proven to be particularly useful for the investigation of CIDP.

High signal intensity in DWI sequences and increased values of the apparent diffusion coefficient (ADC) were detected in 55.6% of cases in a small cohort of 13 CIDP patients, which might be strictly correlated with proliferating layers of Schwann cells and increased endoneurial collagen surrounding the axons [49].

With the clinical availability of 3T MR units, DTI is increasingly used to detect microstructural abnormalities of nerves in CIDP patients.

FA has been shown to be significantly reduced in tibial nerves of patients with variable disease duration, correlating with the amplitude of compound motor action potentials, thus with the axonal damage [50], whereas RD is increased in nerves of CIDP patients and may represent a specific biomarker of demyelinating neuropathy, inversely correlated with nerve conduction velocities (NCV) [51].

DTI may reveal low FA in sciatic nerves that are correlated with clinical impairment in CIDP patients treated with subcutaneous immunoglobulin, in whom MRN is unable to identify abnormalities [52].

These preliminary results suggest a role of DTI as a research tool for identifying quantitative measure of microstructural abnormalities, although further testing is needed to validate the method (Fig. 5.8d).

5.4.2 Multifocal Motor Neuropathy

Multifocal motor neuropathy (MMN) is a chronic, immune-mediated demyelinating neuropathy, affecting motor fibers, characterized by progressive, predominantly distal, asymmetric limb weakness, mostly affecting upper limbs, minimal or no

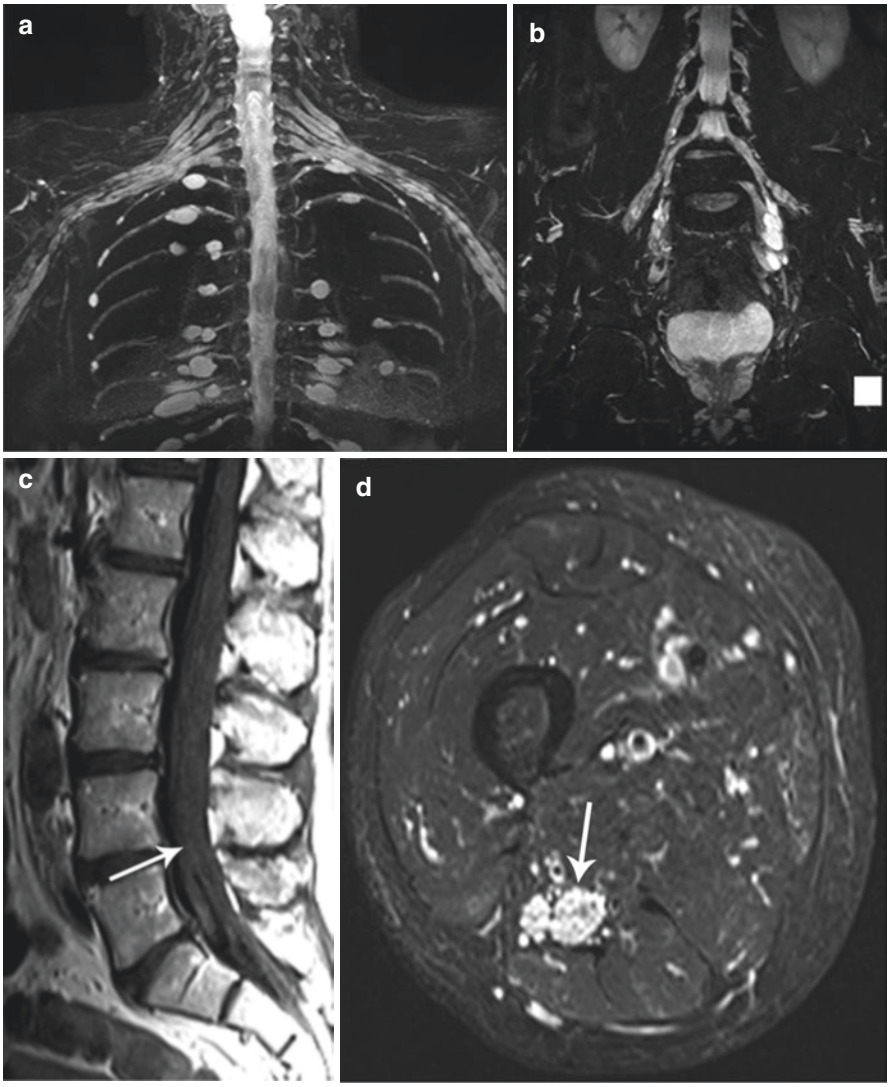

Fig. 5.10 CMT 1A, 3D MRN (1.5T) (**a**, **b**), sagittal T1-W section of the lumbar spine (**c**), axial MRN, T2-STIR section at mid-thigh (**d**). Diffuse symmetric hypertrophy and hyperintensity of the brachial plexus and intercostal nerves (**a**) and lumbosacral plexus (**b**). Hypertrophy of the cauda equina (arrow in **c**). Hyperintensity and hypertrophy of the right sciatic nerve (CSA = 270 mm^2) (arrow), with excellent visualization of the tibial and peroneal divisions

sensory impairment, and the presence of multifocal persistent partial conduction blocks (CB) on motor nerves [53]. Increased levels of serum IgM antibodies to the ganglioside GM1 is another typical feature of the disease.

MRI may be of value in the differential diagnosis between CIDP and MMN, classified as a variant of CIDP in the past and now considered a different disease [54].

A recent revision of the European Federation of Neurological Societies/Peripheral Nerve Society on Multifocal Motor Neuropathies (MMN) also included MRI as a supportive criterion for the differential diagnosis with other neuropathies such as CIDP or multifocal acquired demyelinating sensory and motor (MADSAM) neuropathy (Lewis-Sumner syndrome) and motor neuron disease (MND) [55].

About 40–50% of the patients with MMN show asymmetric hypertrophy and signal intensity abnormalities or contrast-enhancement on MR of the brachial plexus and the pattern of signal alterations closely correlates with the distribution of muscle weakness [56] (Fig. 5.11).

Diffuse nerve swelling and hyperintensity of the affected nerves on the T2-weighted images are usually found in areas outside the expected confines of entrapment neuropathy and reflect demyelination and proximal conduction blocks [57].

The clinical presentation of MMN may mimic motor neuron disease (MND), particularly in patients with predominant lower motor neuron impairment and the differential diagnosis is important, as the prognosis and treatment of these diseases are different.

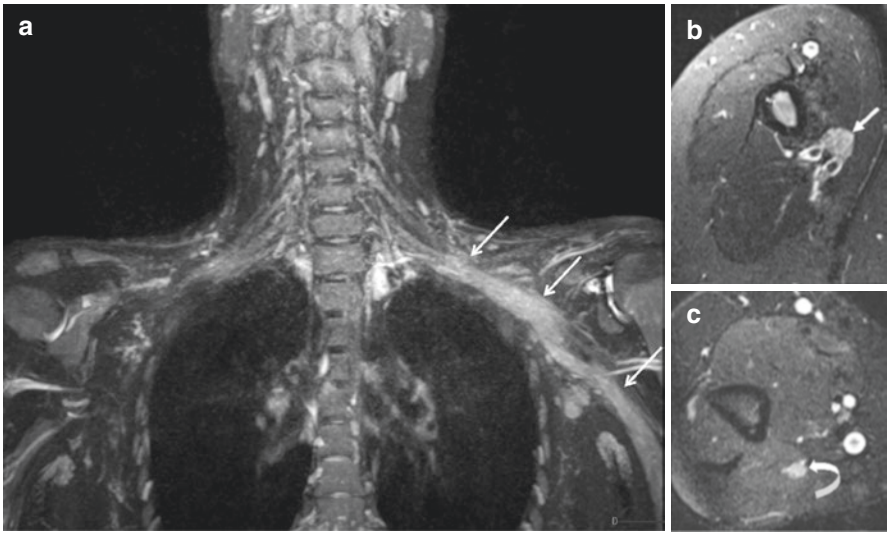

Fig. 5.11 MMN, 3D MRN (1.5T) of the brachial plexus (**a**), axial 2D MRN at mid-arm (**b**), and elbow (**c**). Asymmetric hypertrophy and hyperintensity of the left brachial plexus with prevalent involvement of the trunks and cords (arrows in **a**). Hypertrophy and hyperintensity of the left median (arrow in **b**) and ulnar nerves (curved arrow in **c**)

MRI can be used to help differentiate between MMN and MND, with brachial plexus MRI being normal in the latter [58].

High-resolution ultrasound can provide complementary information to MRI, showing significantly increased cross-sectional area values of the median and ulnar nerve in the forearm in patients with MMN as compared to MND [59].

DTI has been recently demonstrated to be useful for the differential diagnosis between MMN and MND. Besides the known enlargement of median and ulnar nerves, AD values of median and ulnar nerves at forearm arm are significantly lower in MMN patients compared to MND patients and healthy controls, especially in the proximal segments [60]. Thickening of nerves is compatible with changes in the myelin sheath structure, whereas lowered AD values suggest axonal dysfunction.

Axonal multifocal motor neuropathy is a rare entity, which was first described in 2002, and is characterized by a slowly progressive multifocal motor phenotype with neither conduction blocks nor other features of demyelination [61].

MR Neurography has been recently reported to be helpful in the diagnostic workup of the axonal form of MMN, showing mildly increased signal intensity and size of the involved nerves at the arm [62].

5.5 Diabetic Polyneuropathy

Diabetic peripheral neuropathy (DPN) is the most common form of the diabetic neuropathies seen in either type 1 (T1D) or type 2 (T1D) Diabetes Mellitus, with similar frequency. DPN is a common late complication of diabetes and has been defined as a symmetric, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvascular alterations as a result of chronic hyperglycemia exposure and cardiovascular risk covariates [63]. Diabetic neuropathy is believed to be related to damage to the vascular supply of the nerves (vasa nervosa) and/or deposition of advanced glycosylation products in the intraneural space [64].

A combined MRN and DTI MR investigation at 3 Tesla has demonstrated larger sciatic nerve volume and multifocal fascicular lesions in diabetic patients with polyneuropathy with higher neuropathy deficit score (NDS), as compared to non-neuropathic diabetic patients and healthy control subjects [65]. Lower FA and higher MD and RD were found in sciatic nerves of patients with DPN compared with both non-neuropathic patients and healthy controls, correlating closely with the severity of neuropathy, and possibly reflecting loss of axons and/or myelin.

According to a recent MR study, the predominant type of nerve lesion in the sciatic nerves of patients with DPN differs between T1D and T2D [66]. An increased volume of T2w-hyperintense lesions is observed in T1D compared to T2D, whereas T2w-hypointense lesion volume is higher in T2D compared to T1D. Predominant nerve lesions in T1D are associated with poor glycemic control and loss of nerve conduction, whereas predominant lesions in T2D are associated with changes in lipid metabolism.

DPN includes diabetic radiculoplexus neuropathies also known as diabetic amyotrophy, which affect roots, plexus, and individual nerves in the cervical, thoracic, or lumbosacral segments.

Diabetic lumbosacral radiculoplexus neuropathy (DLRPN), which is characterized by debilitating pain, weakness, atrophy of the proximal thigh muscles, and abnormal protein content in CSF, is the best studied subtype [67].

MR imaging is very useful in demonstrating increased signal intensity in nerve roots and trunks which is invariably associated to denervation changes in the affected muscles [68].

The occurrence of a cervical diabetic radiculoplexus neuropathy (DCRPN) sharing many of the clinical and pathological features of DLRPN has also been demonstrated [69] (Fig. 5.12).

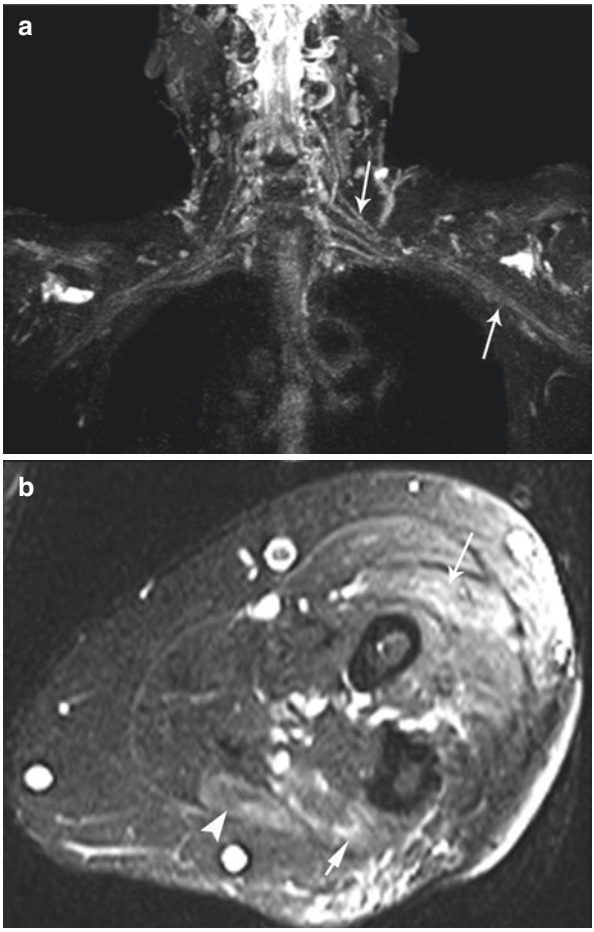

Fig. 5.12 Diabetic cervical radiculoplexus neuropathy in a 64-year-old female with type 2 diabetes, 3D MRN (1.5T) (**a**), 2D MRN, axial T2-STIR section at left mid-forearm (**b**). Mild enlargement and hyperintensity of the left supra and infraclavear brachial plexus (arrows). Denervation changes into the left extensor carpi ulnaris (short arrow), extensor digitorum (arrowhead), and extensor carpi radialis (long arrow)

5.6 Amyloid Neuropathy

Amyloid neuropathies occur in a context of hereditary or acquired amyloidosis. They present usually as severe and progressive polyneuropathy involving sensory, motor, and/or autonomic fibers and carry a poor prognosis [70].

Acquired amyloid neuropathy is almost exclusively represented by immunoglobulin light chain amyloidosis (AL) and is frequently associated with renal manifestations and monoclonal protein

in serum or urine. Peripheral neuropathy occurs in about 35% of cases of AL but is a rare presenting symptom [71].

On MR imaging, both focal amyloidoma or diffuse enlargement of unilateral/bilateral nerves with associated multifocal lesions have been reported [30, 72].

The lesions most commonly involve segments of the lumbosacral plexus or the sciatic nerve and are characterized by increased nerve T2 signal of the affected nerves, with a proximal-to-distal gradient, correlating with the clinical severity [30].

Transthyretin familial amyloid polyneuropathy (TTR-FAP) is the most common form of inherited amyloidosis. Endemic areas of TTR-FAP are Portugal, Japan, Sweden, and Brazil. Patients with FAP may experience different patterns of neuropathy including focal neuropathies, sensorimotor polyneuropathy, autonomic neuropathy, or combinations of the three. The median nerve at the wrist is a common and early site of involvement in FAP [73].

The diagnosis relies on a positive family history and requires TTR gene analysis showing Met30TTR mutation and positive labial salivary gland biopsy (LSGB) for amyloidosis [74].

High-resolution 3T MRN has been recently shown to be able to identify and quantify the distribution of peripheral nerve injury in TTR-FAP patients within the fascicles of the sciatic nerves from proximal to distal (Fig. 5.13), even before

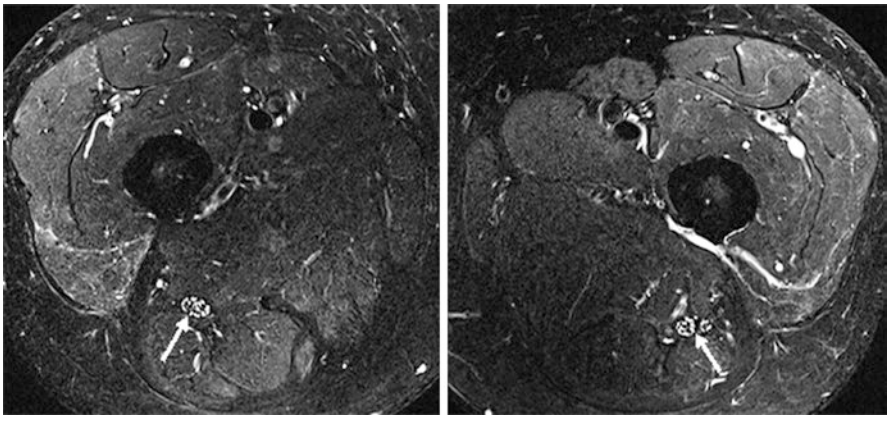

Fig. 5.13 Transthyretin familial amyloid polyneuropathy. 2D MRN at proximal thigh, showing increased signal intensity and mild increase of the fascicular size of both sciatic nerves (arrows)

the manifestation of symptomatic disease in asymptomatic gene carriers, in whom imaging detection may precede clinical and electrophysiological manifestation [75].

5.7 Sarcoidosis

Sarcoidosis is a granulomatous, multisystem disease of unknown etiology. Approximately 3.5–5% of the patients with sarcoidosis have involvement of the central and peripheral nervous system (Neurosarcoidosis) although peripheral nerve manifestations are rare and usually seen late in the disease.

Acute or chronic peripheral neuropathy occurs in about 2–40% of patients with neurosarcoidosis. The most common form is represented by symmetrical axonal sensory motor polyneuropathy. Other patterns include mononeuritis multiplex and Guillain-Barre-like syndrome [76].

Sarcoid granulomas locate themselves in the perineurium and epineurium, while the endoneurium is mostly spared.

Although CNS sarcoidosis can be diagnosed using contrast-enhanced MR imaging [77], the diagnosis of PNS sarcoidosis is more difficult.

MRN has been reported as a useful tool in the diagnostic workup of sciatic nerve sarcoidosis, which can manifest as a mass within the sciatic nerve (sarcoid granuloma), characterized by low signal intensity in T2-weighted images and marked enhancement after contrast administration [78].

Key Points

- Magnetic Resonance Neurography is an imaging technique, directed at identifying and evaluating specific characteristics of nerve morphology, such as internal fascicular pattern, and longitudinal variations in signal intensity and caliber.
- Advanced 3D MR Neurography techniques allow oblique and curved-planar reformations along the course of peripheral nerves and are particularly suitable for imaging brachial and lumbosacral plexus.
- Diseased nerves are hyperintense to muscle on MRN images and appear focally or globally enlarged. The signal intensity variations are not specific and are due to increased water content in the epineurial space, blockade of axoplasmic flow, inflammation and distal Wallerian degeneration.
- Muscle denervation imaging is part of an MR Neurography examination and represents a useful sign of peripheral nerve disease.
- The most frequent MRI finding in patients affected by CIDP is represented by bilateral and symmetric hypertrophy and hyperintensity of both brachial and lumbosacral plexus, better displayed by MR Neurography.
- Atypical variants of CIDP and MMN can be noninvasively characterized with 3D MRN of the brachial and lumbosacral plexus, showing symmetric or asymmetric longitudinal morphological changes from roots to nerve trunks and variable contrast-enhancement.

- DTI provides quantitative measurements of some microstructural properties of peripheral nerves, which are potentially useful for more precise imaging characterization of immune-mediated neuropathies.
- MRI of the brachial plexus can be used in the differential diagnosis between MMN and MND, with MRI being normal in the latter.
- In diabetic cervical and lumbosacral radiculoplexus neuropathies MR Neurography is useful in demonstrating increased signal intensity in nerve roots and trunks which is invariably associated to denervation changes into the affected muscles.

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Part II

Clinical Myology Entities

6 The Spectrum of Inflammatory Myopathies: Dermatomyositis, Polymyositis, Necrotizing Myositis, Anti-synthetase Syndrome-overlap Myositis, and Inclusion-Body Myositis

Marinos C. Dalakas

6.1 Introduction

Since 1991, when a major review on inflammatory myopathies was published [1], there has been tremendous progress in our understanding of these disorders. Although they still comprise a heterogeneous group of muscle diseases, unique clinical, histologic, and immunopathologic characteristics have emerged to classify them in five distinct subsets, *dermatomyositis (DM)*, *polymyositis (PM)*, *necrotizing autoimmune myositis (NAM)*, *inclusion-body myositis (IBM)* [2–4]. Identification of the correct subtype and distinction from disease mimics are important because each subset has different prognoses and responses to therapies [1–4] as, overall, DM responds better than PM and PM better than NAM while IBM remains difficult to treat. The chapter reviews current knowledge in the field; highlights how best to avoid erroneous diagnoses; describes the main clinical, histopathologic, and immune features; and offers a practical update on therapies.

6.2 General Clinical Features Relevant to All IM

Patients with IM experience difficulty with tasks requiring the use of proximal muscles, such as getting up from a chair, climbing up steps, or lifting objects [1–4, 6]; tasks requiring the strength of distal muscles, such as buttoning or holding objects like a pen or golf clubs, are affected early in IBM but only in advanced cases of PM, DM, and NAM. Ocular muscles are spared in all subtypes and, if affected, the diagnosis of IM should be questioned. Facial muscles are unaffected in DM, PM, and NAM, but they are commonly involved in IBM even early in the disease [2–4]. In all subsets, pharyngeal and neck extensor muscles can be affected, resulting in

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dysphagia or difficulty holding up the head. In advanced and rarely acute cases, respiratory muscles may be affected. Extramuscular manifestations may also occur and include (a) systemic symptoms, such as fever, malaise, arthralgias, and Reynaud's phenomenon when associated with another systemic disease or with antisynthetase antibodies (the so-called Jo-1 syndrome); (b) cardiac arrhythmias or ventricular dysfunction if the cardiac muscle is rarely affected; and (c) breathing difficulties rarely due to respiratory muscle involvement but more often due to interstitial lung disease as often seen with antisynthetase antibodies [1].

6.3 Dermatomyositis

Seen in both children and adults, DM presents with distinct skin manifestations in face and extremities, accompanying or preceding muscle weakness. These changes include a heliotrope (blue-purple) rash on upper eyelids with edema; an erythematous rash on the face, knees, elbows, malleoli, neck, anterior chest (in V sign), back, and shoulders (in shawl sign) and knuckles with a violaceous eruption (Gottron's rash) that evolves into a scaling discoloration. Dilated capillary loops at the base of the fingernails, irregular and thickened cuticles, and cracked palmar fingertips (mechanic's hands) are characteristic [1–7]. Subcutaneous calcifications, sometimes extruding to the surface causing ulcerations and infections, may occur especially in children but less often today owing to early initiation of better therapies. Dermatomyositis can be limited to the skin if strength appears normal (*amyopathic dermatomyositis*) although there is often subclinical muscle involvement [2–4]. In children, an early symptom is "misery," defined as an irritable child with red flush on the face, fatigued, and reluctant to socialize [2–4].

Dermatomyositis may overlap with systemic sclerosis and mixed connective tissue disease [1–4]. In up to 15% of adult patients, there may be an underlying malignancy, most commonly ovarian, breast, or colon cancer, melanoma, non-Hodgkin lymphoma, and nasopharyngeal cancer in Asians, necessitating a thorough annual workup, especially the first 3 years from disease onset [1–4, 6, 7]. Although tumors are usually uncovered by abnormal findings in physical examination and not by extensive blind searches, whole-body PET imaging might be considered if malignancy is strongly suspected.

6.4 Polymyositis

In contrast to DM where the skin manifestations unravel the disease, polymyositis has no unique clinical features that point to disease onset. PM is rare as a standalone entity and very often misdiagnosed; it is most frequently encountered in patients with systemic, autoimmune, or viral diseases [1–4] and remains a very rare diagnosis and always one of exclusion. It is a subacute myopathy occurring in adults who do not have rash, family history of neuromuscular disease, exposure to myotoxic drugs (d-penicillamine, zidovudine), involvement of facial and extraocular muscles, endocrinopathy, or the clinical phenotype of IBM, as described later.

6.5 Necrotizing Autoimmune Myositis (NAM)

It is a distinct and previously overlooked entity, occurring more frequently than PM [2–4]. It is seen in all ages but mostly adults and starts either acutely, reaching its peak over days or weeks, or subacutely progressing steadily resulting in severe weakness and very high CK levels. Interstitial lung disease may coexist. The disorder is most of the times autoimmune and antibody-mediated, but it may also sometimes occur after viral infections or in association with cancer. It has been suggested that may be also induced by statins, but this connection may be serendipitous considering that NAM is now recognized as a common myopathy while statins are prescribed in more than 30% of patients above 50 and more than 40% above 60–65; further, a temporal connection with statin initiation has not been demonstrated [2, 8–10]. Up to 60% or more of NAM patients have antibodies against signal recognition particle (SRP) or the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) [2, 9, 10], as discussed later. NAM should be distinguished from muscular dystrophies especially if encountered in young patients.

6.6 Inclusion-Body Myositis (IBM)

It is the most common and disabling IM above the age of 50 [1–4, 11–14]. It starts insidiously, over years, and progresses steadily simulating a late-life muscular dystrophy or slowly progressive motor neuron disease. Most patients will require an assistive device such as cane, walker, or wheelchair several years after onset. Although IBM is commonly suspected when a patient with presumed PM did not respond to therapy, involvement of distal muscles almost from the outset, especially foot extensors and deep finger flexors with atrophy of the forearms, frequent falls due to early involvement of the quadriceps muscle causing buckling of the knees and mild facial muscle weakness are clues to early clinical diagnosis [1–4, 11–14]. Axial muscles may be affected resulting in camptocormia or head drop.

6.7 Diagnosis

The diagnosis of the exact subset is based on the combination of clinical history, tempo of disease progression, pattern of muscle involvement, determination of serum muscle enzymes, electromyography, muscle biopsy, and, for some conditions, certain autoantibodies [2–5].

6.7.1 Serum Muscle Enzyme Levels

The most sensitive indicator is creatine kinase (CK), which is elevated in almost all IM subsets when they have active disease. The higher levels, up to thousands, are seen in NAM and the lowest (less than ten-fold in IBM). Although serum CK

usually parallels disease activity, it can be normal or only slightly elevated, in several cases of active IBM as well as cases of active DM and overlap myositis reflecting pathology predominantly in the interstitial tissue and between the fascicles. Along with serum CK, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are also elevated, a sign sometimes erroneously interpreted as denoting liver disease leading to an investigation with liver biopsy instead of muscle biopsy. Serum aldolase may also be elevated, especially if the fascia is involved as often seen in overlap myositis or myofasciitis.

6.7.2 Electromyography

This shows myopathic motor unit potentials (short-duration, low-amplitude polyphasic units on voluntary activation) and increased spontaneous activity with fibrillations, complex repetitive discharges, and positive sharp waves. These findings are useful to demonstrate active or chronic myopathy and exclude neurogenic disorders, but it is insensitive to differentiate an inflammatory myopathy from toxic or dystrophic myopathies [1–4].

6.7.3 MRI

This is increasingly utilized and, in certain clinical settings, may provide useful information about the extent and selectivity of muscle involvement, identify disease activity, and guide the selection of the most active muscle to perform a diagnostic biopsy. It is helpful in demonstrating fasciitis and in identifying the pattern of muscle involvement in IBM, especially if fat-suppression techniques are utilized to differentiate inflammation from chronic atrophy and fatty replacement. In spite of some reports that MRI can be diagnostic for IBM, it cannot distinguish these changes from dystrophies and caution should be exercised in its use as a definitive diagnostic test.

6.7.4 Muscle Biopsy

Although not always typical, muscle biopsy is the most sensitive and specific diagnostic tool because it shows features distinct for each subset, provided the biopsy site is properly chosen, the specimen is processed by an experienced laboratory, and the findings are interpreted in the context of the clinical picture [1–4, 6]. In DM, there are inflammatory infiltrates perivascularly, in the interfascicular septae or the periphery of the fascicles along with phagocytosis, and muscle-fiber necrosis prominently at the periphery of the fascicles due to microinfarcts that eventually lead to muscle ischemia, hypoperfusion, and perifascicular atrophy [1–4, 6]. Perifascicular atrophy, characterized by layers of atrophic fibers at the periphery of the fascicles, is diagnostic of DM [1–4, 6] (Fig. 6.1). In PM and IBM, the inflammation is in multiple foci within the endomysial parenchyma and consists predominantly of CD8+ cells invading healthy, nonnecrotic, muscle fibers expressing the MHC-I antigen (Fig. 6.2). The MHC/DC8

complex is useful to confirm the diagnosis and exclude disorders with nonimmune inflammation, as seen in some muscular dystrophies [2–4]. In IBM, in addition to the inflammatory features and the CD8/MHC complex, there are also chronic myopathic changes with increased connective tissue and fiber-size variability, autophagic vacuoles with bluish-red material (Fig. 6.3), "ragged-red" or cytochrome oxidase-negative fibers due to abnormal mitochondria, and congophilic amyloid deposits next to the vacuoles best visualized with crystal violet or fluorescent optics [1–4, 11–14]. By electron microscopy, 12–16 nμ tubulofilamentous inclusions in the vicinity of the vacuoles are recognized [11]. In about 15–20% of patients with typical clinical IBM phenotype, the biopsies do not show vacuoles or convincing amyloid deposits but only inflammation, leading to erroneous diagnosis of PM [2, 15]. Such patients have "clinical IBM," a concept that emphasizes the need to diagnose IBM based on clinicopathologic correlations, beyond the microscope [2, 15, 16]; the combination of selective finger flexor or quadriceps weakness, endomysial inflammation with MHC-I expression, and cytochrome-oxidase-negative fibers is diagnostic of IBM even if

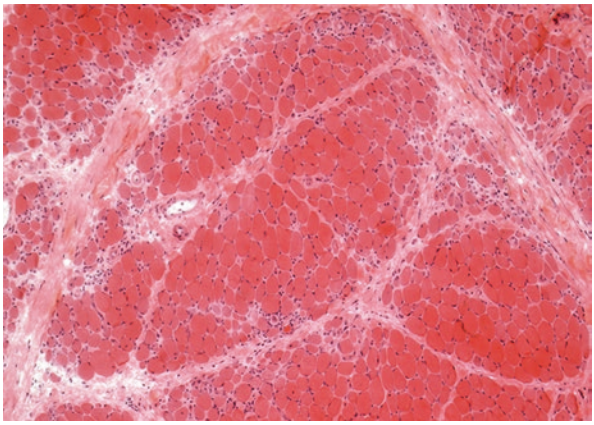

Fig. 6.1 Cross section of a muscle biopsy demonstrates the classic for DM perifascicular atrophy (layers of atrophic fibers at the periphery of the fascicle) with some inflammatory infiltrates

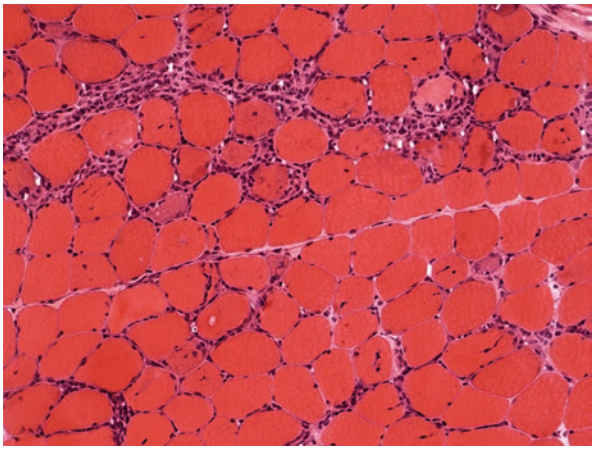

Fig. 6.2 Cross section of a muscle biopsy from a patient with polymyositis demonstrates scattered inflammatory foci with lymphocytes invading or surrounding healthy-appearing muscle fibers

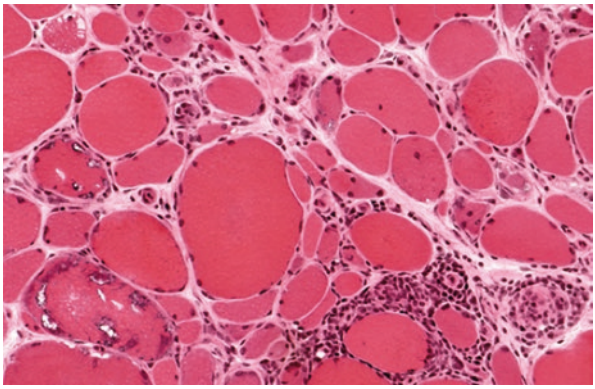

Fig. 6.3 Cross section of a muscle biopsy from a patient with IBM demonstrates inflammatory infiltrates invading or surrounding healthy muscle fibers along with chronic myopathic features based on increased connective tissue, atrophic and hypertrophic fibers, and the typical autophagic vacuoles with bluish-red material, in areas noninvaded by T cells

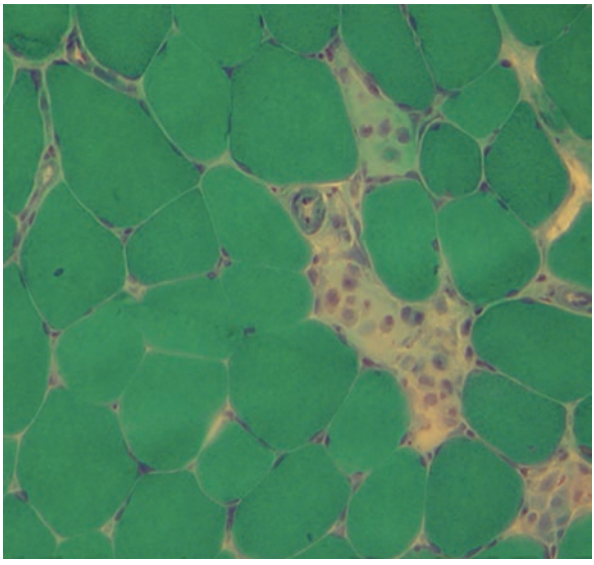

Fig. 6.4 Section of muscle biopsy from a patient within NAM shows scattered necrotic fibers invaded by macrophages

vacuoles or congophilic deposits are absent [2, 15–17]. In NAM, there are abundant necrotic fibers invaded or surrounded by macrophages (Fig. 6.4). There are no lymphocytic infiltrates or vacuoles, but MHC-I is upregulated in some areas, even beyond the necrotic fibers [2].

6.7.5 Autoantibodies

Autoantibodies, directed against nuclear or cytoplasmic antigens, are found in as many as 65–70% of IM patients depending on the series and methodology used

[2, 8–10, 18]. Among those, the most specific or useful for diagnosis are the antibodies against ribonucleoproteins involved in protein synthesis (antisynthetases) or translational transport (anti-signal-recognition particles (SRP)). The antibody against histidyl-transfer RNA synthetase, called *anti-Jo-1*, accounts for 75% of all antisynthetases and is useful because it identifies patients with interstitial lung disease, especially those with overlap myositis [2, 8]. In NAM, two antibodies are diagnostic: one against HMGCR, the pharmacological target of statins, found more often in statin-naive NAM patients, [2, 9, 10, 18–20] and another against SRP [2, 8, 18]. Antibodies against cytosolic 5′-nucleotidase 1A are detected in about 50% of IBM patients [21, 22]; their presence simply highlights B-cell activation, but they lack specificity as they are also seen in other conditions. Other autoantibodies, proposed as markers of malignancy-associated DM, such as melanoma differentiation-associated protein-5 (MDA-5) and nuclear matrix protein NXP-2 [2, 8–10, 18, 23], have not yet shown the sensitivity and specificity needed for routine use.

6.8 Pathogenesis

Although the factors triggering IM are unknown, autoimmune mechanisms—different for each subtype—are strongly implicated.

6.8.1 Dermatomyositis

In DM, there is early activation of complement C5b-9 membranolytic attack complex (MAC) which is deposited on the endothelial cells, leading to necrosis, reduction of endomysial capillaries, ischemia, and muscle-fiber destruction [1–6, 24, 25]; the remaining capillaries have dilated lumen to compensate for the ischemia [2] (Fig. 6.5). The residual perifascicular atrophy reflects the endofascicular hypoperfusion, which is most prominent at the periphery of the fascicles [1–4, 6]. The MAC activation triggers the release of proinflammatory cytokines, induces expression of adhesion molecules on endothelial cells, and facilitates migration of activated lymphoid cells to perimysial and endomysial spaces. The inflammatory infiltrates consist of B cells, CD4+ cells, and plasmacytoid-dendritic cells [2]. Innate immunity also plays a role based on increased expression of type I interferon-inducible proteins in the perifascicular regions [27]; this effect may have a role in enhancing local inflammation after the primary complement-mediated ischemic damage has taken place [26] (Fig. 6.5).

6.8.2 Polymyositis and IBM

In PM and IBM, CD8+ cytotoxic cells surround and invade healthy, nonnecrotic muscle fibers that aberrantly express MHC-I (Fig. 6.6) [1–4, 6, 28–32]. MHC-I

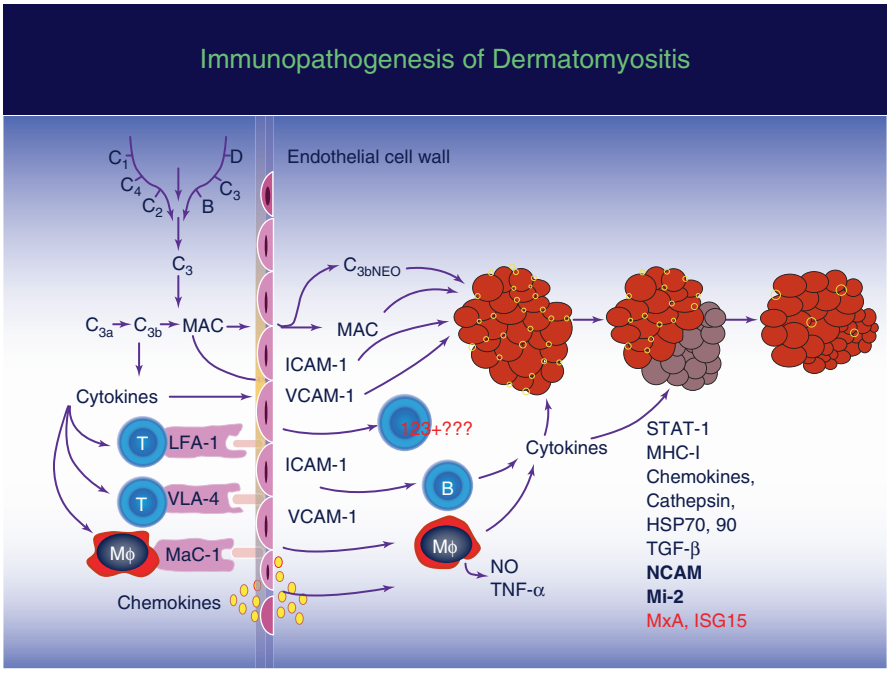

Fig. 6.5 Proposed immunopathogenic scheme of dermatomyositis. Activation of complement C3 is an early event leading to the formation of C3b, C3bNEO, and membrane attack complexes (MAC), which are deposited on the endothelial cell wall of the endomysial capillaries, resulting in destruction of capillaries, ischemia, or microinfarcts, most prominent in the periphery of the fascicles, and perifascicular atrophy. Cytokines released by activated complement lead to activation of CD4+ T cells, macrophages, B cells, and 123+ plasmacytoid-dendritic cells (PDC); enhance the expression of vascular cell adhesion molecules (VCAM) and intercellular adhesion molecule (ICAM) on the endothelial cell wall; and facilitate lymphoid cell transmigration to endomysial tissue by their integrins, late activation antigen (VLA)-4, and lymphocyte function-associated antigen (LFA)-1 which bind VCAM and ICAM. The perifascicular regions contain fibers in a state of remodeling and regeneration (expressing TGFβ, NCAM, Mi-2), cell stress (expressing HSP 70, 90), immune activation (expressing MHC-1, chemokines, STAT-1), and molecules associated with innate immunity (such as MxA, ISG15, Rig-1). The role of innate immunity in inflammation and perifascicular atrophy appears secondary to hypoxic and ischemic damage sensed by the retinoic acid-inducible gene-1 (Rig-1) signaling, which in turn leads to auto-amplification of the local inflammation via β-interferon [26]

expression, absent from the sarcolemma of normal muscle fibers, is probably induced by cytokines secreted by activated T cells [1–4, 6]. The CD8/MHC-I complex is characteristic for PM and IBM [2, 29–33] and its detection aids in confirming the histologic diagnosis [2, 3, 32, 33]. The CD8 cells contain perforin and granzyme granules directed toward the surface of the muscle fibers resulting in myonecrosis upon release [2, 34]. Analysis of T-cell receptor molecules expressed by the infiltrating CD8+ cells reveals clonal expansion and conserved sequences in the antigen-binding region, suggesting an antigen-driven T-cell response [34–39]. This is further supported by the rearrangement of the T-cell receptor genes, the

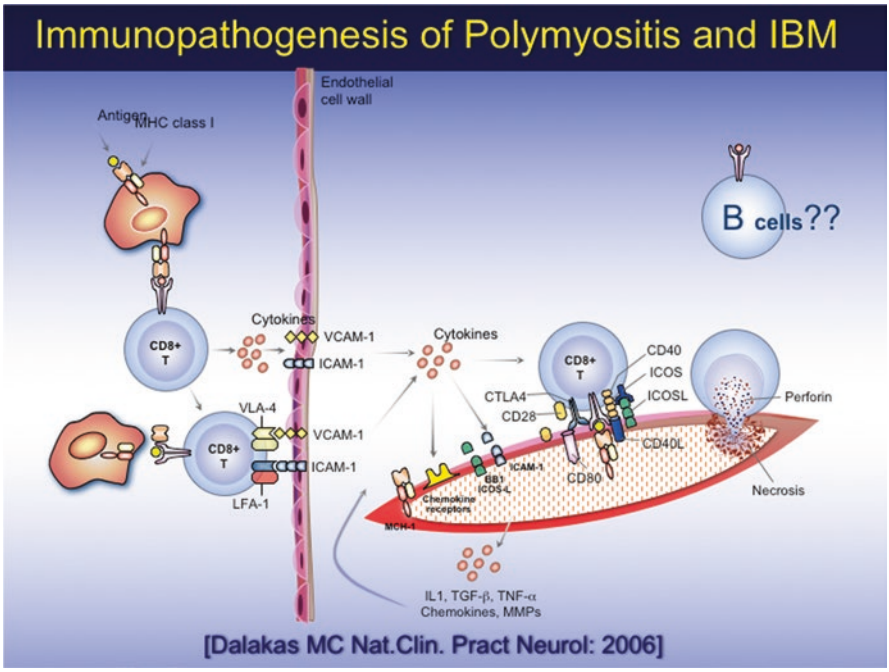

Fig. 6.6 Proposed mechanism of T-cell-mediated muscle damage in polymyositis (PM) and inclusion-body myositis (IBM). Antigen-specific CD8+ cells, expanded in the periphery and subsequently endomysially, cross the endothelial cell wall and bind directly to aberrantly expressed MHC-I on the surface of muscle fibers via their T-cell receptors. Upregulation of co-stimulatory molecules (BB1 and ICOSL) and their ligands (CD28, CTLA-4, and ICOS), along with ICAM-1/LFA-1, stabilizes the synaptic interaction between CD8+ cells and MHC-1 on muscle fibers. Perforin granules released by the autoaggressive T cells mediate muscle-fiber necrosis. Cytokines, such as interferon (IFN-γ), interleukins (IL- 1), and tumor necrosis factor (TNF-α) released by the activated T cells, may enhance MHC-I upregulation and T-cell cytotoxicity. Activated B cells or plasmacytoid-dendritic cells are clonally expanded endomysially and may participate in the process by a still undefined role, either as antigen-presenting cells (APC) or by release of cytokines and antibody production

expression of co-stimulatory molecules and their counterreceptors, and the upregulation of adhesion molecules and cytokines [2–4, 35–42] (Fig. 6.6). There is also B-cell activation, most prominent in IBM [43], as supported by B-cell and plasma cell infiltrates and the presence of autoantibodies against nuclear antigens, initially reported 15 years ago [44], and recently identified as being against 5′-nucleotidase 1A [21, 22].

What triggers disease remains unknown. Genetic risk factors regulating immune responses against undefined environmental agents have been proposed [8]. Viruses may be responsible for breaking tolerance but attempts to amplify viruses from these muscles, including coxsackieviruses, influenza, paramyxoviruses, mumps, cytomegalovirus, and Epstein-Barr, have failed [2–4, 6]. The best evidence for a viral connection is with retroviruses because individuals infected with HIV or

human T-cell lymphotropic virus-I develop typical PM or IBM [45–47]; PM is also seen in primates infected with simian immunodeficiency virus [2, 45]. Retroviral antigens are only detected in endomysial macrophages but not within the muscle fibers themselves. The autoinvasive T cells are clonally driven and some retroviral specific [45]. HIV-PM and HIV-IBM should be distinguished from a toxic mitochondrial myopathy, induced by antiretrovirals, which improves upon drug discontinuation [48].

The complexity of IBM: *the role of nonimmune factors and* "*cross talk*" *between inflammation and degeneration.* In IBM, in addition to autoimmunity, there is an important neurodegenerative component, evident by the presence of congophilic amyloid deposits within some fibers [2, 3, 11–13, 49]. Similar to Alzheimer's disease, these deposits immunoreact against amyloid precursor protein (APP), amyloid β-42, apolipoprotein E, α-synuclein, presenilin, ubiquitin, and phosphorylated-tau attesting to protein aggregation [2, 11, 49]. Immunostaining for the ubiquitin or tau components, TDP43 and p62, has been advocated as diagnostic markers [11, 49, 50]. There is in vitro evidence that β-amyloid is involved in the pathway of intracellular toxicity [49] but remains unclear how these proteinaceous aggregates, which are also seen in other vacuolar myopathies, induce an inflammatory vacuolar myopathy and what triggers disease, inflammation, or protein aggregation [2]. Aging, abnormal proteostasis (the network controlling proteins) [49], cell stress induced by MHC-1 or nitric oxide [51, 52], and long-standing inflammation with proinflammatory cytokines, such as interferon-γ and IL1-β [51–54], may cumulatively play a role either in triggering the disease process or enhancing degeneration and further accumulation of stressor molecules and misfolded proteins [2, 53].

6.9 Treatment of DM, PM, and NAM

Oral prednisone is the first-line drug based on experience, but not controlled trials [1–4, 55, 56]. In patients with severe or rapidly worsening disease, intravenous methylprednisolone for 3–5 days is preferable before starting the oral regimen. After 3–4 weeks, the daily dose is switched to alternate days [2, 54–56]. If by the third month there are no objective signs of increased strength and activities in daily living, tapering should be accelerated to start the next in-line agent. A tactical error is the practice of "chasing" the CK level as a sign of response, especially in patients reporting a sense of "feeling better" but not necessarily stronger. When the strength improves, the serum CK drops, but fall in CK alone, should not be interpreted as a sign of improvement [2–5, 33]. In steroid-responsive patients, the drugs most commonly used for "steroid-sparing" are empirical and include *azathioprine*, *mycophenolate mofetil*, *methotrexate*, or *cyclosporine* [1–4, 54, 55]. *Cyclophosphamide* or *tacrolimus* may be helpful when interstitial lung disease coexists [1–4, 57]. When corticosteroids fail to induce remission, or in rapidly progressive cases, *intravenous immunoglobulin* (IVIg) at 2 g/kg is the most appropriate next in-line drug [2, 55, 56, 58, 59]. In a double-blind study, IVIg was effective in refractory dermatomyositis [58]. The improvement is noticeable after 15 days, but monthly infusions are required to maintain remission. In open-label trials, IVIg is also

effective in several PM and NAM patients [2, 55]. Subcutaneous Ig, a possibly more practical or cost-effective means of infusing IgG, has shown promise in sustaining remission [60].

If corticosteroids and IVIg have failed to improve strength, it is prudent to reevaluate the patient, challenge the diagnosis, or reconsider a repeat muscle biopsy. If the diagnosis is reconfirmed, new biologics approved for other autoimmune diseases may be considered, as discussed [2, 55]. Among those the most promising is *rituximab,* which at 2 g (divided into 2 biweekly infusions) can be effective in several DM, PM, and NAM patients [61–63]. A placebo-controlled study in 200 patients however did not meet the primary end point largely because of study design [64]. Although at week 8 there was no difference between placebo and rituximab, at week 44 when all patients had received rituximab, 83% met the definition of improvement [64]. Patients with autoantibodies against Jo-1, Mi-2, or SRP were more likely to improve [65]. Anti-*TNF-α inhibitors* (infliximab, adalimumab, etanercept) anecdotally used [66] have been ineffective and may even worsen or trigger disease [67]. Other biologics for future experimental trials may include *alemtuzumab*, reportedly effective in PM [68]; *anti-complement C3* (*eculizumab*), which is effective in other complement-mediated diseases and has been approved for Myasthenia Gravis and neuromyelitis. Anticomplement agents are quite appropriate for DM which is a complement mediated microangiopathy and, on this basis, a controlled study has began [2]. New agents against immunoregulatory cytokines, such as *anti-IL-6*, *anti-IL17*, *or IL17A could be other future options* [2, 55]. Because most trials up to now have been small, better-designed studies with more adequate power are needed. Overall, the long-term outcome of IM has substantially improved, with a 10-year survival rate in one recent study to be at >90% [69, 70].

6.10 Treatment of IBM

Because a T-cell-mediated cytotoxicity coexists with degeneration and proinflammatory cytokines enhance amyloid aggregates [2, 51–54], strong anti-inflammatory agents should theoretically halt disease progression. All such agents however have failed probably because IBM starts long before patients seek medical advice, when the degenerative cascade is already advanced [2, 54]. Corticosteroids, methotrexate, cyclosporine, azathioprine, and mycophenolate are ineffective although some patients may initially respond to some degree [2, 54]. IVIg is ineffective in controlled trials but may transiently help some patients, especially the dysphagia [1–73]. Alemtuzumab may provide short-term stabilization [74], but a controlled study is needed. Anti-ILI receptor (Anakinra) failed [75]. Trials targeting muscle-inhibiting TGF-b molecules or muscle growth factors are in progress.

At present, symptomatic therapies are the best options. For life-threatening dysphagia not responding to IVIg, cricopharyngeal myotomy may be considered [76]. Because of coexisting mitochondrial dysfunction, co-enzyme Q_{10} may enhance endurance, although never properly tested. Non-fatiguing, resistance exercises can

be beneficial [77]. Like in all IM, occupational and rehabilitation therapies are useful to improve ambulation, prevent falling, avoid disuse atrophy, and prevent joint contractures.

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Necrotizing Autoimmune Myopathy

7

Charles Kassardjian and Margherita Milone

7.1 Introduction

Necrotizing autoimmune myopathy (NAM) is an idiopathic immune-mediated myopathy, along with dermatomyositis (DM), polymyositis (PM), and sporadic inclusion body myositis (sIBM). NAM manifests with subacute proximal-predominant muscle weakness and elevated serum creatine kinase (CK). Pathologically, it is characterized by prominent muscle fiber necrosis and regeneration, but, contrary to the other idiopathic immune-mediated myopathies, it is accompanied by minimal or no inflammation [1–3]. For this reason, although in the literature NAM or other immune-mediated myopathies are sometimes referred to as "idiopathic inflammatory myopathies" (IIM), we prefer to avoid the term IIM.

The literature around NAM has expanded in recent years with the discovery of serum antibodies associated with this disease, including antibodies to signal recognition particle (SRP) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). As will be discussed in this chapter, the presumed immune basis of NAM rests on the subacute onset, clinical response to immunotherapy, and associated serum antibody profile. The etiology of NAM is unknown, but the disease can occur in isolation, in association with statins, connective tissue diseases, or malignancy. Similar to other rare diseases, the epidemiology, risk factors, and response to treatment remain incompletely understood.

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7.2 Clinical Features and Epidemiology

Most patients with NAM present during adulthood between the second and the sixth decade, but the disease can also manifest in the elderly and childhood. Overall, there seems to be no clear male or female predominance, and different studies have shown conflicting results in this regard. The typical clinical presentation of NAM is of relatively symmetric proximally predominant weakness that progresses subacutely (over days, weeks, or a few months) in most cases [4–7]. NAM often manifests in a more fulminant manner than typically seen in inflammatory myopathies, and more than 50% of patients develop severe weakness. However, some reported NAM cases have presented insidiously over many months, mimicking muscular dystrophy and making the diagnosis challenging [8].

Lower limb weakness tends to be more severe than upper limb weakness, and patients often complain of difficulties arising from a chair, climbing stairs, or ambulating [4]. Distal weakness is common although less severe than proximal weakness. We observed distal weakness in 41% of our patients, mainly affecting wrist and finger extensors and/or foot dorsiflexors [4]. Axial weakness occurs in 30–80% of patients and neck flexor are more commonly involved than neck extensor muscles. Occasionally, neck muscle weakness can be the predominant clinical feature [4]. Bulbar muscles may be affected with one-third of patients experiencing dysphagia, whereas dysarthria is uncommon (only 6% in one series) [4]. Myalgias occur in approximately one-third of the patients. Table 7.1 lists many of the clinical features and frequencies for our large cohort of NAM patients [4].

In their early report of SRP antibody-associated necrotizing myopathy, Pestronk and colleagues noted a predilection for symptoms to start in the fall, but several subsequent reports did not confirm this observation. Antecedent events that could potentially trigger a dysimmune response and NAM are rare, with upper respiratory tract illnesses and surgical procedures noted within 2 weeks prior to weakness onset in few cases [4]. However, the role of these events in the pathogenesis of NAM is unknown.

Rash is extremely rare in NAM. The characteristic skin manifestations of DM are not observed. However, if a patient has a coexisting connective tissue disease,

Table 7.1 Clinical features and frequencies in 63 NAM patients evaluated at Mayo Clinic

Clinical feature	Frequency (%)
Proximal muscle weakness	100
Distal muscle weakness	41
Neck flexor weakness	78
Neck extensor weakness	31
Facial muscle weakness	14
Dysphagia	35
Dysarthria	6
Dyspnea	37
Myalgia	35
Weight loss	29

the dermatological or systemic features of the specific connective tissue disease (e.g., scleroderma) can be observed. Raynaud phenomenon can occur. Weight loss around the time of presentation of NAM is not uncommon and observed in about 30% of cases [4].

7.3 Pulmonary and Cardiac Manifestations

The frequency of cardiac or respiratory involvement in NAM varies greatly in the literature, likely a reflection of inconsistent assessment and definition of restrictive lung disease, interstitial lung disease (ILD), and critical consideration of comorbidities. Many patients with NAM may have risk factors for coronary artery disease or cardiomyopathy, which may account for any detected arrhythmias or cardiac conduction defects seen on electrocardiogram. Nevertheless, respiratory muscle involvement and cardiac manifestations occur in NAM.

In our series, 37% of patients complained of dyspnea and 5 of 63 had hypercapnic respiratory failure requiring mechanical ventilation [4]. In addition, among patients who underwent complete pulmonary function testing, approximately the third had a restrictive pattern suggestive of respiratory muscle weakness [4]. Data on the frequency of ILD in NAM are conflicting. In some series, ILD was very rare in NAM [4, 9] (only 2 of 63 patients had findings consistent with ILD in our cohort [4]), whereas there was a higher incidence of ILD in NAM patients with SRP antibodies (21% of SRP-IgG-associated NAM in one series [10]). There may be a higher incidence of ILD in patients with immune-mediated myopathies associated with connective tissue disease, especially in systemic sclerosis [11].

Cardiac involvement is less common than pulmonary impairment. Patients can have palpitations and chest pain. Similarly to other immune-mediated myopathies, the cardiac conduction system appears preferentially involved in NAM, since arrhythmias and conduction defects, such as bundle branch blocks and prolonged QTc interval, are more commonly observed than structural cardiomyopathy [12].

7.4 Risk Factors and Etiologies

Although several risk factors or disease associations have been reported in NAM, the majority of NAM patients are “idiopathic” [4]. One of the earliest identified associations was of cancer (paraneoplastic necrotizing myopathy), described in several case reports and case series [13, 14]. The cancers reported in association with NAM include non-small cell lung cancer, bladder (transitional cell), gastric, colon, prostate, and pancreatic adenocarcinomas. In our series of 63 NAM cases, six were paraneoplastic, with gastrointestinal tumors being most common (two colon adenocarcinomas, one esophageal adenocarcinoma), followed by one case each of lung adenocarcinoma, ovarian adenocarcinoma, and thymoma [4]. Other authors have detected cancer in association with NAM, including anti-HMGCR antibody-positive NAM cases [15]. In many cases, the underlying cancer is discovered after the onset

of the myopathy, during the malignancy screen prompted by the diagnosis of NAM. These data suggest that patients with NAM should be screened for an underlying malignancy by physical examination (prostate, breast), imaging studies (chest, abdomen, pelvis, breast), and gastrointestinal tract endoscopy. However, one should also be aware that no increased risk of cancer was detected in another large series of NAM cases in comparison with the general population [7].

Rarely, NAM has been associated with connective tissue diseases, including systemic lupus erythematosus [7], scleroderma [4], Sjogren syndrome [4], or an undifferentiated connective tissue disease [16]. In these cases, patients often have multisystemic symptoms or clinical signs suggestive of the underlying connective tissue disease and a compatible serological autoantibody profile. There is no convincing evidence that NAM patients with an underlying connective tissue disease have a different clinical course than other NAM patients, but the small numbers of reported cases preclude reliable conclusions.

Statins appear to be the most common risk factor association in patients with NAM. Several studies have demonstrated an increased risk of myopathy, including NAM, in patients taking statin medications [17–19]. In patients exposed to statins, who develop myopathic symptoms and elevated serum CK, NAM should be suspected if there is no clinical or serological improvement after discontinuation of the statin. Patients may be on statins for months or years (2 months to 10 years in one study) before developing NAM, and in some cases NAM has occurred many months after statin discontinuation [17]. In these cases, it may be difficult to identify the statin as the underlying trigger.

Recently, an antibody directed against HMGCR (the enzyme inhibited by statin medications) has been identified in some NAM patients [19, 20]. Interestingly, not all statin-exposed NAM patients have HMGCR-IgG antibodies, and conversely not all HMGCR-IgG-positive patients have a history of statin exposure [4, 18, 19]. Thus, the exact pathogenesis and role of HMGCR antibodies in NAM remains unknown (see section below on autoantibodies in NAM).

Interestingly, regardless of disease or drug association (paraneoplastic, connective tissue disease, or statin), there appear to be no significant differences in clinical severity, extra-muscular manifestations, serum CK, or clinical outcome [4]. Statin-associated NAM patients tend to be older and are more likely to have myotonic discharges on EMG, whereas idiopathic cases are more likely to have dysphagia.

7.5 Diagnostic Workup and Laboratory Testing

7.5.1 Antibodies in NAM

Several autoantibodies have been recognized to occur in NAM, with the most well-characterized being SRP-IgG and HMGCR-IgG antibodies.

SRP-IgG is most commonly associated with NAM but has been rarely reported in other immune-mediated myopathies, such as DM and sIBM [10, 21, 22]. In a study on antibody profiles of myositis patients (PM, DM, and IBM), approximately

5% of cases were SRP-IgG positive [21]. Characteristically, patients with SRP-IgG-related NAM have relatively rapid onset of severe weakness (significant disability within 6 months) that is often refractory to commonly used immunotherapy (e.g., monotherapy with oral prednisone) [5, 10]. Some investigators believe that SRP-IgG-positive NAM is more resistant to treatment than non-SRP antibody-positive NAM. Although patients with SRP-IgG-associated NAM rarely have an underlying malignancy, the presence of SRP-IgG does not exclude an associated cancer [10, 23].

Antibodies targeting HMGCR have recently been discovered in the sera of NAM patients [19, 20]. Similar to other NAM patients, HMGCR-IgG-positive cases demonstrate severe proximal weakness and markedly elevated serum CK, associated with axial weakness and dysphagia in many cases. A restrictive pattern of respiratory weakness occurs in up to 25% of cases, while cardiac conduction defects have been rarely reported in HMGCR-IgG-positive NAM [18]. Interestingly, statin drug exposure is not required for the development of HMGCR-IgG-associated NAM, and 20–70% of HMGCR-IgG-positive NAM cases are statin naïve [4, 18, 19, 24]. Phenotypic differences are present between HMGCR-IgG-positive patients exposed to statin and those not exposed to it. HMGCR-IgG-positive NAM patients with a history of statin medication exposure tend to be older, are less likely to be African American, and may be more responsive to immunotherapy [24]. In regard to statin-naïve NAM patients, one should consider that these patients may be exposed to statins contained in aliments (e.g., oyster mushrooms contain lovastatin), and therefore they may not be truly statin naive. Underlying malignancies have also been reported in HMGCR-IgG-positive NAM, with the malignancy usually discovered after the onset of myopathy [15, 18]. Thus far, only a single patient has been reported to be dual positive for SRP-IgG and HMGCR-IgG [4].

Other autoantibodies that have been reported in NAM patients include PL-12 [25], PL-7 [20], Ku [7, 26], and Jo-1 [4, 20].

7.5.2 Serum CK

The serum CK is usually markedly elevated in most NAM cases, but ranging from 600 to 30,000 IU/L. When following patients clinically over time, rises and declines in serum CK level occur with clinical relapse or improvement, respectively. So, the serum CK may be used in conjunction with clinical weakness as a surrogate marker of overall disease activity when following patients [6].

7.5.3 Electromyography (EMG)

Nerve conduction studies (NCS) and needle EMG are useful tests to confirm a myopathic process and rule out other neuromuscular causes of weakness. Needle EMG demonstrates fibrillation potentials and short-duration, low-amplitude (myopathic) motor unit potentials in clinically weak muscles [1, 2, 4, 5]. Myotonic discharges

may be recorded and are significantly more common in patients with statin-associated NAM [4]. Although prospective studies are lacking, our 11 patients who underwent posttreatment follow-up EMG studies showed a reduction in the number of muscles with fibrillation potentials and myopathic motor unit potentials [4].

7.5.4 Muscle Imaging

Magnetic resonance imaging (MRI), the preferred method of imaging muscle, can provide useful information but often is not necessary for patient care. Increased signal on short tau inversion recovery (STIR) sequences suggests muscle degeneration and necrosis although does not indicate its etiology. MRI has the potential to improve the diagnostic yield of a muscle biopsy by allowing the clinician to target a weak muscle that is abnormal but not end-stage and completely replaced by fatty connective tissue [27].

7.5.5 Muscle Biopsy

A muscle biopsy is critical to confirm the diagnosis of NAM raised by the clinical history, while excluding other myopathies. The histopathological hallmark of NAM is the presence of necrotic and regenerating muscle fibers, with minimal or no inflammatory infiltrate (Fig. 7.1) [1, 2]. In many biopsies, necrotic muscle fibers are invaded by CD-68-positive macrophages, which may give the false impression of an inflammatory exudate. Nevertheless, approximately 20% of NAM biopsies show some degree of inflammatory infiltrate [4]. This usually consists of small collections of mononuclear cells located at perivascular sites in the perimysium or in proximity to necrotic muscle fibers. The autoaggressive inflammatory exudate with mononuclear cells invading non-necrotic muscle fibers, typical of PM and sIBM, does not occur in NAM. Likewise, the perifascicular preference of the structural abnormalities, classically observed in DM, is not present in NAM. Thickening of the capillary basement membranes ("pipestem capillaries") and capillary depletion has been rarely reported in NAM [5, 16]. Non-rimmed vacuoles can be observed [4]. Often the pathological features of NAM are those of a nonspecific myopathic process and alone cannot confirm the diagnosis of NAM, which should be supported by the clinical history and serological data. For this reason, if clinically indicated, immunohistochemical studies to localize sarcolemmal, cytosolic, or nuclear proteins responsible for muscular dystrophies may be warranted trying to exclude inherited myopathies.

In SRP-IgG-positive muscle biopsies, some authors found a reduced capillary density compared to controls, and membrane attack complex (MAC) deposition in most endomysial capillaries but not diffusely on muscle fibers [5]. However, these findings were not confirmed by others [10].

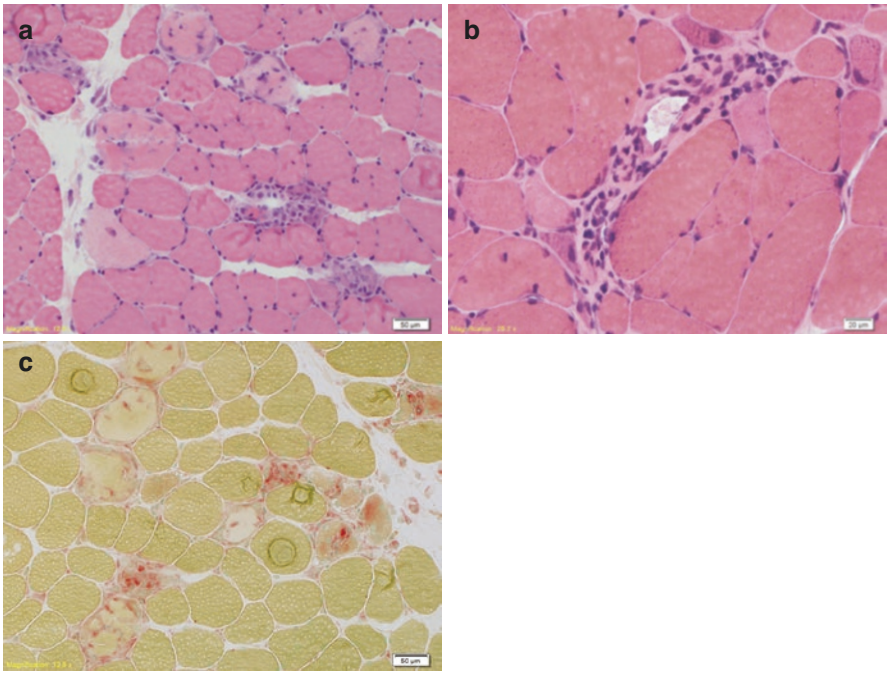

Fig. 7.1 Muscle biopsy of patients with HMGCR-IgG antibody-positive necrotizing autoimmune myopathy. Hematoxylin-eosin stained section shows scattered necrotic and fewer regenerating muscle fibers (**a**) and rare inflammatory cells a perivascular site in the perimysium of another NAM patient (**b**). Some necrotic fibers have been invaded by macrophages, which stain red in acid phosphatase-reacted section (**c**) (**a**, **c**: magnification 20×; **b**: magnification 40×)

In HMGCR-IgG-positive NAM muscle biopsies, several authors have reported upregulation of the major histocompatibility complex class I antigen (MHC-1) [15, 28], while there are discrepant results about the presence of complement deposition on the sarcolemma of non-necrotic muscle fibers and endothelial cells [15, 28]. Some authors have also described enlargement and irregular shape of the myonuclei [15]. The pathological changes seem independent of statin exposure. Characterization of the limited inflammatory exudate in muscle biopsies of HMGCR-IgG-positive NAM patients identified CD-68-positive cells (macrophages) as the most common mononuclear cell type in both endomysium and perimysium [28]. Scattered CD-4- and CD-8-positive T-cells were also noted in the endomysium and perivascular sites; CD-20-positive cells were rare. Plasmacytoid dendritic cells (CD-123 positive) were found in 63% of biopsies, suggesting a role for innate immunity and interferons in the disease pathogenesis. Based on these results, the authors hypothesize that HMGCR antibodies may bind to the cell surface and activate complement to initiate muscle fiber lysis [28].

7.6 Diagnosis and Differential Diagnosis

There is no gold standard diagnostic test for NAM. Clinical history plays a critical role in the diagnosis and results of serological, EMG, and muscle pathological studies all contribute to the correct diagnosis. Response to immunotherapy corroborates it [1, 2].

Basic blood tests are useful for excluding alternate diagnoses as causes of proximal weakness and elevated serum CK, as well as other forms of necrotizing myopathy, such as necrotizing myopathy associated with HIV infection [29], hypothyroidism [30], or drug toxicity [31].

The EMG helps to confirm that the weakness has a myopathic basis, and the presence of myotonic discharges (more common in statin-associated NAM) may be a clue to the etiology.

The muscle biopsy is critical to confirm that the predominant pathological features are muscle fiber necrosis and regeneration and to exclude inflammatory immune-mediated myopathies. The biopsy is also helpful in excluding hereditary myopathies with specific structural abnormalities or reduced protein expression.

There is an increasing tendency to use serum antibody profile to diagnose NAM, particularly with the recent identification of HMGCR-IgG. Although HMGCR-IgG and SRP-IgG are valuable in supporting the autoimmune etiology of a necrotizing myopathy, they do not have a very high sensitivity, and their absence does not exclude the diagnosis of NAM [7, 21, 26, 32]. Additionally, SRP-IgG is not very specific and has been reported in other inflammatory myopathies and in patients without myositis [10, 33].

In light of the data linking NAM to various malignancies, a malignancy screen is recommended for all NAM patients. This could include imaging of the chest, abdomen, and pelvis, mammogram, as well as esophagogastroduodenoscopy and colonoscopy.

7.7 Pathophysiology

The pathogenesis of NAM is not fully understood. The identification of autoantibodies in many patients, such as SRP-IgG and HMGCR-IgG, may suggest an antibody-mediated mechanism, with complement activation leading to cell lysis and fiber necrosis. However, the pathogenicity of the antibodies remains undefined and the subject of active investigation.

SRP is a family of proteins that participate in the protein synthetic pathway, assisting in the docking of newly formed proteins to the endoplasmic reticulum. In one study, in vitro human myoblast cultures incubated with serum from patients with SRP-IgG-positive NAM and complement demonstrated lower survival, and aberrant expression of SRP on the sarcolemmal surface [34]. The authors hypothesize that patients with circulating SRP-IgG antibodies may have abnormal surface expression of SRP that results in antigen-antibody complex formation and activation of complement-mediated cell lysis and necrosis.

The pathogenic role of statins and of the HMGCR-IgG antibodies also remains unclear. HMGCR-IgG is thought to be highly specific for NAM, rather than for a self-limited statin-associated myopathy that resolves with discontinuation of the drug [35] although rare self-limited HMGCR-IgG-positive cases have been reported [18].

The correlation between the HMGCR-IgG titer drop and the improved muscle strength in statin-exposed patients provides indirect evidence of the possible pathogenicity of these antibodies [18, 24]. Similarly, the serum SRP-IgG titer was found to correlate with the serum CK, and both declined with treatment and clinical improvement [6].

In cases of statin-associated NAM, there is likely an interplay between an agenetic predisposition and the environmental trigger (the statin medication). Support for this hypothesis comes from a study showing that the DRB1*11:01 haplotype was more common in patients with HMGCR-IgG-associated NAM compared to normal controls [36]. This haplotype may thus confer an enhanced ability of the antigen-presenting cell to activate an immune reaction in response to statin exposure.

The lack of prominent T-cell infiltrates in NAM points away from the cytotoxic T-cell-mediated mechanism invoked PM and sIBM. The MHC-1 upregulation is not very helpful in defining disease mechanism because it can also occur in hereditary myopathies.

7.8 Treatment and Prognosis

There are no prospective treatment trials to guide treatment of NAM, and thus recommendations are based on retrospective case series and expert opinion [37]. In general, most experts believe that NAM is more refractory to treatment than PM or DM, requiring more aggressive and prolonged courses of immune therapy [4, 5, 17, 22, 38]. However, there are some data suggesting that many NAM patients may not require more aggressive treatment [39].

In most series, patients were treated initially with corticosteroids (often orally at 1 mg/kg) but required the addition of one or more immunotherapies to control the disease [4, 5, 10, 18, 37, 40]. These additional therapies are most commonly steroid-sparing oral agents (methotrexate, azathioprine, or mycophenolate mofetil) and IVIG. The patient's response to therapy, severity of weakness, and the presence of severe complications like respiratory muscle weakness often guides the type of treatment. In our cohort of NAM patients, 56% required "triple therapy" to control the disease, consisting of corticosteroids (oral or IV), IVIG, and an oral steroid-sparing agent [4, 40], while only 2 of the 63 patients could be treated with corticosteroids alone [4]. Early treatment with IVIG seems to increase the likelihood of strength improvement [4]. In refractory cases, other treatment options have included plasmapheresis, rituximab, cyclosporine, and cyclophosphamide. Several case reports suggest that rituximab in particular may be beneficial in highly refractory patients with SRP-IgG [18, 37, 41, 42].

Thus, based on the available literature, it is reasonable to treat NAM patients with a combination of IVIG, corticosteroids, and a steroid-sparing immunosuppressant for at least 3–4 months, followed by long-term treatment with a steroid-sparing agent. However, in the absence of prospective treatment trials, therapy must be individualized on the basis of disease severity and the response monitored carefully. In cases of paraneoplastic NAM, treatment of the underlying malignancy is necessary; however, these patients usually also require immunotherapy [4, 13, 43].

The risk of clinical relapse with drug tapering or discontinuation is high (over 50% of cases) [4, 18, 40]. Many studies have shown that patients are rarely able to discontinue all immunotherapy and most require long-term immunosuppression [4, 18]. A study suggested that the greatest improvement in CK values and muscle strength after relapse occurs after reintroduction of high-dose steroids or IVIG [40].

Despite the need for aggressive and long-term immunotherapy, outcomes in NAM can be favorable. In one large cohort of NAM patients, 17 of 32 patients had marked improvement of weakness or returned completely to baseline with aggressive treatment [4]. Some authors found that although many patients had residual weakness, 75% had normal or near-normal ambulation by the end of their study [10]. Factors that have been identified as possible predictors of favorable outcome include male gender and aggressive treatment with two or more immunosuppressive agents within the first 3 months of presentation [4]. Interestingly, the presence of SRP-IgG or HMGCR-IgG antibodies was not a significant predictor of outcome or treatment response in our study although some investigators have found that SRP-IgG-positive NAM is more resistant to treatment and may have a poor outcome [44]. There are few data directly comparing treatment efficacy or response rate among NAM cases based on etiology or presence of antibodies, but overall there appear to be few differences [45].

Key Points

- Necrotizing autoimmune myopathy is an immune-mediated muscle disease characterized by subacute, often severe, predominantly proximal muscle weakness and elevated serum CK.
- Dysphagia and respiratory muscle weakness are common and potentially life-threatening complications.
- Muscle biopsies demonstrate predominant necrotic and regenerating muscle fibers, with minimal or absent inflammatory infiltrate.
- NAM may be idiopathic or associated with statin exposure, connective tissue disease, or malignancy.
- Autoantibodies most commonly associated with NAM are SRP-IgG and HMGCR-IgG although the presence of these antibodies is not required for the diagnosis.
- NAM can be difficult to treat and usually requires aggressive and prolonged immunosuppression, often with IVIG, corticosteroids, and oral steroid-sparing immunosuppressant medications.

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Statin Myopathy

8

Jochen Schaefer and Sandra Jackson

8.1 Epidemiological Impact of Statins in Cardiovascular Disease

Statins inhibit 3-OH-3-methyl-glutaryl-CoA (HMG-CoA) and are the most potent drugs currently available to treat hypercholesterolaemia [1]. These agents form a mainstay of primary and secondary prevention of coronary artery disease and atherosclerosis and effectively reduce cardiovascular mortality [2]. It is estimated that the incidence of heart attacks and strokes is reduced by 20% for each reduction of 1 mM in LDL cholesterol levels [3]. The average cholesterol-lowering effect of the highest approved statin doses is 33% for fluvastatin (80 mg) and up to 55% for atorvastatin (80 mg) [4, 5]. Besides simply reducing plasma cholesterol levels, statins also have pleiotropic (cholesterol-independent) effects, mainly anti-inflammatory and pro-apoptotic, which contribute to the beneficial actions of statins, but also to their side effects [6].

Shortly after their introduction in 1987, the first cases of statin-associated rhabdomyolysis were published in 1988 [7]. Despite this serious adverse event, the risk-benefit ratio remains very much in favour of statin therapy, and indeed statins are now amongst the most widely prescribed drugs worldwide. So far, only one statin, cerivastatin, had to be removed from the market due to an excess of

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rhabdomyolysis—50 times greater than other statins [8]. Most deaths, however, had occurred with concomitant administration of other drugs, in particular fibrates and gemfibrozil.

8.2 Definition of Statin Myotoxicity

Amongst all side effects of statins, their muscular side effects remain most important and are the crucial factor for patient adherence to statin treatment. In one study [5], the muscle-related side effects were the reason for discontinuation of statin treatment in 65% of cases.

There is still currently no consensus on the definition of statin myopathy which confuses the interpretation of observational studies of statin-associated muscle symptoms. Moreover, most studies on statin myotoxicity have been performed by non-neuromuscular experts.

In the literature, the most widely accepted definitions are those of the National Lipid Association (NLA) Statin Muscle Safety Task Force [9]—these will therefore be used throughout this chapter (Table 8.1).

Further confusion is added by the fact that many observational trials rely on patient self-reporting of statin side effects without objective proof of the muscular origin of these symptoms. Non-neuromuscular side effects, such as tendinopathies or arthropathies, with associated pain are fairly common and have been reported by up to 24% of patients in the PRIMO study, an observational investigation [10].

Table 8.1 Definitions of statin-associated muscle symptoms

Myalgia	Muscle discomfort (soreness, stiffness, tenderness, heaviness, cramps), with normal CK levels. Apparent weakness may occur secondary to pain
Myopathy	Weakness and/or myalgia with elevation of serum CK
Rhabdomyolysis	Myopathy with serum CK >10x ULN plus evidence of associated renal failure or serum CK >10,000 U/L (50-fold elevation)
Asymptomatic myopathy	CK elevation without clinical symptoms—this was not defined by the task force

8.3 Epidemiology of Statin-Related Myotoxicity

Information on statin-associated muscle symptoms (SAMS) has been derived from both controlled clinical trials and observational studies of statins in everyday clinical use. Whilst the latter indicates that 7–29% of patients complain of SAMS [5, 10–12], the randomized controlled trials yield very different results: in RCTs, adverse event rates are similar in statin and placebo groups [1, 13, 14]. A large meta-analysis of 42 randomized trials covering almost 60,000 patients [15] found that 12.7% reported muscle problems in the statin groups vs. 12.4% in the placebo groups, a non-significant difference. In two studies, CARDS and SPARCL, the placebo groups even had a higher rate of myalgia than the verum group [15]. Only two trials [16, 17], however, had questioned participants systematically about muscle symptoms and reported an incidence for myalgia of 3% [16] and 9.4% [17], respectively.

The frequency of myopathy, as defined above, was much lower at less than 0.5% [1, 18] with standard doses of statins, but extended up to 2% at high-dose treatment (atorvastatin 80 mg) [19, 20]. Thus, the overall frequency of myopathy is low, but increases at higher statin doses and with concomitant use of interacting medication (Table 8.2). Moreover, in the above meta-analysis [15], no myopathy was reported with fluvastatin, which was also associated with the least number of muscle symptoms in the PRIMO study [10].

Rhabdomyolysis, as defined above, was even rarer with an incidence of 0.03% in two meta-analyses [15, 21] and its frequency was not different from the placebo groups (0.02%) (Table 8.2). In all trials, rhabdomyolysis was not seen in patients who did not have additional risk factors (Table 8.3).

Why do the outcomes of clinical trials not reflect clinical practice, where SAMS are reported much more frequently (Table 8.2)?

First, clinical trials usually exclude patients with a history of muscle problems and other risk factors for myopathy. Second, most statin trials were not primarily designed to assess muscle complaints. Third, the lack of a placebo group in many observational studies often precludes verification of a causal relationship between statins and muscle symptoms, which are therefore overestimated. This particularly applies to patients with anxiety or depressive disorders who frequently complain of

Table 8.2 Frequency of statin-associated muscle problems

	Verum groups	Placebo groups	Significant
Any muscle symptom			
Meta-analyses	12.7%	12.4%	No
Observational trials	7–29%	–	–
Myopathy (CK >3x ULN)	0.5%	0.3%	Yes
Myopathy (CK >10x ULN)	0.2%	0.16%	No
Rhabdomyolysis	0.03%	0.02%	No

Adapted from Kjekshus et al. [16]

Table 8.3 Risk factors for statin myopathy

Patient features	Drug features
Age >80 years	Statin dose
Comorbidity (renal, hepatic, hypothyroidism, diabetes, trauma, neuromuscular disease)	Type of statin (lipophilicity) *Higher risk*: simvastatin, lovastatin, atorvastatin *Lower risk*: pravastatin, fluvastatin, rosuvastatin
Diet (grapefruit juice)	Interacting drugs:
Pre-existing hyperCKemia	CYP3A4 inhibitors (macrolides, azoles, HIV-drugs, amlodipine, amiodarone, cyclosporine, tetracyclines)
Vitamin D deficiency	Fibrates, gemfibrozil, niacin
Previous statin intolerance	Steroids
Alcohol abuse	
Genetic predisposition (SLCO1B1, CYP3A4 polymorphisms)	

muscle soreness. Fourth, physical activity, an important trigger of "statin myopathy", has not been taken into account in most studies.

Considering the results from both randomized and observational studies, one may conclude that myalgias are not uncommon (2–10%), but clinically significant myopathy with CK elevations and weakness is much rarer (~0.5%) and rhabdomyolysis is even less frequent (<0.05%) and almost always associated with concomitant medication.

8.4 Pathogenesis of Statin-Induced Myopathy

Several mechanisms have been proposed for the myotoxic effects of statins. Inhibition of the mevalonate pathway, the central step in cholesterol biosynthesis, by statins also interacts downstream with other important pathways (Fig. 8.1):

- Impaired production of coenzyme Q10, which forms an essential part of the respiratory chain, may lead to mitochondrial dysfunction. Although statin therapy has been shown to decrease CoQ10 plasma levels [22], the results of muscle CoQ10 analyses have been inconclusive [23, 24]. Some, but not all, patients show mitochondrial pathology in muscle biopsies, such as COX-negative and ragged-red fibres [24, 25], and depletion of mtDNA [26].
- Secondary mitochondrial changes, such as altered membrane fluidity, or changes in calcium homeostasis probably play a minor role.
- Prenylation of proteins is implicated in cell differentiation, signalling and proliferation and is also involved in immune responses. Impaired prenylation will therefore ultimately increase apoptosis. Since inhibition of cholesterol biosynthesis downstream of HMG-CoA reductase at the level of squalene synthase does not cause myotoxicity, impaired protein prenylation is regarded by many as being one of the most important pathogenic mechanisms [27].
- A subgroup of statin-associated myopathy may be triggered by an autoimmune process targeted against HMG-CoA reductase, clinically manifesting as a necrotizing myopathy [28]. However, in a large cohort of patients with HMGCR-

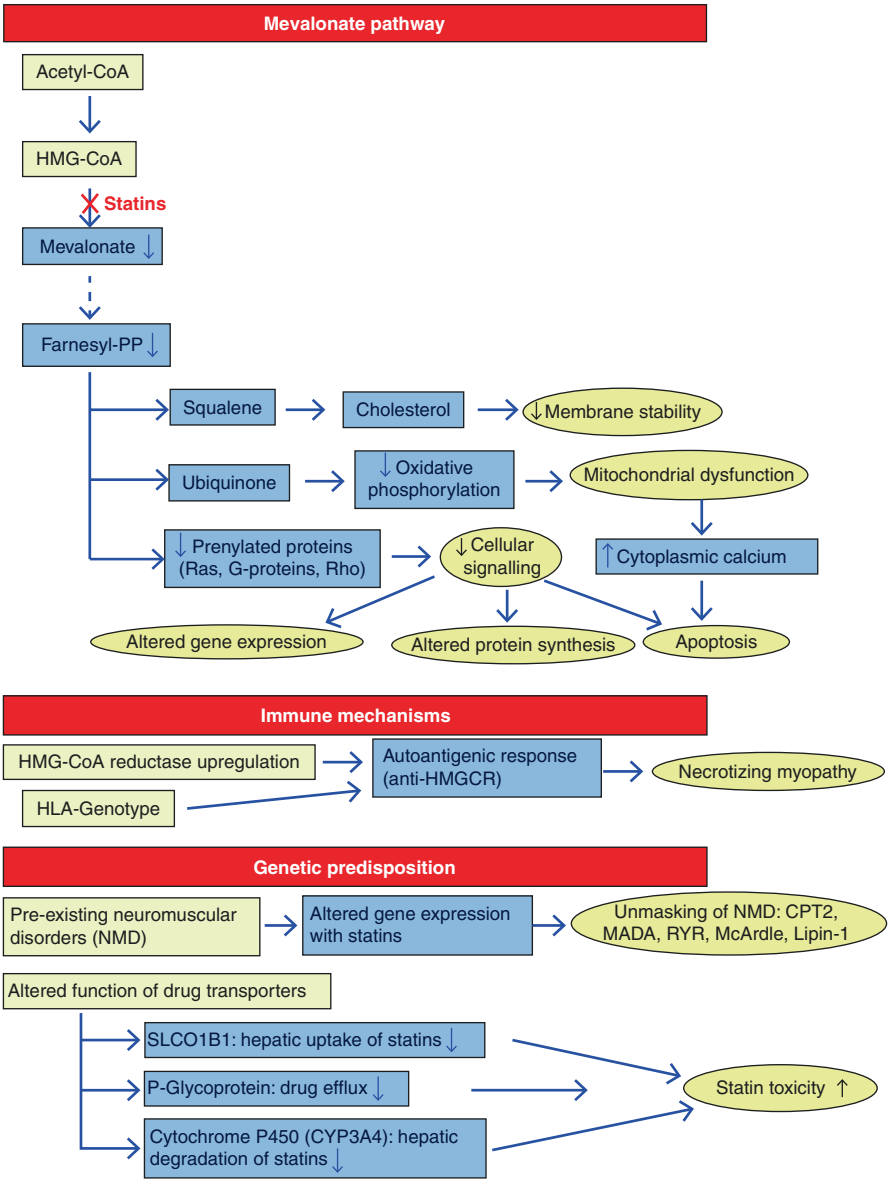

Fig. 8.1 Pathogenic mechanisms of statin myotoxicity

antibodies, only two-third were ever exposed to statins [29]; interestingly, the latter responded better to immunosuppressive therapy than the statin-naïve patients [30]. The antibody was never found in asymptomatic statin users.

- Genetic predisposition to statin myotoxicity may be caused by pathogenic heterozygous variants in muscle disease-related genes [31]. These include CPT2-deficiency, Pompe disease, McArdle disease, Lipin1-deficiency and malignant

hyperthermia. The most significant associations of statin myopathy with genetic polymorphisms have been reported for a hepatic transporter, encoded by SLCO1B1 [32], and for genetic variants in the detoxifying cytochrome P450 system [33]. A particular SLCO1B1 polymorphism is associated with an 18% risk of developing a statin myopathy in homozygotes, a 3% risk in heterozygotes and a 0.6% risk in wild-type carriers [32]. A further association was verified for polymorphisms in the CoQ2-gene, which is involved in CoQ10 biosynthesis [34].

It should, however, be kept in mind that the benefit of reduced cardiovascular mortality far outweighs the risk of statin-related myopathy, even in those with the highest genetic risk known so far, i.e., the SLCO1B1 variants. Therefore, routine genotyping cannot be recommended.

8.5 Clinical Evaluation of Suspected Statin Myopathy

8.5.1 Clinical Features

Statin-induced myalgia and myopathy typically present as proximal, symmetric muscle pain and/or weakness, especially in the legs; nocturnal cramping is also common [17, 35]. The mean duration of statin therapy before symptom onset was 6 months; in one-third of patients, symptoms started within 1 month [35]. The mean interval to recovery after cessation of treatment was 2 months; 57% of patients reported resolution of symptoms after 1 month and 93% after 6 months [35]. The symptoms appear more frequently in exercising individuals and are often triggered by physical activity [10].

If symptoms persist for more than 6 months after discontinuation of the statin, alternative causes should then be investigated, i.e., underlying necrotizing or metabolic myopathies or heterozygous genetic variants thereof (Sect. 8.4).

A proportion of statin users shows an asymptomatic elevation of CK, usually <4x ULN, which resolves quickly after withdrawal of the drug. Some of these patients have mild morphological changes in a muscle biopsy [36].

Rhabdomyolysis, the most severe form of statin intolerance, is very rare (<0.1%) and usually occurs with short delay after initiation of statins. It is characterized by severe muscle pain, weakness, very high CK and may lead to renal failure.

8.5.2 Diagnosis and Monitoring

The diagnosis of statin myotoxicity is usually straightforward and is based upon the temporal correlations of clinical symptoms and CK levels with initiation and termination of statin therapy (Sect. 8.5.1); sometimes a rechallenge of statin exposure may be necessary to firmly establish statin intolerance.

Current European guidelines [2] recommend to obtain a baseline CK in case symptoms develop, but there is no need for routine monitoring of CK, unless problems arise. In this case, CK should be measured to evaluate the severity of muscle damage and to decide whether treatment should be discontinued.

Electromyography, nerve conduction studies and MR imaging may be normal or show non-specific abnormalities; their main purpose is exclusion of other differential diagnoses.

Muscle biopsy is not routinely performed [2], except in those with persisting symptoms or hyperCKemia despite cessation of statin medication. It is then necessary to investigate for autoimmune necrotizing myopathy because this specific complication requires immunosuppressive therapy [30].

8.5.3 Risk Factors for Statin Myopathy

The risk factors predisposing to statin-induced myopathy or myalgia can be classified into patient-related and drug-related factors (Table 8.3):

Amongst the patient characteristics, increased physical activity is probably the most important aspect to consider when statin patients complain of acute muscle symptoms [10, 37]. Other important risk factors are comorbidities, genetic polymorphisms and ethnicity (Asian descent carries a 3–4x increased risk).

In ALS, high cholesterol levels are associated with prolonged survival [38], and it seems prudent to stop statins in patients who develop ALS. Statins are also known to trigger muscle symptoms in cases of pre-existing muscle disease (Sect. 8.4), including myasthenia gravis, but at least in the latter case statin treatment is still regarded as safe, if required.

Amongst the drug characteristics, the statin dose is probably the most important predictor of side effects. Data from the SEARCH trial [19], comparing 80 mg of simvastatin with 20 mg of simvastatin, showed a minor decrease in efficiency with the lower dose but a 40 times higher frequency of myopathy with the higher dose. On the basis of this data, the FDA recommended not to use the higher dose any longer. The risk of myopathy appears to be lower with hydrophilic statins (fluvastatin, pravastatin, rosuvastatin) compared to lipophilic statins (simvastatin, lovastatin) because penetration into muscle tissue is related to lipophilicity. In the PRIMO study [10], fluvastatin appears to carry the lowest risk for myopathy.

In common with half of all the drugs which we take, most statins are metabolized by the cytochrome P450 (CYP3A4) system. Concurrent medication which inhibits CYP3A4 (Table 8.3) will therefore reduce clearance of CYP3A4-dependent statins (simvastatin, lovastatin, atorvastatin), thus increasing toxicity. Fluvastatin, pravastatin and rosuvastatin are metabolized via CYP2C9 and demonstrate less interactions with other drugs.

Increased susceptibility to myopathy is also seen in combination with fibrates [39], in particular gemfibrozil, niacin and drugs which are independently myotoxic (steroids, cyclosporine, zidovudine).

8.6 Management of Statin-Induced Myopathies

Following a recent consensus statement of the European Atherosclerosis Society [39], the first step should always be to reassess the indication for statin use and to evaluate whether the risk factors can be minimized (Table 8.3). Thereafter, the following scenarios are possible:

8.6.1 Creatine Kinase <4x ULN

(a) Tolerable symptoms:
The statin may be continued; symptoms and CK should be monitored regularly and used as guideline for possible discontinuation of treatment.

(b) Intolerable symptoms:
The statin should be discontinued regardless of CK levels because compliance for taking the drug will be low.

If symptoms persist after a 4-week washout phase and the risk-benefit ratio warrants further treatment, the following options exist:

- Restart with lower dose of statin [19]
- Restart with less myotoxic statin (pravastatin, fluvastatin, rosuvastatin) [10]
- Try alternate-day dosing with long half-life statin (atorvastatin)
- Vitamin D deficiency should be corrected: some evidence supports vitamin D supplementation [40].
- CoQ10 administration (600 mg/day): a recent large meta-analysis [41] failed to show any benefit of CoQ10 supplementation.

If symptoms improve after discontinuation of the statin, treatment can be recommenced with either the same statin dose or according to the above protocol.

8.6.2 Creatine Kinase >4x ULN and <10x ULN

The statin should always be stopped and the need for treatment be reassessed. If considered necessary the statin may be restarted following the above options, once CK and symptoms have normalized. CK levels should then be continuously monitored and that particular treatment regimen be stopped if the levels exceed 10x ULN. An alternative regimen may be tried again or a non-statin-based therapy may be employed.

If CK persists to be high, the possibility of an underlying neuromuscular disease, in particular a necrotizing myopathy (Sect. 8.5.2), should be considered.

8.6.3 Creatine Kinase >10x ULN

Statin treatment should be stopped and renal function and risk factors be checked. If the CK level returns to normal, a second attempt with a lower dose of a different statin may be undertaken with careful monitoring of CK.

In case of rhabdomyolysis (affecting renal function), no further statins should be tried, and a non-statin-based lipid-lowering therapy be considered.

Following this algorithm (Sects. 8.6.1–8.6.3), 43% of patients with statin intolerance were eventually able to continue statin treatment with another lower dose statin [35].

8.6.4 Complete Statin Intolerance

Even though rare (<0.2% of statin users), alternative non-statin-based lipid-lowering therapies may become necessary in case of complete statin intolerance.

Ezetimibe, an intestinal cholesterol uptake inhibitor, is the first choice for these patients and may sometimes permit the use of statins concomitantly at low enough doses to limit muscle damage.

Other less efficient alternatives are bile acid sequestrants (cholestyramine), fenofibrate or niacin, which should be used in combination with ezetimibe.

Recently, PCSK9 inhibitors (alirocumab) which target LDL receptors for degradation, were approved by the FDA and EMA. Studies have consistently shown large LDL reductions with a very low rate of muscle symptoms [42] which makes these drugs a good alternative for statins.

Bempedoic acid represents a new class of cholesterol-lowering drugs. It antagonizes ATP citrate-lyase, a cytosolic enzyme upstream of HMGCoA reductase thus reducing cholesterol biosynthesis. The conversion of bempedoic acid to its active metabolite is restricted to the liver, which greatly reduces the risk of skeletal muscle side effects. In one study, myalgias were reported in 3.1% of cases. In combination with ezetimibe, bempedoic acid might therefore be a promising alternative for patients with statin intolerance. Finally, LDL apheresis may pose an option for statin-intolerant patients with very high LDL cholesterol levels, but this is reserved for the most severe cases.

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Myasthenia Gravis

9

Amelia Evoli and Raffaele Iorio

9.1 Introduction

At the normal NMJ, the motor nerve ending (presynaptic region) and a specialized portion of muscle membrane (postsynaptic region) are juxtaposed, being separated by a ~50 nm width, termed synaptic cleft. This space comprises the basal lamina that has a central role in NMJ formation, securing a stable concentration of synaptic proteins, both nerve-derived (as agrin, neuregulin) and muscle-derived (as laminin β-2) and of the enzyme acetylcholinesterase (AChE) [1]. AChE is expressed in an asymmetric form composed of tetramers of catalytic subunits attached to a collagen tail ColQ that anchors the enzyme through binding both perlecan and the muscle protein MuSK (muscle-specific tyrosine kinase receptor) [2].

In the nerve terminal, synaptic vesicles accumulate at the active zones where P/Q-type voltage-gated calcium channels (VGCC) are clustered. Each vesicle contains 5000–10,000 molecules of acetylcholine (ACh) and is referred to as a quantum. The postsynaptic membrane is folded into secondary synaptic folds which greatly increase its area. At the crest of the folds, the acetylcholine receptors (AChRs) are assembled at a high density (10,000–20,000/μm^2), anchored to the dystroglycan complex through rapsyn [1]. The AChR clustering and the maintenance of NMJ require MuSK activation by agrin through its co-receptor LRP4 (low-density lipoprotein receptor protein 4) [3].

When an action potential (AP) depolarizes the nerve terminal, the opening of VGCCs results in a rapid increase of the intra-nerve Ca^{2+}concentration, which triggers the exocytosis of 20–200 quanta. The binding of two ACh molecules leads to a conformational change in the AChR and opens the ion channel; the influx of Na^+ results in a local membrane depolarization, end plate potential (EPP), which is

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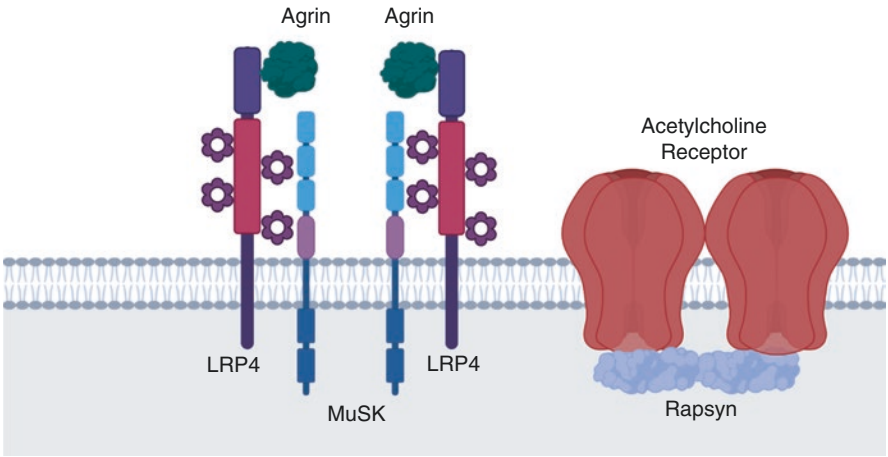

Fig. 9.1 Antibody targets in myasthenia gravis: structure and interactions with related molecules

greatest in the depths of secondary folds where voltage-gated sodium channels (VGSC) are highly concentrated. When the EPP is adequate to open these channels, a muscle AP ensues.

At the normal NMJ, the EPP largely exceeds the threshold for the generation of a propagated muscle AP. This corresponds to the safety factor of neuromuscular transmission (NMT), that depends on presynaptic (number of quanta released per each nerve depolarization) and postsynaptic (AChR and VGSC density) factors. NMT diseases are characterized by an alteration, generally a reduction, of the safety factor.

Myasthenia gravis (MG), the most common of these disorders, is caused by antibodies (Abs) to proteins of the postsynaptic membrane or the extracellular matrix (Fig. 9.1). Abs to the AChR are detected in the great majority of patients, 5–8% have Abs against MuSK, and a lower proportion of patients harbor Abs to LRP4 or agrin [4]. The autoimmune attack causes morphologic and functional alterations, responsible for NMT impairment, which results in fatigable weakness of voluntary muscles.

9.2 Epidemiology

MG affects all races and can onset at any age, from the first year of life to the 90s. Epidemiological investigations have mostly been focused on the AChR-positive disease (AChR-MG). On the whole, these studies show a broad variability both in incidence that varies from 4.3 to 18.0 per million, and in prevalence rate, ranging from 70.6 to 163.5 per million [5]. In Western countries, AChR-MG typically shows a bimodal age-of-onset, with predominance of women among early-onset cases (between the second and fourth decade) and of men in a more advanced age; childhood MG with purely ocular symptoms is common in Asian populations.

The positivity rate of MuSK Abs in AChR-negative patients varies across populations, with higher rates in Mediterranean countries in Europe, and among Afro-American patients in the USA [6]. In two nationwide studies, MuSK-MG prevalence was 1.9 per million in the Netherlands and 2.9 per million in Greece [5]. The disease shows a marked prevalence in women with an average age at onset in the mid-30s.

The positivity rate of LRP4 Abs varies in different studies. In a large series of 635 patients, the overall frequency of LRP4 Abs in AChR- and MuSK-negative patients was 18.7%, with some variability among populations from different countries [7]. LRP4-MG appears to be prevalent in women (male/female ratio of 1:2) with mean age of onset in the fourth decade.

9.3 Pathogenesis

9.3.1 MG with Abs to AChR

The AChR is a pentameric ion channel with a stoichiometry $2\alpha1\beta1\gamma\delta$ in embryonic/denervated muscle and $2\alpha1\beta1\varepsilon\delta$ in normal adult muscle. Although AChR Abs are polyclonal and can recognize all receptor subunits, epitope mapping studies have shown that a high proportion of patients have serum Abs to the so-called main immunogenic region (MIR) on the extracellular domain of each α1 subunit [8]. Abs to MIR are highly pathogenic, and their serum level was shown to correlate better with disease severity than total AChR Ab titer [8].

AChR Abs mostly belong to IgG1 and IgG3 subclasses and impair NMT through complement-mediated destruction of the postsynaptic membrane, increased AChR degradation by receptor cross-linking, competition with ACh binding [4]. Their pathogenicity has been fully demonstrated in experimental MG studies, both by active immunization and patients' IgG injection.

AChR-MG is frequently associated with alterations of the thymus, the organ where T cell maturation and establishing of central tolerance occur. Most patients with early-onset MG (age of onset <50 years) have thymic follicular hyperplasia characterized by expansion of the perivascular spaces with prominent B cell and plasma cell infiltration and germinal center formation. The hyperplastic thymus is thought to be the site where the auto-sensitization against AChR occurs and Ab production is initiated [9]. An intra-thymic inflammatory milieu, possibly induced by infectious agents, together with immune-regulatory defects and a predisposing genetic background concur to the establishing of the autoimmune response [10]. Early-onset AChR-MG is associated with human leucocyte antigens (HLA) B8 and DR3 [10]. A thymoma is present in 10–20% of AChR-MG patients, with the highest frequency between the fifth and seventh decades of life. Thymomas are tumors of thymic epithelial cells harboring variable proportions of non-neoplastic lymphocytes. Thymomas associated with MG are prevalently of "cortical" types with a rudimental medulla and retain the capacity to export mature T cells [10]. Tumor tissue does not produce AChR Abs, but through a defective T cell selection can contribute to MG pathogenesis by the export of auto-reactive $CD4^+$ T cell and a

reduced production of regulatory T cells [9]. Lastly, in patients with late-onset disease, the thymus parenchyma is mostly replaced by fat, even though some B cell infiltration and occasional germinal centers can be found [9].

9.3.2 MG with Abs to MuSK

MuSK is a transmembrane protein, made of an extracellular region consisting of three immunoglobulin-like (Ig-like) domains and a cysteine-rich domain, a transmembrane helix, and a cytoplasmic region harboring the kinase activity. MuSK activation by neuronal agrin triggers an intracellular signaling cascade leading to AChR and rapsyn clustering [3]; in addition, its binding to ColQ carboxyl-terminal anchors AChE to the basal lamina [2].

Abs to MuSK are prevalently IgG4 and target mostly the first two Ig-like domains in the extracellular region [11]. Although IgG4 do not activate complement and are inefficient in cross-linking adjacent antigens, IgG4 MuSK Abs were found to correlate with disease severity in patients [12], and induced MG weakness when injected into mice [13]. These Abs were shown to interfere with MuSK-LRP4 binding, thus inhibiting MuSK activation and AChR clustering [14], and to block MuSK-ColQ binding, causing a reduced AChE concentration at the synaptic cleft [15]. In addition, immunized animals showed a presynaptic dysfunction as lack of compensatory increase in ACh release, which is a homeostatic response in AChR-MG [13].

The thymus does not appear to be involved in MuSK-MG pathogenesis, as pathological examination of specimens from thymectomized patients did not show hyperplastic changes and the association with thymoma has rarely been reported [9]. An association with HLA DR14/16 and especially with DQ5 has consistently been reported in these patients [10].

9.3.3 MG with Abs to LRP4

LRP4 belongs to the low-density lipoprotein (LDL) receptor family and is expressed in several tissues. At NMJ, LRP4 acts at both pre- and postsynaptic level, as it enhances MuSK activation through binding agrin and, in a retrograde manner, stimulates nerve terminal differentiation [16]. LRP4 is a transmembrane protein consisting of a large extracellular region with multiple LDL repeats, epidermal growth factor (EGF)-like and β-propeller domains, a transmembrane helix, and a short cytoplasmic region. The extracellular region binds both agrin and MuSK [17].

Abs to LRP4 are mostly IgG1 and were shown to interfere with agrin binding [18]. Immunization with LRP4 ectodomain induced muscle weakness, AChR cluster fragmentation, and both pre- and postsynaptic NMT dysfunction [19]. A thymoma has never been found in Lrp4-MG. Though some of these patients were reported to have thymic hyperplasia, there is no evidence of a pathogenic link with the thymus.

Abs to agrin [20], ColQ [21], and Cortactin [22] have been reported in MG patients, often in association with either AChR or MuSK Abs. Their pathogenicity has not been proved, so far, in animal models.

9.4 Clinical Features

The hallmark of MG is fatigable weakness of skeletal muscles. Fatigability is the most consistent feature; weakness is usually present on examination but, in mildly affected cases, may be evident only on exertion. Clinical fluctuations, both daily and over longer periods, are typical. Although all voluntary muscles can be affected, some muscle groups are more commonly involved than others, and clinical presentation is quite characteristic. However, there is a marked variability in weakness extension and severity, from purely ocular symptoms to severe life-threatening disease.

The extrinsic ocular muscles (EOM) are affected in the great majority of patients, and ptosis and diplopia are the most common presenting symptoms. Ptosis is generally asymmetrical (Fig. 9.2, section A) and frequently alternating; it typically fluctuates in severity over short periods. Binocular diplopia can be caused by weakness of a single muscle or of any EOM combination. It is usually intermittent in the early stages of the disease, then tends to become constant. The association of variable diplopia and asymmetrical ptosis is useful in differentiating ocular MG from oculopharyngeal dystrophy, chronic progressive ophthalmoplegia, and thyroid myopathy.

In around 15% of patients, MG remains confined to EOM; in the other cases, usually within 2 years from the onset, weakness spreads to other muscle groups [23]. Facial weakness is very common, with the inability to close the eyes tightly and to whistle, and development of a vertical smile (Fig. 9.2, section B). In limbs, proximal muscles are prevalently involved; weakness of finger extensors is relatively frequent, while ankle dorsiflexion is more rarely affected. Weakness of "bulbar" muscles (masseter, tongue, pharyngeal and laryngeal muscles) is responsible for difficulty in chewing, dysphagia with regurgitation of fluids through the nose, and dysarthria (nasal speech). Among axial muscles, both neck flexors and extensors are involved. Respiratory failure requiring assisted ventilation (the so-called myasthenic crisis) is due to weakness of the diaphragm and intercostal muscles

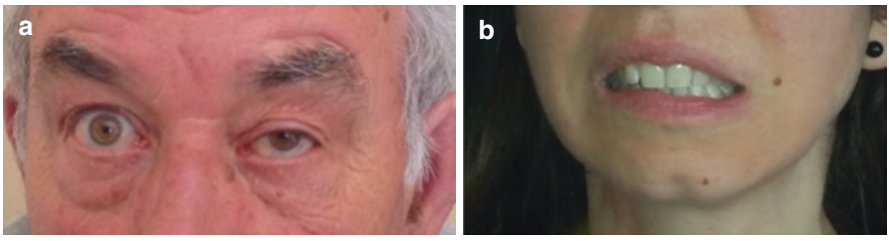

Fig. 9.2 Asymmetrical ptosis in (**a**). Facial weakness with a vertical smile in (**b**)

together with upper airway obstruction by bronchial secretions. Crises occur in 15–20% of patients, and, in spite of improvement in MG treatment and critical care, the related mortality rate is still 5% [24]. Although AChR-MG encompasses the whole clinical spectrum, weakness pattern shows some differences in patient subgroups. Leg muscle involvement is often predominant in younger patients; bulbar and neck weakness are frequent in late-onset disease; early respiratory crises are more common in thymoma-associated MG.

MuSK-MG is nearly always a generalized disease. In most patients, clinical phenotype is characterized by a prevalent involvement of bulbar and axial muscles, with dysarthria, dysphagia, weakness of the tongue, facial, and neck muscles. Limbs are mildly affected and can be totally spared [25]. Ocular symptoms are common at presentation, but diplopia is generally transient, and ptosis is less asymmetrical than in AChR-MG. Myasthenic crises and muscle atrophy are more frequent than in other forms of MG [26]. Muscle atrophy mainly affects facial, tongue, and masseter muscles and can lead to fixed weakness with permanent dysarthria and a myopathic face. Lastly, daily symptom fluctuations are uncommon in these patients, who, however, suffer from frequent MG deteriorations, especially in the first years from the onset [26].

The characteristics of LRP4-MG are not fully defined, but the clinical phenotype in these patients seems to be similar to AChR-MG. In the largest population reported so far, around 22% of patients had purely ocular symptoms and those with generalized MG were prevalently affected by mild to moderate weakness [7].

9.5 Diagnosis

Once MG is suspected on clinical grounds, diagnosis confirmation is achieved through serum Ab detection, electrophysiological evidence of a postsynaptic defect of NMT, clinical response to acetylcholinesterase inhibitors (AChE-I).

9.5.1 Serum Ab Assay

AChR Abs are detected in 85–90% of patients with generalized MG, in around 50% of those with ocular disease and in nearly all cases of thymoma-associated MG [4]. Therefore, these are the first Abs to be tested when MG is suspected. All patients with negative results on this assay, should be tested for MuSK Abs, taking into account that the latter are rarely associated with isolated ocular symptoms. AChR and MuSK Abs are very specific [4], and, in practice, their detection in patients with congruent symptoms confirms the diagnosis.

The positivity rate of AChR Abs has been further increased by the demonstration that some patients have serum IgG unable to bind the AChRs when in solution as in the standard radio-immunoassay (RIA), but able to bind to the receptors when concentrated on cell surface, as they are in vivo at the NMJ. With a cell-based assay (CBA), using human embryonic kidney cells transfected with AChR subunits and

Rapsyn serum Abs to "clustered" AChR were found in a proportion of patients negative on the standard assay, predominantly associated with ocular myasthenia and onset in childhood [27].

Abs against LRP4 have been detected with different techniques at varying frequencies in AChR- and MuSK-negative patients. The recent report of these Abs in a high proportion of patients with amyotrophic lateral sclerosis (ALS) casts doubt upon their specificity for MG [28].

The diagnostic value of Abs against agrin and ColQ is not yet defined. Moreover, while the standard RIA for AChR and MuSK Abs is largely available, other Abs can be tested only in selected laboratories.

AChR-MG is associated with striated muscle (striational) Abs that recognize intracellular proteins, as titin and the ryanodine receptor (RyR). These Abs are strongly associated with thymoma (titin Abs are positive in 95% and RyR Abs in 70% of thymoma patients), are present in nearly 50% late-onset non-thymoma patients, while are very uncommon in early-onset MG. Striational Abs are not diagnostic of MG and presumably not pathogenic, but are markers of thymoma in younger MG patients, and seem to correlate with disease severity [29]. Abs to Kv1.4 that target the muscle voltage-gated potassium channel were found to be associated with severe MG and myocarditis in Japanese patients [30].

9.5.2 Electrophysiological Studies

Repetitive nerve stimulation (RNS) is the most frequently used technique in the electrophysiology of NMT. In MG, low-frequency (2–3 Hz) RNS is typically associated with a decrement, greater than 10%, of the compound muscle AP (CMAP) amplitude between the first and fourth or fifth stimulus. RNS diagnostic yield depends on testing weak muscles and is thus related to weakness pattern and severity. The rate of positive results is close to 75% patients with generalized MG and less than 50% in those with isolated ocular symptoms [31]. In MuSK-MG, on account of the predominant bulbar involvement, diagnostic sensitivity is low, unless facial muscles are examined [32]. A decremental response on low-frequency RNS is not specific for MG as it is found in other primary disorders of NMT and in some patients with ASL or radiculopathy [31].

Single fiber electromyography (SF-EMG) records APs from single muscle fibers and measures jitter during voluntary activation or nerve stimulation. In volitional SF-EMG, jitter corresponds to the time interval variations between pairs of APs from 2 or more muscle fibers belonging to one motor unit. When NMT is impaired as in MG, increased jitter and "impulse blocking" (when EPP does not reach the threshold to generate an AP) occur [31]. SF-MG is the most sensitive diagnostic test for MG, as, provided that appropriate muscles are examined, positive results are recorded in 98% of cases, including patients with ocular myasthenia [33] or MuSK-MG [26]. However, an increased jitter is far from specific as, apart from other diseases of NMT, it can be found in neurogenic and myopathic conditions [31].

9.5.3 Pharmacological Test (Response to AChE-Is)

In MG, AChE-Is improve NMT by increasing the lifetime of ACh that can bind repeatedly to AChRs. Short-acting agents, as edrophonium chloride i.v. and neostigmine i.m., are generally used for diagnostic purposes. Response should be evaluated on selected weak muscles and compared with reaction to placebo. With these prerequisites, a definite clinical improvement, although not specific, strongly supports the diagnosis. As edrophonium injection can be associated with broncho-constriction and severe bradycardia, atropine should always be kept at reach.

In patients with MG, the overall rate of positive responses of edrophonium/neostigmine testing is around 90% [34]. However, in MuSK-MG, improvement upon AChE-I injection is much less common (50–70%), side effects, such as muscle cramps and fasciculations, are frequent, and symptom worsening can be observed [26]. Cholinergic hypersensitivity in MuSK-MG can be ascribed to a relative deficiency of AChE at the synaptic cleft as a result of Ab interference with MuSK-ColQ binding [15].

A positive reaction to edrophonium/neostigmine test is observed in congenital myasthenic syndromes (CMS) and, less frequently, in Lambert-Eaton myasthenic syndrome. “False” responses have been reported in ALS and Guillain-Barrè syndrome [35].

Upon MG confirmation, all patients should undergo a radiological study of the thymus to rule out a thymoma, together with a screening for other autoimmune diseases (especially thyreopathies) and medical conditions that could interfere with treatment.

9.6 Treatment

Treatment decisions are based on weakness extension and severity, pathogenic aspects (associated Abs, thymus pathology), and patient’s characteristics. Current treatment, although largely unspecific, has dramatically reduced mortality and restored lifestyle to normal in many patients.

9.6.1 Symptomatic Treatment

Oral AChE-Is represent the first-line treatment, pyridostigmine bromide (Mestinon) being the agent most commonly used. In general, MG patients respond to AChE-Is, even though a satisfactory control of symptoms can be achieved in a minority of cases. Treatment is usually well tolerated and adverse effects (gastric discomfort, diarrhea, salivation, and cramps) are mild and can be reversed by dose reduction. On the other hand, MuSK-MG patients might often show both unresponsiveness to and intolerance of AChE-Is, as—with Mestinon standard doses—develop signs of cholinergic hypersensitivity [36] that may progress to weakness worsening (due to

depolarization block) and respiratory failure [26]. Cholinergic crises are unusual or rare in AChR-MG, appear associated with AChE-I overdosage.

Both 3,4-diaminopiridine and albuterol proved effective and well tolerated in MuSK-MG animal models [37]. A preliminary study has reported positive responses to 3,4-diaminopiridine in patients with MuSK Abs [38], and this agent is currently being evaluated in a randomized controlled trial (RCT) (ClinicalTrials.gov Identifier: NCT03304054). Albuterol has not been tested so far, in formal studies, in these patients.

9.6.2 Thymectomy

Thymectomy, when feasible, is indicated in all thymoma cases irrespective of MG severity. In the absence of a thymoma, thymectomy has been in use for several decades in patients with generalized MG as an option to increase the probability of remission and improvement [39]. Its efficacy in adult-onset non-thymomatous AChR-MG has been recently proved in an RCT comparing thymectomy plus prednisone with prednisone alone. Patients undergone thymectomy achieved significantly better results in both primary endpoints (reduced disease severity and alternate-day prednisone requirement) and in several secondary outcomes [40]. The indication to surgery in the other disease subtypes is highly controversial. In particular, in MuSK-MG clinical studies failed to show significant differences in outcome measures between thymectomized and unthymectomized patients [41].

Lastly, it is worth pointing out that thymectomy, even in patients with thymoma, is never to be considered an emergency treatment, and should be performed once stable control of the disease has been achieved.

9.6.3 Short-Term Immunomodulation

Plasma exchange (PE) and intravenous immunoglobulin (IVIg) that induce a rapid albeit temporary improvement are mostly used in the treatment of MG exacerbations. In addition, both (particularly IVIg) are used as periodic treatment in selected cases unresponsive to immunosuppression. In two randomized trials, PE and IVIg were shown to have comparable efficacy in an acute setting [42, 43]; there is no evidence for IVIg superiority over steroids in chronic treatment.

PE protocol consists of 3–5 exchanges performed every other day. Serious complications are mainly related to central venous catheters [44]. Semiselective immunoadsorption, that does not remove albumin and coagulation factors, can be a safer alternative in patients requiring frequent PE. IVIg is administered at a dose of 400 mg/kg/day for 2–5 days. It is generally well tolerated although serious complications have occasionally been reported [45]. Subcutaneous Ig (SCIg) can be a valid alternative to IVIg, especially in patients requiring chronic treatment. Encouraging results have been reported by retrospective studies [46] and an open-label trial [47].

9.6.4 Immunosuppressive Therapy

Immunosuppressive therapy is performed when symptoms are not adequately controlled with AChE-Is. The initial goal is to improve MG as quickly as possible; thereafter, medications should be reduced to the minimum effective dose to minimize side effects. From these principles, steroids are still the first treatment because of their rapid-onset effect, in chronic administration immunosuppressants are associated as steroid-sparing agents.

9.6.4.1 Steroids

Prednisone and prednisolone are the agents mostly used in MG. They are generally administered on a daily basis at the start of treatment, then shifting to an alternate-day regimen with slow dose reduction. In most cases, ocular myasthenia can satisfactorily be managed with low-dose prednisone (25 mg/day as starting dosage), while in patients with generalized MG higher doses (0.75–1 mg/kg/day) are employed. As steroids may induce a temporary MG deterioration, in patients with generalized disease treatment should be started in the hospital, and PE or IVIg may be given to reduce symptom severity. The association of high-dose steroids plus P-E or IVIg is also the standard treatment for severe bulbar symptoms or respiratory crises.

Steroids are effective in around 80% of MG patients [4], but symptom relapses are frequent on dose tapering and chronic administration entails the risk of several side effects.

9.6.4.2 Immunosuppressants

Several immunosuppressants are used in the treatment of MG and appear to be effective in the great majority of patients although class I evidence is still limited. All these agents have a long-latency effect; they can be administered in combination with steroids from the beginning and can replace prednisone in long-term treatment. Close monitoring of side effects is recommended and because of the potential risk of infections and malignancy, the lowest maintenance dose should be determined in each patient [4].

In many countries, the purine analog azathioprine is the first-choice immunosuppressant in MG, at a starting daily dose of 2.5–3 mg/kg and a maintenance dose of 1 mg/kg. Leukopenia and hepatotoxicity are the main adverse effects, which usually subside with dose reduction or withdrawal. As patients with thiopurine methyl transferase (TPMT) deficiency may develop severe bone-marrow toxicity, TPMT activity should be measured before treatment.

Mycophenolate mofetil (MMF) inhibits T and B cell proliferation, with higher specificity than azathioprine for activated lymphocytes. At the standard daily dosage of 2–2.5 g, it resulted effective in retrospective analyses and open-label trials. Although, these results were not confirmed in two randomized studies [48, 49], MMF, also in view of its favorable toxicity profile, is largely used in patients unresponsive to or intolerant of azathioprine.

Of calcineurin inhibitors, both cyclosporine and tacrolimus were shown to improve MG in small randomized trials [4]. The use of cyclosporine (at an initial dose of 4–5 mg/kg and a maintenance dose ≤3–4 mg/kg) is limited by side effects, as nephrotoxicity and hypertension. Tacrolimus seems to be relatively safe at the doses used in MG and can be used as third-line drug. In a recent single-blinded study, methotrexate was found to be effective as steroid-sparing agent, with similar efficacy and tolerability to azathioprine [50]. The use of cyclophosphamide on account of significant toxicity is mostly reserved to patients with severe refractory disease.

Immunosuppression in MG as in other autoimmune diseases has been rapidly evolving with the introduction of biologics, mostly monoclonal Abs (mAbs). These agents are promising in view of their specific immune targets. Their use is limited so far to MG refractory to conventional treatment.

In uncontrolled studies, rituximab, an anti-CD20 mAb that causes a marked depletion of B cells, was generally found to be effective in MG. A small phase 2 RCT (NCT02110706) failed to show outcome differences between rituximab and placebo in AChR-MG patients (who were mostly affected by mild to moderate disease) [51]. In clinical studies and metanalyses, rituximab appears to be highly effective in MuSK-MG [52–54] and has been proposed as an early option in these patients after failure of first-line immunosuppression [55].

Add-on treatment with eculizumab, a humanized mAb that inhibits cleavage of C5 in the terminal phase of complement activation, was evaluated in a phase 3 RCT. This study did not meet the primary endpoint, while most secondary outcomes and a pre-specified-sensitive analysis clearly favored eculizumab [56] that has since been approved for the treatment of refractory AChR-MG.

9.6.4.3 Therapies in Developmental Stage

Second-generation C5 inhibitors, ravulizumab and zilucoplan, are currently being evaluated in a phase 3 (NCT03920293) and a phase 2 RCT (NCT04025632), respectively.

Antagonists of the neonatal Fc receptor (FcRn) constitute a new class of drugs under investigation in MG. FcRn has a crucial role in prolonging the half-life of serum IgG preventing its lysosomal degradation. These agents compete with IgG in binding FcRn, leading to an increased serum IgG catabolism that includes pathogenic Abs [57]. Efgartigimod, an anti FcRn IgG1 Fc fragment, was evaluated in a phase 2 RCT including AChR-MG patients on stable standard-of-care treatment. This agent proved to be well-tolerated and associated with a rapid decrease in total IgG and anti-AChR Ab levels [58] and is currently the object of a phase 3 RCT (NCT03669588). Two additional FcRn inhibitors Rozanolixizumab (NCT03052751) and M281 (NCT03772587) are under evaluation in phase 2 trials. Second-generation and third-generation anti-B cell agents have not been tested, so far, in MG.

Targeted immunotherapy with biologic agents is a promising development in MG. Well-designed RCTs and careful assessment of side effects are required to justify these agents' high cost particularly in chronic treatment.

Highlights

- Myasthenia gravis (MG) is a heterogeneous disease, in which different antibodies affect neuromuscular transmission.
- MG with antibodies to AChR is frequently associated with thymus pathology.
- MuSK antibodies should be tested in all AChR-negative patients.
- In patients without detectable antibodies, other conditions that can mimic MG must be carefully ruled out.
- Treatment strategy should be individualized, taking into account weakness severity, patient's characteristics, and pathogenic aspects.
- The increasing availability of biologic agents may result in targeted immunotherapy for different disease subtypes.

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Endocrinological Myopathies

10

Corrado Angelini

Disorders in endocrinological pathways lead to manifest acquired or endogenous forms of myopathy. Imbalance disorders of protein synthesis, electrolytes, and carbohydrate can lead to severe forms of myopathies. The severity of endocrinopathies is important for the long-term outcome. In general, the main neuromuscular symptom is proximal weakness, sometimes in addition to myalgia and fiber atrophy. Endocrine myopathies are usually reversed by treatment.

10.1 Thyroid-Related Myopathies (Table 10.1)

Abnormalities in thyroid function are common endocrine disorders that affect 5–10% of the general population, with hypothyroidism occurring more frequent that hyperthyroidism [1].

10.1.1 Thyrotoxic Myopathy

The initial descriptions by Graves and von Basedow included muscle weakness and atrophy in the clinical picture of thyrotoxicosis. Engel [2] reviewed the histopathological changes in thyrotoxic myopathy, thyrotoxic periodic paralysis, and the clinical features of myasthenia gravis associated with thyrotoxicosis. Females tend to be more affected than males (ratio 3:1).

The incidence of weakness in thyrotoxic patients is estimated to be about 67%. In oriental races, 82% of thyrotoxic patients over 40 years of age have weakness, compared with only 50% of younger patients [3].

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Table 10.1 Metabolic changes in muscle in hyperthyroidism and hypothyroidism

Hyperthyroidism	Hypothyroidism
High basal metabolic rate	Decreased oxygen consumption
Increased mitochondrial oxidation	Decreased mitochondrial oxidation
Glycogen depletion	Increased glycogen
Accelerated protein degradation	Decreased protein turnover
Increased cathepsin activity	Decreased lysosomal proteases
	Reduced acid alpha-glucosidase

Thyrotoxic myopathy may occur in acute or chronic form [4]. In the acute form, the patient presents sudden onset of muscle weakness, dysphagia, dysarthria, sometimes associated with vomiting, diarrhea, tachycardia, and atrial fibrillation. The weakness of the patient is more severe than the atrophy of muscles and the thyrotoxicosis may be present for only a few weeks before the onset of weakness.

In the chronic form, the thyrotoxicosis may be relatively mild and of long duration. The onset is usually characterized by weakness and atrophy of the limbs and is commonly evident first in the upper arms. Proximal muscle weakness is observed in 63% of cases, while distal muscle weakness is uncommon (18% of cases). Severe cases may present also a bulbar muscle involvement. The EMG changes are myopathic and there is no elevation of serum creatine kinase, in contrast with hypothyroid myopathy. Cases of reversible motor neuron dysfunction in thyrotoxicosis were described [5].

Ocular involvement is sometimes associated with thyrotoxicosis. The main clinical signs of thyroid-associated ophthalmopathy include exophthalmos, eyelid retraction, double vision, and optic neuropathy. Elevation and abduction are the most severely compromised eye movements. Magnetic resonance imaging reveals muscle enlargement in the majority of patients with Graves' disease. Orbital myositis might be distinguished from Graves' ophthalmopathy by tendon expansion but less prominent muscle enlargement.

Physiological and metabolic mechanisms. The changes are more prominent in slow-twitch muscle and result in a shift of the myosin ATPase activity toward that characteristic of fast-twitch muscle; there is an increased rate of calcium uptake by the sarcoplasmic reticulum. Thyroid hormone might increase potassium efflux and causes potassium depletion in muscles. The loss of muscle potassium could account for depolarization of thyrotoxic muscle fibers [6]. Thyrotoxic muscle tends to be depleted of glycogen. Thyroid hormones increase skeletal muscle heat production and the mitochondrial consumption of oxygen, pyruvate, and malate. Although this increased oxidation and accelerated metabolism contributes to heat production, there is no direct evidence that thyrotoxicosis uncouples muscle mitochondria. Thyrotoxic patients also develop insulin resistance [7]. Satoyoshi et al. [3] found decreased creatine and creatine phosphate in hyperthyroid muscle. In addition, thyroid hormone stimulates protein degradation by increasing lysosomal protease activity, particularly cathepsins B and C [7].

Excessive amounts of thyroid hormone have the following effects (Table 10.1):

- Increased mitochondrial respiration, without uncoupling
- Accelerated protein degradation
- Enhanced calcium uptake by sarcoplasmic reticulum (SR)
- Glycogen depletion and decreased glucose uptake may cause fatigability
- Shortening of contraction time derives from accelerated myosin ATPase and fiber type adaptation
- Thyrotoxicosis induces depolarization of muscle fibers due to potassium depletion

All these mechanisms may contribute to the development of thyrotoxic myopathy in which histopathological changes are mild and consist only of simple muscle atrophy. Neuropathy associated with thyrotoxicosis is rare.

10.1.2 Thyrotoxic Periodic Paralysis (TPP)

The first descriptions of the association of periodic paralysis with hyperthyroidism were by Japanese authors [8]. Similar cases have been reported in China [9]. There seems to be a racial susceptibility, given that TPP is much more frequent in Orientals, and only sporadic cases have been observed in Caucasians. The disease is characterized by a triad of muscle weakness, hypokalemia, and thyrotoxicosis. The attacks have many similarities to the familial hypokalemic disorders. There is a profound weakness of the pelvic girdle and leg muscles lasting several hours. Cranial, speech, and swallowing muscle are not affected. The most important criteria used to differential diagnosis are the remission of paralytic attacks in TPP with appropriate antithyroid measures, including partial thyroidectomy if the attacks are due to a toxic adenoma. Hypokalemia is due to migration of potassium from the extracellular space into the myocyte [10, 11]: a constant positive arterio-venous difference of potassium during the development of paralysis was found in six TTP patients.

In TPP, the total exchangeable potassium was reduced slightly although there was no significant difference from the normal and thyrotoxic controls [10, 11]. Au and Yeung [12] found a periodic variation in the muscle sarcoplasmic reticulum (SR) calcium pump activity: both the calcium pump ATPase activity and the total amount of calcium uptake showed a decrease during TPP, and a reversion to normal following the attack. TTP has emerged as a primary channelopathy related to mutations in the *KCNJ18* gene, encoding Kir2.6 channel, some of which cause decreased potassium current density [13].

The morphological abnormalities observed by Schutta and Armitage [14] consisted of a vacuolar myopathy, with diffuse evidence of structural damage, proliferation of the T-tubule system, and vacuoles which were connected with it, and marked distension of the terminal sacs of SR with granular material. These vacuoles do not represent dilatation of the SR but may represent sequestrated areas of focal myofiber necrosis. Atypical mitochondria showing linear inclusions in the cristae, were also seen. Electrophysiological changes in TPP include sarcolemmal depolarization and inactivation of sodium channels leading to sarcolemmal inexcitability. The

paradoxical depolarization associated with hypokalemia in TPP remains unexplained. TPP resolves in over 90% of cases, with normalization of thyroid function, while in acute paralytic attacks the administration of i.v. potassium chloride is the treatment of choice.

10.1.3 Hypothyroidism

Clinical symptoms and signs are often nonspecific, but it is underestimated that muscle symptoms may represent the predominant or the only clinical manifestation, raising the issue of a differential diagnosis with other causes of myopathy [1].

Hypothyroidism is characterized by muscle weakness, stiffness, myalgia, cramps, easy fatigability, slow movements, delayed reflex relaxation, and the characteristic myxedema. Muscle disease can also occur in the absence of overt myxedema. There are two clinical syndromes characteristically described:

- Muscle hypertrophy in calves, thighs, neck, tongue, facial, hand muscles with weakness, and painful spasms occurring in adults (Hoffmann's syndrome).
- Painless muscle enlargement and slow movements have been observed in cretins. The features of this syndrome include intellectual disability, physical and osseous development, a peculiar *facies* and generalized muscle hypertrophy (Kocher-Debré-Sémélaigne syndrome or infantile hypothyroidism) [15, 16]. Myopathy of hypothyroidism is manifested therefore by muscle hypertrophy in lower extremity or generalized, myxedema, short stature. Occasionally, hypothyroidism may produce proximal weakness and atrophy.

Serum creatine kinase is elevated in almost all patients, whether or not other evidence of muscle disease is present. Rhabdomyolysis and acute compartment syndrome may be rarely observed [1]. EMG may be normal and demonstrates that the spasms are not manifestations of myotonia. Fibrillation and fasciculation are unusual, unless there is a coincident neuropathy. Biopsy of hypothyroid muscles usually shows mild structural changes, consisting in atrophy, necrosis, and hypertrophy of single muscle fibers and increased internal nuclei. Glycogen accumulation is usually found. Histochemical staining for oxidative enzymes in mitochondria may show prominent abnormalities (Fig. 10.1) and, at the ultrastructural level, mitochondrial swelling and inclusions have been found as well as lipoid granules. In the Kocher-Debré-Sémélaigne syndrome, type 1 fiber atrophy and abnormalities of oxidative enzymes were prominent [15]. Congenital and neonatal hypothyroidism can be detected early by neonatal screening test, and soon corrected otherwise they may cause cranio-stenosis and developmental delay.

Metabolic changes in hypothyroidism. Hypothyroidism causes reduced metabolism and protein synthesis. Proteotoxicity may be due to autophagy failure and/or dysregulation [17]. There is a decreased metabolic rate and oxygen consumption as a result of decreased mitochondrial oxidation capacity [7]. In human embryos, the exposure of thyroid hormone stimulated mitochondrial replication and energy

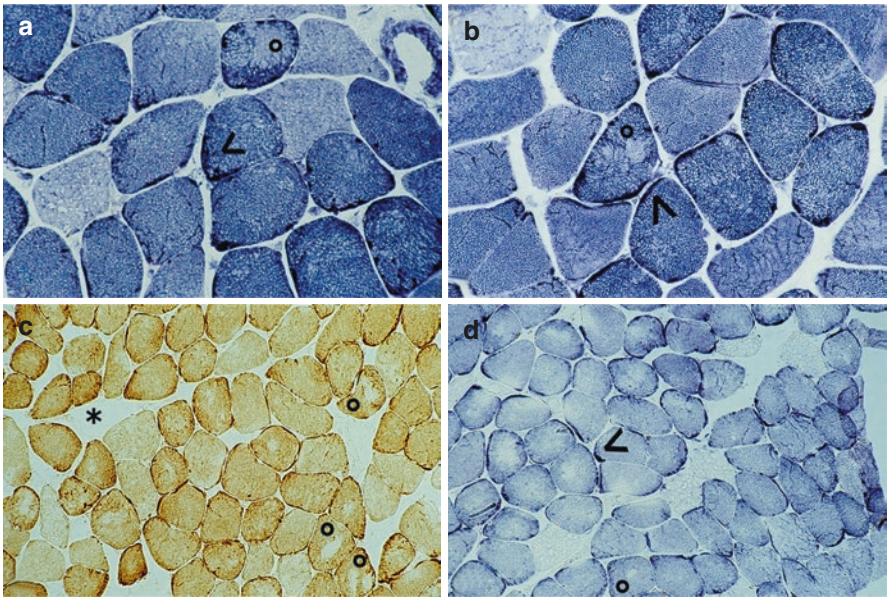

Fig. 10.1 Skeletal muscle pathology (deltoid) in a 77-year-old woman with hypothyroid myopathy, showing prominent abnormalities using histochemical stains for mitochondrial enzymes (**a**, **b**: NADH-TR reductase; **c**: cytochrome C oxidase; **d**: succinate dehydrogenase), consisting in rims of mitochondria in subsarcolemmal region (arrows), irregular distribution of mitochondria resembling moth-eaten, centralcore or minicore (circles), and rare COX-negative fibers (asterisk). Microscope magnification: 400× (**a**, **b**), 200× (**c**, **d**)

production by switching metabolism from the glycolytic pathway to more efficient oxidative phosphorylation [18]. Muscle glycogenolysis is also impaired, resulting in reduced ischemic lactate production, and contributing to muscle cramps and fatigability. The acid maltase level is decreased [19]. Reduced protein degradation is associated with decreased lysosomal protease activity, both of which normalize with thyroid hormone replacement.

Recently, reduced serum concentrations of a new adipo-myokine called irisin (encoded by the *FNDC5* gene) were found in patients with prolonged hypothyroidism, suggesting that they might primarily constitute the results of prolonged myopathy [20].

10.2 Parathyroid-Related Myopathy

10.2.1 Hyperparathyroidism

Hyperparathyroidism is mostly caused by a single benign parathyroid adenoma. Classic symptoms involve renal and/or skeletal muscle. Hyperparathyroid myopathy is more frequent in women than men. The first muscle symptoms are usually

easy fatigability and muscle hypotonia. Painful cramps in upper and lower limbs and back may be present. Muscle weakness usually involves proximal pelvic girdle muscles. In severe forms, there may be an involvement of muscle in hands, neck flexors, and facial. Gait may be rigid and slow.

Muscle biopsy shows type 2 fiber atrophy and occasional regenerative features.

10.2.2 Hypoparathyroidism

Muscle symptoms consist in rigidity and cramps in muscles of the hands, feet, and lower limbs. Contracture rigidity may cause falls, and tetanic spasm of laryngeal muscles may impair respiration. Muscle biopsy shows nonspecific type 2 fiber atrophy, increased central nuclei, rims of mitochondrial accumulation, myofibrillar disorganization, ring fibers with cytoplasmic bodies.

10.3 Steroid Myopathy

Steroid myopathy may be endogenous (Cushing's syndrome) or iatrogenic (prolonged steroid therapy). The onset of weakness is usually insidious, starting in the proximal muscles of the legs and arms. Myalgia may accompany the weakness. The levels of serum lactate dehydrogenase, aspartate aminotransferase, creatine kinase, and aldolase are usually normal.

Patients suffering from steroid myopathy usually have other clinical signs of glucocorticoid excess. The diagnosis of steroid myopathy is simple when significant muscle weakness occurs in a patient receiving steroids for a condition unrelated to the skeletal muscle system. The differentiation of steroid myopathy from inflammatory myopathy is more difficult. The following criteria can be used: steroid myopathy takes time to develop and the weakness that occurs at the onset of a steroid treatment in polymyositis is probably still related to the inflammatory process. If the relevant serum enzymes are elevated, the weakness is probably not steroid-induced. Among patients with steroid myopathy, there is a wide range in dose and duration of steroid treatment received. Patients who have received steroids for less than 4 weeks rarely develop steroid myopathy. Women are twice as likely as men to develop steroid myopathy with the same glucocorticoid dose.

Lowering the steroid dose will usually correct the weakness. On EMG needle insertion, activity is normal and the motor unit potentials are of low amplitude and short duration [21]. Although many commonly used steroids can cause myopathy, the fluorinated steroids such as dexamethasone, betamethasone, and triamcinolone are more likely to produce muscle weakness [21].

Muscle pathology. Histological studies in either iatrogenic steroid myopathy or Cushing's disease [22] show a selective type 2 fiber atrophy, which stain darkly for myofibrillar alkaline ATPase. Sometimes the atrophy is so intense that it can resemble a neurogenic atrophy, except that other features of neurogenic disorders

("target fibers" or "type grouping") are absent. Lipid droplets are frequently seen in type 1 fibers and electron microscopy shows mitochondrial aggregates and vacuolization.

10.4 Myopathy Due to Excess of Adrenocorticotrophic Hormone (ACTH)

In 17 patients who had been treated for Cushing's syndrome by adrenalectomy between 6 months and 14 years previously, Prineas et al. [23] observed the association in six patients between the appearance of skin pigmentation and the occurrence of myopathy. These authors therefore suggested that an extra-adrenal action of ACTH might be a factor predisposing these patients to myopathy. All six patients developed weakness more than 1 year after adrenalectomy, and all were receiving glucocorticoid replacement treatment (hydrocortisone 20–40 mg/day). These patients had proximal muscle weakness and atrophy, but other features differed from those seen in typical steroid myopathy: four patients exhibited sharp waves and fibrillation on EMG and in three patients muscle biopsy showed marked subsarcolemmal lipid deposition. Similar changes can be provoked by ectopically produced ACTH. In muscle the early changes include the accumulation of subsarcolemmal mitochondria. Many regenerating muscle fibers showed unusual aggregation of filaments, deranged Z-disc with filaments attached and T-system with tubular networks.

10.5 Drug-Related Myopathies (Table 10.2)

10.5.1 Valproate

Children with epileptic seizures treated with valproate may present fatal hepatotoxicity and hyper-ammonemia with a syndrome similar to Reye's syndrome [24]. Because of its structural resemblance to fatty acids, valproate-induced toxicity is due to interference with mitochondrial beta-oxidation, inhibition of complexes I and IV of the respiratory chain, and reduced oxidative phosphorylation, which finally cause energy deprivation [25].

Table 10.2 Drug related myopathies

Agent	Effects
Valproate	Hepatotoxicity, hyperammonemia
Heroin	Myonecrosis, myoglobinuria
Clofibrate	Myonecrosis, myoglobinuria
Chloroquine	Increased autophagy
Emetine	
Colchicine	

Valproate conjugates with carnitine to form valproyl-carnitine, which is preferentially excreted in the urine, and this process may lead to low plasma carnitine. Lowered levels of free carnitine and high levels of short-chain and long-chain acyl-carnitines in plasma have been observed by us after a load of valproic acid in adults and children.

Valproate may unmask mitochondrial disorders, such as MELAS syndrome, acyl-CoA dehydrogenase deficiency.

10.5.2 Opioids and Clofibrate

The occurrence of acute rhabdomyolysis as a complication of the i.v. injection of heroin was first reported by Richter et al. [26]. It is possible that adulteration of the heroin mixtures was responsible. They described four young men presenting severe generalized muscle edema and weakness, within hours after administration of the heroin preparation. Muscle biopsy showed necrosis of muscle fibers with severe oedema. Creatine kinase and other serum enzymes were elevated, and there was massive myoglobinuria. Since this first report numerous other observations have followed.

Clofibrate (Atromid-S) is a branched fatty acid ester used therapeutically as a hypolipidemic agent. A toxic syndrome characterized by severe muscle pain, cramps, and weakness has been described. Tenderness of muscle was accompanied by an elevation of serum transaminases and creatine kinase [27]. Muscle biopsy showed some atrophic fibers (associated with damaged neuromuscular junctions) and massive degenerative changes [28].

The clinician should be aware that the use of clofibrate may cause a severe sense of exhaustion, due to its toxic effect on muscle.

10.5.3 Chloroquine, Hydroxychroloquine, Emetine, Vincristine, and Colchicine

These drugs may cause a severe but reversible myopathy. They have a direct effect on mitochondria, and result in type 1 fiber atrophy and a vacuolar myopathy by stimulating autophagic mechanisms.

Chloroquine (CQ) and hydroxychloroquine (HCQ) are used in the treatment of malaria, amoebiasis, rheumatoid arthritis, and systemic lupus erythematosus. Whisnant et al. [29] reported the development of widespread muscular weakness in four patients requiring long-term CQ treatment of collagen vascular diseases. Muscle biopsies showed vacuolar myopathy. Itabashi and Koykmen [30] observed a reversible granulo-vacuolar myopathy in a 46-year-old woman with abdominal sarcoidosis after 9 months of CQ treatment. McDonald and Engel [31], in a study of experimental CQ myopathy, observed the formation of diffuse autophagic vacuoles in muscle (somewhat similar to those observed in type 2 glycogenosis), affecting

more type 1 oxidative fibers than type 2 glycolytic fibers, frequent splitting of muscle fibers, proliferation of membrane systems, and abundance of myeloid bodies reactive for acid phosphatase. Autophagic vacuoles contain cytoplasmic degradation products and exhibit acid phosphatase activity, indicative of lysosomal dysfunction. Since CQ and HCQ are amphiphilic drugs, once inside acidic lysosomes, these molecules become cations, increasing intra-lysosomal pH, inhibiting lysosomal enzymes, and interfering with lysosomal protein degradation [32–34]. CQ and HCQ muscle toxicity is usually self-limiting after discontinuation of the drug [35].

Colchicine has been used for centuries for the treatment of gout. Since colchicine destabilizes microtubule formation and cytoskeletal network, it blocks the mitotic division, and impairs the endosomal and autophagosomal maturation [36]. Its neuromuscular toxicity is largely unrecognized. Kunge et al. [37] reported 12 patients with colchicine myopathy and neuropathy who presented with elevation of serum creatine kinase and a mild axonal polyneuropathy. Colchicine myopathy is characterized by myalgias, muscle weakness, and occasional rhabdomyolysis [38]. EMG may show myotonic changes in proximal limb muscles. Muscle biopsy typically shows the features of a vacuolar myopathy, with marked accumulation of lysosomes and autophagic vacuoles, necrotic fibers, and large complex membranous bodies [39].

Recent studies aimed to clarify the pathogenetic mechanism have demonstrated that in muscle there is an increased accumulation of autophagic substrates and ubiquinated proteins, suggesting that colchicine toxicity results in a failure of autophagic vacuole build-up, due to enhanced autophagosome formation and impaired autophagic degradation [40].

Emetine, a constituent of ipecac, has been used as an amoebicidal agent to treat diarrhea, and also by bulimic patients to stimulate emesis. An adverse aspect of its use is weakness, pain, tenderness, and stiffness of skeletal muscles, but it is toxic also to cardiac muscle where it may produce electrical disturbances and heart failure. In rat muscles, emetine produces myofibrillar degeneration, areas of mitochondrial loss, necrosis, and regeneration [41]. In patients, a severe disruption of the sarcomeres, Z-band streaming, formation of cytoplasmic bodies, abnormalities of the sarcotubular system [42], loss of myosin ATPases and dehydrogenase, accumulation of PAS-positive material, decrease or loss of mitochondria [43] have been observed. It has been proposed that emetine inhibits protein synthesis and mitochondrial oxidative phosphorylation in muscle, causing decreased ATP formation, release of calcium into the sarcoplasm, which activates proteases causing Z-band lysis and myofilament disorganization [44].

Vincristine, an alkaloid used in leukemia, is a well-recognized cause of peripheral neuropathy; however, there is also a direct effect on muscle. In an experimental model, Anderson et al. [45] and Clarke et al. [46] have demonstrated that vincristine may cause spheroid-membranous degeneration, suggesting that its toxicity impairs the biodegradation of muscle phospholipids [45].

10.6 Nutritional and Secondary Deficiency States (Table 10.3)

10.6.1 Carnitine

Carnitine deficiency may be seen in cirrhosis [47], in pregnancy [48], during total parenteral nutrition in infants and in some adults [49] undergoing surgery. In these patients, inadequate exogenous supplement, hormonal factors, and defective endogenous synthesis contribute to the carnitine-deficient state. The low carnitine content in liver may explain the steatosis observed in infants on total parenteral nutrition and suggests an essential role during early stages of life, when endogenous synthesis and storage are probably not adequate. Similar observations apply to the plasma carnitine deficiency found in undernourished patients with schistosomiasis worm infection [50].

Carnitine changes in various metabolic states: fasting, diabetes. In fasting man, the increase in plasma long-chain acyl-carnitines parallels that of plasma-free fatty acids [51], reflecting the conversion of increased fatty acids first to acyl-CoA and then to carnitine derivatives in many tissues. A similar marked increase in serum acyl-carnitines and decreased free carnitine was observed by Genuth [52] in diabetic ketoacidosis: he reported that insulin reverses this pattern toward normal. Plasma insulin levels decline in fasting subjects [51], accompanied by a qualitative redistribution of plasma carnitine fractions while re-feeding restores the plasma carnitine fractions to normal, as plasma insulin rises. It therefore appears that the increased ratio of acyl-carnitines to free carnitine may be related to reduced secretion of insulin, a modulator which might negatively influence fatty acid oxidation in tissues [53]. A high plasma glucagon concentration causes a decrease in total plasma carnitine, particularly of the free carnitine fraction [53]. McGarry et al. [54] demonstrated that the liver carnitine concentration increases following glucagon administration. The hormonal variations that occur in patients undergoing chronic hemodialysis are similar to those found in diabetes or fasting subjects. Hyper-glucagonemia has been observed in hemodialyzed patients [55, 56]. Furthermore, a decreased peripheral tissue sensitivity to insulin in uremic patients is a well-known feature since these patients have a normal or increased secretion of insulin [57, 58].

Table 10.3 Myopathies caused by nutritional deficiency

Compound	Effect
Vitamin E (α-tocopherol)	High CK, neuromyopathy
Vitamin B_t (carnitine)	Protein-energy malnutrition
Selenium	Myopathy
Magnesium	
Iron	Anemia, myelo-neuropathy
Copper	

10.6.2 Myopathies Caused by Nutritional Deficiency (Table 10.3)

It has been known for many years that deficiencies of vitamins E and D can cause myopathy [59]. The deficiency of vitamins B do not affect muscle although they cause profound central and peripheral nervous system changes. Deficiency of vitamin B_t (carnitine) may result in a lipid storage myopathy. Protein-energy malnutrition, which causes Kwashiorkor in infants and children, can produce secondary carnitine deficiency but is not associated with overt pathological changes in muscle.

The only mineral nutritional deficiencies described in association with myopathy are those of selenium and, possibly, magnesium [60, 61]. A deficient intake of Selenium, a constituent of glutathione, peroxidases, and selenoprotein-P may cause reduced oxidative capacity [62].

10.6.3 Vitamin E Deficiency

In animal models, a diet deficient in vitamin E produced lesions in muscle. In rabbits, vitamin E deficiency resulted in muscle necrosis, and necrotic fibers are invaded by macrophages and polymorphonuclear leukocytes [63]. Elevated serum creatine kinase was observed [63], and it returned to normal on reintroduction of vitamin E, when regeneration of muscle fiber occurs.

Vitamin E deficiency causing a primary muscle disease in man has, however, not been fully established. Thomasi [64] reported a patient who developed jaundice and pruritus in infancy because of biliary atresia; he was treated with high doses of cholestyramine and had a reduced plasma vitamin E level. At 7 years, the patient had severe generalized muscle weakness with high serum creatine kinase. Muscle biopsy disclosed angulated atrophic fibers. The patient improved after 4 months of treatment with massive doses of vitamin E. Muscle strength and plasma level of α-tocopherol returned to normal. Vitamin E deficiency following fat malabsorption can cause a spinocerebellar type of syndrome with neuropathy, muscle inclusions, retinal degeneration, and ophthalmoplegia [65]. A case of Friedreich's disease with normal fat absorption and low plasma vitamin E has been reported [66]. However, re-feeding vitamin E did not improve the neurological picture.

10.6.4 Iron and Copper Deficiency

Given their similar physico-chemical properties, whole-body iron and copper homeostasis is linked to dietary and copper absorption in the upper small bowel. Perhaps, the first detailed description of iron–copper interactions in humans dates back to the mid 1800 in Italy and France. Young women suffered from a disease called Chlorosis, with pale complexion, amenorrhea, lethargy. It is conceivable that these young women suffered from copper deficiency. Iron assimilation from the diet must be tightly controlled. In fact, there are mechanisms that induce trans-activation of gene in erythrocytes by a hypoxia inducible factor (HIF2α) by iron deprivation. Lack of iron

decreases hemoglobin production which impairs oxygen delivery to several tissues leading to hypoxia. Several genes encoding iron transport-related proteins are up-regulated by the transcriptional mechanism involving HIF2α. Molecular mediators governing iron–copper interaction and total body stores are described by Gulec and Collins [67]. Several questions and foci for future research remains unanswered, for instance, what is the biological significance of increased copper levels in enterocytes during iron deficiency and why does copper deficiency cause anemia.

Copper deficiency is an under-recognized cause of myelo-neuropathy and cytopenias. Damage to mitochondria and alteration of several copper transport proteins was documented in muscle by Spinazzi et al. [68]. Usually, copper deficiency might be easily missed [69], but it causes a neurological dysfunction, almost indistinguishable from sub-acute combined degeneration. The neurological sequelae of copper deficiency can become debilitating and irreversible, making early recognition a crucial point. Risk factors of copper deficiency appear to be: upper gastrointestinal tract surgery, bariatric surgery, zinc overload, ingestion of zinc-containing dental fixative, malasorption syndrome. Laboratory indicators of copper deficiency include cytopenia and low copper. In clinical practice, guidelines recommended testing for copper deficiency in post-bariatric surgery patients and anemia, neutropenia, and myelo-neuropathy. Spinal cord magnetic resonance imaging is abnormal in half the patients and neurophysiological studies might show axonal sensory-motor polyneuropathy. The reversibility of the condition depends on the stage of neuronal deficiency.

Highlights

- Thyroid action is important to maintain metabolism in muscle; often insidious hypothyroidism can be caused by Hashimoto autoimmune disease or thyroid removal.
- Steroids action is particularly evident in type 2 fibers in muscle biopsy; fluorinated steroids should be avoided in treating inflammatory muscle disease.
- Malabsorption syndrome can cause secondary carnitine deficiency, iron, and vitamin B deficiency.
- Copper deficiency is an under-recognized cause of cytopenia and myelo-neuropathy.
- In patients with unexplained proximal muscle weakness in the context of previous surgical procedures or malabsorption, clinicians should have a low threshold for screening in blood the following: T3, T4, TSH, Cu^{+}, Fe^{++}, Zn^{+}, carnitine, valproic acid, vitamin D3.

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Vitamin D Deficiency in Muscle

11

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11.1 Epidemiology of Vitamin D Deficiency

Vitamin D deficiency is an extremely common condition, especially in the elderly. It is estimated that more than 60% of nursing home residents in the US are vitamin D deficient [1]. However, vitamin D deficiency can be present among young and healthy individuals as well. According to the National Health and Nutrition Examination Survey (NHANES) of 2877 US children and adolescents, an estimated 10.3% had insufficient vitamin D levels (12–20 ng/mL), and 4.6% had vitamin D deficiency (<12 ng/mL) [2]. Various studies demonstrated that vitamin D deficiency has a bimodal distribution as it is mainly encountered in children and elderly [3].

These results are comparative to those encountered among Canadian and European populations [4]. Interestingly, the prevalence of vitamin D deficiency is drastically higher in the Middle Eastern countries, which might be attributed to their cultural and religious practices [5]. Moreover, it has been noted that vitamin D deficiency is more common among women [6]. Different ethnic groups display variations in prevalence of vitamin D deficiency. For instance, the prevalence of vitamin D deficiency among dark-skinned individuals is higher compared to other ethnicities, this may be attributed to the excess of melanin, which interfers with ultraviolet B-rays (UVB) role in activation of vitamin D synthesis [3, 6].

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There has been considerable debate in the medical community regarding what is considered low vitamin D level. The general consensus follows the National Academy of Medicine, which defines vitamin D insufficiency as serum concentration levels of 25-hydroxycholecalciferol falling between 12 and 20 ng/mL, while deficiency is defined when levels are below 12 ng/mL [7].

11.2 Biochemistry of Vitamin D

Vitamin D is a fat-soluble steroid hormone synthesized in the skin or found in dietary sources. There are two forms of vitamin D: vitamin D2 (ergocalciferol) derived from plants and vitamin D3 (cholecalciferol) derived from animals or synthesized in the epidermis layer of the skin from 7-dehydrocholesterol conversion in response to ultraviolet light (UVB). Both forms are transported to the liver via vitamin D-binding protein in order to undergo their first hydroxylation process by 25-hydroxylase enzyme, which gives rise to **25-hydroxycholecalciferol**. 25-hydroxycholecalciferol undergoes another hydroxylation (rate-limiting step) mainly in the proximal tubules of the kidney by 1-α-hydroxylase enzyme giving rise to 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3 (**calcitriol**), respectively [8, 9].

It is the action of calcitriol on vitamin D receptors (VDR) in target tissues that produces the desired effects of vitamin D. Calcitriol binds to VDR inducing conformational changes within it, allowing it to heterodimerize with the retinoid X receptor (RXR). This complex binds specific genomic sequences known as vitamin D response elements (VDREs) in order to influence gene transcription and produce its desired effects [10]. It has been shown that this binding site is located near autoimmune and cancer-associated genes, which might explain the higher tendency of **autoimmune diseases** and cancers among vitamin D-deficient individuals [10]. However, the effects of vitamin D are not entirely "genomic." Vitamin D plays an essential role in MHC class I assembly, molecular chaperoning, among others through its nonnuclear effects on VDR [9, 11].

The 24-hydroxylase (CYP24A1) enzyme, which is found in nearly all tissues of the body, especially the kidneys, limits the amount and catabolizes calcitriol in target tissues by converting it to inactive metabolites, including 1,24,25-hydroxycholecalciferol and calcitroic acid [9].

11.3 Causes of Vitamin D Deficiency

As stated previously, the main sources of vitamin D are cutaneous production following sunlight exposure (80–90%) and intake via dietary sources (10–20%) [7]. The cutaneous production of vitamin D accounts for the majority of vitamin D production in the body, and any interruption in the process of its synthesis can lead to insufficiency or deficiency [12].

The widespread awareness of the association between sunlight exposure and skin cancer, as well as the development of premature wrinkles, has led many to use

Table 11.1 Common causes of vitamin D deficiency

Causes of vitamin D deficiency
Inadequate exposure to sunlight
Malabsorption syndromes (e.g., *celiac disease, bariatric surgery*)
Liver diseases (e.g., *cirrhosis*)
Renal disease (e.g., *renal failure, nephrotic syndrome*)
Exclusively breast milk-fed infants
Steroids
Pregnancy
CYP450 inducers (e.g., *phenytoin, rifampicin*)
Season and latitude
Obesity
Inflammation
Dark skin pigmentation

sun-protecting factor (SPF) containing products and sunscreens. A minimum SPF of 15 is enough to prevent vitamin D synthesis in the skin [3]. Therefore, with increased rate of utilization of these products, the prevalence of vitamin D deficiency continues to rise.

Vitamin D deficiency can manifest as a result of various etiological factors. A few of these causes and conditions are summarized in Table 11.1.

11.4 Effects of Vitamin D on Muscles and Nervous System

The effects of vitamin D are not limited to **calcium** and **phosphate** homeostasis and **bone health**. There is increasing evidence supporting the role of vitamin D in the nervous system and muscle function. Studies have shown that muscles and numerous areas within the central nervous system harbor VDR including the pituitary gland, forebrain, hindbrain, and spinal cord [13]. In addition, 1-α-hydroxylase enzymes have been detected in the brain; and some vitamin D metabolites have been retrieved from the cerebrospinal fluid [14].

In the nervous system, vitamin D promotes neural differentiation, growth, and maturation. It also protects the nervous system from toxicity and decreases the rate of neurological injury. Among vitamin D deficient individuals, this effect has been noted by the increased rate of multiple sclerosis, Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease. In addition, vitamin D and its metabolites contribute to the synthesis of various neurotransmitters including serotonin, dopamine, acetylcholine, and catecholamines. Its role in neuroplasticity is crucial as it stabilizes the cellular structures and maintains the mitochondrial functions [15].

Grossly, vitamin D-deficient animals demonstrated increased overall brain size and hemispheric length, cortical layer thinning, and big lateral ventricles [16]. Histologically, deficient animals showed increased cellular proliferation, higher rates of mitosis, and reduced cellular apoptosis [17].

As in the nervous system, vitamin D stimulates **mitogen-activated protein kinases (MAPK)** family in myoblasts, increasing the expression of genes responsible for cellular proliferation and differentiation [18]. It has also been shown to have a role in the regulation of muscle contractile elements, cytoskeleton, and phospholipid membranes [19, 20].

On muscle histology, vitamin D-deficient individuals display nonspecific findings, which include atrophy of type II (fast twitch) muscle fibers, fatty infiltration, presence of glycogen granules, and fibrosis. In addition, degenerative changes on electron microscopy with small foci of fiber necrosis, Z-band degeneration, and lytic vacuoles have been noted [21, 22].

11.5 Myopathy and Myalgia

The effects of vitamin D are beyond calcium and phosphate homeostasis and bone health. Vitamin D deficiency is implicated in many neuromuscular disorders.

Muscle weakness (**myopathy**), muscle pain (**myalgia**), bone pain, and hypotonia are commonly encountered among patients with vitamin D deficiency (e.g., **rickets** and **osteomalacia**). The muscle weakness noted in vitamin D deficiency is progressive and occurs mostly in the proximal musculature [23, 24]. The muscle weakness will lead to difficulty rising from a seated position (Gower's sign), climbing stairs, and lifting objects. In addition, it may lead to changes in gait (e.g., waddling and reluctant gait) as well as imbalance [24, 25]. It is important to note that it is sometimes difficult to distinguish between the giving away weakness due to bony pain and the muscle weakness encountered in vitamin D deficiency. In vitamin D deficiency, the muscles are not painful or tender to move. Chronic cases of vitamin D deficiency may even develop skeletal abnormalities such as bowing and pathological fractures.

The pathogenesis of myopathy and myalgia in vitamin D-deficient individuals remains elusive. However, low calcium, phosphate, and calcitriol along with high parathyroid hormone levels have been attributed to the development of these symptoms [26]. The myopathy and myalgia encountered in these conditions respond rapidly to vitamin D therapy [25–27].

However, it is important to note that the pattern of muscular weakness among vitamin D-deficient individuals is not specific and is commonly encountered among myopathies of other etiologies, as well as endocrine disorders exemplified by Cushing's syndrome and hyperthyroidism [24]. In addition, vitamin D-deficient individuals with muscle weakness commonly demonstrate electromyographic changes reflecting myopathy, but without specific topographies [28].

11.6 Myotonic Dystrophy

Myotonic dystrophy types 1 and 2 are group of autosomal dominant-inherited disorders characterized by the presence of early-onset cataract, myotonia, and muscle weakness and atrophy [29]. Vitamin D levels among patients with myotonic dystrophies are generally low. Interestingly, 18% of patients with myotonic dystrophy

type 1 have a concurrent hyperparathyroidism, and the severity of their disease correlates with the parathyroid hormone level [30]. Patients with myotonic dystrophy type 2 commonly have simultaneous high parathyroid hormone level. However, its exact prevalence has not yet been established. In addition, it has been demonstrated that a positive correlation between **CTG repeats** and parathyroid hormone levels exists among myotonic dystrophy type 1 patients [30, 31].

Vitamin D deficiency among patients with myotonic dystrophy type 1 has been attributed to insufficient cutaneous synthesis, altered gastrointestinal absorption, and abnormal hepatic hydroxylation. However, studies have delineated that patients given oral cholecalciferol showed a normal increase of circulating vitamin D levels, ruling out malabsorption and liver dysfunction. Thus, impaired cutaneous synthesis remains the most plausible mechanism of vitamin D deficiency [29, 32]. The relation between vitamin D deficiency and myotonic dystrophies remains elusive. It is yet to be proven whether the deficiency has a causal relationship with the development of these diseases or is the result of the disease course.

11.7 Myasthenia Gravis

Myasthenia gravis is an autoimmune disease resulting from the formation of autoantibodies against the postsynaptic acetylcholine nicotinic receptors. The hallmark of myasthenia gravis is fluctuating weakness in multiple muscle groups such as bulbar and proximal muscles. Interestingly, vitamin D deficiency has been reported in up to 88% of myasthenia gravis patients [33, 34]. The association between vitamin D deficiency and myasthenia gravis is not entirely understood. It is hypothesized that vitamin D deficiency in myasthenia gravis might be attributed to lack of physical activity and decreased sunlight exposure among these patients. Additionally, muscle strength among these patients has been correlated with bone mineral density and vitamin D levels [33]. Vitamin D supplementation has been shown to improve symptoms in the majority of patients as reflected by the reduction in the myasthenia gravis composite scale score [35]. It is thought that these effects are mediated by regulating the autoimmune process involved in myasthenia gravis and by maintaining and stimulating muscle function through the effects of vitamin D on muscles' VDR [35, 36].

Vitamin D has been shown to exert positive effects on the immune system. It has a role in the production of T regulatory cells (T-regs) and regulating the proliferation of T-cells [37, 38]. Studies have shown that T-cell education in the thymus and the involvement of T-regs are linked to the development of myasthenia gravis. However, the exact mechanism and relationship between vitamin D deficiency and T-regs is yet to be understood among myasthenia gravis patients.

11.8 Drug-Induced Myopathy

According to the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (**SEARCH**) collaborative group's trial, myopathy can be either incipient or definite. Incipient myopathy is defined by a CK level >3 times the upper

normal limit and >5 times the patient's baseline in addition to an alanine aminotransferase (ALT) level >1–7 times the baseline. Definite myopathy is defined as a CK level ≥10 times the upper normal limit plus unexplained muscle pains. Rhabdomyolysis is defined by a CK level ≥10 times the upper normal limit plus evidence of end-organ damage (elevated serum creatinine). The presence of myoglobinuria and electrolyte abnormalities supports the diagnosis of **rhabdomyolysis** [39].

Statin-induced myopathy ranges from simple myalgia to life-threatening rhabdomyolysis [40]. The risk of myopathy is mainly attributed to the drug dosage with the more lipophilic statins (e.g., **simvastatin**) having the highest risk [41]. Other factors that play a role in the development of statin-related myopathy include old age (>80 years), Asian ethnicity, female gender, low body mass index, frailty, and having concurrent systemic diseases (e.g., diabetes mellitus, hypothyroidism, renal or hepatic disease) [42]. The development of myalgia among these patients is an indication of drug intolerance [43]. Vitamin D deficiency significantly augments the myopathy among patients on statin therapy. Therefore, cessation of statin therapy in conjunction with high-dose vitamin D supplementation in those patients results in the resolution of symptoms in the majority of patients [9].

Various other medications can cause myopathy and myalgia, either as a side effect or due to increased vitamin D metabolism. Antiepileptic medications (eg., phenytoin), antibiotics (eg., rifampicin), and other drugs have been shown to induce liver p450 enzymes increasing the catabolism of vitamin D, resulting in a picture similar to vitamin D-deficient myopathy [12–14].

11.9 Painful Peripheral Diabetic Neuropathy

Patients suffering from diabetes have clinically demonstrated several forms of neuropathy as one of the microvascular complications. Diabetic neuropathy affects more than 50% of type 2 diabetic patients [44]. Diabetics typically experience paresthesias, numbness, autonomic dysfunction, among other symptoms of peripheral neuropathy. The nocturnal exacerbations of these symptoms significantly impact sleep and quality of life in these patients.

The most significant of these manifestations is painful **peripheral diabetic neuropathy (PPDN)**, which presents as electric shocks, burning, cold, or pins and needles sensations. It is estimated that 21% of diabetics experience PPDN, especially in patients suffering from type 2 diabetes mellitus [45]. Interestingly, over half of diabetic patients with PPDN have a concurrent vitamin D deficiency [45].

While pain control remains the mainstay treatment for these patients, some patients remain refractory to these medications or cannot tolerate their dose-related side effects [46, 47]. Therefore, alternative measures may be valuable and are of interest in this patient population. Studies demonstrated that a single 600,000 IU dose of vitamin D might improve symptoms of PPDN over a period of 20 weeks [45]. However, placebo effect cannot be completely excluded, and despite the clear association between vitamin D deficiency and PPDN, a causation relationship has not been established. Animal studies on rodents suggest that vitamin D deficiency

may have some contribution to sensory hyper-innervation via hormonal pathways, which might explain the development of paresthesia and various other symptoms of peripheral neuropathy [48, 49]. Further research is still needed to elucidate a causation relationship between vitamin D deficiency and peripheral neuropathy in humans.

11.10 Treatment and Complications

Vitamin D supplementation can have significant benefits in treating and preventing the development of neuromuscular symptoms. These symptoms tend to increase the risk of **falls**, **fractures**, injuries, and affect the **quality of life**, especially in the elderly. It is estimated that 30% of community-dwelling people over the age of 65 suffer from repeated falls each year, 20% of which require medical attention [50, 51]. Vitamin D supplementation in conjunction with calcium has shown to be effective in the prevention of falls and improvement of neuromuscular functions among the elderly.

Therefore, vitamin D and calcium supplementation are recommended for people over the age 65 years [9]. In addition, vitamin D supplementation should be given to all patients with neuromuscular conditions to prevent the development of the aforementioned undesirable complications.

The general dietary recommendation for vitamin D is based upon bone health. It is proposed that a serum concentration of 20 ng/mL of vitamin D is sufficient to maintain physiological requirements in healthy individuals [3]. It is advocated to give 200 IU/day of vitamin D for children and adults up to the age of 50 years, 400 IU/day for adults from the age of 50–70 years, and 600 IU/day for those older than 70 years [52]. It has been noted that daily supplements of ≥800 IU/day vitamin D plus calcium has the potential to reduce vertebral and nonvertebral fractures in up to 26% among the elderly [53, 54]. Alternatively, giving 100,000 IU oral vitamin D every 4 months is equally effective [55].

The **complications** of vitamin D therapy (**hypervitaminosis D**) are minimal and related to extremely high doses. Vitamin D toxicity can cause high calcium levels (hypercalcemia), which can lead to kidney stones, bone loss, and organ calcification (e.g., heart and kidneys). However, the toxicity is very unlikely in healthy individuals at intake levels lower than 10,000 IU/day [52, 56].

11.11 Conclusions

Vitamin D plays an integral role beyond calcium and phosphate homeostasis and bone health. It is an essential component for the integrity and function of nerves, muscles, and the neuromuscular junction. Vitamin D deficiency is common in patients with neuromuscular disorders. **Screening** patients with these disorders for vitamin D deficiency is therefore essential, as early detection and intervention might aid in preventing the development of debilitating symptoms and improving the quality of life in these patients. Patients with neuromuscular conditions must receive vitamin D **supplementation**, once diagnosis is established. It is yet to be discovered

whether vitamin D deficiency plays a role in the development of these diseases or is just a result of the poor nutrition and limited sun exposure commonly encountered in such patients. Future research is needed to delineate this association and to answer these questions.

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Intensive Care Unit-Acquired Weakness

12

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12.1 Introduction

Intensive care unit-acquired weakness (ICU-acquired weakness) is a severe acquired muscle weakness during critical illness and for which there is no other explanation than the critical illness itself [1]. Patients who have quadriparesis or quadriplegia because of spinal cord injury (or disease), those with a previous history of neuromuscular diseases and acute neuromuscular disorders, for example, Guillain-Barré syndrome are excluded. The condition delays rehabilitation and may not be completely reversible. The acute outcome and long-term functional outcome is strongly dependent on age, comorbidities and the length of intensive care unit stay [2].

12.2 Prevalence and Risk Factors

The first description of ICU-acquired weakness was reported in 1977 [3], in a patient treated for status asthmaticus: she was mechanically ventilated for 8 days and received large doses of 9α-fluorinated steroids (up to 3 g in 24 h by constant infusion) and intermittent doses of neuromuscular blocking drug (pancuronium). After ceased airway obstruction, she had great difficulties in weaning from the

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ventilator and was also unable to lift her limbs against gravity. She gradually improved and was able to walk unaided after 3 weeks but had residual muscle weakness after 2 months.

The prevalence of ICU-acquired weakness is strongly dependent on the type of patient population studied. ICU-acquired weakness occurs more frequently in patients with longer exposure to mechanical ventilation: 33% of patients mechanically ventilated up to 5 days and 43% of patients mechanically ventilated up to or more than 7 days develop it [4], while the frequency rises to 67% in patients mechanically ventilated up to or more than 10 days [5]. Despite incomplete understanding of the underlying mechanisms, it has been proposed that the duration of mechanical ventilation is a major risk factor, acting together with sedation and neuromuscular blockade. It causes immobility, i.e. mechanical silencing of skeletal muscles, including silencing of respiratory muscles. Some other independent risk factors are sepsis, bacteraemia, systemic inflammatory response syndrome, multiorgan dysfunction/failure, malnutrition, hyperglycaemia/insulin resistance, advanced age [6]. After the first case of ICU-acquired weakness in a patient treated for status asthmaticus with corticosteroids and neuromuscular blocking agent [3], some studies indeed suggested that these agents may contribute to ICU-acquired weakness but other studies could not confirm this. The role of corticosteroids and neuromuscular blocking agents to trigger ICU-acquired weakness remains somehow inconclusive, depending also on other factors as dose, timing and concomitant glycaemic control [7].

12.3 Clinical Signs

Symmetrical and flaccid weakness of limb muscles, more pronounced in proximal than distal muscles and weakness of respiratory muscles, which is responsible for difficulties in weaning from mechanical ventilation, are the main features. The time dependent and early development of diaphragmatic atrophy and weakness is also named "ventilator-induced diaphragmatic dysfunction" (VIDD) [8]. Septic shock before awakening is significantly associated with VIDD [9]. VIDD might be the initial or the only clinical presentation of ICU-acquired weakness. Using twitch tracheal pressure in response to bilateral anterior magnetic phrenic nerve stimulation (a pressure < 11 cm H_2O defined dysfunction) and ultrasonography of diaphragm (excursion of diaphragm and thickening fraction), it was observed that VIDD is *twice as frequent* as ICU-acquired weakness of limb muscles and delays weaning from mechanical ventilation [10]. "Safe anaesthesia table" [11] is available and should be used to prevent anaesthesia complications (difficulties in weaning from mechanical ventilation included) in pauci- to mildly symptomatic patients with clinically undiagnosed myopathy.

VIDD strongly impacts clinical outcomes: it prolongs time to liberation from mechanical ventilation, increases the frequency of reintubation, tracheostomy or death [12].

The diaphragm is also an important regulator of expiration and its expiratory activity seems to preserve lung volume and protects against lung atelectasis [13]; the loss of diaphragmatic expiratory contraction during mechanical ventilation may be a contributing factor to unsuccessful respiratory control as demonstrated experimentally, in mild acute respiratory distress syndrome induced in pigs [13].

Facial and ocular muscles are often spared, tendon reflexes are generally reduced, but may be normal. Sensory loss, if present, is usually localized in distal parts of the limbs and is an argument for CIP, but may be due to other causes, such as diabetes. Autonomic dysfunction may be present [7]. While ICU-acquired weakness is relatively obvious in patients with a primary non-neurological disorder, it may be difficult to notice in patients with the primary lesion in the central nervous system [14] who are initially spastic: affection of the peripheral neuromuscular compartment was considered in them when a previously spastic patient developed a flaccid weakness and an absence of myotatic reflexes, and/or weaning from mechanical ventilation could not be achieved. Patients with head trauma, head surgery or cerebrovascular insult are at high risk for ICU-acquired weakness since they are sedated and mechanically ventilated for prolonged period.

12.4 Diagnosis

12.4.1 Manual Muscle Testing

Manual muscle testing [4, 15, 16] including simplified version of the Medical Research Council manual muscle testing [17] and handgrip dynamometry [15, 18, 19] are applicable to detect ICU-acquired weakness only in an awake and fully cooperative patient. Since 10–75% of patients during intensive care unit stay do not fulfil this criterion, these outcome measures have limited value in detecting ICU-acquired weakness [7, 20].

12.4.2 Electrophysiological Testing

Electrophysiological testing is usually used in making a diagnosis of ICU-acquired weakness: concentric needle EMG in 90% of studies, nerve conduction studies in 84% of studies, direct muscle stimulation [21, 22] in 19% of studies [4]. In intensive care, these tools are difficult to perform, are time consuming, technically challenging, expensive and require subspecialists. Nevertheless, electrophysiological tests are minimally invasive, easily reproducible, possibly bedside performed and the results are available immediately [7].

The most common cause of ICU-acquired weakness is critical illness myopathy (CIM); CIM may be found in a combination with a neuropathy (critical illness myopathy and neuropathy CRIMYNE, named also critical illness polyneuromyopathy or polymyoneuropathy); the incidence of a debilitating axonal sensory-motor

polyneuropathy as an independent injurious event, i.e. critical illness polyneuropathy (CIP) was overestimated in the past, based on EMG only [23]; some cases remain undetermined [24, 25].

CIP and CIM have some common electrophysiological characteristics: reduced compound muscle action potential (CMAP) amplitude and abnormal spontaneous activity; if reduced CMAP amplitude is associated with prolonged duration of CMAP, this is an argument for CIM [14]. CMAP on direct muscle stimulation (dmCMAP) [21, 22] may also help to differentiate CIM and CIP. With this technique, both stimulating and recording electrodes are placed in muscle, in case of myopathy the dmCMAP will be reduced or absent after stimulation through the nerve and direct muscle stimulation [26]. Unfortunately, direct muscle stimulation is fairly rarely used (19% of studies) [4]. Reduced sensory nerve action potential (SNAP) amplitude is an argument for CIP, but reduced SNAP amplitude can be detected in CIM if polyneuropathy or limb oedema co-exists in CIM. A shorter duration (5–15 days) of abnormal spontaneous activity is an argument for myopathy since it would need more time to evolve in the case of axonal lesion [14]. A pattern of recruitment of MUPs and analysis of MUP parameters may help to differentiate CIM and CIP [14], but this requires alert and motivated patient. If MUPs cannot be estimated, electrophysiological diagnosis is invariable CIP even if CIM is ongoing (revealed by muscle biopsy findings) [23, 24]. Nerve conduction studies are usually normal in both CIM and CIP or might be slightly prolonged in CIP [14].

Electrophysiological tests are important for indicating that the lesion is peripheral, despite that they cannot always differentiate CIM or CIP.

In spite of the limitations, a simplified electrophysiological test, i.e. peroneal nerve test, using peroneal nerve CMAP, has been proposed to be used as a *screening* test for *probable* CIM or CIP [24, 25] or at least to identify patients who require more extensive electrophysiological testing. Recently [27], peroneal nerve test was confirmed to have high sensitivity (94%) and good specificity (91%) compared to complete EMG and nerve conduction studies. Peroneal nerve test is also very "economic" in terms of time since it can be performed in 10 min [25] and, since it does not require patient's cooperation, it is a valuable objective method in detecting probable CIM or CIP. Since 80% of subjects with EMG/NCS abnormalities had moderate to severe muscle weakness [4], correlation between electrophysiological studies and clinically detected muscle weakness is considered good. Most studies used manual muscle testing (when applicable) and electrophysiological tests sequentially, not comparing the two diagnostic approaches; in spite of this, electrophysiology has aided our understanding of the mechanisms of ICU-acquired weakness and, can aid in determining a patient's ability to respond to certain treatments [4]. Electrophysiological alterations can be detected earlier than the clinical signs and have predictive power, for example, a reduction of the CMAP amplitude can precede ICU-acquired weakness for 48 h in patients with sepsis [28]. Electrophysiological tests are also important with respect to acute outcome: hospital mortality is higher in patients with abnormal NCS-EMG than in those with normal findings as well as 1-year mortality [7] .

The prevalence of electrophysiological abnormalities in ICU patients is strongly dependent on the population of patients enrolled: it varies from 46 to 76% [15], if mostly patients with sepsis, multiorgan failure and prolonged mechanical ventilation are recruited.

12.4.3 Muscle Ultrasound

In ICU-acquired weakness, muscle ultrasound allows objective outcome measure of muscle atrophy in limb muscles which develops quite early during ICU-acquired weakness: rectus femoris cross-sectional area decreased for about 20% from days 1 to 7 [29]. In a follow-up study, quadriceps muscle atrophy was present in all patients 7 days after intensive care unit discharge, and most patients still had atrophy 6 months later [30]. Muscle atrophy quantified by ultrasound may also be utilized as a rapid bedside modality for risk stratification of critically ill patients [31].

Diaphragm ultrasound is also a promising new method to evaluate the diaphragm during mechanical ventilation. The assessment of diaphragm excursions and diaphragm thickening fraction are particularly useful for detecting VIDD: cut off excursion less than 11 mm during tidal breathing and thickening fraction less than 20% are diagnostic of VIDD [10, 20]. Loss of diaphragm thickness over time can indicate atrophy; tidal diaphragm thickening during inspiration provides a non-invasive methodology useful to quantify inspiratory effort (diaphragm contractile activity) and maximal diaphragm thickening, during maximal inspiratory effort can be used to assess diaphragm function [32].

Diaphragm thickness may decrease by as much as 10–12% over the first week in 40% patients regardless of ventilator mode, and this is predicted by lower levels of inspiratory effort and higher levels of ventilatory support [32].

Interestingly, diaphragm thickness *increases* in approximately 10% of patients in association with excess inspiratory effort and lower levels of ventilatory support [32]. Increased diaphragm thickness may be related to load-induced diaphragm injury although the threshold for (concentric) load-induced diaphragm injury in humans is usually relatively high [33]. However, critically ill patients may be much more susceptible to load-induced injury because of systemic inflammation. Patients who develop this increase in thickness had impaired diaphragm function and were more likely to require prolonged mechanical ventilation, which supports the possibility that such increases in diaphragm thickness reflect concentric load-induced muscle injury [33].

Eccentric load-induced diaphragm injury may occur when the diaphragm contracts eccentrically during certain forms of patient-ventilator dyssynchrony when the patient is unable to trigger the ventilator and during reverse triggering where neural inspiration results from passive mechanical inflation and often extends into mechanical expiration [33].

The diaphragm may become weak because of excessive unloading (leading to atrophy) or because of excessive loading (either concentric or eccentric) owing to excessive ventilator assistance [33] and these may be recognized by ultrasound.

12.4.4 Muscle Biopsy

Muscle biopsy (and nerve biopsy) are also used for the diagnosis of CIM or CIP, in 26% and 6% of studies [4].

Muscle biopsy in ICU-acquired weakness has a continuum of pathological presentations [34] which partly depend when the biopsy is performed. Early biopsies, performed during the first week in sedated and mechanically ventilated patients, who had non-excitable muscle membrane after direct muscle stimulation, may show preferential atrophy of type 2 fibres as the main finding [35]. Some described selective atrophy of type 2 fibres also in late biopsies, if sarcolemma was non-excitable [36, 37], others found concomitant atrophy of type 1 fibres [23, 38]. Myofibrillar ATP-ase activity may be reduced (Fig. 12.1a), but immunostaining for myosin heavy chains does not show attenuation. In 40% of septic patients, necrotic fibres

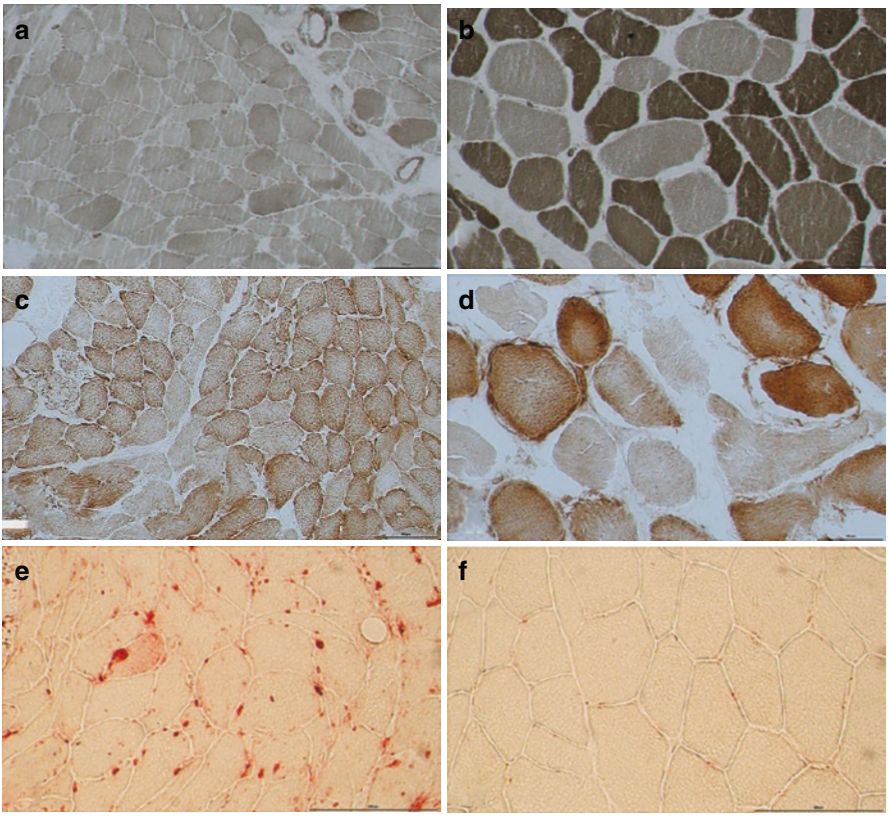

Fig. 12.1 Histochemical demonstration of myofibrillar ATP-ase activity pH 9.4 (**a**), cytochrome oxidase (**c**) and acid phosphatase (**e**) in CIM compared to control (**b**, **d**, **f**). Vastus lateralis muscle biopsy. Enzyme activities of myofibrillar ATP-ase and cytochrome oxidase are reduced, acid phosphatase activity is increased below the sarcolemma and in the endomysium. Cytochrome oxidase activity is nearly absent in necrotic fibres. Bar 100 μm

and CD68-positive macrophage infiltrate were found at early biopsies [29] contrary to the common finding that muscle fibre necrosis is usually detected mostly in late biopsies [23, 39]. It seems that qualitative changes in muscle fibres may occur quite early.

On late biopsies, i.e. 2/3 weeks after the admission to intensive care unit histochemical activity of cytochrome oxidase may be reduced (Fig. 12.1c) and activity of acid phosphatase increased (Fig. 12.1e). Necrotic muscle fibres (Fig. 12.2a), as well as scattered atrophic angular fibres and small group atrophy may be present (Fig. 12.2b). By electron microscopy on longitudinal view, loss of myosin filaments is observed (Fig. 12.3). A specific pathomorphological lesion in CIM is selective loss of myosin myofilaments relative to actin [40]; Electrophoresis of total muscle

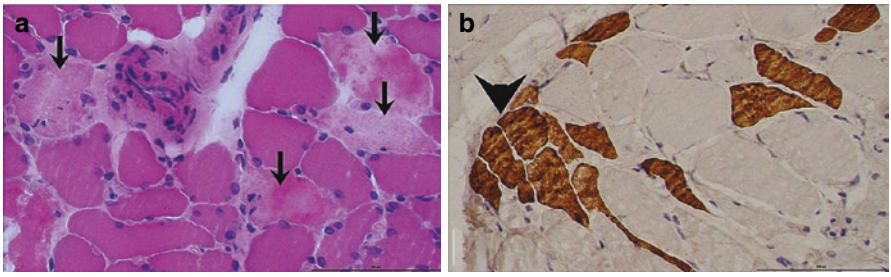

Fig. 12.2 General histopathology of CIM. Vastus lateralis muscle biopsy. Haematoxylin and eosin (**a**) and myosin heavy chain 2A (**b**). Necrotic fibres are marked by arrows (**a**). A small cluster of atrophic fibres (arrowhead) which express myosin heavy chain 2A (**b**). Bar 100 μm

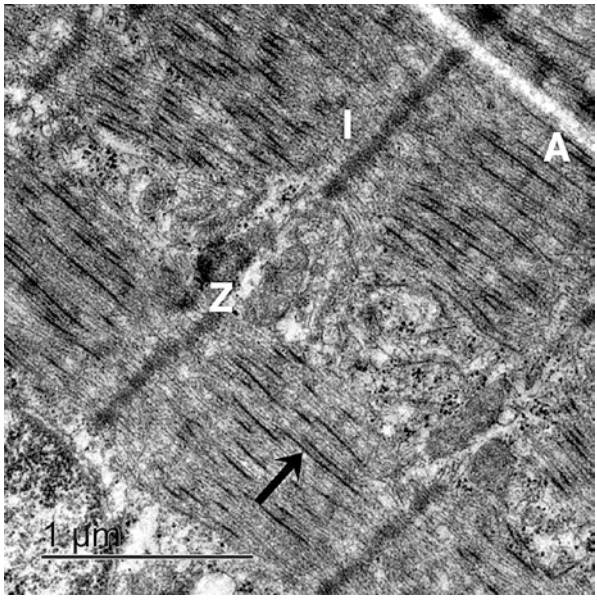

Fig. 12.3 Electron microscopy shows severe loss of myosin filaments (arrow) which causes nearly disappearance of A band. Actin filaments are preserved and I band and Z line look normal. Vastus lateralis muscle biopsy

homogenate detects a reduction of myosin in relation to actin (Fig. 12.4) [41, 42]. There is no predilection for the loss of the specific myosin heavy chain isoform [43], but more severe muscle atrophy is usually observed in type 2 fibres (Fig. 12.2b).

No inflammatory changes are detected in CIM [34]. Increased macrophages in endomysium may be found, usually around necrotic fibres [29].

In addition to myopathy neurogenic alterations may be observed: Fibre type grouping indicates denervation and reinnervation and is a late phenomenon unrelated to the acute muscle weakness/paralysis; it may reflect a pre-existing chronic condition such as an axonal neuropathy due to diabetes or a previous denervation and reinnervation due to radiculopathy. Small group atrophy or scattered angular fibres may be related to distal concomitant axonal damage, if CIM and CIP co-exist. Despite that developmental myosins which are re-expressed during muscle regeneration and functional denervation are generally accepted as a specific marker of regenerating fibres in the pathologic skeletal muscle [44], i.e. an argument for myopathy, they can appear also in neurogenic disorders, for example, motor neuron disease [45]. Since up to now no reliable marker of acute denervation exists, it is impossible to state whether the scattered angular fibres result from acute or chronic neuropathy or from segmental muscle fibre necrosis (myopathy).

Both nerve and muscle can be injured during "multiorgan failure" at critical illness and mixed myopathic and neuropathic alterations can in fact be part of combined neurogenic and myopathic changes of varying severity in response to critical illness.

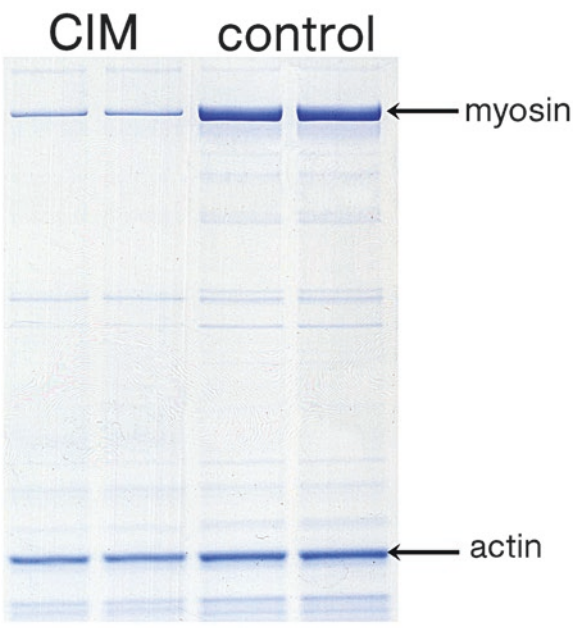

Fig. 12.4 Electrophoresis of total muscle homogenate. Severe loss of myosin in relation to actin. The same patient as shown in Fig. 12.3

Acute necrotizing myopathy of intensive care unit [34] is diagnosed if necrotic fibres are the outstanding feature; necrotic fibres may be related to concomitant toxic myopathy, due to adverse effect of pharmacotherapy, or muscle fibre necrosis may be considered as an advance stage of CIM; acute necrotizing myopathy of intensive care unit is often associated with myoglobinuria [34]. As stated above, necrotic fibres are usually detected in late biopsies, some reported scattered necrotic fibres also in early biopsies [29].

Muscle biopsy is useful for the demonstration of the characteristic myosin loss and is important with respect to prognosis since CIM has more favourable short- and long-term outcomes than CIP [46]. An exception is the prognosis for recovery from weakness of acute necrotizing myopathy of intensive care unit, which is very poor [34].

However, myopathy in pure sepsis, i.e. septic myopathy, does not produce severe myosin loss [6]. Pathomorphologically muscle fibre necrosis is outstanding (Fig. 12.5) but invasion of leucocytes into muscle may not be detected on muscle biopsy due to focal changes. According to [6], septic myopathy should be considered as "an independent injurious event", in addition to CIP and CIM.

Muscle biopsy is not universally available, is invasive and time consuming. In addition. Unspecific, mixed myopathic-neuropathic changes, may be detected and

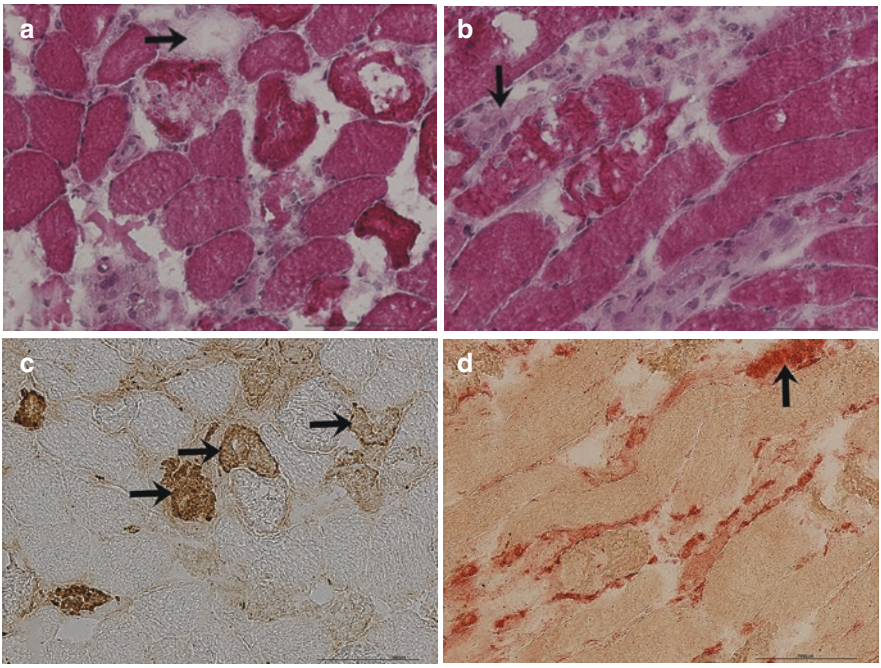

Fig. 12.5 Histopathology of septic myopathy. Vastus lateralis muscle biopsy. Haematoxylin and eosin (**a**, **b**), final component of the complement (**c**) and acid phosphatase (**d**). Necrotic fibres including those invaded by macrophages are marked by arrows (**a**–**d**). Bar 100 μm

caution in the interpretation is needed, since neuropathic signs can be chronic, not related to ICU-acquired weakness. Morphological analysis also takes time and is fairly inconvenient for the demands of an intensive care. Quantification of the myosin–actin ratio in electrophoresis is more appropriate with respect to time, since it can be performed in 1 or 2 days, but further studies are needed in this field to understand the clinical significance of different degrees of myosin loss.

12.4.5 Nerve Biopsy

Nerve histology is initially preserved. Most sensory nerves in early biopsies (day 15 of sepsis) look normal, despite having reduced SNAP [23]. Late biopsies (day 56) demonstrate large axonal loss [23]. Small fibre neuropathy was recently demonstrated in skin biopsies of the critically ill [47], and this may be responsible for neuropathic pain, stocking and glove sensory loss, cool extremities, and burning pain in the survivors of CIP [47].

Axonal degeneration was also observed in autopsy samples of sympathetic chain and vagal nerve [48] and autonomic dysfunction is frequently observed in the critically ill [47].

12.5 Pathophysiology

CIM and CIP are not isolated events but are an integral part of multiorgan dysfunction syndrome in severe illness and a shared pathogenesis of microcirculatory, cellular and metabolic pathophysiological mechanisms for CIM and CIP is likely [2]. A comprehensive review of proposed pathophysiological mechanisms from clinical studies and animal experiments can be found in [6]. Increasing awareness of central nervous sequels in survivors of critical illness supports a possible role for central neuroimmune regulation of both CIM and CIP through IL-1β signalling which can evoke a catabolic programme in muscle by activation hypothalamic-pituitary-adrenal axis [2].

12.5.1 CIM

Skeletal muscle dysfunction in CIM is a combination of reduced muscle mass (muscle wasting or atrophy) and impaired contractility [2].

12.5.1.1 Muscle Wasting

In the critically ill, several processes, such as immobility—muscle unloading, inflammation, endocrine stress response, cellular energy stress, rapidly developing nutritional deficit, impaired microcirculation and denervation, can cause muscle atrophy [2, 7]. Tumour necrosis factor-α, interleukin-1, interleukin-6, and growth and differentiation factor-15 are key proinflammatory mediators involved [7].

Muscle wasting may contribute to weakness, premature fatigue, and glucose intolerance [49]. Muscle wasting in CIM can occur quite early [29]*:* Rectus femoris cross-sectional area decreased for about 20% from days 1 to 7, detected on ultrasound. Muscle wasting was more pronounced in multiorgan failure compared to single organ failure and also correlated with hypoxemia; these findings strongly suggest that muscle atrophy in ICU-acquired weakness is not only the result of inactivity [50].

A net catabolic state could be the consequence of diminished protein synthesis and increased muscle proteolysis which were both demonstrated in muscle wasting in CIM [29]. Protein synthesis also remained refractory to increased protein delivery. The ubiquitin-proteasome system, studied mostly in patients with sepsis, [51, 52] and calpain activation [43, 53, 54], mediate enhanced proteolysis in the critically ill. The role of the caspase family of cysteine proteases in muscle proteolysis in the critically ill is suggested from animal studies [6]. Lysosomal proteases, cathepsins, have been evaluated for their contribution to muscle loss in sepsis [55], but there is no current consensus on the role of cathepsins in CIM [6]. Increased lysosomal (and proteasomal) activation was observed in the diaphragm of prolonged (15–276 h) mechanically ventilated patients [56], and it was concluded that activation of both systems is responsible for fibre atrophy in the critically ill. Increased lysosomal activity is easy to demonstrate on acid phosphatase stain on muscle biopsy. However, in adult *prolonged critically ill* patients, insufficient autophagy [57] may cause inadequate removal of damaged proteins and mitochondria and may explain prolonged recovery or lack of recovery.

Immobility per se causes a decrease in muscle protein synthesis and is associated with the so-called anabolic resistance, i.e. diminished protein synthesis as a response to infusion of amino acids [58]. Older critically ill patients display in addition "anabolic resistance" due to age per se, diminished suppression of muscle proteolysis by insulin [58] and diminished mitochondrial respiratory capacity [59]. It follows that advance age represents high risk for ICU-acquired weakness.

12.5.1.2 Muscle Contractile Dysfunction

Impaired contractility [2] is brought about by decreased sarcolemmal excitability during early phase of ICU-acquired weakness, later by the loss of motor protein myosin and suppression of contractility by free radicals, abnormalities of Ca^{2+} sequestering and depletion of cellular energy by mitochondrial dysfunction.

Decreased Excitability of Sarcolemma

Excitability of skeletal muscle is decreased early during critical illness and predicts ICU-acquired weakness in sedated and mechanically ventilated patients [35]. Direct muscle stimulation in critically ill detects reduced dmCMAP, compatible with the decreased excitability of sarcolemma [21, 22, 60]. Assessment of dmCMAP has led to the concept that most patients with ICU-acquired weakness have in fact a myopathy [61].

Decreased excitability of sarcolemma could be induced by sodium channel dysfunction, as demonstrated in vitro in patients with sepsis [62] and experimentally, in

sepsis and steroid-denervation experiments in rats [6]. Sodium channelopathy is an early event during ICU-acquired weakness and contributes to muscle weakness.

Myosin Loss

Preferential loss of the molecular motor protein myosin reduces muscle contractility and contributes to muscle weakness during second phase of ICU-acquired weakness. A reduction of myosin in relation to actin can be demonstrated by electron microscopy [63] and/or electrophoresis of total muscle homogenate [41, 42]. There is either no predilection for the loss of the specific myosin heavy chain isoform [43] or more pronounced reduction of "fast myosin" can be detected [64]. More severe atrophy is usually observed in type 2 fibres. Myosin loss is also accompanied by decreased myosin heavy chain mRNA expression [42, 64].

Inflammation

Systemic inflammation has been linked to the development of muscle weakness in humans [2]. Inflammation has an effect both on muscle mass (see above) and muscle contractility, i.e. chronic inflammatory states can reduce muscle contractile force by increasing free radicals, which depress myofibrillar function [65].

Uncoupling of Excitation-Contraction

Uncoupling of excitation-contraction has a negative impact on contraction and might be an accompanying mechanism of CIM for the subpopulation of intensive care unit patients with comorbidities, such as *chronic obstructive pulmonary disease* and *congestive heart failure* in whom the pre-existent abnormalities of Ca^{2+} sequestration exist [2], and these might worsen by stress-induced elevated sympathetic nerve activity in intensive care unit [2].

Mitochondrial Dysfunction/Abnormalities

The loss of normal mitochondrial function results in depletion of cellular energy and increased production of free radicals [2]. Complex I and IV of the respiratory chain in particular are depleted in CIM [66]. Activation of mitochondrial biogenesis seems to be important for short and late outcome: if compensatory mechanisms of increased mitochondrial biogenesis are activated early, this has a positive effect on survival in critical illness [66]; in critically ill patients with a prolonged clinical course markers of mitochondrial biogenesis are not upregulated [67].

12.5.2 CIP

CIP is a distal axonal sensory-motor polyneuropathy affecting limb and respiratory muscles. CIP is in fact less frequent as an independent injurious event than previously thought: it was overestimated in past, based on EMG only, particularly if MUPs could not be estimated; in such cases, electrophysiological diagnosis was invariable CIP even if CIM was ongoing (revealed by muscle biopsy findings) [23, 24]. Abnormalities in CMAP and sensory nerve action potential may occur within

hours in humans [25]. Reversible inactivation of sodium channels was demonstrated in a rat ICU model of CIP [68], but this was not confirmed in humans [2]. In some patients, weakness subsides when the global health is restored, but a subgroup of patients does not regain normal function even after 1–2 years [2]. As already stated, the current view is that CIP is not an isolated event but an integral part of multiorgan dysfunction syndrome and the precise mechanisms are not known. Diabetes mellitus as a pre-existing morbidity predisposes to CIP and the severity of CIP corresponds to serum glucose levels [7].

12.5.2.1 Microvascular Injury and Membrane Depolarization Defect

Microvascular injury of nerve, mediated by endotoxins, drugs, hyperglycaemia, reactive oxygen species (ROS) and proinflammatory mediators (tumour necrosis factor-α, serotonin and histamine) released during sepsis or systemic inflammatory response syndrome, causes hypoperfusion, endoneurial oedema and lack of oxygen. Accumulation of potassium and acidic metabolites in the endoneurium leads to depolarization of the nerve membrane and nerve dysfunction [2]. The hypothesis of (micro)vascular injury is supported by increased expression of E-selectin in the endothelial cells of endoneurial microvessels and epineural small-calibre vessels of critically ill patients [69]. E-selectin mediates the initial step of leukocyte adhesion and extravasation to the endoneurial space, which leads to endoneurial cytokine production and tissue injury during sepsis [69].

12.6 Biomarkers

At present, no validated biomarkers for CIM/CIP are available [7]: Creatine kinase may be raised in CIM and slightly also in CIP, but is not a good biomarker; biomarkers of axonal injury, plasma levels of neurofilaments, are elevated in patients with ICU-acquired weakness, but early diagnosis of ICU-acquired weakness, before muscle strength assessment, is not possible using neurofilament levels in plasma and the marker also does not differentiate between CIP and CIM; a possible future candidate may be stress-induced cytokine, growth, and differentiation factor-15 (GDF-15) [70]. MicroRNAs, as MiR-181a [71], may be the solution for the increasing need for biomarkers in intensive care unit-acquired weakness [72].

12.7 Prevention and Therapy

- Aggressive treatment of sepsis is considered to be a cornerstone in the prevention of ICU-acquired weakness [7].
- Insulin treatment for normalizing glycaemia is complex and difficult to perform optimally. It seems that absolute normoglycaemia is not the optimal choice since patients treated to strict normoglycaemia had a worse outcome than patients treated to slightly higher blood glucose levels [73]. Continuous monitoring of

blood glucose versus intermittent is under discussion and additional research is needed, if continuous monitoring of blood glucose is to become a routine part of daily practice in the management of critically ill patients [7].
- Reducing the duration of immobilization can be achieved by decreasing the levels of sedation and overall beneficial effects of decreased sedation have been demonstrated [74].
- Early passive and active exercise training (such as bedside ergometer) improve muscle strength at hospital discharge [75]; however, evidence of long-term efficiency remains uncertain [20].
- Functional electrical muscle stimulation may be used to activate muscles during the period when patients are not able to cooperate. In bed cycling also increased thigh circumferences of rectus femoris but adding functional electrical stimulation did not show difference [76]. The evidence remains inconclusive and more research is necessary [77].
- Diaphragm-protective mechanical ventilation has been studied in a porcine animal model [78], but has not yet been studied in humans [33]. Optimal diaphragm load may be similar to that of healthy subjects breathing at rest [12, 32].
- Drugs that inhibit pathways of proteolysis or enhance protein synthesis are under way [20].
- Late parenteral nutrition accelerates recovery compared to early parenteral nutrition [79] since it reduces muscle weakness (but not atrophy), but this effect was of short duration. Accelerated recovery may be mediated by more efficient activation of autophagic quality control of myofibres. The interactions between nutrition and ICU-acquired weakness remain incompletely understood [20].
- Respiratory muscle training is feasible and improves both inspiratory and expiratory muscle strength; the impact on clinical outcomes requires further confirmation [80].

12.8 Long-Term Disability After ICU-Acquired Weakness

Survivors of critical illness often experience permanent functional disability. Assessing patients 6 months after intensive care unit discharge, it was concluded that long-term disability is not explained by ongoing muscle proteolysis, inflammation, or diminished mitochondrial content [30]; sustained muscle atrophy and impaired voluntary contractile capacity might be associated with diminished regenerative capacity resulting from the loss of satellite cells.

12.9 Update on Experimental Pharmacological Interventions in Intensive Care Unit-Acquired Weakness

It seems logical to seek pharmacological interventions that may attenuate muscle injury or help recovery, however none of the interventions implemented so far can be recommended; the research area in pharmacological therapies in ICU-acquired

weakness needs strong preclinical biological rationale and early phase evidence of safety before critically ill patients are included in clinical trials [81].

12.9.1 Experimental Rat Model of CIM and Ventilator-Induced Diaphragmatic Dysfunction

Experimental rat model induces muscular atrophy and weakness with preferential myosin loss in limb muscles in response to mechanical silencing [82, 83] and VIDD [84–86]. This model induces an identical limb muscle genotype and phenotype observed in ICU patients with CIM [82]. In the experimental rat model, controlled mechanical ventilation is used, which is less often applied in clinical practice than assisted mechanical ventilation. As difficulties in weaning from the ventilator are not prevented by the assisted mechanical ventilation, it is assumed that both types of mechanical ventilation induce similar pathology in the diaphragm. The latter may be more pronounced at controlled mechanical ventilation [86].

Two weeks of controlled mechanical ventilation in rats causes markedly decreased diaphragmatic function assessed by in vitro contractility and motility measurements, oxidative stress, post-translational modifications of myosin, activation of proteolytic pathways, muscle atrophy, vesicular or swollen mitochondria with less organized cristae and less electron-dense matrix and intracellular lipid accumulation, i.e. VIDD [84, 86]. In contrast to limb muscles, there is no preferential myosin loss or transcriptional down-regulation of myosin synthesis in VIDD after 2 weeks of controlled mechanical ventilation. Qualitative, not quantitative alterations of myosin characterize VIDD in a rat experimental ICU model, i.e. a rodent model not limited by early mortality. These post-translational myosin modifications reduce myosin–actin interactions as demonstrated in "in vitro" motility assay [86]. Mitochondrial dysfunction, intracellular lipid accumulation and ROS production are important in the pathogenesis of VIDD [84].

12.9.2 Vamorolone

Vamorolone (or VBP15) is a synthetic prednisolone derivate originally designed to replace prednisolone in Duchenne muscular dystrophy [87]. It has reduced genomic activity-related side effects but retains anti-inflammatory effect by potent inhibition of NF-κB pathway, mediated through protein interactions of the cytoplasmatic glucocorticoid receptor. Vamorolone is a hydrophobic substance and is expected to have physicochemical effects also on lipid bilayers. Contrary to prednisolone, it is a membrane stabilizer. In the live single-cell laser injury model, vamorolone protected injury to the plasma membrane, indicated by the uptake of fluorescent dye (FM1-43), while prednisolone exacerbated it [87].

Vamorolone increases maximal force (but not specific force), measured in vitro, of slow-twitch soleus muscle fibres in the experimental rat model of CIM [88]. The effect could be mediated by its stabilization membrane effect. As low level of

immune activation seems to be ongoing in CIM [89], some of its beneficial effects in CIM may also be anti-inflammatory.

Vamorolone also demonstrated an improved survival at 5 days experimental period, significantly less decrease of body weight and slow-twitch soleus weight, but not of fast-twitch extensor digitorum longus, were observed with respect to untreated [88].

The use of steroid therapy in critically ill ICU patients is controversial since it has been forwarded as an important factor triggering CIM [90, 91], at least when given in high doses; however, low to moderate doses may be beneficial [88]. In a recent Cochrane's review [92] of the use of corticosteroids in sepsis, it was concluded that corticosteroids may result in little or no difference in long-term mortality, reduce the length of intensive care unit and hospital stay, but increase the risk of muscle weakness. However, it was demonstrated [88] that in the experimental rat ICU model the effects of classical corticosteroid (prednisolone) and the new generation glucocorticoid (vamorolone) differ: Prednisolone demonstrated significant depression of size and contractility (maximum and specific force) of fast-twitch extensor digitorum longus fibres compared to vamorolone treated; in slow-twitch soleus fibres, prednisolone caused significantly higher activation of ubiquitin/proteasome degradation pathway at the level of transcription of atrogenes (MURF1 and atrogin-1) than vamorolone. It seems that next-generation glucocorticoids might in fact be suitable for the treatment of critically ill immobilized ICU patients; in addition, three serum cytokine markers (IL-18, IP-10 and fractalkine) were significantly higher in prednisolone treated group than in vamorolone. The main concern with vamorolone might be that it did not increase specific force of muscle fibres [88]. This indicates that in spite of having positive effect on muscle fibre size, the quality of muscle fibres, i.e. contractility seems to be compromised in spite of vamorolone treatment.

12.9.3 BGP-15

Hallmark of stressed cells and organisms is the elevated synthesis of the ubiquitous and highly conserved heat shock proteins (HSP)s [93]. HSPs, as HSP70 and HSP72, are important for proper protein folding and repairing of damaged proteins and polypeptides during stress [94]. Masticatory muscles, which are spared in CIM, have a higher level of upregulated of HSPs than limb muscles [95]. A positive correlation exists between HSPs and specific force in rat and porcine ICU models [83, 96], suggesting that HSPs are also necessary for the maintenance of muscle force.

HSP co-inducer is a substance that cannot enhance HSP production alone but can enhance HSP induction in combination with mild stress [93]. HSP co-inducers might have indications in many diseases as they target only stressed cells and potentially have fewer side effects than drugs without this selectivity [97].

Besides protein denaturation or alteration in nucleic acid conformation, stress protein signals may originate also from the cellular membranes according to the "membrane sensor" hypothesis [98, 99]. There is a growing body of evidence

linking the production of HSPs, triggered by exposure to different kinds of environmental stress conditions, to subtle membrane changes, i.e. changes in the lipid composition and in the architecture of membranes. In pathophysiological conditions, these changes may cause dysregulated HSPs induction and expression due to abnormal signal transduction [93].

In experimental models of CIM, impaired upregulation of HSP has deleterious effects on muscle structure and function [85, 95, 100].

Multi-target compound, a hydroximic acid derivate, HSP72 co-inducer BGP-15 is a "membrane lipid therapy" pharmaceutical, that alters the organization of cholesterol-rich membrane domains so-called lipid rafts [93]. It fluidizes yet stabilizes membranes [101]. BGP-15 activates stress signal transduction pathways for HSPs induction by remodelling plasma membrane rafts. BGP-15 also facilities Rac-1 signalling [93, 101], a membrane bound protein which regulates redox-dependent HSP activation [102]. It seems that BGP-15 restores the HSP stress response, i.e. aids organs in tolerating stress [101]. As poly ADP ribose polymerase-1 (PARP-1) inhibitor it down-regulates multiple inflammatory pathways [103] and preserves the cytoplasmic NAD+ availability needed to activate silent information regulator T1 (SIRT1) [101]. SIRT-1 deacetylases, thereby activates, heat shock factor-1, promoting prolonged HSP transcription, thereby prolonging HSP response [104]. BGP-15 also protects mitochondria by reducing the production of free radicals [105–107] and stimulates mitochondrial biogenesis [108]. By suppressing ROS levels, BGP-15 decreases protein-, lipid-, and DNA oxidation ultimately decreasing single-stranded DNA breaks formation and PARP-1 activation [101]. Mitochondrial dysfunction, ROS production and deficiency of HSPs are important in the pathogenesis of CIM and BGP-15 seems to be an appropriate pharmaceutical to be investigated in CIM.

BGP-15 administrated intravenously at a dose of 40 mg/kg per day for 10 days improved the activity of complex III and IV of respiratory chain enzymes, increased mitochondrial content and reduced post-translational myosin modifications as demonstrated by mass spectroscopy and restored the force generating capacity of muscle fibres to 75% of normal [86]. BGP-15 significantly improved soleus muscle fibre force after 5 days exposure to the intensive care unit conditions and had a strong positive effect on survival compared to untreated animals [109].

BGP-15 (a) improved some markers of mitochondrial fusion, fission, mass, and biogenesis which were all down-regulated in untreated; (b) reduced sumoylation, oxidation, deamidation, carbonylation and nitration of myosin compared to untreated; (c) upregulated HSP 72 which was not upregulated in untreated rats and (d) reduced the ratio cleaved/inactive PARP compared to untreated [86]. As protein carbonylation is an indicator of ROS [84], reduced carbonylation induced by BGP15 indicates reduced ROS production and mitochondrial protection by BGP15 in the experimental rat ICU model. It seems that the improvement in specific force was mediated by improved quality of myosin, i.e. less myosin post-translational modifications due to decrease in ROS production. However, the effect of BGP-15 was age dependent: In old rats, BGP-15 did not induce a concomitant increase in HSP72 in identical doses as in young rats [110]. An age-related impairment in the induction

of HSP by stressors is well known [111]. Ageing increases membrane stiffness [101] and stiff membranes limit the cellular stress response [112, 113]. As good tolerability and safety of BGP-15 was reported in clinical trials related to treatment of type 2 diabetes mellitus [114], higher doses in older animals than the one used in the study of Salah et al. [86] are pending.

Nevertheless BGP-15 is a promising drug to combat VIDD at least in young, as it improves mitochondrial dysfunction, decreases ROS production and thus reduces post-translational myosin modifications.

Highlights

- Clinicians should be aware that ICU-acquired weakness can be due to different concomitant causes.
- A myopathy or a polyneuropathy can be the underlying pathogenic mechanism of this flaccid weakness.
- Although the myopathy is acute, the time of onset is difficult to determine.
- Critical illness myopathy can be part of generalized reduction of sarcolemma excitability in the early phase and due to loss of myosin thick filaments in the second phase of ICU-acquired weakness.
- The role of early mobilization and rehabilitation should be studied.
- The predictive values of EMG and muscle ultrasound should be confirmed in further studies.
- Specialized nutritional strategies need further evaluation.
- Optimal pain treatment, minimal sedation and daily spontaneous breathing should be implemented and evaluated.

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Acquired Immune-Mediated Rippling Muscles With and Without Myasthenia Gravis

13

Carl F. Ansevin

13.1 Introduction

The hallmarks of rippling muscle diseases are peculiar stretch and percussion-induced contractions that roll in a wave-like manner across muscles [1]. These mechanosensitive contractions are "electrically silent" with no evidence of myotonia, neuromyotonia, or myokymia [2]. Rippling muscle diseases are neuromuscular disorders that are either genetic or immune-mediated. The rippling muscle phenomena is clinically and electrophysiologically identical in inherited and immune-mediated rippling muscle disease [1–6].

Inherited rippling muscle disease (hRMD) is usually an autosomal dominant disorder and presents in childhood or early adulthood with myalgias and cramps [2]. This is due to a genetic defect in the gene encoding caveolin-3 on chromosome 3p25 [7].

An acquired autoimmune form of rippling muscles is usually associated with myasthenia gravis either with or without thymoma [3–5]. Immune-mediated rippling muscle disease (iRMD) also occurs without myasthenia gravis although this has been less often reported [6].

The genetic form of rippling muscle disease will be first described in this chapter as background for the acquired immune-mediated rippling muscle diseases both with and without myasthenia gravis.

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13.2 Inherited Rippling Muscle Disease

Rippling muscle disease is rare and usually inherited in an autosomal dominant pattern. Rippling muscle disease (RMD) is a myopathy with symptoms and signs of mechanosensitive muscle contractions. The disorder was first described in 1975 in five members of a Scandinavian family over three generations [1]. They had stiffness after periods of rest and muscle mounding after percussion. The most unusual manifestation was the peculiar rolling wave-like phenomena in muscle after percussion or stretch. There was no electrical evidence of myotonia and the pedigree suggested an autosomal dominant inheritance.

In 1989, six patients in two German families were described and the disorder was aptly named rippling muscle disease because of the peculiar wave-like phenomena propagated across skeletal muscle [2]. The waves of rippling muscles could be induced by stretch or percussion and traveled at 6 m/s which was about ten times slower than muscle action potentials. Because the phenomenon was "electrically-silent" (i.e., no evidence of myotonia)—an intracellular calcium-mediated pathogenesis was postulated.

Five large German and Norwegian families with rippling muscles were reported in 2001 and the disorder was localized to chromosome 3p25 with missense mutations in the gene encoding caveolin-3 (CAV3) [7].

Most cases that are inherited are autosomal dominant although a more severe homozygous autosomal recessive form has also been described [8–10].

13.2.1 Symptoms and Signs

The onset of symptoms in the inherited form is usually in childhood or early adulthood. Myalgia and pain appear to be the most common and distressing symptoms while patients usually do not complain of the rippling phenomena. Pain is associated with cramps (both spontaneous and exertional) and can range from mild to severe. Interference with glycogenolysis due to problems with caveolae-associated phosphofructokinase has been postulated to contribute to the pain. Other signs include muscular hypertrophy, weakness, and percussion-induced muscle mounding and rapid contractions of muscle.

13.2.2 Phenotypic Variability

The rippling muscle phenomenon is the most common manifestation of caveolin-3 disorders, but there is significant phenotypic variability [11]. Defects in the caveolin-3 gene were initially shown to cause a form of limb girdle muscular dystrophy [12]—which was classified as LGMD 1C. The onset of symptoms in these disorders can be in childhood, adolescence, or as an adult. Symptoms

include myalgias, cramps, and weakness and serum CPKs are elevated. The CPKs can be elevated up to 10–40 times normal, and myoglobinuria has been described. Next-generation sequencing has continued to expand the phenotypic spectrum with cases of CAV-3 mutations associated with exercise intolerance, myalgias, and rhabdomyolysis [13].

Defects in the caveolin-3 gene cause rippling muscle disease. Caveolin-3 gene defects cause four different clinical pictures [14]. Besides rippling muscle disease and LGMD 1C, caveolin-3 defects can cause a distal myopathy and hyperCKemia. Patients with cardiomyopathies and long QT intervals have also been reported.

Phenotypic variability of genetic defects in the caveolin-3 gene are thus capable of causing both dystrophic and non-dystrophic neuromuscular problems. LGMD with a defect in the caveolin-3 gene was described before hRMD was localized to chromosome 3p25. The same genetic defects in caveolin-3 associated with LGMD 1C cause RMD. Over 30 defects in the caveolin-3 gene have been described.

13.2.3 Caveolin-3 and Caveolae

Caveolins are a family of structural proteins that form caveolae on cell surfaces including sarcolemmal membranes. Caveolae are 50–80 nm diameter bulb-shaped invaginations in plasma membranes of most cell types including the sarcolemma [15]. Caveolae are lipid-rich and the structures formed by caveolins have been referred to as “lipid rafts” in cell membranes [16]. An additional family of integral structural proteins in caveolae called cavins has also been described.

There are three isoforms of the caveolin family—CAV -1, -2, and -3. Caveolin-1 and -2 are in most tissues, but caveolin-3 is specific and exclusive to striated and smooth muscle. Caveolin-3 is the major structural protein in caveolae in muscle. In addition to structural scaffolding, caveolin also has roles in membrane transport and signal transduction [17].

Caveolin-3 has also been associated with the transverse (T) tubule system [18]. Caveolin-3 hot spots have been described at the necks of transverse tubules after the plasma membrane has been removed from rat skeletal muscle [19]. Disorganized T-tubules are seen in human caveolin-3 limb girdle muscular dystrophy [20].

Initially, caveolin-3 was thought to be an integral part of the dystroglycan complex [21]. However, by a variety of biochemical and histochemical techniques the association with the sarcolemmal dystroglycan protein complex was not as strong as initially thought [22]. Caveolin-3 directly interacts with the C terminal tail of β-dystroglycan on the cytoplasmic side of the sarcolemmal membrane [23].

Caveolae and the caveolin-cavin system play a critical role in skeletal muscle mechanoprotection [24]. Caveolae can flatten in response to stretch and provide mechanoprotection to muscle cells [15]. This is particularly important in skeletal muscle—a tissue that is constantly undergoing contraction and stretching and requires extreme mechanical flexibility and durability.

13.2.4 Caveolin-3 Immunohistochemistry and Western Blots

Patients with the inherited form of RMD have a homogeneously decreased pattern of staining for caveolin-3 on muscle histochemistry and decreased or absent caveolin-3 on western blots. Dysferlin is also often somewhat decreased homogeneously on histochemistry but is quantitatively normal on western blots. Dysferlin is associated with caveolin [25].

13.2.5 Electron Microscopy

Ultrastructurally, caveolae in hRMD are diminished or absent in sarcolemmal membranes but present in small blood vessels. Caveolin-3 is specific for skeletal and smooth muscle, while caveolae in blood vessels and most other tissues utilize caveolins 1 and 2.

13.3 Acquired Immune-Mediated Rippling Muscles with Myasthenia Gravis

An autoimmune form of rippling muscles associated with myasthenia gravis was first described in 1996 [3]. The initial patient was 56 years old when he developed myalgias and noticed the rippling muscle phenomena. He had nine siblings and no family history of RMD. His disorder was characterized by a stretch and percussion-induced electrically silent (i.e., no myotonia or neuromyotonia) rippling phenomena that propagated across the muscle in an organized wave-like manner. The rippling muscle phenomena was identical to the rippling muscles described in the inherited forms of RMD, and like the inherited forms of RMD he had muscular hypertrophy and mild weakness when initially examined. The patient was lost to follow-up for 5 years. When he finally returned, he had florid symptoms and signs of myasthenia gravis including ptosis, diplopia, dysarthria, dysphagia, severe weakness, and fatiguability. He had positive acetylcholine receptor antibodies, decremental responses on repetitive nerve stimulation, a dramatic improvement with anticholinesterases, and a thymoma. His CPKs were elevated and he had myopathic and inflammatory changes on muscle biopsy. He was treated with plasmaphereses, thymectomy, steroids, and immunosuppression and improved.

The next two cases of rippling muscles with myasthenia gravis were described in 1999 and they also responded to immunosuppressive therapy [26].

To date there have been over 20 cases of rippling muscles associated with myasthenia gravis reported [3–5, 26–31]. Cases have also been reported with thymomas [3, 29, 32].

13.3.1 Symptoms and Signs

Like the initial case of autoimmune rippling muscles with myasthenia gravis, subsequent patients have presented with myalgias and the stretch and percussion

activated rippling muscle phenomena. These patients generally present in adulthood and have elevated CPKs. There is no family history of neuromuscular disorders in the acquired immune-mediated form of rippling muscles. The rippling muscle phenomena can present prior to the myasthenic symptoms. The rippling muscles can be so dramatic as to sometimes overshadow more subtle myasthenic symptoms such as mild ptosis, intermittent diplopia, and/or dysphagia [3]. Weakness may also be mild, especially in the early stages of the disorder.

13.3.2 Laboratory Findings

13.3.2.1 Muscle Enzymes

Mildly elevated CPKs are usually present.

13.3.2.2 Autoantibodies to NMJ

Patients with autoimmune rippling muscles with myasthenia gravis have positive anti-acetylcholine receptor antibodies. Thus far, immune-mediated rippling muscle disease has not been reported in patients with seronegative myasthenia gravis, or in those with anti-MuSK or LRP4 antibodies.

13.3.2.3 Anti-skeletal Muscle Antibodies in Acquired Immune-Mediated Rippling Muscles with Myasthenia Gravis

In the patients with iRMD and myasthenia gravis in whom it has been reported: 10/11 patients had antibodies to skeletal muscle; 5/8 had antibodies to titin; and 4/7 had antibodies to the ryanodine receptor [3, 4, 26, 31–33]. Voltage-gated potassium and calcium channel antibodies have been negative [4]. Anti-skeletal muscle and titin antibodies have have long been known to be associated with myasthenia gravis both with and without thymomas. Patients with iRMD with myasthenia gravis have antibodies to titin that bind near the PEVK epitope [33]. The PEVK region is felt to be responsible for the elasticity of titin and is distinct from the main immunogenic region previously identified in patients with myasthenia and thymomas [34]. Because of the association of acquired rippling muscles with myasthenia gravis—antibodies to skeletal muscle antigens have long been suspected in the pathogenesis of immune-mediated rippling muscles.

13.3.2.4 Electrophysiological Studies

EMGs in patients with both the inherited and iRMD show the rippling muscle phenomena to be electrically silent [2–6, 26–32]. The term “electrically silent” has been used to differentiate the organized wave-like phenomena of rippling muscles from myotonia, neuromyotonia, and the more vermiform myokymia—all of which are quite visible and noisy on electromyography. There have been isolated reports electrical activity in rippling muscle disorders [31] which have been difficult to characterize [35] and distinguish from normal insertional activity [29, 32]. Myotonia, neuromyotonia, and myokymia are easily distinguishable and are not present in rippling muscle disease.

13.4 Muscle Biopsies in Immune-Mediated RMD

Myopathic and inflammatory changes have been noted in muscle biopsies from patients with immune-mediated rippling muscle disease [3–6]. The myopathic changes reported are usually mild and include fiber size variability from 28 to 140 um and central nuclei in about 20% of fibers [4, 30]. Inflammatory changes are also usually minimal with scattered rare endomysial islets of lymphohistiocytic cells [4–6]. Interstitial infiltrates consist of both lymphocytes and plasma cells. In cases in which it has been described, the majority of lymphocytes express T-cell antigens with more CD4-positive cells than CD8-positive cells. MHC 1 upregulation has also been described. In most cases, the inflammatory changes are mild although in more advanced cases with severe myasthenia and thymoma the changes can be more marked [3].

13.4.1 Immunohistochemistry in iRMD

Sera from patients with acquired immune-mediated RMD binds to the sarcolemmal membrane, the T-tubules, and the triad [32, 33]. Decreased caveolin staining in a mosaic pattern on muscle immunohistochemistry in acquired immune-mediated rippling muscle disorders has repeatedly been described in patients both with and without myasthenia [4–6, 30, 32]. This is often accompanied by a decrease in dysferlin staining in a mosaic pattern. Other immunostaining shows the sarcolemmal membrane intact.

13.4.2 Western Blots in iRMD

Caveolin-3 is present on western blots in iRMD although slightly decreased. In some cases, dysferlin has also been reported to be slightly decreased but less frequently and to a lesser extent. Genetic screening for caveolin-3 and dysferlin abnormalities are negative in patients with immune-mediated rippling muscle disease.

13.4.3 Electron Microscopy in iRMD

Ultrastructural studies have shown decreased (or undetectable) caveolae on the sarcolemmal membranes of patients with immune-mediated rippling muscles [32]. Caveolae remain intact on electron microscopy of blood vessels of the same patients since caveolin-3 is present only in muscle and not the endothelial lining of blood vessels. Electron micrographs have shown dilated t-tubules with electron densities around dilated triads [32].

13.5 Immune-Mediated Rippling Muscles Without Myasthenia Gravis

Immune-mediated rippling muscles without clinical evidence of myasthenia gravis or acetylcholine receptor antibodies have been reported. Lo et al. reported three patients with immune-mediated rippling muscles and a mosaic pattern of caveolin-3 staining on muscle biopsy without evidence of myasthenia gravis or acetylcholine receptor antibodies [6]. This mosaic pattern of caveolin-3 staining had previously been reported in a patient with immune-mediated rippling muscles and a thymoma [32] and confirmed in additional patients with iRMD and myasthenia gravis [4, 5, 30]. Dysferlin staining was also decreased in a mosaic pattern, with normal dysferlin levels in these patients. As in patients with iRMD and myasthenia gravis, Lo et al. reported mild myopathic changes in all three of their patients of which two had mild inflammation on muscle biopsies [6]. Genetic screening for caveolin-3 and other inherited disorders has been negative in all patients who have been tested with immune-mediated rippling muscles both with and without myasthenia.

13.5.1 Isolated Presumably Immune-Mediated Cases of Rippling Muscle Disease

Sporadic forms of rippling muscles not associated with myasthenia gravis with benign courses and spontaneous recovery have been reported [6, 36].

13.6 Comparison of Pathology on Muscle Biopsies of hRMD and iRMD with and without Myasthenia Gravis

13.6.1 Homogeneous Vs. Mosaic Pattern of Caveolin-3 Immunohistochemistry

The regularly seen mosaic pattern of caveolin-3 immunostaining in immune-mediated rippling muscle disorders contrasts with the homogeneous decrease in caveolin immunostaining in the inherited form of rippling muscle disease.

13.6.2 Caveolin-3 Absent or Markedly Decreased with hRMD and Decreased or Slightly Decreased with iRMD

The mild decrease in caveolin-3 on western blots in immune-mediated rippling muscles is much less severe than in the inherited forms where caveolin-3 is markedly decreased or absent.

13.6.3 Caveolae Absent in Sarcolemma of hRMD and Decreased in iRMD

On electron microscopy, the caveolae are absent in the sarcolemma of the genetic form of rippling muscle disease and decreased in immune-mediated rippling muscle disease.

13.7 Other Autoimmune Disorders Reported with iRMD

13.7.1 Other Autoimmune Disorders Reported with iRMD with Myasthenia Gravis

Individual cases of acquired autoimmune rippling muscles with myasthenia gravis have been preceded by or co-incidentally diagnosed with the following immune-mediated disorders: Yersinia enterocolitica infection [26], bronchial asthma [26], thymitis [37], alopecia areata [29], viral infections, and thymoma [3, 30, 32]. One patient had colon cancer diagnosed and treated 7 years prior to the diagnosis of iRMD with myasthenia gravis [4]; and in another prostate cancer was diagnosed 6 months after the onset of MG with the onset of the symptoms of rippling muscles [4].

13.7.2 Other Autoimmune Disorders Reported with iRMD Without Myasthenia Gravis

A single case of immune-mediated rippling muscles was reported with an autoimmune hemolytic anemia [38]. Another case was reported 3 years prior to diagnosis of a malignant (lymphoplasmacytoid) lymphoma [39]. Breast cancer was diagnosed 6 months prior to iRMD in a patient with biopsy proven pulmonary sarcoidosis and pernicious anemia [40]. A case of iRMD was reported following a severe viral infection with respiratory involvement [6].

13.8 Treatment of Acquired Immune-Mediated Rippling Muscle Disease with Myasthenia Gravis

Acquired immune-mediated rippling muscles with myasthenia gravis is treatable with immunotherapy.

Interestingly, the acetylcholinesterase inhibitors edrophonium and pyridostigmine used to diagnose and treat myasthenia gravis can worsen the rippling phenomena in patients with iRMD with myasthenia gravis [4, 26].

Patients with rippling muscles and myasthenia gravis respond to immunosuppressive treatment. Immunotherapy is used primarily to treat these patients for myasthenia gravis, as the symptoms of rippling muscles are often mild. The commonly used immunosuppressive treatments for myasthenia gravis improve the symptoms of rippling muscles [3–5, 26]. Typical treatments that have been used include steroids, plasmapheresis, azathioprine, and mycophenolate [3–5, 26]. IV immunoglobulins were tried in one patient with iRMD and myasthenia without benefit on the rippling phenomena [4].

13.8.1 Treatment of Immune-Mediated RMD Without Myasthenia Gravis

Patients with immune-mediated rippling muscles without myasthenia gravis also have been reported to respond to immunotherapy including steroids and plasmapheresis [6].

13.9 Where and What Are the Immunogenic Targets in Immune-Mediated Rippling Muscles?

The precise mechanism of immune-mediated rippling muscles is not known, but there are interesting clues from the clinical and pathologic data available.

13.9.1 Post-synaptically Near the Neuromuscular Junction?

Myasthenia gravis is caused by antibodies to different proteins of the post-synaptic membrane, and antibodies to the acetylcholine receptor are detected in the great majority of cases [41]. Immune-mediated rippling muscle disease is most commonly associated with myasthenia gravis, suggesting that the immunogenic target is post-synaptic and near the neuromuscular junction [35]. Exacerbation of the rippling with cholinesterase inhibitors suggests that the electrically-silent muscle contractions may originate at the neuromuscular junction [4, 26]. Caveolin-3 promotes acetylcholine receptor clustering and regulates neuromuscular junction activity [42]. Interestingly, Iwasa et al. [43] have observed patchy or partly deficient caveolin-3 immunoreactivity in muscle biopsies of patients with myasthenia gravis—i.e., without rippling muscles or other reported neuromuscular disorders. There is inflammation at the neuromuscular junction in myasthenia gravis [44] and skeletal muscle is “not passive” during the autoimmune attack [45]. Indeed, muscle is constantly trying to repair itself—and caveolin-3 is a major structural player in setting up caveolae and docking sites for cell transport and signaling. It is perhaps no surprise that in Duchenne’s muscular dystrophy caveolin-3 is upregulated [46].

13.9.2 On or Near Caveolar Lipid Raft?

Schoser et al. have suggested based on the mosaic pattern of decreased caveolin staining in skeletal muscles with largely normal levels of caveolin and dysferlin that the immunogenic target may be in the caveolar lipid rafts of the sarcolemma. The mosaic pattern of decreased caveolin staining of the sarcolemma was initially described by Schulte-Mattler et al. [32] and confirmed by others in iRMD with myasthenia gravis [4, 5, 30]. Lo et al. also reported the mosaic pattern of caveolin staining in their three cases of iRMD without evidence of myasthenia gravis [6].

13.9.3 In the Triad or T-Tubules or Near Their Openings in the Sarcolemmal Membrane?

Because the mechanically induced rippling muscle phenomena is electrically silent, an abnormality in the T-tubule coupling system has long been suspected [2, 3]. In iRMD antibodies to a stretch-activated (or inactivated) channel in skeletal muscle have been suggested [47, 48]. Schulte-Mattler et al. have demonstrated immunostaining of the sarcolemma and triad regions with sera from their patient with iRMD [32]. They also described electron dense material around the triads on electron microscopy.

13.9.3.1 Caveolin-3 and T-Tubules

Caveolin-3 is present in the sarcolemma of mature sarcomeres and associated with the T-tubules of mature skeletal muscle [18]. After plasma membrane removal in rat skeletal muscle, caveolin-3 hot spots have been described around the openings of the T-tubules [19]. Vassilopoulos et al. have reported that caveolin-3 is functionally associated with the calcium-release complex in skeletal muscle and is modified via in vivo triadin modification [49].

13.9.3.2 Caveolin-3 LGMD and T-Tubules

Minetti et al. have shown T-tubule system disorganization in addition to impairment of caveolae formation in human muscular dystrophy with caveolin-3 deficiency (a.k.a. LGMD 1C) [20]. Weiss et al. have shown that expression of a muscular dystrophy-associated caveolin-3 mutant specifically alters the calcium channel function of the dihydropyridine receptor (DHPR) in adult mouse skeletal muscle [50]. The molecular architecture of human caveolin-3 has been shown to interact with the skeletal muscle ryanodine receptor [51].

13.9.3.3 Ryanodine and Dihydropyridine Receptors

Schoser et al. have reported antibodies to the ryanodine receptor (RyR) in three of the six patients who were tested for this [4]. Mygland et al. reported RyR antibodies

in patients with myasthenia gravis but noted that the titers were particularly high in a patient with rippling muscles, myasthenia, and a thymoma [52]. That patient was the initial patient that we described in 1996 with rippling muscles and myasthenia gravis [3]. Watkins et al. described a striational immunofluorescent banding pattern with sera from this patient corresponding to the striational banding pattern seen for the DHPR and the RyR suggesting the presence of autoantibodies to their cellular location [33].

13.9.4 Skeletal Muscle Autoantibodies

Because the vast majority of patients with acquired iRMD reported to date have been associated with myasthenia gravis, autoantibodies to skeletal muscle have long been suspected in its pathogenesis. The study of autoantibodies is complicated because many of the structures involved are heterogeneous combinations of proteins, phospholipids, cholesterol, and glycolipids. Many of the issues in search of specific autoantibodies in neuromuscular disorders were described by Querol et al. [53] in the first edition of this book. The potential roles of anti-skeletal muscle antibodies in myasthenia gravis have also been summarized by Meriggioli and Sanders [54].

13.9.4.1 Titin

There can be autoantibodies to different portions of the same protein or structure.

Titin has been described as a giant protein in charge of ultrastructure and elasticity in skeletal muscle [55]. Antibodies to to the main immunogenic region(MIR) of titin have long been known to occur in patients with myasthenia gravis (MG) and thymomas [34]. Until recently, titin antibodies to the MIR were found almost exclusively in patients with MG and acetylcholine receptor antibodies [56]. Titin antibodies have more recently been identified in triple seronegative (tSN) patients with myasthenia (tSN-MG, without detectable AchR, MuSK, or LRP4 antibodies) using a sensitive radioimmunoprecipitation assay (RIPA). Titin antibodies measured by this RIPA have been proposed as a useful tool in the serologic MG diagnosis of tSN patients [57]; and the clinical characteristics of titin-MG have been described [58].

Antibodies to titin have been identified in patients with iRMD with myasthenia gravis [4, 33] and most recently in iRMD patients **without myasthenia** (see addendum) [59].

In immune-mediated rippling muscles with myasthenia gravis, Watkins et al. demonstrated autoantibodies to titin near the elastic PEVK region [33]. The PEVK region of titin is near the Z band along which the T-tubules align and provides elasticity to muscle allowing it to be stretched. The PEVK region is a different region when compared to the main immunogenic region to which antibodies to titin have been previously described in patients with myasthenia gravis [34]. These

autoantibodies were cloned with a fusion protein and the product shown to react with the same immunogenic area of titin as the original autoantibodies [60].

13.10 Why Are Rippling Muscle Diseases with Immune-Mediated and Genetic Etiologies Important?

The clinical and electrophysiological rippling muscle phenomena and the immunohistochemical studies with caveolin-3 suggest a similar pathophysiology for iRMD and hRMD. There are very few areas of medicine where clinical, electrophysiologic, pathologic, and immunochemical findings overlap in disorders with clearly different etiologies. This offers a rare opportunity to use both immunological and genetic techniques to study mechanisms of muscle contraction and the pathophysiology of both dystrophic and non-dystrophic neuromuscular disorders.

13.10.1 Rippling Muscle Diseases Are Also Important from a Scientific Viewpoint Because

13.10.1.1 Mechanoprotection in Dystrophic and Non-dystrophic Myopathic Skeletal Muscle

Caveolae have been shown to play a role in mechanoprotection of skeletal muscle [15, 17, 24]. Caveolae and caveolin-3 or caveolin-3-associated proteins are affected in both hRMD and iRMD. Caveolin-3 is absent or severely reduced in the genetic forms of RMD with dystrophic changes in striated muscle. Immune-mediated rippling muscle diseases have mild decreases in caveolin-3 with patchy immunostaining and mild myopathic changes. These disorders provide an opportunity to study both dystrophic and myopathic changes in disorders that share clinical, electrophysiologic, and pathologic and likely pathophysiologic mechanisms.

13.10.1.2 Clinical and Electrophysiologic Evidence for Mechanosensitive Stretch-Activated Channels in Neuromuscular Disorders in Humans

Theories about the pathophysiology of the muscular dystrophies have for decades postulated the presence of the so-called stretch-activated or mechanosensitive channels in skeletal muscle [61]. This however has been difficult to prove. Most of the evidence regarding mechanosensitive channels in skeletal muscle involves patch-clamp studies in animals [62]. The stretch and mechanically induced contractions described in patients with hRMD and acquired iRMD suggests the possibility of a stretch-activated channelopathy [47, 48]. The evidence for mechanosensitive channels in skeletal muscle has continued to evolve [63–65] as have the roles of caveolae, caveolin-3, and the T-tubules [16–24, 48–50]. Rippling muscle diseases demonstrate clinical evidence of mechanosensitivity in human skeletal muscle and the potential role of mechanosensitive channels in myopathies and muscular dystrophies.

13.10.1.3 May Use Both Genetic and Immunological Clues and Tools to Unravel Pathophysiologic Mechanisms in Diseases with Clearly Different Etiologies

Clinical, electrophysiological, and pathological studies, including histochemistry suggest a similar pathophysiology for iRMD and hRMD—but with clearly different etiologies. Because of their remarkable similarities—these disorders offer an opportunity to apply both genetic and immunological techniques to unravel mechanisms of skeletal muscle contraction. Hopefully, a combination of genetic and immunological techniques may be used synergistically in the study of these disorders.

13.10.2 Immune-Mediated Rippling Muscle Diseases Are Treatable

Clinically, recognition and differentiation of iRMD from the inherited form is important because immune-mediated rippling muscle diseases are treatable. Immune-mediated rippling muscles with myasthenia gravis responds to the same immunotherapy routinely used for myasthenia gravis including corticosteroids, azathioprine, mycophenolate, and plasmapheresis. It should be noted however that the acetylcholinesterase inhibitors used to diagnose and treat myasthenia gravis (edrophonium and pyridostigmine) can worsen the rippling phenomena. When other immunological problems are identified with immune-mediated rippling muscles—they can be addressed. Thymomas can be removed and other autoimmune disorders, malignancies, and infections can be treated.

13.11 Summary

Rippling muscle diseases can be either genetic or immunological in etiology. These disorders share clinical and electrophysiological features. Their muscle biopsies share common features at the light and electron microscopic levels and on histochemical examination. Acquired immune-mediated rippling muscle disorders both with and without myasthenia gravis are important to recognize because they are treatable and respond to immunotherapies. Rippling muscle diseases are also important because they offer rare opportunities to study both dystrophic and non-dystrophic myopathies that appear to share similar pathophysiological characteristics. Further study of both genetic and immune-mediated rippling muscle disorders may lead to a better understanding of the role of mechanosensitivity in muscle physiology and the pathophysiology of myopathies and muscular dystrophies.

Addendum:
As this chapter was going to press, 10 cases of iRMD were reported, 3 with and 7 without myasthenia gravis. Autoantibodies to caveolae-associated protein 4 (cavin-4) were identified as possible biomarkers of immune-mediated rippling muscle disease [59]. A cell-based assay for cavin-4 antibodies was positive in 8 of their

10 patients. Cavin-4 antibodies were positive in 5/7 of their patients without concurrent myasthenia.

Antibodies to titin were positive in 6/9 patients tested, including 4/7 patients **without concurrent myasthenia**. Antibodies to the main immunogenic region (MIR) of titin have long been associated with myasthenia gravis and thymomas [34]; and, until recently, these antibodies were found almost exclusively in patients with myasthenia gravis and positive acetylcholine receptor antibodies [56]. Anti-titin antibodies have more recently been identified in seronegative patients with myasthenia (without detectable AchR, MuSK, or LRP4 antibodies) using a sensitive radioimmunoprecipitation assay (RIPA) [57]; and the clinical characteristics of titin-MG have been described [58].

Anti-titin antibodies in 4/7 patients with **iRMD without myasthenia** suggests that titin antibodies may have role as a complimentary biomarker linking these immunological disorders.

Mosaic patterns of caveolin-3 and cavin-4 immunostaining were present and congruent in muscle from 7/8 patients tested. A mosaic pattern of caveolin-3 labeling had previously been well-described in iRMD.

This report suggests a role for caveolae-associated protein 4 antibodies as a biomarker in patients with iRMD with and without myasthenia gravis.

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Part III

Neurogenic Disorders

14 Idiopathic Chronic Immune-Mediated Neuropathies: Chronic Inflammatory Demyelinating Polyradiculoneuropathy and Multifocal Motor Neuropathy

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14.1 Chronic Inflammatory Demyelinating Polyradiculoneuropathy

14.1.1 Introduction

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a chronic demyelinating neuropathy that is deemed to be caused by an immune attack against peripheral nerve myelin [1–3]. It is a rare neuropathy with a prevalence ranging from 0.8/100,00 in Japan [4] to 8.9/100,000 in Olmstead County, USA [5]. The different figures in prevalence and incidence can be at least partially explained by the different set of diagnostic criteria used among studies [6]. CIDP usually presents with a relatively symmetric, distal and proximal, sensory-motor impairment that evolves over the years and that, if untreated, may lead to a consistent disability. A few different clinical presentations have been reported broadening the spectrum of CIDP [3, 7, 8]. These include multifocal acquired demyelinating sensory and motor (MADSAM) neuropathy also known as Lewis–Sumner syndrome, distal acquired demyelinating symmetric (DADS) neuropathy, pure motor CIDP, and pure sensory CIDP including chronic immune sensory polyradiculopathy (CISP). These forms are currently considered to be variants of CIDP, even if the presence of some differences in their response to therapy does not permit to exclude that they represent different forms of demyelinating neuropathies.

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14.1.2 Clinical Presentation

CIDP may have a chronic progressive or relapsing course [1]. Initial symptoms may progress over several weeks to months even if a rapid progression over a few days or weeks is possible (up to 16% of all CIDP patients) and may initially lead some patients to the diagnosis of Guillain-Barré syndrome (GBS) [9]. Diagnosis of CIDP in patients initially diagnosed with GBS should be suspected when deterioration continues >2 months from onset or when ≥3 treatment-related fluctuations occur [9]. Prominent sensory symptoms and signs at presentation should also raise this suspicion.

Typical CIDP presents with symmetric sensory and motor symptoms. Cranial nerve symptoms occur in a minority of patients. Gait unbalance and upper limb tremor may occur in some patients while pain is relatively infrequent. Respiratory failure and symptoms of dysautonomia seldom occur in CIDP. In over 50% of the patients, fatigue is a relevant symptom.

On examination, both proximal and distal weakness is common, usually in a symmetric manner. The presence of proximal weakness is one of the clues to the diagnosis of CIDP in patients with chronic neuropathy. Distal weakness is however more common and severe than proximal weakness. Reflexes are historically deemed to be absent in CIDP even if total areflexia only occurs in 70% of patients. Sensory deficits are present in over 80% of patients, with vibratory impairment more common than deficits to pinprick. Occasional patients may have clinical or Magnetic Resonance Imaging (MRI) evidence of central nervous system (CNS) demyelination.

CIDP is a severe disease with over 50% of the patients having at least temporary severe disability in the course of the disease including temporary restriction to a wheelchair or inability to walk without support and approximately 10% become persistently disabled or die because of the illness [10, 11]. A few patients have however a disturbing but functionally indolent course with minimal weakness and minor sensory symptoms.

14.1.3 Variants of CIDP

The variants of CIDP have been defined as "atypical CIDP." Diagnostic criteria for these variants have been recently proposed although they are still not validated [8]. In a variable proportion of patients, atypical CIDP progress into typical CIDP with time [8], while other patients maintain an atypical presentation during the follow-up.

Sensory CIDP. Some patients with CIDP may present with a pure sensory syndrome with a prevalence ranging from 3.5 to 35% [3, 7, 8]. Most of these patients have however electrodiagnostic evidence of demyelination also on motor nerves [8, 12]. A few patients have a clinically and electrodiagnostically pure sensory neuropathy responding to immune therapy and are considered to have a purely sensory CIDP although the EFNS/PNS diagnostic criteria does not allow diagnosis of CIDP in these patients [8, 13]. A particular form is chronic immune sensory

polyradiculopathy (CISP) characterized by the presence of sensory symptoms without weakness, normal nerve conduction studies, delayed somatosensory evoked potentials, increased cerebrospinal fluid (CSF) proteins, and response to immune therapy. Lumbar MRI may reveal an enlargement of lumbar roots and the inflammatory nature of the process can be confirmed by root biopsy [14].

Motor CIDP. Up to 10% of the patients have a purely motor impairment throughout the course of the disease [7, 8]. Of these patients, some have abnormal sensory nerve conduction studies despite the absence of sensory symptoms while in others sensory nerve conduction studies are normal. Response to steroids seems different in these two subgroups, with only patients with abnormal sensory nerve conduction studies being apparently responsive [8]. Most of the patients with motor CIDP show a good response to intravenous immunoglobulins (IVIg) [8, 15].

Lewis–Sumner syndrome (MADSAM). Lewis and colleagues [16] reported five patients with a chronic, acquired, asymmetric sensorimotor demyelinating polyneuropathy. Electrodiagnostic studies demonstrated multifocal conduction block in motor nerves with normal conduction velocities. Two patients improved after therapy with prednisone. This disease is currently known as Lewis–Sumner syndrome or MADSAM neuropathy and is considered a variant of CIDP. There is still disagreement on whether Lewis–Sumner syndrome should include also patients with an asymmetric form of CIDP or only patients with a multifocal neuropathy. Only this latter group of patients seems to have a different response to therapy compared to typical CIDP [8]. The different definition of this phenotype may also explain the differences in its reported response to treatment [8]. A reduced response to IVIg was indeed reported by some authors [8] but not by others [8]. Focal CIDP, characterized by a focal distribution of weakness and sensory loss that are restricted to one or both arms, should also be included under this form [8, 17].

Distal Acquired Demyelinating Symmetric (DADS) neuropathy. This term refers to a slowly progressive, distal, symmetric, predominantly sensory, demyelinating neuropathy often associated with upper limb tremor and ataxia [18]. This phenotype is frequently observed also in patients with neuropathy associated with IgM monoclonal gammopathy and antibodies to the myelin-associated glycoprotein (MAG) [19]. Patients without IgM monoclonal gammopathy or anti-MAG antibodies and a DADS clinical phenotype are considered to have a variant of CIDP. DADS seems to be less clinical disabling and to have a less satisfactory response to therapy, specifically to IVIg, than typical CIDP [8].

14.1.4 CIDP with Associated Diseases

CIDP may co-exist with a number of other diseases [20, 21]. About 75% of the patients with CIDP have at least one comorbidity and about half of them at least two comorbidities [21]. In almost 50% of the patients with CIDP, comorbidities may possibly influence treatment choice, leading to a less frequent use of corticosteroids [21]. Some of these comorbidities seem to be more frequent in CIDP than in general population. Diabetes, which association with CIDP has been a matter of controversy

for a long time, seems to be a risk factor for CIDP although the pathogenic mechanisms underlying this association are yet-to-be discovered [21, 22]. Monoclonal gammopathy of undetermined significance (MGUS), and in particular the IgM-MGUS, is also more prevalent in CIDP than in general population [21]. IgM-MGUS is known to be more frequently associated with peripheral neuropathy compared with IgG or IgA MGUS but so far only anti-MAG antibody specificity has shown a clear relationship with a specific clinical phenotype. The prevalence of other autoimmune disorders was reported to be three times more frequent in CIDP than in the general population in Europe [21], suggesting that CIDP shares common pathogenic mechanisms with other immune disorders. An association of CIDP with other conditions was also noted, but there is no evidence that prevalence of these disorders in CIDP is different from that of general population [21]. In most of these conditions, the pathogenesis of the neuropathy is considered to be the same of CIDP. These diseases include cancer, HIV infection, chronic active hepatitis, sarcoidosis, thyroid disease, renal diseases, gastrointestinal diseases, arterial hypertension, or organ transplantation [21].

In CIDP associated with these comorbidities, treatment is the same for idiopathic CIDP, with the only caution derived from the possible effect of treatment on the associated condition. Only CIDP associated with diabetes seems to have a reduced response to treatment and a more severe course compared to idiopathic CIDP, while the other comorbidities do not seem to have an impact on disease severity and treatment response [21].

In other conditions, the pathogenesis and pathology may be different from CIDP and therapy is directed at treating the associated disease. These include infection with Borrelia burgdorferi, IgM monoclonal gammopathy with anti-MAG antibodies, POEMS syndrome, osteosclerotic myeloma, and other hematological and non-hematological malignancies.

14.1.5 Etiology and Pathogenesis

There is a general consensus that CIDP is an immune-mediated disorder affecting peripheral nerve myelin [2, 3, 7] as mainly confirmed by the fact that the vast majority of patients improve with immune therapies. Pathological studies on nerve biopsy of affected patients revealed the presence of infiltrates of macrophages and T cell infiltrates and of deposits of Ig and experimental studies on animal have demonstrated a similarity of CIDP with chronic experimental allergic neuritis induced in animals by immunization with nerve antigens [2]. Antibodies against a several myelin antigens have been reported in patients with CIDP, but none of them was consistently associated with CIDP [2, 3]. More recently, attention has been devoted to antibodies directed against myelin or axonal proteins at the node of Ranvier including contactin-1 (CNTN1) [23] and neurofascin-155(NF155) [24]. Even if these antibodies are only found in approximately 5% of the patients [7], they are associated with some distinctive features including a severe motor weakness, poor

response to IVIg and, in those with anti-NF155 antibodies, the frequent presence of tremor. These data were recently confirmed on a large series of Japanese patients with CIDP [25] supporting the hypothesis that different antibodies may underlie different forms of CIDP and that these forms may have different response to therapy.

Causes of CIDP are still unknown. Two recent studies showed that some dietary habits may influence the risk of CIDP and its severity, but further studies are needed to confirm these findings [26, 27]. There have been many attempts to find an association between human leukocyte antigen (HLA) or other putative genes with CIDP, with little success [28]. Possible explanations for these insuccesses encompasses the small number of patients included in these studies and the likely immune and genetic heterogeneity of CIDP [28], as recently also confirmed by the finding of an association between DRB1*15 alleles and the presence of anti-NF155 antibodies in CIDP [29].

14.1.6 Diagnosis of CIDP

Even if the diagnosis of CIDP is often considered easy in the clinical practice, misdiagnosis is not uncommon [30]. Several diagnostic criteria, with different sensitivities, have been proposed for CIDP, but currently the most frequently adopted are those of the EFNS/PNS [31] for their best combination of sensitivity and specificity (Tables 14.1 and 14.2) [32]. These diagnostic criteria allow diagnosis of CIDP when demyelinating abnormalities are present in only one nerve. Other ancillary tests are considered supportive for the diagnosis of CIDP although in the majority of patients the diagnosis is possible solely with motor nerve conduction studies [33]. These supportive criteria include elevated protein levels in the cerebrospinal fluid (CSF) with normal leukocyte count, enhancement and/or hypertrophy of nerves, plexus or nerve roots on MRI or ultrasound, evidence of demyelination on sensory nerve conduction studies, delayed somatosensory evoked potentials (SSEP), evidence of demyelination on nerve biopsy, and response to immune therapy [31].

In most reported series, there is a consistent proportion of patients who have the clinical features compatible with a diagnosis of CIDP but who do not fulfill the EFNS/PNS electrodiagnostic criteria and, therefore, might be denied the access to effective therapy. These patients have similar clinical findings, frequency of abnormal supportive criteria, and response to therapy compared to patients fulfilling EFNS/PNS [34]. In these patients, a clinical diagnosis of CIDP is often supported by the presence of abnormal ancillary investigations [34], but this is still outside the EFNS/PNS guidelines. Addition of nerve ultrasound to nerve conduction studies for the diagnosis of chronic inflammatory neuropathy improves detection of a 21–25% of patients that may respond to treatment at the expense of some false positive [35–37]. Recent evidence suggests that nerve ultrasound and nerve conductions studies may be complementary, with nerve ultrasound having superior sensitivity but less specificity than nerve conductions studies [35].

Table 14.1 EFNS/PNS (2010) clinical diagnostic criteria for CIDP [31]

1. Inclusion criteria
(a) Typical CIDP
– Chronically progressive, stepwise, or recurrent symmetric proximal and distal weakness and sensory dysfunction of all extremities, developing over at least 2 months; cranial nerves may be affected
– Absent or reduced tendon reflexes in all extremities
(b) Atypical CIDP (still considered CIDP but with different features): One of the following, but otherwise as in (a) (tendon reflexes may be normal in unaffected limbs)
– Predominantly distal (distal acquired demyelinating symmetric, DADS)
– Asymmetric (multifocal acquired demyelinating sensory and motor neuropathy [MADSAM], Lewis–Sumner syndrome)
– Focal (e.g., involvement of the brachial or lumbosacral plexus or of one or more peripheral nerves in one upper or lower limb)
– Pure motor
– Pure sensory (including chronic immune sensory polyradiculopathy affecting the central process of the primary sensory neuron)
2. Exclusion criteria
– Borrelia burgdorferi infection (Lyme disease), diphtheria, drug, or toxin exposure likely to have caused the neuropathy
– Hereditary demyelinating neuropathy
– Prominent sphincter disturbance
– Diagnosis of multifocal motor neuropathy (MMN)
– IgM monoclonal gammopathy with high titer antibodies to myelin-associated glycoprotein (MAG)
– Other causes for a demyelinating neuropathy including POEMS syndrome, osteosclerotic myeloma, diabetic and non-diabetic lumbosacral radiculoplexus neuropathy. PNS lymphoma and amyloidosis may occasionally have demyelinating features

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14.1.7 Treatment of CIDP

Several controlled studies and retrospective studies on large series of patients have shown the efficacy of steroids, plasma exchange, and IVIg in CIDP (reviewed in [38–40]) with approximately 50–70% of the patients responding to each of these treatments. In addition, almost 50% of the patients not responding to one of these treatments respond to the second therapy used, leading to about 80% of patients improving with these therapies [41].

It is often difficult for the clinician to decide what therapy should be first used in CIDP. This decision should consider the efficacy, cost, side effects, and duration of the benefits of each therapy. A few randomized trials have shown a comparable short-term efficacy of IVIg and oral corticosteroids [42] and of IVIg and plasma exchange [43] and that both IVIg and steroids have prolonged efficacy in CIDP. A randomized controlled trial comparing the 6-month efficacy of IVIg and intravenous methylprednisolone (IVMP) showed that IVIg were more frequently effective and tolerated than steroids during the first 6 months of treatment, although, when

Table 14.2 EFNS/PNS (2010) electrodiagnostic criteria for CIDP [31]

1. Definite: at least one of the following
(a) Motor distal latency prolongation >50% above ULN in 2 nerves (excluding median neuropathy at the wrist from carpal tunnel syndrome)
(b) Reduction of motor conduction velocity >30% below LLN in 2 nerves
(c) Prolongation of F-wave latency >20% above ULN in 2 nerves (≥50% if amplitude of distal negative peak CMAP <80% of LLN values)
(d) Absence of F-waves in 2 nerves if these nerves have distal negative peak CMAP amplitudes >20% of LLN + >1 other demyelinating parameter[a] in >1 other nerve
(e) Partial motor conduction block: >50% amplitude reduction of the proximal negative peak CMAP relative to distal, if distal negative peak CMAP >20% of LLN, in 2 nerves, or in 1 nerve + >1 other demyelinating parameter[a] in >1 other nerve
(f) Abnormal temporal dispersion (>30% duration increase between the proximal and distal negative peak CMAP) in >2 nerves
(g) Distal CMAP duration (interval between onset of the first negative peak and return to baseline of the last negative peak) increase in >1 nerve (median >6.6 ms, ulnar >6.7 ms, peroneal >7.6 ms, tibial >8.8 ms) + >1 other demyelinating parameter[a] in >1 other nerve
2. Probable
>30% amplitude reduction of the proximal negative peak CMAP relative to distal, excluding the posterior tibial nerve, if distal negative peak CMAP >20% of LLN, in 2 nerves, or in 1 nerve + >1 other demyelinating parameter[a] in >1 other nerve
3. Possible
As in (1) but in only 1 nerve

CMAP compound muscle action potential, *ULN* upper limit of normal values, *LLN* lower limit of normal values

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[a] Any nerve meeting any of the criteria (a–g)

effective, steroids were less frequently associated with deterioration than IVIg after therapy discontinuation [44]. This data were confirmed in the follow-up extension of the study [45], showing that a similar proportion of patients eventually deteriorated after discontinuing IVIg (87%) or IVMP (79%) but that the median time to deterioration was longer after discontinuing IVMP (14 months) than IVIg (4.5 months). Steroids are associated with more side effects and less tolerance compared to IVIg, making them less suitable for long-term treatment. Pulsed high dose steroid therapy seems however to have fewer side effects than daily steroid dose therapy [46]. Plasma exchange is often considered the third choice since it is more invasive for the patients and has a higher prevalence of side effects that makes it less suitable for the long-term treatment of the patients [39]. IVIg is often considered the treatment of choice for CIDP given its rapid efficacy and low frequency of side effects, even if it is consistently more expensive than steroids. It is still unclear what is the best strategy to use IVIg as maintenance therapy. There is a general consensus that maintenance IVIg treatment needs to be individualized trying to find the lowest effective and most delayed dose, periodically attempting dose reduction or interval lengthening trials. The periodic use of outcome measures is recommended to monitor IVIg treatment response and dependency given the lack of a specific biomarker

for the disease [31]. A multimodal approach using different outcome measures seems the best option to accomplish this task [47].

The possibility to reduce the inconvenience of repeated hospital admissions for maintenance IVIg therapy can be resolved in most patients with home injection of subcutaneous immunoglobulin (SCIg), which efficacy as maintenance therapy was recently confirmed in a RCT and its extension [48, 49]. One third of the patients are reported to relapse after passing from IVIg to SCIg [48, 49] possibly reflecting a lower SCIg bioavailability [50] and suggesting that an increase in the equivalent dose might be necessary when switching from IVIg to SCIg. An increase in patients' satisfaction [51–53], disability [52], and muscle strength [53] after switching from IVIg to SCIg have been reported, and some studies report a more favorable systemic side-effect profile of SCIg compared to IVIg. Unfortunately, there are still no head-to-head trials that compare the two therapies. SCIg should be however preferred in patients with intolerable recurrent adverse events from IVIg, difficult venous access, desire of greater autonomy and flexibility, and working difficulties related to absenteeism.

No specific immunosuppressive and immunomodulatory agent has been proven to be effective on randomized controlled trials (RCT) for the treatment of CIDP [54]. The recent RCT comparing Fingolimod with placebo was prematurely stopped for futility [55]. In the recent years, there has been an increasing attention on the possible beneficial effect of Rituximab, also boosted by the recent reports that CIDP patients with antibodies against proteins at the node of Ranvier have a poor response to IVIg but seem to respond to Rituximab [56], even at low doses [57]. Several anecdotal reports and small case series suggest that also seronegative CIDP patients and patients with CIDP and associated disorders may respond to Rituximab, and a review of uncontrolled studies estimated an efficacy around 70% [54]. Rituximab seems promising also in the treatment of refractory CIDP [58, 59]. Currently, two Italian and one Japanese clinical trial are ongoing to determine the efficacy and safety of Rituximab in patients with CIDP responsive to IVIg and in patients with refractory CIDP with or without nodal antibodies.

14.2 Multifocal Motor Neuropathy

14.2.1 Introduction

The term multifocal motor neuropathy (MMN) was first introduced in 1988 by Pestronk et al. [60] who described two patients with a progressive, purely motor, predominantly distal, asymmetric neuropathy with multifocal persistent conduction blocks (CB) on motor but not sensory nerves. Even if a few similar patients had been previously reported, Pestronk et al. first highlighted the frequent association with anti-GM1 IgM antibodies and the response to immune therapies. MMN was originally related to CIDP or to motor neuron disease (MND), but it is now considered a well-defined separate clinical entity [61, 62].

14.2.2 Clinical Features

MMN is a rare neuropathy with a prevalence ranging from 0.29 to 0.7 per 100,000 inhabitants [63, 64]. MMN almost invariably presents with progressive, usually distal, multifocal, or asymmetric weakness in the upper limbs [65, 66]. Some patients may however present with more proximal weakness or with symptoms in their legs. The disease usually has a progressive course affecting other nerves but may also have a stepwise progression with intervals of months or even years or occasionally a relapsing course. Localized muscle atrophy may be mild or irrelevant in the early stage of the disease while in the later stages may be very severe and pronounced. Fasciculations, cramps, and myokymia have been variably reported in these patients making a differential diagnosis with MND sometimes very difficult. This clinical distinction may become even more difficult in the 20–30% of patients who have brisk tendon reflexes, while in the majority of patients reflexes are reduced in a patchy way or diffusely. Cranial nerve involvement or respiratory failure due to unilateral or bilateral phrenic nerve palsy may seldom occur. One of the typical features of this neuropathy is the absence of sensory impairment even in the territory of affected sensorimotor nerve. Some patients may report mild sensory symptoms but only a minority of them has a definite though minor sensory loss. Vibration sense abnormalities may be however found in about half of the patients [67]. Taking into account its clinical presentation, it is not surprising that several patients with MMN are mistakenly diagnosed with MND or entrapment neuropathy, before receiving the correct diagnosis [67].

Most patients with MMN become disabled in their daily life due to a reduced dexterity in manual activities, while very few of them become disabled in walking [67].

14.2.3 Diagnosis of MMN

The most commonly used diagnostic criteria for MMN have been proposed by the EFNS/PNS (Tables 14.3 and 14.4) [66]. As in the case of CIDP, these criteria allow the diagnosis of MMN only with clinical and electrophysiological studies. In particular, the presence of persistent, multifocal, partial CB in motor nerves outside the usual sites of nerve compression is the mainstay of this diagnosis. CB is not specific for MMN but can also be found in other demyelinating neuropathies including CIDP and GBS. Sensory nerve conduction studies are usually normal or only minimally affected in MMN, even in the nerves with motor CB [65–67].

Other tests may help in the diagnosis of MMN when clinical and electrophysiological findings are inconclusive. Moderately increased serum creatin kinase activity are found in up to two third of patients while CSF proteins are moderately increased (usually up to 80 mg/dl) in one third of the patients [61]. The most typical laboratory finding is the presence of increased levels of serum IgM antibodies to the ganglioside GM1, with frequency in most laboratories of 40–50% [61]. These antibodies are not specific for MMN as they can be found in 5–10% of patients with

Table 14.3 EFNS/PNS (2010) clinical diagnostic criteria for MMN [66]

Core criteria (both must be present)
1. Slowly progressive or stepwise progressive, asymmetric limb weakness, or motor involvement having a motor nerve distribution in at least two nerves, for more than one month[a]
2. No objective sensory abnormalities except for minor vibration sense abnormalities in the lower limbs
Supportive clinical criteria
1. Predominant upper limb involvement[b]
2. Decreased or absent tendon reflexes in the affected limb[c]
3. Absence of cranial nerve involvement[d]
4. Cramps and fasciculations in the affected limb
Exclusion criteria
1. Upper motor neuron signs
2. Marked bulbar involvement
3. Sensory impairment more marked than minor vibration loss in the lower limbs
4. Diffuse symmetric weakness during the initial weeks

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[a] Usually more than 6 months

[b] At the onset, predominant lower limb involvement account for nearly 10% of the cases

[c] Slightly increased tendon reflexes, in particular in the affected arm have been reported and do not exclude the diagnosis of MMN provided criterion 7 is met

[d] 12th nerve palsy reported

Table 14.4 EFNS/PNS (2010) electrophysiological criteria for conduction block[a] [66]

1. Definite motor CB
Negative CMAP area reduction on proximal versus distal stimulation of at least 50% whatever the nerve segment length (median, ulnar, and peroneal). Negative CMAP amplitude on stimulation of the distal part of the segment with motor CB must be >20% of the lower limit of normal and >1 mV (baseline negative peak) and an increase of proximal negative peak CMAP duration must be ≤30%
2. Probable motor CB
Negative CMAP area reduction of at least 30% over a long segment of an upper limb nerve with an increase of proximal negative peak CMAP duration ≤30%
Negative CMAP area reduction of at least 50% (same as definite) with an increase of proximal negative peak CMAP duration >30%
3. Normal sensory nerve conduction in upper limb segments with CB and normal SNAP amplitudes (see exclusion criteria)

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[a] Evidence for conduction block must be found at sites distinct from common entrapment or compression syndromes

MND. Positivity of antibodies anti-GM1 in MMN seems to predict evolution to MADSAM and better response to treatment, but these findings need to be confirmed [68]. More recently, the combination of GM1 with galactocerebroside has been reported to increase the sensitivity of anti-GM1 testing to approximately 75% of MMN patients with only a marginal reduction of their specificity [69]. Testing for anti-GM1 antibodies may help the diagnosis in patients in whom the clinical and electrophysiological data are not conclusive, even if their absence does not exclude the diagnosis of MMN.

MRI and ultrasound study of the nerves may help revealing nerve abnormalities, particularly in the proximal segment of nerves not easily accessible to nerve conduction studies [66], and MRI has been included in the supportive EFNS/PNS criteria. A recent study, however, casts doubts on the real utility of MRI as supportive criteria in the diagnosis of MMN, while suggests that nerve ultrasound is more appropriate [70]. Nerve biopsy is seldom useful as it is routinely performed on the sural or other sensory nerves, which are typically normal in MMN. Biopsy of motor nerves may help in the distinction of MMN from MND showing a significantly higher density of clusters of regenerative small myelinated fibers in MMN than in MND [71].

14.2.4 Etiology and Pathogenesis

The frequent association of MMN with anti-GM1 antibodies, and the frequent improvement after IVIg support the opinion that the disease is immunologically mediated and possibly caused by anti-GM1 IgM binding to neural structures [61, 62]. It remains however unclear what may cause the disease in patients without these antibodies and what impairs motor nerve conductions in experimental studies using sera of patients with MMN not only with, but also without, high anti-GM1 antibodies [72]. It is likely that sera of patients with MMN contain soluble factors able to affect the neural transmission, and the role of anti-GM1 antibody in this blocking effect remains unclear.

14.2.5 Therapy

Almost 80% of patients with MMN respond to IVIg, which short-term and long-term efficacy and safety have been confirmed in five randomized controlled studies [73] and in one multi-center open-label study [74]. IVIg induces a rapid improvement which often occurs within 1 week of treatment and is usually more evident in recently affected regions. Only a few patients have persistent improvement after a single or few courses of therapy while in most patients their effect has to be maintained with periodic IVIg infusions for long periods of time, if not indefinitely [75, 76]. Several patients become however progressively less responsive to IVIg and require increasing dosage or frequency of IVIg to maintain improvement. In most of the patients, however, muscle strength and disability deteriorate over time despite

treatment [67, 76, 77]. A lower Medical Research Council (MRC) sum score and the absence of reflexes at clinical onset seem to predict a more progressive disease course [67].

An alternative to IVIg is SCIg, which efficacy and safety in MMN have been confirmed by several studies [78–80]. A two-year follow-up study showed that SCIg are able to maintain long-term clinical stability [81]. As in CIDP, also in MMN the required equivalent dose of SCIg should be 30% greater than that of IVIg [81].

There are not real alternatives to the use of IVIg and SCIg in MMN [82]. Steroids are ineffective and potentially harmful with almost 20% of the patients worsening after this therapy. Similarly ineffective and sometimes harmful is plasma exchange. This highlights the importance of a correct distinction of MMN from CIDP and Lewis–Sumner syndrome where instead steroids and plasma exchange are effective. Cyclophosphamide was initially reported to be effective in MMN [60], but it has several side effects that make it unsuitable for a non-fatal disorder such as MMN [66]. A few anecdotal or open-label studies report the efficacy in some patients of azathioprine, interferon -β1a (IFN-β1a), methotrexate, Rituximab, and eculizumab (reviewed in [82]). In an open-label study, eculizumab failed to allow for a reduction in the amount of IVIg needed for maintenance clinical stability although an improvement in self-evaluated functional rating scale and muscle strength was seen [83]. An open-label trial of six patients on maintenance IVIg given rituximab and followed for 12 months did not demonstrate a reduction in IVIg administration or change in grip strength, MRC sum score or disability score [84]. The only randomized controlled trial with immune suppressants in MMN showed that mycophenolate mofetil did not permit to increase the effectiveness or to reduce the dose of IVIg [85]. A recent Cochrane review concluded that there is so far little evidence that any immunosuppressant may be useful in MMN [82], confirming that immunoglobulin therapy remains the gold standard for its treatment.

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Immune Neuropathies

15

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Abbreviations

AIDP	Acute inflammatory demyelinating polyradiculoneuropathy
AMAN	Acute motor axonal neuropathy
AMSAN	Acute motor and sensory axonal neuropathy
ANNA-1	Type 1 antineuronal nuclear antigens
CANDA	Chronic ataxic neuropathy with disialosyl antibodies
CANOMAD	Chronic ataxic neuropathy, ophthalmoplegia, M protein, agglutinins, and disialosyl antibodies
CASPR1	Contactin-associated protein-1
CIDP	Chronic inflammatory demyelinating polyradiculopathy
CIMP	Chronic inflammatory motor polyradiculopathy
CISMP	Chronic inflammatory sensorimotor polyradiculopathy
CISP	Chronic immune sensory polyradiculopathy
CMAN	Chronic motor axonal neuropathy
CMAP	Compound muscle action potential
CNTN1	Contactin-1
CSA	Cross-sectional area
CSF	Cerebrospinal fluid
fSCIG	Subcutaneous immunoglobulin with recombinant human hyaluronidase
FOSMN	Facial-onset sensory and motor neuropathy
GBS	Guillain-Barré syndrome
HIV	Human immunodeficiency virus
ICC	Immunocytochemistry

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IFNγ	Interferon-gamma
Ig	Immunoglobulin
IP	Immune-mediated polyradiculoneuropathy
IV	Intravenous
IVIG	Intravenous immunoglobulin
mAb	Monoclonal antibody
MADSAM	Multifocal acquired demyelinating sensory and motor neuropathy
MAG	Myelin-associated glycoprotein
MFS	Miller-Fisher syndrome
MGUS	Monoclonal gammopathy of undetermined significance
MMN	Multifocal motor neuropathy
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MRS	Modified Rankin Scale
NF	Neurofascin
POEMS	Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin abnormalities
SCIG	Subcutaneous immunoglobulin
SNAP	Sensory nerve action potential
SS	Sjögren syndrome
SSA(ro)	Sjögren's-syndrome-related antigen A
SSEP	Somatosensory evoked potential
UPSS	Ultrasound pattern sum score
VEGF	Vascular endothelial growth factor
VLA4	Very late antigen 4

15.1 General Introduction

Immune-mediated neuropathies are a heterogeneous group of disorders affecting the peripheral nerves. The first immune-mediated neuropathy was reported in France during the First World War in 1916 when Guillain, Barré, and Strohl described two patients with acute onset weakness that was progressive and predominantly motor, both of which recovered spontaneously. Though similar cases were previously described in 1859 by Landry [1], Guillain and colleagues were the first to demonstrate the presence of areflexia and albuminocytological dissociation in the cerebrospinal fluid (CSF). The disease was subsequently named Guillain-Barré syndrome (GBS).

Since the first description of GBS, numerous forms of immune-mediated neuropathies have been described. These neuropathies are defined as a group of diseases arising from autoimmunity to components of the peripheral nervous system where autoantibodies attack the proteins, glycoproteins, and glycolipids of myelin and neuronal cell membranes leading to damage. Common clinical features include muscle weakness, sensory deficits, and gait unsteadiness. The disease may be acute or chronic, demyelinating, or axonal.

15.2 Diagnostic Guidelines

The diagnosis of immune-mediated neuropathy primarily relies on clinical symptoms and abnormalities observed during electrophysiological studies, which may indicate conduction block or peripheral nerve demyelination. When electrophysiologic results are equivocal, further types of testing may help inform a diagnosis, including the presence of albuminocytologic dissociation (i.e., elevated protein level with normal cell count) in the CSF; magnetic resonance imaging (MRI), nerve ultrasound, and nerve biopsy to detect histopathologic changes [2]. Determining the type of nerve fibers that are affected can also help lead to an accurate diagnosis. Demyelinating immune neuropathies are more large-fiber predominant, as large fibers have the most myelin, while small fibers containing little to no myelin are not affected [3]. Large-fiber abnormalities include symptoms such as weakness and ataxia compared to small-fiber abnormalities that are generally associated with symptoms of autonomic issues and pain.

Laboratory testing must be performed in order to rule out other diseases such as diabetes mellitus or hematological malignancies associated with a monoclonal gammopathy. These laboratory assessments can include electrolytes (and fasting glucose), complete blood count, liver, renal, and thyroid function, vitamin B12, and screening for the presence of a monoclonal gammopathy in the serum and urine. In addition, tests for human immunodeficiency virus (HIV), neuroborreliosis, or antinuclear antibodies may be conducted. Genetic testing may also be performed to rule out genetic neuropathies (e.g., Charcot-Marie-Tooth disease) [4].

In cases where atypical test findings are observed, MRI can be used to identify hypertrophy or contrast enhancement of the cervical nerve roots, brachial plexus, lumbosacral plexus, and cauda equina [4]. Alternatively, nerve ultrasound has demonstrated better ability to distinguish between subtypes of immune neuropathies and segmental spinal muscular atrophy than MRI [5]. Nerve ultrasound is used to evaluate the cross-sectional area (CSA) and diameter of peripheral nerves and detect morphological alterations, especially in the upper limbs. Nerve CSA and diameter can become enlarged in certain neuropathies, most prominently in demyelinating neuropathies. The ultrasound pattern sum score (UPSS) and its sub-scores, based on the CSA and/or diameter of peripheral sensorimotor nerves, cervical spinal nerves C5 and C6, the vagus nerve, and the sural nerve can be used to differentiate acute and subacute onset neuropathies [6]. Nerve CSAs are also inversely correlated with motor conduction velocity as measured by nerve conduction studies [7]. Nerve enlargement measured by ultrasound can distinguish inflammatory neuropathies from axonal neuropathies and motor disease [8]. However, there was a paucity of data describing the use of nerve ultrasound in diagnosis of immune neuropathies suggesting further investigations are needed.

Autoantibodies present in a patient with suspected immune neuropathy can also aid in diagnosis. Antibodies are directed towards the proteins of the node of Ranvier or the myelin of peripheral nerves (Fig. 15.1). Identification of antibodies can also help avoid misdiagnosis. It has been reported that nearly half of patients in the United States (US) diagnosed with chronic inflammatory demyelinating

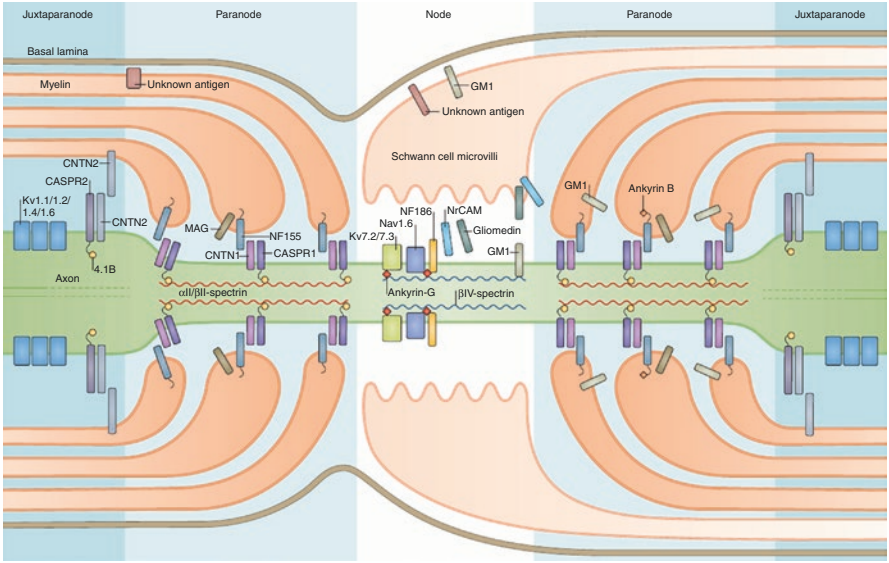

Fig. 15.1 The Node of Ranvier. The figure shows the structure and key molecular components of the node of Ranvier, including those targeted by autoantibodies in autoimmune neuropathies. Adhesion molecules (NF186, NF155, NrCAM, CNTN1, CNTN2, CASPR1, CASPR2, and MAG) mediate axoglial attachment. Ion channels (Kv7.2/7.3, Kv1.1/1.2/1.4/1.6, and Nav1.6) mediate action potential propagation. Adhesion molecules and ion channels are all linked to the cytoskeleton by proteins, including ankyrins and spectrins. Gliomedin is an extracellular matrix constituent that stabilizes the structure of the nodal area. *CASPR* contactin-associated protein, *CNTN* contactin, *Kv* voltage-gated potassium channel, *MAG* myelin-associated glycoprotein, *Nav* voltage-gated sodium channel, *NF* neurofascin, *NrCAM* neuronal cell adhesion molecule. (Source: Adapted from Stathopoulos et al. [9] and Querol et al. [10])

polyradiculopathy (CIDP; Sect. 15.3.2) have been misdiagnosed [11, 12]. Detection of specific autoantibodies, and knowledge of their target antigens can also play a role in the selection of treatment options as discussed further in Sect. 15.4.

15.2.1 Electrodiagnostic Features

Motor and sensory nerve conduction studies are critical in the diagnosis of immune neuropathies. For the assessment of motor nerves, distal motor latency, nerve conduction velocity, and compound muscle action potential (CMAP) amplitudes are measured, in addition to mean F-wave latencies. Assessment of sensory nerves include measures of the sensory nerve action potential (SNAP) and conduction velocity. Neuropathies are typically characterized as being predominantly axonal if there is a reduction in CMAP amplitude and conduction velocity is reduced by <20% in at least two nerves. Predominantly demyelinating neuropathies are typically characterized by a reduction in conduction velocity >20% and prolongation in distal motor latency (≥130%) and F-wave latency (≥130%) [13].

Nerve conduction studies can also aid in the differentiation between neuropathies with anti-myelin-associated glycoprotein (MAG), CIDP, or other types of neuropathies. Neuropathies with anti-MAG are characterized by prolonged distal motor latency or reduced terminal latency index compared to CIDP neuropathies that are typically characterized by conduction blocks and F-wave pathology [13]. Conduction block occurs when an action potential fails to propagate through an axonal segment. Motor conduction block is defined as negative CMAP area reduction on proximal versus distal stimulation of at least 50% the nerve segment length. Negative CMAP amplitude on stimulation of the distal part of the segment must be >20% of the lower limit of normal and >1 mV. An increase of proximal negative peak CMAP duration must be ≤30% [14].

Electrodiagnostic criteria do not usually include somatosensory evoked potential (SSEP) testing, which is a more sensitive diagnostic tool [15]. SSEP may be specifically useful when demyelination is only proximal (or predominantly proximal), as SSEP testing allows the isolation of conduction slowing at the nerve root level [3]. Although not widely used, the diagnostic utility of SSEPs in evaluating CIDP patients has been demonstrated in the scientific literature [15].

In some cases of mild immune neuropathy, standard nerve conduction studies and imaging with MRI are unable to detect abnormalities. In this case, single-fiber conduction velocity can be performed, which is more sensitive than routine electrodiagnostic testing [16].

15.2.2 Histologic Features

Given the distinct histological features that have been observed between the different types of neuropathies, nerve biopsy can be used to confirm diagnosis in immune neuropathies in cases where clinical symptoms and nerve conduction studies do not provide a clear diagnosis or if there is high suspicion of an alternative diagnosis (e.g., malignant neoplasm). Increased rates of myelin remodeling (i.e., segmental demyelination, remyelination, or reduplication of myelin) can be observed through the teased fiber analysis of a nerve biopsy and are indicative of CIDP. Onion bulb formation, consisting of stacks of Schwann cell cytoplasmic processes, indicative of recurrent demyelination and remyelination can also be observed in biopsy, as can inflammatory infiltrates [3].

15.3 Immune Neuropathies

15.3.1 Guillain-Barré Syndrome

GBS refers to a group of acute, acquired, immune-mediated polyradiculoneuropathies characterized by acute, flaccid paralysis, and areflexia or hyporeflexia. Diagnosis of GBS requires the presence of progressive weakness of more than one limb and areflexia. The two major subtypes of GBS are acute inflammatory

demyelinating polyradiculoneuropathy (AIDP) and acute motor axonal neuropathy (AMAN) (Sect. 15.3.1.1.1). AIDP is more common in western countries and AMAN is more frequently described in East Asia, Central America, and South America [17, 18]. The incidence of GBS is 0.81 to 1.89 per 100,000, affecting more men than women [19]. GBS arises due to infectious, inflammatory, or systemic diseases. Common preceding infections include Campylobacter jejuni, Cytomegalovirus, Epstein–Barr virus, Zika virus, and HIV with a latency period of 10 days on average. Influenza vaccination and surgery may also activate the immune system and precede GBS. COVID-19-associated GBS has also been observed [20–22]. Weakness usually plateaus by the fourth week after clinical features arise [23]. Other variants also include Miller-Fisher syndrome (MFS) (Sect. 15.3.1.1.2), pharyngeal-cervical-brachial variant (Sect. 15.3.1.1.3), and acute small fiber sensory neuropathy (Sect. 15.3.1.1.4).

Variants of GBS differ in their autoantibody presentation against different glycolipids of the ganglioside family. However, the antibodies in AIDP, which accounts for the large majority of GBS cases, have not been fully characterized. The precise mechanisms of pathogenesis are not completely understood but are believed to develop due to cross-reaction between peripheral nerve antigens and microbial or viral components through molecular mimicry [24].

Nerve ultrasound of 27 GBS patients (25 of which had AIDP and 2 of which had AMAN) showed enlargement in the vagus nerve in carotid sheath; median nerve in the upper arm, elbow, and forearm; ulnar nerve in upper arm and forearm; tibial nerve in poplitea and ankle; and peroneal nerve in popliteal fossa; the sural nerve was not enlarged in the same patients. Vagus nerve enlargement was associated with autonomic dysregulation. The vagus nerve and fifth/sixth cervical spinal nerve diameters decreased significantly after 6 months [7]. However, another study from the same group found that nerve enlargement occurred particularly in the nerve roots and the vagus nerve of 33 GBS patients [25]. The same study showed that nerve enlargement decreases or disappears after 6 months.

Childhood GBS is similar to that in adults in terms of electrophysiological abnormalities: decreased conduction velocity, increased distal motor latencies, increased F-, H-, or T-wave latencies, and conduction block in at least one motor nerve. However, children tend to recover faster, recovery is more complete, and mortality is lower. The clinical presentation of GBS is different in children, with pain and difficulty walking being the most common features. Cranial nerve involvement is also more common in children than adults with GBS [26]. Furthermore, the incidence of pediatric GBS has been shown to correlate with seasonal variation, with increased occurrences reported in the summer and autumn months, and in those living in rural areas and with a prior history of pulmonary infection [27].

The clinical presentation, electrodiagnostic features, and antibodies present of the GBS variants that have been reported in the literature are provided in Table 15.1. Further details of each are provided in Sect. 15.3.1.1 with the exception of AIDP given the focus of this review on non-classical, emerging forms of immune neuropathies.

Table 15.1 Comparison of features of Guillain-Barré syndrome variants

Variant	Clinical presentation	Electrodiagnostic features	Antibodies present	References
AIDP	Acute, flaccid paralysis Areflexia or hyporeflexia Progressive weakness of more than one limb	Prolonged F-wave latency Reduced conduction velocity Absent SNAPs	Not fully characterized IgG GQ1b	[7, 28]
AMAN/ AMSAN	Acute, flaccid paralysis Respiratory distress Bulbar abnormalities	Reduced CMAP Normal SNAPs	IgG1 and IgG3 GM1, GD1a, GD1b, GalNAc-GD1a	[18, 29]
MFS	Ophthalmoplegia Ataxia Areflexia	NCS often normal Reduced SNAP amplitude	IgG GQ1b, GM1, GM2, GD1a, GD1b, GT1a	[30–32]
Pharyngeal-cervical-brachial variant	Acute weakness of oropharyngeal, neck, and shoulder muscles Dysphagia Facial paresis	Reduced conduction velocity	IgG GT1a, GQ1b	[32]
Acute small fiber sensory neuropathy	Pain (may be severe) Impaired pinprick sensation, hyperesthesia, brush allodynia in glove-and-stocking distribution	NCS normal	Not fully characterized	[33]

AIDP acute inflammatory demyelinating polyradiculoneuropathy, *AMAN* acute motor axonal neuropathy, *AMSAN* acute motor and sensory axonal neuropathy, *CMAP* compound muscle action potential, *IgG* immunoglobulin G, *MFS* Miller-Fisher syndrome, *NCS* nerve conduction study, *SNAP* sensory nerve action potential

15.3.1.1 Variants of Guillain-Barré Syndrome

Acute Motor Axonal Neuropathy and Acute Motor and Sensory Axonal Neuropathy

AMAN is an axonal variant of GBS, similar to acute motor and sensory axonal neuropathy (AMSAN). Axonal conduction failure occurs due to immune-mediated disruption of the nodal-paranodal region of the axolemma leading to failure of saltatory conduction [29]. Reports of this GBS variant were observed in northern China in the 1950s and 1990s and in Mexico in the 1960s in cases of acute flaccid paralysis. In 1989, the abnormally high prevalence of Chinese paralytic syndrome was characterized by symmetric, rapidly ascending flaccid paralysis of the four limbs and often progressing to acute respiratory distress and bulbar abnormalities. In electrophysiology assessments, reductions in CMAP, normal SNAPs, and denervation potentials in limb muscles were observed. Upon autopsy, motor fibers showed Wallerian-like degeneration with no or minimal demyelination and lymphocytic

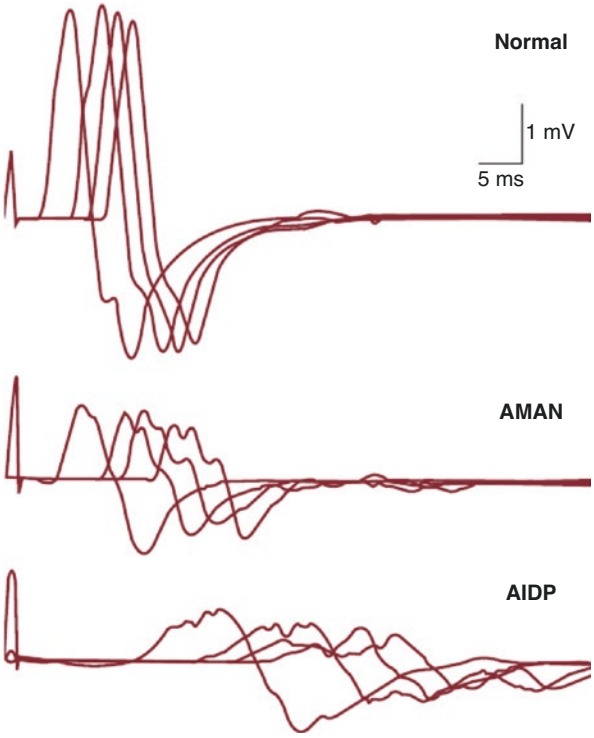

Fig. 15.2 Compound muscle action potentials in normal subject, AMAN, and AIDP patients. *AIDP* acute inflammatory demyelinating polyneuropathy, *AMAN* acute motor axonal neuropathy. Ulnar nerve conduction studies in a normal subject and in patients with AMAN or AIDP. Compound muscle action potentials are recorded from the abductor digiti minimi with stimulation at the wrist, below the elbow, above the elbow, and at the axilla. Note the simple reduction of compound motor action potential amplitudes in AMAN and prolonged distal latency and increased compound motor action potential duration in AIDP. (Source: Kuwabara [35])

infiltration [34]. Representative CMAPs are shown in Fig. 15.2 in a normal subject, in an AMAN patient, and an AIDP patient.

AMAN is often preceded by C. jejuni enteritis [17]. Infection may result in antibody cross-reactivity between the lipooligosaccharides on the bacterium and gangliosides on the axons, known as molecular mimicry.

Antiganglioside immunoglobulin G (IgG) antibodies of the IgG1 and IgG3 subclass are present in AMAN. AMAN is considered a nodo-paranodopathy based on the observation that specific antibodies bind at the node and paranode, which results in disorganized sodium channels and detachment of myelin. IgG antibodies bind the ganglioside GM1 or GD1a on the axolemma of motor fibers at the nodes of Ranvier, disrupting paranodal myelin. Anti-GD1b and GalNAc-GD1a IgM antibodies have also been reported [18, 29].

Several atypical variants have been reported for AMAN. For example, AMAN with conduction block has been observed, which is a feature of demyelination [17].

AMAN with brisk reflexes or preserved reflexes has also been reported [17, 36, 37]. These atypical types of AMAN were treated successfully with IVIG or plasmapheresis [17, 36, 37].

Miller-Fisher Syndrome

MFS is a variant of GBS, accounting for approximately 5–10% of GBS cases. MFS is associated with cranial nerve dysfunction and presents clinically with a triad of symptoms, including ophthalmoplegia, ataxia, and areflexia [38]. It is more common in Japan than in the United States and also occurs more frequently in men than women [30]. MFS arises following infection, usually upper respiratory tract or gastrointestinal infection that initiates the process of molecular mimicry, leading to humoral attack on GQ1b gangliosides on peripheral and cranial nerves [30, 31]. MFS is clinically diagnosed, but serological confirmation can be achieved through the identification of anti-GQ1b antibodies in approximately 90% of MFS patients [30, 31]. Less often, patients with MFS have antibodies against GM1, GM2, GD1a, or GD1b [30].

In MFS patients, motor nerve conduction and F-wave latencies are often normal while abnormalities in sensory nerve conduction studies may include reduced SNAP amplitude [31]. MRI may show thickening and/or enhancement of intrathecal spinal nerve roots and cauda equina [24]. A pilot nerve ultrasound study has also suggested enlarged facial nerve width at the onset of MFS when compared to healthy controls [39].

In some patients, MFS rapidly progresses to include weakness of the limb and respiratory muscles, resulting in overlapping symptoms between patients with MFS and GBS [40]. It is thought that the neurotoxic effect of the anti-GQ1b antibodies in MFS contribute to the disease progression and MFS-GBS-overlap syndrome. Based on CMAP scans, a neurophysiological method based on CMAP recordings induced by a range of stimulus intensities, patients with preserved limb muscle strength have subclinical dysfunction of the limb motor nerves (Fig. 15.3) [40, 41]. It has been speculated that this subclinical dysfunction may progress to overt limb weakness in some patients and indicates that MFS may be a more general neuropathy that is not only confined to the cranial nerves.

MFS has been reported in several case reports as occurring with overlapping myasthenia gravis, an autoimmune disorder of the neuromuscular junction [31]. Myasthenia gravis is characterized by fatigability and voluntary muscle weakness, often with symptoms of diplopia, ptosis, and limb weakness. In a case report, an 81-year-old male patient presented with signs of ocular myasthenia gravis who was diagnosed with MFS based on the presence of the anti-GQ1b antibodies. He presented with acute onset of diplopia following mild gastrointestinal illness. Clinically, the patient had complete bilateral external ophthalmoplegia and left-sided ptosis, mild left-sided facial and bilateral orbicularis oculi weakness, no limb ataxia, and a slightly wide-based gait with difficulty walking heel-to-toe [30]. Another case report of MFS and myasthenia gravis occurred in a 79-year-old patient with a history of myasthenia gravis who had worsening diplopia and fatigue occurring after bronchitis and receiving the flu vaccination. The patient's myasthenia gravis had

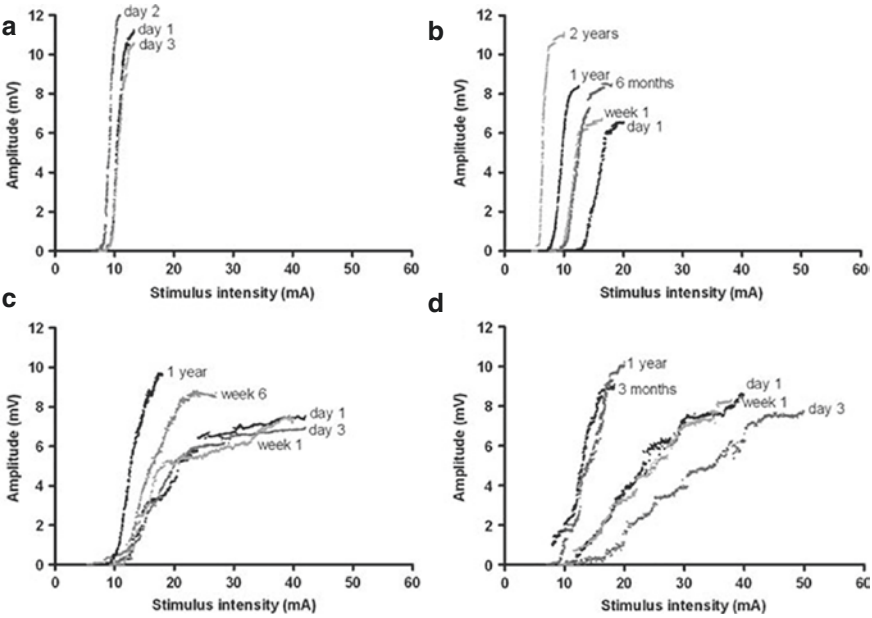

Fig. 15.3 Compound muscle action potential scan in healthy subject and three Miller-Fisher syndrome patients. *CMAP* compound muscle action potential, *MFS* Miller-Fisher syndrome, *S50* stimulus intensity at which 50% of the maximum compound muscle action potential is generated, *SI* stimulus intensity. Serial CMAP scans of thenar muscles after stimulation of the median nerve at the wrist in one healthy control (**a**) and three patients with MFS (**b**–**d**). All MFS patients had normal limb strength and motor nerve conduction studies. Time of follow-up after hospital admission is indicated. (**a**) Reproducibility of the CMAP scan in a 42-year-old healthy control. (**b**) MFS patient 1: increased S50 and SI range on day 1, with improvement at 1 week and normalization after 2 years, indicating a transiently disturbed nerve excitability in the acute phase. Although the other parameters are within normal values at day 1, they improve during follow-up. (**c**) MFS patient 2: increase in all SI parameters in the first week, with improvement at 6 weeks and normalization at 1 year. (**d**) MFS patient 3: a decrease in nerve excitability (shift to higher SI) from days 1 to 3 paralleling clinical deterioration, and subsequent improvement of nerve excitability with general clinical recovery. (Source: Drenthen et al. [40])

been controlled with pyridostigmine, azathioprine, and low dose steroids. A clinical workup of blood tests, electromyography, brain MRI, and CSF analysis indicated acute polyradiculoneuropathy. Plasma exchange stabilized symptoms. The patient was positive for anti-GQb1 antibodies [31].

Bickerstaff's brainstem encephalitis, a variant of MFS, has the same clinical features observed including ophthalmoplegia and ataxia with hyperreflexia or pathological reflexes as well as encephalopathy. In addition, patients with Bickerstaff's brainstem encephalitis have consciousness disturbance. Patients are positive for anti-GQ1b, GT1a, and other antiganglioside IgG antibodies [32].

The clinical course of MFS is self-limiting and usually resolves within weeks to months and immunotherapy is not often required. Thus far, no clinical trials

investigating possible treatments have been reported; however, intravenous immunoglobulin (IVIG) and plasma exchange have been proposed as treatment options [30].

Pharyngeal-Cervical-Brachial Variant

The pharyngeal-cervical-brachial variant of GBS is characterized by acute weakness of oropharyngeal, neck, and shoulder muscles along with dysphagia and facial paresis. Reflexes and strength are preserved in the lower extremities. It is caused by an antecedent infection, most often C. jejuni [32], and in one case report this variant occurred post-vaccination [42]. Electrophysiological findings reveal a denervation pattern and decreased conduction velocity in the peripheral nerves. Patients have elevated CSF protein and are positive for antiganglioside antibodies in the CSF [24, 32]. Antibodies detected are most frequently IgG anti-GT1a and anti-GQ1b [32].

Acute Small Fiber Sensory Neuropathy

GBS normally only involves large motor-sensory fibers; however, in some rarer instances, the small fibers are affected. Similar to traditional GBS, acute small fiber sensory neuropathy has a preceding infectious illness or vaccination, monophasic course, and albuminocytologic dissociation. Patients may or may not report pain, which suggests that there may be two distinct variants of this small fiber neuropathy. In three Chinese patients presenting with severe pain in the extremities after preceding infectious illness, patients exhibited impaired pinprick sensation, hyperesthesia, and brush allodynia in a glove-and-stocking distribution. Pallesthesia, proprioception, and power in all extremities was normal and tendon reflexes were diminished. Nerve conduction studies were normal. There were no IgG antibodies present against GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, or GQ1b. IVIG treatment decreased pain in two patients that received it within a week. The third patient received oral prednisolone and improved gradually [33]. Whether this entity can be truly classified as a GBS variant is debated.

15.3.2 Chronic Inflammatory Demyelinating Polyradiculopathy

CIDP, although rare, is the most common type of chronic acquired demyelinating polyneuropathy of the peripheral nerves and nerve roots. It was described as early as 1890 by Eichhorst [43]. Its incidence is reported to be 0.33 cases per 100,000 person-years [44]. It occurs more frequently in males and incidence increases with age. Its pathogenesis has not yet been elucidated. Unlike GBS, a preceding infection to CIDP is not typically identified [3].

The classic CIDP phenotype includes symmetric proximal and distal weakness, hyporeflexia, or areflexia, and predominantly large fiber sensory deficits. Patients may present with neuropathic pain, usually radicular pains or paresthesia, occurring in up to half of patients [45, 46]. CIDP is progressive, with symptoms developing over a period of at least 8 weeks, leading to progressive disability in walking and

climbing the stairs, while the cranial district is not usually involved [15]. Presentation can also be relapsing-remitting, stepwise progressive, or gradually progressive.

Diagnosis of CIDP is not always straightforward, as some patients do not show the typical progression, clinical symptoms, or common electrodiagnostic criteria, and as a result misdiagnosis is common [4]. CIDP is clinically and immunologically heterogeneous and includes a number of variants. There is still some debate as to whether all clinical phenotypes similar to CIDP are atypical CIDP or whether they are CIDP-like but having a different pathogenesis.

To confirm a diagnosis of CIDP, nerve conduction studies must show features of a demyelinating neuropathy. To assist with the diagnosis of CIDP, at least 15 criteria have been developed [47]. Currently, the most frequently used criteria have been reported to be the revised European Federation of Neurological Societies/Peripheral Nerve Society 2010 criteria [48], which include clinical and electrodiagnostic criteria (Table 15.2).

Table 15.2 European Federation of Neurological Societies/Peripheral Nerve Society 2010 clinical and electrodiagnostic criteria for CIDP

Criteria
Electrodiagnostic criteria
1. Definite: at least one of the following:
(a) Motor distal latency prolongation ≥50% above ULN in two nerves (excluding median neuropathy at the wrist from carpal tunnel syndrome), or
(b) Reduction of motor conduction velocity ≥30% below LLN in two nerves, or
(c) Prolongation of F-wave latency ≥30% above ULN in two nerves (≥50% if amplitude of distal negative peak CMAP <80% of LLN values), or
(d) Absence of F-waves in two nerves if these nerves have distal negative peak CMAP amplitudes ≥20% of LLN + ≥1 other demyelinating parameter[a] in ≥1 other nerve, or
(e) Partial motor conduction block: ≥ 50% amplitude reduction of the proximal negative peak CMAP relative to distal, if distal negative peak CMAP ≥20% of LLN, in two nerves, or in one nerve + ≥1 other demyelinating parameter[a] in ≥1 other nerve, or
(f) Abnormal temporal dispersion (>30% duration increase between the proximal and distal negative peak CMAP) in ≥2 nerves, or
(g) Distal CMAP duration (interval between onset of the first negative peak and return to baseline of the last negative peak) increase in ≥1 nerve (median ≥ 6.6 ms, ulnar ≥ 6.7 ms, peroneal ≥ 7.6 ms, tibial ≥ 8.8 ms) + ≥1 other demyelinating parameter[a] in ≥1 other nerve
2. Probable
≥30% amplitude reduction of the proximal negative peak CMAP relative to distal, excluding the posterior tibial nerve, if distal negative peak CMAP ≥ 20% of LLN, in two nerves, or in one nerve + ≥1 other demyelinating parameter[a] in ≥1 other nerve

Table 15.2 (continued)

Criteria
3. Possible
As in (1) but in only one nerve To apply these criteria, the median, ulnar (stimulated below the elbow), peroneal (stimulated below the fibular head), and tibial nerves on one side are tested. If criteria are not fulfilled, the same nerves are tested at the other side, and/or the ulnar and median nerves are stimulated bilaterally at the axilla and at Erb's point. Motor conduction block is not considered in the ulnar nerve across the elbow and at least 50% amplitude reduction between Erb's point and the wrist is required for probable conduction block Temperatures should be maintained to at least 33 °C at the palm and 30 °C at the external malleolus (good practice points)
Clinical diagnostic criteria
1. Inclusion criteria
(a) Typical CIDP
Chronically progressive, stepwise, or recurrent symmetric proximal and distal weakness and sensory dysfunction of all extremities, developing over at least 2 months; cranial nerves may be affected; and Absent or reduced tendon reflexes in all extremities
(b) Atypical CIDP (still considered CIDP but with different features) One of the following, but otherwise as in (a) (tendon reflexes may be normal in unaffected limbs): Predominantly distal (distal acquired demyelinating symmetric, DADS) or Asymmetric [multifocal acquired demyelinating sensory and motor neuropathy (MADSAM), Lewis-Sumner syndrome] or Focal (e.g., involvement of the brachial or lumbosacral plexus or of one or more peripheral nerves in one upper or lower limb) Pure motor or Pure sensory (including chronic immune sensory polyradiculopathy affecting the central process of the primary sensory neuron)
2. Exclusion criteria
Borrelia burgdorferi infection (Lyme disease), diphtheria, drug or toxin exposure probably to have caused the neuropathy Hereditary demyelinating neuropathy Prominent sphincter disturbance Diagnosis of multifocal motor neuropathy IgM monoclonal gammopathy with high titre antibodies tomyelin-associated glycoprotein Other causes for a demyelinating neuropathy including POEMS syndrome, osteosclerotic myeloma, diabetic and nondiabetic lumbosacral radiculoplexus neuropathy. PNS lymphoma and amyloidosis may occasionally have demyelinating features

CIDP chronic inflammatory demyelinating polyradiculoneuropathy, *CMAP* compound muscle action potential, *LLN* lower limit of normal, *PNS* peripheral nervous system, *POEMS* polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes, *ULN* upper limit of normal

From: Van den Bergh et al. [48]. Erratum in Eur J Neurol. 2011 May;18(5):796

[a] Any nerve meeting any of the criteria (a–g)

The myelin sheaths of peripheral nerves are targeted in CIDP leading to electrophysiologic abnormalities suggesting acquired demyelination [41]. Nerve conduction studies suggest that demyelination in CIDP patients is more prominent at the distal motor axons near the motor nerve terminals than at the nerve trunk [49]. Abnormalities associated with CIDP include slow nerve conduction velocity, prolonged distal motor and F-wave latencies, abnormal temporal dispersion, and (partial) motor nerve conduction block in one or more motor nerves [41].

Nerve biopsies reveal segmental demyelination, onion bulb formation, and endoneurial inflammatory infiltrates. Although not specific to the disease, CSF protein is usually elevated in CIDP [3]. CIDP patients have multifocal nerve enlargement on ultrasound [13, 50]. Enlargement of peripheral nerves, pure sensory nerves, and the roots and vagus nerve have also been demonstrated. Enlarged nerve size does not generally reduce within 6 months, even after treatment [25].

CIDP can be treated; first-line treatment options include IVIG (or subcutaneous immunoglobulin [SCIG]), corticosteroids, or plasma exchange, with IVIG most often used of the three options [3]. Rituximab has also been shown to be effective [4].

The clinical presentation, electrodiagnostic features, and antibodies present of the CIDP variants that have been reported in the literature are provided in Table 15.3. Further details of each variant are provided in Sect. 15.3.2.1.

15.3.2.1 Variants of Chronic Inflammatory Demyelinating Polyneuropathy

Chronic Immune Sensory Polyradiculopathy

Chronic immune sensory polyradiculopathy (CISP) is an atypical form of CIDP with paresthesia, mild pain, numbness, areflexia, gait ataxia, sensory ataxia, and asymmetric distribution progressing to a distal symmetric pattern, accounting for just 0.5% of CIDP [57]. High CSF protein levels are considered a hallmark of CISP. Motor and sensory nerve conduction studies are typically normal in CISP patients, which distinguishes CISP from typical CIDP [15, 52]. Demyelination is demonstrated by prolonged SSEPs [15]. Treatment with IVIG and corticosteroids have been shown to be effective, but not in all cases [15].

In a case report, a patient had a subacute onset of asymmetric paresthesias in the lower limbs, which was relapsing-remitting for the first 4 months then slowly progressed for the next 2 months, resulting in limb ataxia and progressive gait disturbance requiring crutches [15]. Routine blood examinations were normal and there was no abnormal presence of anti-MAG, anti-sulfatide, or anti-peripheral nerve antibodies. Motor and sensory nerve conduction studies and electromyographic evaluation were within the normal limits except for bilateral mild elongation of the tibial F-waves latencies, which were < 15% of the upper normal limit. Tibial SSEPs lacked lumbar and cortical responses while median SSEPs were normal. CSF was acellular with increased protein concentration. Spinal MRI showed thickening of the sacral nerve roots and a tube-shaped enlargement. Left sural biopsy showed slight axonal impairment, sporadic fibers in Wallerian degeneration, minimal

Table 15.3 Comparison of features of CIDP variants

Subtype	Clinical presentation	Electrodiagnostic features	Antibodies present	References
Chronic immune sensory polyradiculopathy (CISP)	Paresthesia, mild pain, numbness, areflexia, ataxia	NCS Normal Prolonged SSEPs	Not described	[15]
Motor CIDP (CIMP)	Lower limb weakness Back pain	Some reduced CMAP amplitudes Some prolonged F-waves	Not described	[51]
Sensorimotor CIDP (CISMP)	Sensory ataxia Lower limb weakness Areflexia	Sensory and motor NCS normal F-waves absent H-reflexes absent	Not described	[52]
MADSAM (Lewis-Sumner syndrome)	Upper limbs affected Pain Paresthesial Weakness and atrophy progress slowly	Single nerves only affected at onset Asymmetric, multifocal abnormal SNAPs	Not described	[53]
CMAN	Weakness, muscular atrophy	Reduced CMAP amplitude	IgG GM1	[54]
CIDP with antibodies to nodal and paranodal proteins	Severe clinical phenotype Tremor Sensory ataxia Paresthesia	Conduction block Prolonged F-waves	IgG4 Contactin 1 NF155 NF140 NF186	[10, 55, 56]

CIDP chronic inflammatory demyelinating polyneuropathy, *CIMP* chronic inflammatory motor polyradiculopathy, *CISMP* chronic inflammatory sensorimotor polyradiculopathy, *CISP* chronic immune sensory polyradiculopathy, *CMAN* chronic motor axonal neuropathy, *CMAP* compound muscle action potential, *MADSAM* multifocal acquired demyelinating sensory and motor neuropathy, *NCS* nerve conduction study, *SSEP* somatosensory evoked potential, *SNAP* sensory nerve action potential

reduction in myelinated fiber density, and some isolated aspects of chronic (cluster) reinnervation. The patient was treated with a high dose course of methylprednisolone, which was ineffective. As a second-line treatment, she was treated with IVIG for five consecutive days every 4 weeks, which enabled prolonged, stable remission for 9 years. The patient then began to have symptom fluctuations. Treatment with plasmapheresis, mycophenolate mofetil, and cyclophosphamide were each tried and discontinued due to lack of benefit or because of side effects [15].

A pediatric case of CISP has been described [58]. A 17-year-old female presented with progressive sensory ataxia and hand clumsiness and had diffuse tendon areflexia and hypokinesthesia. Features in this pediatric case were similar to those in adult patients described above; motor and sensory nerve conduction studies were normal, tibial F-waves were elongated, SSEPs were absent, and CSF was acellular with increased protein concentration [58].

Chronic Inflammatory Motor Polyradiculopathy

Chronic inflammatory motor polyradiculopathy (CIMP) is a form of CIDP confined to the motor roots. Criteria for CIMP include nerve root hypertrophy, elevated CSF protein, onion bulb formations on biopsy, and responsiveness to immunomodulatory therapy.

In a case report, a 68-year-old male had a 10-year history of progressive, symmetric lower extremity weakness [51]. There was predominant involvement of motor fibers with sparing of sensory, bowel, and bladder function. The patient had cauda equina nerve root hypertrophy resulting in progressive bilateral lower limb weakness and back pain without sensory deficits. Symptoms were not present in the upper extremities, nor did he experience sensory symptoms or bulbar symptoms.

In this patient, nerve conduction studies of the lower extremities showed that fibular CMAP amplitudes were reduced but tibial CMAP amplitudes were not. F-waves were prolonged for the tibial nerves and were not obtainable for the fibular nerves. Distal latencies and conduction velocities were normal bilaterally. Sensory nerve conduction studies were normal for sural and superficial fibular nerves. Sensory and motor nerve conduction studies were normal in the upper extremities. Concentric needle examination showed large amplitude, polyphasic motor unit potentials, fasciculation potentials, fibrillation potentials, and neurogenic recruitment pattern in the tibialis anterior muscles bilaterally, which were similarly found in right vastus lateralis, right and left gastrocnemius, and right gluteus maximum muscles. MRI imaging revealed diffuse thickening of the cauda equina nerve roots with some nerve roots demonstrating enhancement with gadolinium. Nerve root hypertrophy is hypothesized to result from demyelination and remyelination of Schwann cell proliferation and onion bulb formation [15].

CSF had elevated protein of 1.0 g/L with six mononuclear white cells. CSF cytological analysis was normal. Laboratory investigations were normal. No anti-MAG antibodies were present. A nerve root biopsy was performed (L3–4 laminectomy) and a nerve that did not elicit a motor response was selected. Toluidine blue staining of a cross-section of the nerve root demonstrated numerous onion bulbs and significant large myelinated fiber loss, and electron microscopy also demonstrated onion bulbs (Fig. 15.4). The findings were consistent with recurrent demyelination and remyelination (i.e., onion bulb formation) [15].

The patient received IVIG and methylprednisolone for 6 weeks without significant improvement and further deterioration of power in the lower extremities with the patient becoming unable to walk. Azathioprine and plasma exchange were subsequently initiated and led to patient ambulation without a wheelchair and improvements in muscle power in hip, knee, and ankle measures [15].

Although the patient lacked the CIDP pathological features of endoneurial lymphocytic infiltrates and segmented demyelination with or without axonal degermation, this does not exclude CIDP. The lack of inflammation on the biopsy could be due to small sample size, multifocality of the disease, or lack of inflammatory changes at that stage of the disease [15].

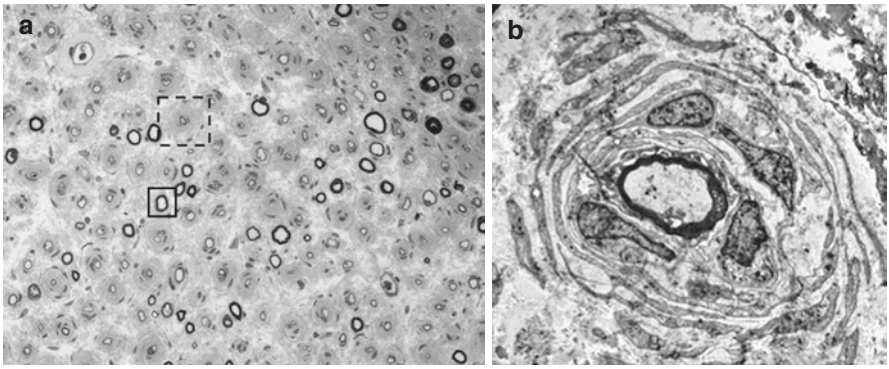

Fig. 15.4 L3–4 Nerve root biopsy demonstrating onion bulbs in a CIMP patient. *CIMP* chronic inflammatory motor polyradiculopathy. An L3–4 laminectomy was performed to obtain a nerve root biopsy. (**a**) Toluidine blue staining of a cross-section of the nerve root demonstrated numerous onion bulbs and significant loss of large, myelinated axons. A single onion bulb is demarcated with a box (dashed line), while an axon lacking onion bulb formation is also demarcated with a box for comparison (solid line). No malignant cells were seen (20× magnification). (**b**) Electron microscopy demonstrated multiple onion bulbs, one example of which is shown here (2200× magnification). (Source: O'Ferrall et al. [15])

Chronic Inflammatory Sensorimotor Polyradiculopathy

Chronic inflammatory sensorimotor polyradiculopathy (CISMP) is the term used to describe the focal, proximal form of immune-mediated polyradiculopathy involving sensory and motor roots. Distal nerve segments are spared while proximal nerves indicate abnormalities in sensory and motor roots. Two patients have been investigated with both sensory ataxia and weakness due to isolated proximal root involvement, with similar clinical workup results [52]. Both cases of CISMP presented with gradually progressive sensory ataxia and lower limb weakness. Assessment of Medical Research Council (MRC) power was reduced (Grade 3) at both hip and knee joints while spared (Grade 4 or 5) at ankle joints. Both patients were areflexic.

In one patient, a 27-year-old woman, knee and ankle reflexes were absent and joint position and vibration sensation were diminished in the lower limbs up to the anterior superior iliac spine. The patient had normal pain, fine touch, and temperature sensation and normal power, sensation, and tendon stretch reflexes in the upper limbs. Sensory and motor nerve conduction studies were normal. F-waves were absent from both tibial nerves with reduced persistence from both ulnar nerves and the tibial/gastrocnemius H-reflexes were absent. There was chronic denervation observed in the vastus medialis, tibialis anterior, gluteus medius, and gastrocnemius muscles. Tibial SSEPs had normal potentials for popliteal nerve (N8), were absent for lumbar root (N22), and prolonged for cortical (P40) bilaterally and symmetrically. Upper limb SSEPs had normal potentials. MRI indicated hypertrophy and contrast enhancement of lumbosacral roots. CSF protein was elevated with normal glucose and cell composition. Treatment with prednisolone, with the addition of

azathioprine as a steroid-sparing agent after 3 months of therapy, improved ataxia but not areflexia, and modified Rankin scale (MRS) score improved from 4 to 1 by 8 months [52].

A second patient, a 17-year-old female, was similarly affected. F-waves were absent from the nerves of the lower limbs, but not upper, and tibial H-reflexes were absent. Electrophysiological studies of sensory nerves were normal for upper and lower extremities. Distal CMAP amplitudes were normal in both common fibular nerves and right tibial nerve but were mildly decreased in the left tibial nerve. Chronic denervation was observed for the vastus medialis, tibialis anterior, gluteus medius, and gastrocnemius muscles. Tibial nerve SSEPs were normal for popliteal nerve potentials, and absent for lumbar root and cortical potentials. SSEPs were normal for the upper limbs. MRI showed hypertrophy and contrast enhancement of lumbosacral roots. Serum biochemistry and autoimmune markers were negative. CSF had elevated protein and normal cell composition. Following treatment with prednisolone and mycophenolate (azathioprine was not tolerated), the MRS score improved from 4 to 2 at 4 months then was stabilized up to 2 years later. Sensory and motor components stabilized but areflexia remained [52].

Multifocal Acquired Demyelinating Sensory and Motor Neuropathy or Lewis-Sumner Syndrome

Multifocal CIDP, also called Lewis-Sumner syndrome or multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) is a variant of CIDP predominantly affecting the upper limbs. It is asymmetrical with focal motor and sensory nerve conduction blocks. The disease is multifocal and often affects only single nerves early in its course causing numbness and pain, but weakness and atrophy develop slowly over time. Patients may experience pain and paresthesias. Reflexes are reduced or absent. CSF protein is slightly elevated. Nerve conduction studies reveal asymmetric, multifocal abnormal SNAPs [53]. Pathology is the same as classical CIDP, with segmental demyelination, epineurial and endoneurial inflammation, and onion bulb formation. Nerve ultrasound studies show regional enlargement compared to controls [59]. MADSAM cases respond to IVIG and corticosteroids such as prednisone [3].

CMAN (Chronic Motor Axonal Neuropathy)

A rare variant of immune neuropathy is chronic motor axonal neuropathy (CMAN), which may sometimes be described as a variant of multifocal motor neuropathy (MMN), as described in Sect. 15.3.3.

In a case report, a 50-year-old male with no previously reported infections was described to have progressive weakness in all four limbs over 5 months [54]. Weakness began in the upper right limb and quickly progressed to the upper left limb. Three monthly courses of IVIG were prescribed, with only partial benefit after one course with progressing weakness observed.

The patient had widespread asymmetric muscular atrophy and weakness in the upper limbs with mild atrophy and weakness in the lower limbs. Tendon reflexes were reduced or absent. There was no sensory involvement or cranial nerve

involvement. Laboratory investigations were normal. IgM anti-GM1 antibodies were detected. CSF protein was mildly elevated (69.9 mg/dL) with pleocytosis (9 cells/mm^2). Spinal MRI revealed widespread enhancement of anterior leptomeninges and anterior roots up to the intervertebral foramina and spinal nerves with no volumetric increase.

Nerve conduction studies showed slight asymmetric reduced CMAP amplitudes without conduction blocks and no signs of demyelination/temporal dispersion. Sensory conduction was normal. There was active denervation and neurogenic motor unit potentials in all limb muscles on electromyography that were not present in paraspinal muscles or the tongue. Motor biopsy of the obdurator nerve showed moderate reduction of myelinated fibers, axonal degeneration, and endoneurial edema, as well as clusters of small myelinated fibers indicated axonal regeneration. There were also isolated thinly myelinated fibers and onion bulb formations suggesting recurrent demyelination and remyelination. Immune infiltration was demonstrated by electron microscopy and immunocytochemistry (ICC). Some nerve fibers were positive for complement activation product C3i and IgM, more prominently at the nodes of Ranvier, by ICC. Intravenous methylprednisolone was initiated but was interrupted due to worsening weakness. Four monthly courses of IVIG produced only minimal transitory response followed by further disease progression. Plasma exchange 9 months after disease onset without benefit and monthly cyclophosphamide was also unable to prevent disease progression. Ten months after disease onset the patient developed respiratory failure and died 15 months after disease onset [54].

CIDP with Antibodies Against Nodal and Paranodal Proteins

Approximately 10% of CIDP patients have been found to harbor antibodies to nodal and paranodal proteins; the term nodo-paranodopathy has been proposed to refer to these patients. Patients with this variant have IgG4 antibodies against cell adhesion proteins located at or near the node of Ranvier. These IgG4 antibodies include the paranodal proteins contactin 1 (CNTN1), neurofascin isoform 155 (NF155), and contactin-associated protein-1 (CASPR1), as well as the nodal proteins NF140 and NF186. Anti-contactin 1 antibodies have been found in 1–7% of CIDP patients [10, 56]. CNTN1 is an axonal adhesion protein that interacts with CASPR1 on the axon side and with NF155 on the glial side to form the paranodal axo-glial junction. NF140 and NF186 are neuronal isoforms that are located at the nodes of Ranvier where they form nodal complexes and cluster sodium channels at the nodes.

Patients with antibodies against a paranodal protein tend to have a severe clinical phenotype, including predominantly motor and early axonal involvement, tremor, sensory ataxia, and paresthesia. Patients do not respond well to the common first-line treatment of IVIG therapy but respond better to corticosteroids and rituximab, the anti-CD-20 monoclonal antibody (mAb) for B-cell depletion treatment [55]. NF155 and CNTN1 are localized at the paranodal region and the antibodies are the IgG4 isotype. The IgG4 isotype does not efficiently activate complement and has a low affinity for Fc receptors of effector cells, which may explain why IVIG does not lead to more favorable response.

Patients with anti-NF140 and anti-NF186 antibodies have subacute onset, severe leg weakness, sensory ataxia, no tremor, conduction block, and involvement of the cranial nerves. Patients with anti-NF140 and anti-NF186 antibodies can respond well to IVIG and steroid treatment [4, 55].

In pathological studies patients with the described IgG4 antibodies do not show the typical features of demyelination usually observed in CIDP, such as onion bulbs, inflammation, or macrophage-mediated demyelination. Instead, widened nodes, detached myelin loops, and axonal degeneration without regeneration have been noted [4]. A retrospective clinical review of 10 CNTN1 IgG seropositive cases demonstrated the electrophysiological features were more representative of POEMS syndrome than CIDP and nerve pathology was distinct from CIDP, including segmental demyelination and the absence of onion bulbs [60].

As a result of the variable phenotypes observed and treatment responses in patients with nodal or paranodal IgG4 antibodies, it has been questioned whether these patients are CIDP variants or whether they should be classified separately [4, 60, 61].

In a recent case report, a 47-year-old female with ascending weakness, sensory deficits, and back pain followed by paresthesias in both lower limbs that ascended to involve the upper limbs 2 weeks after a diarrheal illness was described [62]. Four weeks after onset, she developed right facial weakness. She was found to have multiple cranial neuropathies and a polyradiculopathy with evidence of NF155 in serum and both NF155 and NF140 in CSF. Initially thought to be AIDP, the neurologic involvement in this patient was eventually determined to be sarcoidosis based upon identification of granulomas in the mediastinal lymph nodes. Both sarcoidosis and the presence of these autoantibodies may produce a similar clinical phenotype and respond similarly to treatment, thus, it cannot be confirmed whether one or both pathological entities contributed to her clinical symptoms.

There has been one case described with both anti-CNTN1 and anti-NF140 antibodies with a clinical presentation resembling the phenotypes of both CIDP variants [55]. The patient was a 20-year-old African American female who presented with a 2-month history of bilateral numbness and tingling in the hands and legs, bilateral leg weakness, and gait imbalance that had abruptly worsened requiring assistance with ambulation. Four months prior, she had left-sided facial numbness and tingling, drooling, and dysarthria following flu-like symptoms that resolved after 6 weeks. The patient had areflexia, impaired proprioception, bilateral lower extremity edema, proximal and distal muscle weakness, and decreased vibration and pinprick sensation distal to the knees. Nerve conduction studies revealed prolonged F-wave latencies that were greater in the lower extremities than the upper. The patient improved moderately after one treatment course of IVIG but did not improve upon two subsequent treatment courses. Her symptoms worsened, causing her to become wheelchair bound again. Contrast lumbar spine MRI results, previously normal, showed diffuse enlargement and enhancement of the cauda equina to the sacral plexus. Nerve conduction studies showed severe axonal and demyelinating neuropathy, with greater involvement of the motor than sensory

nerves and greater lower than upper extremity involvement. Further assessment showed normal sural nerve biopsy, indicative of a predominantly proximal demyelination. Methylprednisolone significantly improved symptoms [55].

Novel Variant of CIDP

A case report of a CIDP-like neuropathy with erythromelalgia was reported in a 52-year-old woman that has been suggested to represent a novel variant of CIDP or a novel dysimmune neuropathy. Erythromelalgia can occur due to an underlying medical condition and has been observed in patients with other neuropathies, such as small fiber neuropathy and axonal large fiber peripheral neuropathy. Erythromelalgia is characterized by constant erythema, warmth, edema, and intense burning pain in the distal extremities, which are aggravated by heat [46].

The patient presented with severe erythromelalgia, progressive severe burning pain, progressive weakness, swelling of hands and feet, hyporeflexia, and distal pan-sensory deficits. She had a history of alcohol abuse in the past. The patient had elevated CSF protein (219 mg/dL). Nerve conduction studies revealed extremely (ten-fold) prolonged distal motor latencies, very slow motor nerve conduction velocity, reduced terminal latency index, markedly reduced CMAP amplitudes, distal denervation, and probable conduction block of the median nerve. The patient responded well to IVIG, prednisone, and azathioprine, both clinically and in nerve conduction studies. The patient fulfilled the diagnostic criteria for CIDP but had unique electrodiagnostic features and presentation with erythromelalgia and small fiber involvement that indicate it may be a novel variant of CIDP. The disproportionate distal slowing observed is more often observed in anti-MAG neuropathy; however, the patient did not have the clinical phenotype observed in distal, acquired, demyelinating, and symmetrical (DADS), IgM gammopathy, or anti-MAG antibodies. Alcohol-related peripheral neuropathy, based on the patient's past alcohol abuse, could not be ruled out but was considered unlikely [46].

15.3.3 Multifocal Motor Neuropathy

Multifocal motor neuropathy (MMN) is a chronic motor neuropathy characterized by slowly progressive, asymmetrical muscle weakness in peripheral nerves, usually of the upper extremities, without sensory loss. The prevalence of MMN is less than 1 per 100,000 individuals, with men more frequently affected than women [63, 64]. Sensory nerve conduction velocities and action potential amplitudes are normal in MMN. Cranial nerves are rarely affected [65]. A hallmark of MMN is persistent motor conduction blocks outside of compression sites in radial, ulnar, median, and peroneal nerves [14, 66]. Patients may have elevated CSF protein [14]. MRI with increased signal intensity on T2-weighted imaging may also help in the diagnosis of MMN as a supportive criterion [64].

The clinical presentation, electrodiagnostic features, and antibodies present in MMN are provided in Table 15.4.

Table 15.4 Comparison of features of multifocal motor neuropathy

Subtype	Clinical presentation	Electrodiagnostic features	Antibodies present	References
MMN with conduction block	Slowly or stepwise progressive focal, asymmetric limb weakness No or minimal sensory abnormalities	Motor conduction block outside of compression sites	IgM GM1, GalNAc-GD1a, GM2	[63, 67]
MMN without conduction block	Slowly or stepwise progressive focal, asymmetric limb weakness No or minimal sensory abnormalities	No conduction block	IgM GM1	[68]

MMN multifocal motor neuropathy

The clinical features of MMN include the presence of slowly progressive or stepwise progressive, focal, asymmetric limb weakness with multineuropathic distribution affecting at least two distinct motor nerves lasting longer than 1 month. There must be minimal or no sensory abnormalities except for minor vibration sense abnormalities of the lower limbs [69]. The electrophysiologic feature of MMN is the presence of conduction block outside of typical compression sites. Conduction block may be difficult to detect and may require techniques such as triple stimulation, transcutaneous cervical root stimulation, and transcranial magnetic stimulation [64, 69]. Although conduction block is considered a hallmark of MMN, it is only found in approximately 70% of patients [70]. It is considered controversial as to whether conduction block is essential for the diagnosis of MMN. Cases of MMN without conduction block have been reported to be indistinguishable from MMN with conduction block in terms of clinical course and treatment response to IVIG [68]. Conduction block may be difficult or impossible to detect at very proximal or distal locations, which may account for cases of MMN without block. Alternatively, nerve conduction studies may only have been performed in clinically affected limbs while the conduction block may have been present in the non-clinically affected limbs.

Nerve ultrasound studies show regional enlargement compared to controls [59]. Nerve biopsies of MMN patients show axonal degeneration, axonal loss, and regenerating fibers [71].

GM1 is a glycolipid highly expressed in axonal membranes of motor nerves, abundant at the node and paranodal Schwann cells. Half of adults with MMN are positive for anti-GM1 antibodies and anti-GalNAc-GD1a and anti-GM2 IgM antibodies are also associated with MMN [63]. The majority of MMN patients with anti-GM1 IgM antibodies have only one type of immunoglobulin (Ig) light chain (either IgK or IgL), which suggests that antibodies are produced by only a single or very few anti-GM1 B-cell clones [67]. Anti-GM1 antibodies activate complement. Although the complete details on the pathophysiology of MMN have not yet been elucidated, conduction block is believed to occur as a result of immune-mediated attack of the nodes of Ranvier and paranodal regions [64]. The term

nodo-paranodopathy has been proposed to describe neuropathies where disruption of nodal, paranodal, and juxtaparanodal regions lead to conduction blocks [72].

IVIG has been found to be safe and effective for the treatment of MMN while corticosteroids and plasma exchange are ineffective and may even lead to worsening of weakness. Rituximab has been demonstrated to be safe for patients with MMN when given with IVIG but did not lead to reductions in the amount of IVIG required in an open-label trial with six patients [73]. SCIG trials in MMN have shown promise but require further investigation [74, 75].

The mean age of onset for MMN is 40 years of age; however, although very rare, childhood onset MMN has been reported [63]. In a recent case report, a 12-year-old girl presented with slowly progressive weakness of the upper extremities without sensory loss. MRC scale scores indicated distal weakness of both upper limbs. Motor conduction velocities were reduced in the left ulnar and bilateral radial nerves and the F-wave ratio was also reduced in the same nerves. The patient was found to have definite conduction blocks in the left median and bilateral radial nerves, defined as >50% reduction in CMAP area and < 30% increase in duration characteristic of MMN. After diagnosis with MMN, serology showed IgM antibodies to GM2 and GalNAc-GD1a. High-dose IVIG led to partial improvements in muscle weakness and electrophysiological assessments [63]. Anti-GM2 and anti-GalNac-GD1a antibodies are also associated with GBS occurring following cytomegalovirus or C. jejuni infection although this case had no prodromal infection.

A patient has been described with MMN as well as the presence of anti-MAG antibodies [66], which is a clinically distinct entity described in Sect. 15.3.4. The patient presented with a history of slowly progressive predominantly distal tetraparesis without sensory deficit or pain. Nerve conduction studies showed proximal motor conduction blocks on right ulnar nerve, right tibial nerve, left median nerve, and both fibular nerves. Distal CMAP amplitudes were slightly reduced and SNAP amplitudes and conduction velocities were normal in the lower limbs. Lumbar MRI and CSF analysis were normal. IVIG for 5 days led to mild clinical improvement. The patient refused further treatment. Eight years later, the patient returned with unstable gait requiring assistance, severe bilateral foot drop, and reduced sensibility of lateral aspect of right foot and leg and medial aspect of right hand. The patient had elevated anti-MAG, anti-SGPG, and mildly elevated anti-GM1 IgM antibodies, and skin biopsy indicated minor IgMλ paraprotein. Rather than the deposits being located on myelin sheaths, as is normally observed in anti-MAG neuropathies, the IgMλ deposits were located on the perineurium. Repetitive IVIG was given but patient response was poor. This report suggests that the pathogenicity of anti-MAG antibodies is variable [66].

15.3.4 Other Immune-Mediated Neuropathies

Other types of immune-mediated neuropathies have been described that do not necessarily fall under the three main subtypes already described (CIDP, GBS, and MMN) although in some cases there is an overlap in symptoms or controversy as to how these variants should be classified.

The clinical presentation, electrodiagnostic features, and antibodies present of the other types of immune-mediated neuropathies that have been reported in the literature are provided in Table 15.5.

Table 15.5 Comparison of features of immune-mediated neuropathies

Subtype	Clinical presentation	Electrodiagnostic features	Antibodies present	References
MGUS (not IgM)	Paresthesia Weakness	Absence of SNAPs Absent CMAPs Slowed conduction velocity Conduction block	IgG or IgA	[76]
IgM MGUS with MAG	Highly variable Mild, minimal symptoms Tremor Sensory ataxia	Slowed conduction velocity No conduction block Reduced SNAPs	IgM MAG	[13, 77]
IgM MGUS without MAG	Highly variable Mild, minimal symptoms Tremor Sensory ataxia	Slowed conduction velocity No conduction block Reduced SNAPs	IgM	[13, 77]
POEMS	3 of 5 of: polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin abnormalities	Length-dependent	IgG or IgA	[78, 79]
Paraneoplastic neuropathies with anti-ANNA-1 antibodies	Paraneoplastic, usually SCLC	Reduced conduction velocity	ANNA-1	[80]
CANOMAD and CANDA	Sensory ataxia, gait disorder, areflexia, tremor, ophthalmoplegia	Axonal, demyelinating, or mixed	IgM GD3, GD1b	[81]
FOSMN	Facial paresthesia Dysphagia, dysarthria, fasciculation, muscle atrophy, weakness	Normal in lower extremities No evoked SNAPs in upper extremities Slightly delayed motor and sensory nerve conduction velocity Prolonged distal latencies No evoked F-waves	IgG Sulfatide MAG	[82–84]

Table 15.5 (continued)

Subtype	Clinical presentation	Electrodiagnostic features	Antibodies present	References
SS with neuropathy	Dry eyes and dry mouth Pain Limb weakness	Impaired motor and sensory nerves	SSA(ro)	[85, 86]
Swine abattoir-associated IP	Symmetric, flaccid weakness Limb paralysis Pain, paresthesia	Distal F-wave latencies Reduced CMAP amplitudes (50% of cases Normal conduction velocity No conduction block Normal SNAP amplitudes	IgG	[87–90]

ANNA-1 antineuronal nuclear antigens, *CANDA* chronic ataxic neuropathy with disialosyl antibodies, *CANOMAD* chronic ataxic neuropathy, ophthalmoplegia, M protein, agglutinins, and disialosyl antibodies syndrome, *CMAP* compound muscle action potential, *FOSMN* facial-onset sensory and motor neuropathy, *Ig* immunoglobulin, *IP* immune polyradiculoneuropathy, *MAG* myelin-associated protein, *MGUS* monoclonal gammopathy of undetermined significance, *POEMS* polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin abnormalities, *SCLC* small-cell lung cancer, *SNAP* sensory nerve action potential, *SS* Sjögren syndrome

15.3.4.1 Monoclonal Gammopathies

Monoclonal gammopathies are a heterogeneous group of disorders ranging from monoclonal gammopathy of undetermined significance (MGUS) to malignant systemic disorders. These disorders arise when there is a proliferation of monoclonal plasma cells or B lymphocytes and are characterized by the proliferation and deposition of paraproteins or M proteins formed by a single heavy chain (M, G, or A) with a light chain (kappa or lambda) [91]. Immunoglobulin MGUS is the most common monoclonal gammopathy, with about 60% of cases being IgG MGUS (Sect. 15.3.4.1.1) [76]. However, among the 5–10% of patients with an unexplained polyneuropathy, IgM MGUS (Sect. 15.3.4.1.2) is much more common (40–70% of patients), particularly in those with demyelinating neuropathies [92].

IgG/IgA Monoclonal Gammopathy of Undetermined Significance

Neuropathies related to IgG or IgA are less common than those related to IgM [93] and little information regarding these neuropathies is available in the scientific literature. As a result, there is no consensus on treatment of IgG and IgA paraproteinemic neuropathies; however, immunosuppressants or immunomodulatory have been considered.

A case has been reported with a paraproteinemic polyneuropathy linked to IgG MGUS with electron microscopic features identical to those of IgM neuropathy with anti-MAG antibodies (Sect. 15.3.4.1.2) [76]. This patient, a 51-year-old male, had paresthesia of both hands and experienced several falls. Clinical examination

showed distal motor deficit of the lower limbs without amyotrophia. Deep tendon reflexes were absent from the ankles and there was distal hypoesthesia of the feet. Electrophysiological studies showed severe demyelinating sensorimotor polyneuropathy with no SNAPs in all four limbs and no CMAP in lower limbs. Motor nerve conduction velocities were slowed with distal latencies and conduction block of left median nerve. Serum testing showed that there was serum IgG kappa monoclonal gammopathy without anti-MAG or anti-glycolipid antibodies, and there was slight elevation in CSF protein (70 mg/dL) without leukocytes. The patient was treated with IVIG (0.4 g/kg/day for 5 days each month), but the patient worsened, presenting with severe tetraparesis with diaphragmatic palsy requiring intensive care. The addition of oral corticosteroids (1 mg/kg/day) and one course of plasma exchange progressively improved his motor strength. The corticosteroids and monthly IVIG were continued; azathioprine and subsequently cyclophosphamide were later added without success. The patient again worsened after 4 years. An increase of the steroid dosage to 1 mg/kg/day was associated with clinical improvement. The patient eventually became dependent on IVIG. Sural nerve biopsy revealed significant demyelination with IgG deposits. Lenalidomide, an analog of thalidomide, was later added to the regimen, and the patient was stable. Treatment with lenalidomide was subsequently switched to mycophenolate mofetil, which inhibits inosine monophosphate dehydrogenase to limit B and T lymphocyte proliferation and sustained clinical improvements over the following months were noted. IVIG and steroid treatments were decreased over time with the only treatment currently being mycophenolate mofetil [76].

IgM MGUS (with or Without Anti-MAG Antibodies)

Distal sensory-predominant neuropathy associated with IgM monoclonal proteins are considered by some to be a variant of CIDP [4], while others believe it is distinct due to its different underlying pathophysiology [3]. The clinical features of IgM MGUS with anti-MAG antibodies have been described by the term DADS. However, the clinical pattern of DADS can also be present in patients without anti-MAG antibodies. For example, other cases of IgM MGUS have the presence of IgG or IgA M-protein (Sect. 15.3.4.1.1) and may also have the DADS pattern. The DADS pattern may also arise in a neuropathy without IgM MGUS and tend to have better prognosis [3].

IgM MGUS is highly variable clinically as well as in terms of electrophysiology. IgM MGUS is generally mild, with minimal symptoms that may not interfere with the patient's daily activities. Patients may have tremor and sensory ataxia, and management may only require supportive care with exercise or balance training [77].

Nerve conduction studies show a pattern of demyelination, distal slowing of motor conduction, no conduction block, and reduction of SNAPs. A more CIDP-like phenotype based on nerve conduction studies has been shown to be correlated with a lower titer of anti-MAG antibodies [94]. Ultrasound findings in IgM MGUS patients with anti-MAG antibodies are heterogeneous. Some patients have significantly enlarged nerves while others have no enlargement or only slight enlargement. The CSA is significantly enlarged in the legs of anti-MAG patients but not the arms

or spinal nerves. There is no correlation in the CSA of nerves and anti-MAG antibody titers [13].

IgM MGUS should be treated when there are sensorimotor abnormalities. Reports suggest that patients do not respond well to conventional immunomodulatory therapy of IVIG, corticosteroids, or plasma exchange [3, 95]. Rituximab has been suggested as a promising treatment option although only approximately half of patients with anti-MAG antibodies have been shown to respond to treatment [95–98].

Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal Gammopathy, and Skin Abnormalities

Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin abnormalities (POEMS) is a monoclonal gammopathy and acquired demyelinating peripheral neuropathy that resembles CIDP but has a different pathological cause. The monoclonal gammopathy is usually IgG or IgA lambda light chain [23, 78]. The skin abnormalities observed in POEMS can include hyperpigmentation, hypertrichosis, acrocyanosis, plethora, and hemangioma/telangiectasia. For a diagnosis of POEMS, three of the five of features (i.e., polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin abnormalities) must be present though polyneuropathy is commonly observed and is present in approximately 50% of patients [23]. Some patients with POEMS with a documented clonal plasma cell disorder also have Castleman's disease, a rare lymphoproliferative disorder [78]. CSF protein is highly elevated and vascular endothelial growth factor (VEGF) is present at high levels in the serum. Overproduction of VEGF by monoclonal plasma cells is thought to be the cause of pathogenesis of neuropathy due to increased vascular permeability and neovascularization.

The clinical presentation of POEMS is similar to CIDP including distal neuropathy and pain, usually starting with distal sensory symptoms that evolve proximally over weeks or months. It is a length-dependent sensory and motor neuropathy. However, nerve conduction studies in patients with POEMS have shown a number of clinical differences relative to those with CIDP, including: greater reduction of motor amplitudes, greater slowing of motor and sensory conduction velocities, more uniform demyelination, less temporal dispersion, less conduction block, less sural sparing, and more axonal loss [79]. Furthermore, nerve biopsies show less endoneurial inflammation, more axonal degeneration, no onion bulb formation, and more neovascularization [99]. Unlike CIDP, which typically responds to immunotherapy, POEMS does not show sustained response to IVIG, corticosteroids, or plasma exchange [3].

15.3.4.2 Paraneoplastic Neuropathies (Anti-ANNA-1 [Anti-Hu] Antibodies)

Neuropathies directly related to cancer are known as paraneoplastic neuropathies. They may arise from direct tumor infiltration, compression, or mass effect on the plexus or peripheral nerves. These paraneoplastic neuropathies can involve sensory or motor ganglia, peripheral sensory and/or motor nerves, and autonomic nerves.

Sensory neuronopathy is the most common paraneoplastic neuropathy observed and is associated with antibodies against type 1 antineuronal nuclear antigens (ANNA-1; formerly referred to as anti-Hu antibodies). This neuropathy is characterized by subacute onset, sensory ataxia, neuropathic pain, motor neuropathy, cerebellar degeneration, limbic encephalitis, and brainstem involvement [23]. Sensory neuronopathy may appear up to a year or more before cancer diagnosis and is often found in small-cell lung cancer [80]. Nerve conduction studies reveal peripheral nerve dysfunction with reduced conduction velocity. Treatment for paraneoplastic neuropathies is often focused on treatment of the underlying malignancy; however, corticosteroids, cyclophosphamide, sirolimus, and rituximab can be used to treat paraneoplastic sensory neuronopathy in addition to IVIG and plasma exchange [23].

15.3.4.3 CANOMAD and CANDA

Immune-mediated polyneuropathy with IgM antibodies against disialosyl residues (chronic ataxic neuropathy, ophthalmoplegia, M protein, agglutinins, and disialosyl antibodies syndrome [CANOMAD] and chronic ataxic neuropathy with disialosyl antibodies [CANDA]) are two similar immune neuropathy variants that have not been definitively classified. These neuropathies with antibodies against disialosyl residues are often characterized by sensory ataxia, gait disorder, areflexia, tremor, preserved motor function except for the presence of ophthalmoplegia, difficulty speaking and swallowing, and a chronic or relapsing disease course [3, 81, 100]. Patients are seropositive for IgM anti-GD1b and seronegative for IgM anti-GM1 [81]. This syndrome has several similarities to chronic MFS (Sect. 15.3.1.1.2).

Electrodiagnostic testing in CANOMAD/CANDA patients can reveal a mixed axonal demyelinating pattern, an axonal pattern, or, less frequently, pure demyelinating pattern. Nerve ultrasound in four patients suggested acquired demyelination, even in one patient that had axonal features on electrodiagnostic testing. The ultrasound showed regional enlargement of different nerves at one or more non-entrapment sites [81].

Rituximab has been shown to be effective at preventing disease progression, which has been demonstrated to lead to a reduction or complete abolishment of IgM GD1b antibody titers. IVIG is also effective at preventing relapses in approximately 50% of patients. Although only tested in small numbers of patients (i.e., $n = 1–3$), corticosteroids, mycophenolate mofetil, and plasma exchange have been shown to be ineffective [81].

Peripheral neuropathy with anti-disialosyl IgM antibodies may also occur more rarely in patients with malignant lymphoma [100]. Immune neuropathies as a paraneoplastic syndrome are rarely characterized by the presence of anti-ganglioside antibodies, such as IgG or IgM anti-GM1, anti-GM2, anti-GD1b, or anti-GT1b antibodies typically associated with GBS or its variants (Sect. 15.3.1); However, in a case report of a patient with diffuse large B-cell lymphoma, serum was positive for the presence of IgM antibodies against GD1b and GD3. The patient had elevated CSF protein and a mild sensory disturbance without motor deficit or ataxia. Deep tendon reflexes were almost normal and nerve conduction studies were also normal. Neurological symptoms worsened after initiation of chemotherapy for his

lymphoma (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). Treatment with a high-dose IVIG for 5 days, along with this chemotherapy regimen (except for vincristine), improved motor and sensory disturbances. Anti-GD1b and anti-GD3 antibodies were no longer detectable after treatment [100].

15.3.4.4 Facial-Onset Sensory and Motor Neuropathy Syndrome with Anti-MAG or Anti-sulfatide IgG

Facial-onset sensory and motor neuropathy (FOSMN) syndrome is an extremely rare disease characterized by facial paresthesia and sensory deficits which spread to the scalp, neck, upper trunk, and upper extremities in a rostral-caudal direction. Lower motor neuron signs can appear concurrently or at a later point following diagnosis and can include dysphagia, dysarthria, fasciculation, muscle atrophy, and weakness. The disease slowly progresses over a number of years until death.

In a case report, nerve conduction studies were normal in the lower extremities, while the upper extremities showed no evoked SNAPs, slightly delayed sensory and motor nerve conduction velocities, prolonged distal latencies, and no F-waves that were evoked [82].

There are contradictory reports as to the disease mechanisms underlying FOSMN, with some suggesting a primary neurodegenerative disorder without evidence of inflammation [83, 84]. However, three FOSMN syndrome patients have been reported with anti-sulfatide IgG [83, 84] and one patient with anti-MAG IgG [82], while other patients have shown partial response to immunotherapies such as IVIG and plasma exchange [101, 102]. These results suggest a potential immune-mediated process associated with FOSMN syndrome.

15.3.4.5 Sjögren Syndrome with Neuropathy

Sjögren syndrome (SS) is a systemic autoimmune disease with symptoms of dry eyes and dry mouth and affects 1–2% of the adult population. Peripheral nervous system complications are common, including painful small fiber neuropathy or polyneuropathy with limb weakness [85, 86]. B-cell dysregulation leading to induction of pathogenic and nonpathogenic antibodies is also observed in patients with SS. Lymphocytic infiltration of the dorsal root ganglia or other ganglionic elements, leading to sensory neuronopathies, painful small-fiber neuropathies, and facial pain disorders can also occur [85].

In a systematic review, the prevalence of peripheral neuropathies in SS ranged from 2 to 35%; however, the studies with higher prevalence used older diagnostic criteria that have since been updated, and as a result, it was suggested the true prevalence of peripheral neuropathies is likely closer to 2–10% [85]. Nerve conduction studies reveal impaired motor conduction in all cases and impaired sensory nerves in the majority of cases as well [86]. Critically, over half of the patients with SS with peripheral neuropathy also fulfill the diagnostic criteria for CIDP, demonstrating the challenge of making an accurate diagnosis [86].

Approximately half of SS patients with polyneuropathy and limb weakness are seropositive for anti-Sjögren's-syndrome-related antigen A (anti-SSA[ro]) antibodies. CSF protein is elevated in half of patients. The limbs are often symmetrically

involved, with either tetraparesis or paraparesis. While often purely sensory, there are some instances of pure motor involvement. Nerve conduction studies show a mix of predominantly axonal, predominantly demyelinating, and mixed axonal and demyelinating nerve damage [86].

15.3.4.6 Nodo-Paranodopathy Case Report: Sensory Neuropathy with Anti-GT1a and GQ1b Antibodies Associated with Cold Urticaria

In a case report of a unique nodo-paranodopathy, a 64-year-old female presented with subacute distal paresthesia and cold-induced urticaria, a rare form of physical urticaria, developed after an upper respiratory tract infection [103]. Although the presence of anti-GQ1b antibodies suggest MFS, the patient only had hyporeflexia and lacked other clinical signs of ataxia, ophthalmoplegia, and areflexia. The patient also lacked many of the features of CANOMAD, other than cold-induced urticaria possibly being associated with cold agglutinins.

Cranial district was normal except for a feeling of bitterness on tasting. The reflexes were normal in the upper limbs but hypoelicitable in the lower limbs. Motor function, coordination, and proprioception were normal. There was subjective hypoesthesia for tactile and thermonociceptive stimuli on hands and feet with impaired vibratory sense in the lower limbs. Nerve conduction studies demonstrated that the polyneuropathy was exclusively sensory, with prolonged distal latencies and reduced amplitudes of sensory action potentials; CMAPs were normal. The patient did not have elevated CSF protein or pleocytosis. Serum was positive for anti-GQ1b and anti-GT1a anti-ganglioside antibodies. Blood examinations showed slightly elevated IgG count with normal blood cell counts and without paraproteins or cryoglobulinemia. The sensory disturbance was resolved through treatment with IVIG and steroids with anti-histaminic resolved the urticaria. The features of anti-ganglioside antibodies and the mixture of demyelinating and axonal features of electromyography are suggestive of an early-stage nodo-paranodopathy [103].

15.3.4.7 Immune-Mediated Polyradiculoneuropathy Among Swine Abattoir Workers

A novel sensory predominant, immune-mediated polyradiculoneuropathy (IP) was identified in several areas of the United States among workers at swine abattoirs [87, 88]. Epidemiological investigation indicated a strong link between the disease and those who worked in an area of the plant where a compressed air technique was used to remove porcine brains, which generated aerosolized droplets containing porcine neural tissue antigens resulting in neural damage [87, 89]. First identified in Minnesota in 2007, two additional plants were identified in Indiana and Nebraska that also used this method of brain extraction [87]. A total of 24 patients exposed to the aerosolized porcine brain tissue developed the workplace environment-associated IP from the Minnesota and Indiana plants [89]. Clinical characteristics of this novel IP were new onset of bilateral and relatively symmetric flaccid weakness and/or limb paralysis with or without cranial nerve innervated muscles, and new onset of decreased or absent deep tendon reflexes at least in the affected limbs [87].

Patients also had pain and paresthesia in the limbs [90]. This novel IP shares some similarities with GBS and CIDP [89].

Electrodiagnostic studies had features consistent with axonal and/or demyelinating peripheral neuropathy features, neuroimaging was consistent with radiculitis, myelitis, or encephalitis, and CSF protein >45 mg/dL with or without pleocytosis [87]. Higher levels of interferon-gamma (IFNγ) in cases compared to controls was observed; elevated IFNγ has also been observed in patients with AIDP or CIDP [88]. MRI imaging abnormalities were also noted, with the most frequently observed being enlarged, enhancing cauda equina nerve roots [89].

Electrodiagnostic findings showed distal and F-wave latencies suggestive of demyelination at the nerve root and distal nerve terminals. CMAP amplitudes were only reduced in half of the patients and most patients (10/12) had normal conduction velocity. No patients had conduction block and sural SNAP amplitudes were normal. Immunohistochemical results identified a unique IgG immunofluorescence pattern with axon staining observed without staining of the myelin sheath. The novel IP is considered a primary axonopathy with secondary paranodal demyelination causing conduction changes [90].

All patients improved over time. Seven patients received no treatment and 17 received immunotherapy because of progression of sensory loss and weakness. Immunotherapies given included intravenous (IV) methylprednisolone, oral prednisone, IVIG, and azathioprine [89].

15.4 Available Treatment Options

An overview of currently available treatment option for immune neuropathies is provided in Table 15.6.

15.4.1 Intravenous Immunoglobulin

IVIG is used to treat many immune neuropathies, including GBS, CIDP, and MMN. IVIG is a first-line treatment option for CIDP and has shown favorable safety and tolerability with long-term administration [48, 106, 115]. The current guidelines for the treatment of CIDP recommend the lowest effective maintenance dose should be used, with stable patients undergoing periodic dose reductions or lengthening the interval between treatments to determine whether continued therapy is needed. These measures have been developed to help reduce overtreatment, which is a common issue [2]. For GBS, a single two-day course of IVIG is often sufficient for treatment [61]. IVIG is expensive, labor-intensive, and lengthy although studies have shown that at-home administration of IVIG can be safely done by a nurse or person who has received specific training from the hospital [116, 117].

IVIG is thought to reduce the amounts of pathogenic autoantibodies in addition to reducing their effector functions. This can occur by competition between the

Table 15.6 Summary of available treatment for immune neuropathies

Treatment	Treatment description	Responsive subtypes	References
IVIG	Pros: Rapid clinical improvements Cons: Expensive, labor-intensive, long-term use limits peripheral venous access	CIDP (First-line) (except IgG4 Ab) GBS MMN	[48, 69]
SCIG	Pros: Lower costs, reduced adverse events, at-home use (relative to IVIG) Cons: Reduced bioavailability, more frequent infusions needed in some patients	CIDP MMN	[75, 104, 105]
Corticosteroids	Pros: Inexpensive Cons: Slower response, severe side effects of long-term use	CIDP (First-line)	[48]
Plasma exchange	Pros: Fast response for rapidly deteriorating patients or IVIG and corticosteroid non-responders Cons: Invasive, time-intensive	CIDP (First-line) GBS	[48, 106, 107]
Immunoadsorption	Pros: Can be used in first-line treatment non-responders More studies required	CIDP GBS	[48, 106, 108]
mAb-based Rituximab Alemtuzumab Eculizumab Natalizumab	Effective in small studies and case reports but more studies required	CIDP CIDP with anti-NF155 and anti-CNTN1 Ab GBS MMN	[109–114]
Immunosuppresive drugs Methotrexate Cyclosporine Mycophenolate mofetil Cyclophosphamide	Pros: Steroid-sparing More studies required	Some CIDP variants	[15, 52, 76, 81]

Ab antibody, *CIDP* chronic inflammatory demyelinating polyneuropathy, *CNTN1* contactin 1, *GBS* Guillain-Barré syndrome, *IgG* immunoglobulin G, *IVIG* intravenous immunoglobulin, *mAb* monoclonal antibody, *MMN* multifocal motor neuropathy, *NF155* neurofascin 155, *SCIG* subcutaneous immunoglobulin

therapeutic IgG and pathogenic IgG by saturating neonatal Fc receptors, inhibition of complement deposition, anti-idiotype neutralization, and potentially feedback inhibition of B-cell antibody production [2]. The autoantibody Fc domain structure is defined by class (IgG, IgM, or IgA) and subclass (IgG1 to IgG4). Knowing the structure of the autoantibody can predict the likelihood that FcRn blocking or saturating agents will lead to successful treatment.

Once in the intravascular space, IgG has a half-life of approximately 21–30 days, thus patients often receive monthly infusions [118]. The relatively broad range in

half-life noted for IgG may be due to patient variation in FcRn expression but have not yet been fully elucidated. The catabolism of IgG follows first-order kinetics and higher IgG levels lead to faster catabolism. To achieve clinical benefit, a high and stable trough level should be achieved to keep IgG at a beneficial threshold level while avoiding accelerated catabolism that occurs at higher IgG levels [2]. Patient-to-patient variation also exists with respect to the frequency of dosing; some patients may benefit from IVIG at intervals ≤14 days while others may need dosing at 4- to 6-week intervals.

The rapid clinical improvements often observed with IVIG or plasma exchange (see Sect. 15.4.4) may be driven by reversible nodal or paranodal immune-mediated dysfunction. IVIG temporarily reduces the effects of the autoantibodies that bind nodal and paranodal proteins or ganglioside complexes; however, as the therapeutic IgG decreases the pathogenic autoantibodies and their processes may return and lead to cyclic clinical deterioration following an interval of IVIG [2]. Some patients may undergo years of cyclic IVIG treatment and fluctuations, indicating that treatment can compete with autoantibodies but does not alter their production or function.

In MMN, IVIG is also considered the primary form of treatment. Long-term treatment is often required, and at-home infusion of IVIG or SCIG (see Sect. 15.4.2) may be used in certain patients. Steroids and plasma exchange are not recommended as their efficacy has not been clearly demonstrated [63, 64]. Early treatment with IVIG prevents axonal loss in patients with MMN, thus, early confirmation of diagnosis is important [63].

15.4.2 Subcutaneous Immunoglobulin

SCIG is an effective alternative to IVIG for CIDP and MMN patients and has been recently approved by the FDA (March 2018) for CIDP. When administered IV, IVIG treatment leads to peak serum IgG levels immediately following administration which drop by 50% over 2–4 days. With SCIG the serum IgG levels rise more slowly and peak within 2–3 days [2, 118]. Although effective for treating many immune neuropathies, IV administration (i.e., IVIG) has several downsides. For example, some patients require years of maintenance therapy leading to limited peripheral venous access. Systemic adverse events have also been reported with IVIG, such as headache, malaise, and chills. A meta-analysis showed that there is a significant 28% reduction in the relative risk of moderate and/or systemic adverse events for SCIG compared to IVIG in MMN and CIDP patients [105].

Another benefit of SCIG is that it can be administered at home after appropriate patient training, cutting down on travel time and costs. Additionally, there are fluctuations in muscle strength and physical function at the end of IVIG dosing that may be diminished with SCIG [119]. The non-inferiority of SCIG over IVIG has been demonstrated in an observational study [75]. Higher personal satisfaction and improved quality of life have also been reported after switching to SCIG in MMN and CIDP [104]. SCIG has also been shown to be tolerated in MMN patients in an open label trial in 15 patients. Patients already receiving IVIG were to receive SCIG

at 1.53:1 ratio SCIG to IVIG; the exception was patients who were receiving 2 g/kg/month IVIG, who received SCIG at a 1:1 ratio ($n = 6$). Of the patients receiving 1:1 SCIG, three (50%) developed intolerable weakness by month 3. In total, 11 patients completed the program with minor localized reactions and high satisfaction [120]. Meta-analysis combining 100 MMN and CIDP patients together showed that switching to SCIG led to a significant improvement in the maintenance of muscle strength. The addition of data from patients with a wider range of auto-immune muscular disorders to the MMN and CIDP patients also showed that SCIG led to statistically significant increases in overall strength, health-related quality of life, and patient satisfaction [121].

SCIG is limited in its infusion volumes and has reduced bioavailability that requires more frequent infusions and an increased dose in some patients. A newer treatment is subcutaneous administration of human immune globulin facilitated by pretreatment with 10% recombinant human hyaluronidase (fSCIG) which increases the dispersion and absorption allowing for higher bioavailability and less frequent infusions. Treatment with fSCIG has been approved for primary autoimmune deficiency. The safety of fSCIG and its effects on overall muscle strength, disability, and treatment satisfaction were demonstrated to be comparable to that of IVIG in a study of 18 MMN patients [122]. The effectiveness, feasibility, and safety of fSCIG compared to SCIG was also investigated in a trial comparing fSCIG ($n = 10$) to conventional infusion of multiple small dosages ($n = 10$) in patients with MMN [123]. The primary efficacy outcome, isometric strength, did not differ between the two treatments. fSCIG had a higher frequency of localized side effects at the injection site; however, mild and short-lasting generalized side effects were similar between the two treatments. In addition, patients preferred fSCIG to conventional SCIG for two out of five visual analog scale scores (statistically significant for ease of administration of infusion and maintenance of a normal life; no difference between treatments for preference, side effects, and overall satisfaction) as well as total mean score for all preferences.

15.4.3 Corticosteroids

Corticosteroids suppress inflammation and are used to treat a number of immune-mediated diseases. Alongside IVIG and plasma exchange, corticosteroids are also considered as a first-line treatment option for CIDP [48]. Several corticosteroids are available including oral prednisolone (typical starting dose of 60 mg/day), pulsed high-dose dexamethasone (starting at 40 mg/day for 4 days every 4 weeks), or IV methylprednisolone (1000 mg weekly or monthly) for CIDP, which appear to be equally effective [4].

Corticosteroids are generally not recommended for GBS or its variants except for neuropathic or radicular pain [24]. As described in Sect. 15.3.2.1.6, patients with anti-NF155 or anti-CNTN1 antibodies respond poorly to IVIG but respond well to corticosteroids.

Patients usually do not respond as quickly to corticosteroids as to IVIG and may also experience more frequent and severe side effects after long-term use than with IVIG.

15.4.4 Plasma Exchange

Plasmapheresis, or plasma exchange, is a therapeutic process in which plasma is separated from blood cells, which removes antibodies, immune complexes, inflammatory moderators, paraproteins, or other toxins in the plasma from the blood. The plasma is then substituted by a human albumin solution or fresh frozen plasma. It is considered invasive and time-intensive, with restricted availability as it requires a medical center for its administration. It can be considered the best treatment option in rapidly deteriorating patients [3].

Plasma exchange is the first-line treatment option for CIDP, in addition to IVIG and corticosteroids [48]. Plasma exchange is typically considered as an option for patients who do not respond to IVIG or corticosteroids and in cases with especially severe symptoms. Plasma exchange for patients with CIDP is often given as a course of five sessions on alternate days followed by 1–2 sessions every 3–4 weeks [23]. In GBS, plasma exchange is effective, typically done in 4–6 sessions over 8–10 days [23, 107]. Response is typically faster in GBS (acute) than CIDP (chronic) [107].

Selective immunoadsorption is an equivalent alternative to classic plasma exchange and has been used in GBS and small numbers of CIDP patients who did not have satisfactory response to first-line treatment (IVIG, corticosteroids, and/or plasma exchange) [108]. In immunoadsorption, rather than discarding the plasma, it is passed through an adsorber column after plasma separation and returned to the patient. Immunoglobulins, including IgG and IgM, and immune complexes are removed from the plasma by the adsorber surface in the column. Interestingly, in a retrospective analysis of patients treated with IgG-depleting immunoadsorption, 13/20 patients with GBS and 8/20 patients with CIDP were responsive to treatment, despite the fact that none of the serum samples of these patients tested positive for IgG antibodies against glycolipids or nodal/paranodal proteins [124].

15.4.5 Monoclonal Antibody-Based Treatments

Rituximab is currently approved for the treatment of relapse or refractory low grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma. It has also been investigated in a variety of autoimmune disorders in which B cells and autoantibodies are implicated. In a pooled meta-analysis, the response rate to rituximab for CIDP patients was 48% [125]. Rituximab is more effective than IVIG for the treatment of CIDP with IgG4 anti-NF155 and anti-CNTN1 antibodies [126].

Alemtuzumab is an mAb that targets CD52, expressed by most lymphocytes and monocytes. Only a single case report and one small trial have been conducted but show some potential for its use in CIDP [109, 110]. In the trial, seven patients with

severe CIDP dependent on IVIG were treated with nine courses of alemtuzumab; following treatment mean monthly IVIG and administration frequency decreased [110].

Eculizumab is a mAb that binds complement component 5 and potently inhibits complement activation. It is safe and may be effective in MMN based on results of a small open-label study [111]. It has also been investigated in two randomized trials for treatment in GBS. IVIG plus eculizumab was well tolerated in one study of GBS [112] but did not show a significant difference in another study comparing eculizumab to placebo [113] and requires further study in larger randomized controlled trials [127].

Natalizumab is an mAb directed to the α4 integrin of the very late antigen 4 (VLA4) antigen expressed on the surface of T cells. It has been used to treat CIDP patients after failure of first-line therapies. Of three patients, one experienced long-term improvement, one improved over a significant duration, and the third patient stabilized [128]. It was not effective in one case of CIDP [129].

A number of mAb-based treatments have been tried in immune neuropathies, usually in cases of CIDP. A Cochrane review concluded that regimens including rituximab, cyclophosphamide, alemtuzumab, natalizumab, and hematopoietic stem cell transplantation were effective for CIDP in case reports and small studies (up to 32 patients) but larger randomized controlled trials are needed [114].

15.4.6 Immunosuppressive Drugs

The use of immunosuppressive drugs (e.g., methotrexate, cyclosporine, mycophenolate mofetil, cyclophosphamide) is based on the effectiveness of these treatments for other immune-mediated diseases. These immunomodulatory therapies are considered steroid-sparing as they reduce or eliminate the need for steroid-based treatments; however, they have not yet been widely studied in controlled clinical trials.

There is currently insufficient data to recommend methotrexate for CIDP [61]. Cyclosporine has severe adverse effects and should only be considered for patients resistant to conventional therapies.

Mycophenolate mofetil inhibits inosine monophosphate dehydrogenase, limiting B and T lymphocyte proliferation. It is approved for prevention of acute allograft rejection following organ transplantation and hematopoietic stem cell transplantation but has been shown to be effective for autoimmune diseases. It was developed as a replacement for azathioprine. It has not been studied in a large number of patients with immune neuropathies thus far. Some types of CIDP variants and monoclonal gammopathies have been successfully treated with mycophenolate mofetil, including one patient with CISMP [52] and a patient with IgG MGUS (with similar features to an anti-MAG neuropathy) [76]. However, it was ineffective in CISP variant of CIDP [15] and in a CANOMAD/CANDA patient [81].

15.4.7 Treatments for Immune Neuropathy Subtypes

The first-line treatment options and other available treatment options for the immune neuropathy subtypes (GBS, CIDP, MMN), their variants, and other immune-mediated neuropathies are summarized in Table 15.7.

Table 15.7 Summary of first-line and other treatment options for immune neuropathies by subtype

Subtype	First line treatment	Other treatments	References
Guillain-Barré syndrome			
Typical Guillain-Barré syndrome	IVIG	Plasma exchange, eculizumab	[23, 35, 48, 111, 112]
AIDP	IVIG	Plasma exchange, eculizumab	[23, 35, 48, 111, 112]
AMAN/AMSAN	IVIG	Plasma exchange, eculizumab	[23, 35, 48, 111, 112]
MFS	IVIG	Plasma exchange	[23, 35, 48]
Pharyngeal-cervical-brachial variant	IVIG	Plasma exchange	[23, 35, 48]
Acute small fiber sensory neuropathy	IVIG	Plasma exchange, corticosteroids	[23, 33, 35, 48]
Chronic inflammatory demyelinating polyradiculopathy			
Typical chronic inflammatory demyelinating polyradiculopathy	IVIG, SCIG, plasma exchange, corticosteroids	Methotrexate	[3, 23, 48, 104, 120]
CISP	IVIG, corticosteroids	Plasma exchange	[3, 4, 15, 48]
CIMP	IVIG	–	[48, 51]
CISMP	–	Prednisolone, azathioprine, mycophenolate	[52]
MADSAM (Lewis-Sumner syndrome)	IVIG, corticosteroids	–	[3, 53]
CMAN	IVIG	–	[54]
CIDP with antibodies to nodal and paranodal proteins	IVIG, corticosteroids	Rituximab	[4, 10, 56]
Multifocal motor neuropathy			
MMN with conduction block	IVIG	SCIG, fSCIG, rituximab, eculizumab	[23, 52, 104, 110, 119–122]
MMN without conduction block	IVIG	SCIG, fSCIG, rituximab, eculizumab	[23, 52, 104, 110, 119–122]
Other immune-mediated neuropathies			
MGUS (not IgM)	IVIG	Corticosteroids, mycophenolate mofetil	[76]

(continued)

Table 15.7 (continued)

Subtype	First line treatment	Other treatments	References
IgM MGUS with MAG	–	Rituximab, lenalidomide, mycophenolate mofetil	[13, 23, 76, 95–98]
IgM MGUS without MAG	IVIG, SCIG, plasma exchange, corticosteroids	–	[4]
POEMS	Treatment of underlying plasma cell disorder (surgical excision, irradiation, corticosteroids and melphalan, high-dose chemotherapy and stem cell transplantation)	Bevacizumab, lenalidomide/thalidomide	[23, 78, 79]
Paraneoplastic neuropathies with anti-ANNA-1 antibodies	Treatment of underlying malignancy	IVIG, plasma exchange, corticosteroids, cyclophosphamide, sirolimus, rituximab	[23, 80]
CANOMAD and CANDA	Rituximab	IVIG	[81, 100]
FOSMN	IVIG, plasma exchange	Corticosteroids, azathioprine, mycophenolate mofetil	[82, 84, 101]
SS with neuropathy	IVIG, corticosteroids	Cyclophosphamide, azathioprine, plasma exchange, rituximab	[85, 86]
Swine abattoir-associated IP	–	Methylprednisolone, prednisone, IVIG, azathioprine	[89]

AIDP acute inflammatory demyelinating polyradiculoneuropathy, *AMAN/AMSAN* acute motor axonal neuropathy/acute motor and sensory axonal neuropathy, *ANNA-1* type 1 antineuronal nuclear antigens, *CANDA* chronic ataxic neuropathy with disialosyl antibodies, *CANOMAD* chronic ataxic neuropathy, ophthalmoplegia, M protein, agglutinins, and disialosyl antibodies, *CIMP* chronic inflammatory motor polyradiculopathy, *CISMP* chronic inflammatory sensorimotor polyradiculopathy, *CISP* chronic immune sensory polyradiculopathy, *CMAN* chronic motor axonal neuropathy, *FOSMN* facial-onset sensory and motor neuropathy, *fSCIG* subcutaneous immunoglobulin with recombinant human hyaluronidase, *IgM* immunoglobulin M, *IP* immune-mediated polyradiculoneuropathy, *IVIG* intravenous immunoglobulin, *MADSAM* multifocal acquired demyelinating sensory and motor neuropathy, *MAG* myelin-associated glycoprotein, *MFS* Miller-Fisher syndrome, *MGUS* monoclonal gammopathy of undetermined significance, *MMN* multifocal motor neuropathy, *POEMS* polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin abnormalities, *SCIG* subcutaneous immunoglobulin, *SS* Sjögren's syndrome

Note: First-line treatment was defined as either the preferred, standard, or first choice of treatment. When no consensus first-line treatment was available for a given variant (due to only a small number of case reports available), no first-line treatment is listed; the effective treatments reported in the case reports are included as "Other Treatments" for these variants

15.5 Discussion and Conclusions

Since the first report of acute monophasic paralysis a century ago by Guillain, Barré, and Strohl, and even earlier reports in the 1800s, the spectrum of immune-mediated neuropathies has continued to grow. Our understanding of the associated clinical characteristics and pathophysiology have continued to grow since these first observations. This heterogeneous group of disorders arises due to autoantibodies against the proteins of the node of Ranvier or the myelin sheath of peripheral nerves. Still additional antibodies and their targets are likely to be identified among the immune neuropathy variants.

There are numerous subtypes of neuropathies. GBS, CIDP, and MMN are typically considered to be the most widely recognized types; however, monoclonal gammopathies and other unique, atypical, or overlapping disorders are becoming more recognized and understood. The most common type, CIDP, also consists of the broadest array of typical and atypical variants. Further characterization of these variants will help define whether they are appropriately categorized as a CIDP variant or should be considered as a CIDP-like disease with a different pathogenesis. Although MMN and GBS are generally more well-defined entities than CIDP, there are case reports of patients with atypical symptoms, electrophysiology, pathology, and treatment response. Thus, continued honing of diagnostic criteria of all types of immune neuropathies is necessary.

Diagnosis is complex and challenging but must be accomplished rapidly in order to ensure proper disease management with IVIG, plasma exchange, corticosteroids, or newer emerging therapeutic options such as monoclonal antibody-based treatments. Early initiation of appropriate treatment can help to prevent further nerve damage.

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Amyotrophic Lateral Sclerosis: Neurochemical Biomarkers

16

Federico Verde and Nicola Ticozzi

16.1 Introduction

Amyotrophic lateral sclerosis (ALS) is the most common, adult-onset, neurodegenerative motor neuron disease (MND) characterized by progressive loss of upper motor neurons (UMNs) of the cerebral cortex and lower motor neurons (LMNs) of the brainstem and spinal cord. This causes relentless paralysis of voluntary muscles, which starts focally in one body region and then spreads to contiguous segments, ending with death, usually due to respiratory failure, after a median of 3–5 years [1]. The only two approved drugs for ALS treatment, riluzole and edaravone, provide a very modest benefit on survival and functional decline. ALS has an incidence of ~2 cases/100.000 persons/year and a prevalence of ~6 cases/100.000 persons [1]. 90–95% of ALS cases are sporadic (sporadic ALS, sALS), while 5–10% are familial (familial ALS, fALS), usually with autosomal dominant inheritance. About two thirds of fALS cases are due to mutations in known genes [2]; these mutations are also present in a small proportion of apparently sporadic cases, whereas in the vast majority of sALS patients, etiology and pathogenesis remain largely unknown. Notably, up to 50% of ALS patients manifest cognitive-behavioral alterations of the frontotemporal spectrum and up to 15% fulfill diagnostic criteria for frontotemporal dementia (FTD). Indeed, ALS and FTD share the common neuropathological substrate of TDP-43 proteinopathy as well as several genetic abnormalities causing familial forms of both diseases (often occurring together in the same kindred or in the same individual), the most important of which is the $(G_4C_2)_n$ hexanucleotide repeat expansion in the *c9orf72* gene [3]. Neuropathologically, ALS—as well as

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about half of FTD cases—is characterized by neuronal and glial cytoplasmic inclusions composed of ubiquitinated and phosphorylated TAR DNA-binding protein of 43 kDa (TDP-43) [4]. A staging of TDP-43 pathology in ALS has been proposed, with pathological inclusions first found in motor cortex, motor nuclei of cranial nerves of the lower brainstem and spinal cord α-motor neurons and then progressively spreading to connected cortical and subcortical structures of the central nervous system (CNS), suggesting a mechanism of corticofugal axonal spread as a way of propagation of pathology [5, 6].

The diagnosis of ALS is mainly clinical, with neurophysiological examinations (most importantly, electromyography) confirming physical signs of motor neuron degeneration and neuroradiological and laboratory investigations ruling out alternative diagnoses. The diagnosis is made according to the revised El Escorial criteria, requiring evidence of signs of UMN and LMN dysfunction in distinct body segments [7]; a simplification of these criteria has recently been proposed [8]. Neurochemical biomarkers still do not have an established role for ALS diagnosis in the clinical scenario according to current guidelines. Nevertheless, for neurofilaments measured in cerebrospinal fluid (CSF) and blood, solid evidence has accumulated in support of their use in the clinical field, while other potentially meaningful candidates are those reflecting neuroinflammation [9]. The introduction of ALS biomarkers in clinical practice would be important both for facilitating early and differential diagnosis, which are sometimes particularly challenging, and for predicting disease course. Other strengths of biomarkers pertain to the research field: firstly, they reflect putative pathophysiological processes and therefore help to elucidate disease mechanisms. Secondly, they can help in patient selection and stratification in therapeutic trials; thirdly, in these trials they can be used as pharmacodynamic biomarkers indicating target engagement and measuring treatment effect [10].

16.2 Neurofilaments

16.2.1 Structure of Neurofilaments and Biological Rationale as Biomarkers

Neurofilaments are cytoskeletal proteins belonging to the class of intermediate filaments and represent the main structural components of the scaffold of large myelinated axons of the CNS and peripheral nervous system (PNS). They are polymers composed of the following subunits: heavy, middle, and light chains (NFH, NFM and NFL, respectively), plus alpha-internexin in the CNS and peripherin in the PNS [11]. The neurofilament subunits normally used as ALS biomarkers are the phosphorylated form of NFH (pNFH) and NFL. Their role as ALS biomarkers is due to the fact that they are released in the CNS intercellular space, and hence in the CSF, from degenerating motor neuron axons [12]. However, some biological data suggest that the quantitative alterations of neurofilaments in ALS could be not simply the consequence of this release but rather reflect their involvement in disease

pathophysiology. As an example, early neuropathological investigations demonstrated accumulation of phosphorylated neurofilaments in the perikaryon and in the proximal axon of spinal motor neurons of ALS patients [13]. Several experimental data on ALS animal models point to a possible role of neurofilaments in disease pathogenesis. Genetic deletion of NFL in mutant *SOD1* (m*SOD1*) mice delays onset of disease and slows loss of motor neuron axons, but also reduces the selectivity of the effect of *SOD1* mutation towards motor neurons, as demonstrated by a heavier loss of sensitive axons in the lumbar nerve roots [14]. Moreover, genetic manipulation producing absence of the phosphorylation-prone tail of NFH and NFM subunits in m*SOD1* mice delays disease onset, extends survival, and reduces degeneration of motor neurons and whole motor units [15].

16.2.2 CSF Neurofilaments for the Diagnosis of ALS

The foundation of the role of neurofilaments for ALS diagnosis is represented by their raised concentrations in the CSF. Steinacker et al. analyzed CSF levels of pNFH and NFL in a large cohort composed of 253 MND patients (222 sALS patients, 20 fALS patients, 11 patients with the UMN-only MND primary lateral sclerosis [PLS]), 85 patients with MND mimics (i.e., conditions clinically mimicking MND), and 117 neurological controls. pNFH and NFL were highly correlated with each other and were higher in MND patients than in the other categories. pNFH discriminated between MND and mimics with an area under the ROC curve (AUC) of 0.841, showing 83% sensitivity and 80% specificity at a diagnostic cut-off of 560 pg/mL. For NFL, the AUC was 0.866, with 77% sensitivity and 88% specificity at a cut-off of 2200 pg/mL [16]. In this study, no statistically significant difference was reported in neurofilament levels between sALS, fALS, and PLS, but according to Zucchi et al. and to our data, CSF pNFH is significantly higher in ALS than in PLS, which is promising for differential diagnostic purposes [17, 18]. Importantly, both CSF pNFH and NFL have a good diagnostic performance for ALS also in patients sampled within 6 months from symptom onset [19].

In the work by Poesen et al., conducted on 220 ALS patients, 50 ALS mimics, and 316 neurological controls, pNFH had a better diagnostic performance, in that it discriminated between ALS and ALS mimics with an AUC of 0.912, a sensitivity of 90.7%, and a specificity of 88.0%, whereas NFL showed an AUC of 0.863, a sensitivity of 85.4%, and a specificity of 78.0%. In the differentiation between ALS and other neurological diseases, pNFH proved more selective for ALS than NFL, as several patients with FTD displayed NFL, but not pNFH, levels in the ALS range, while both neurofilaments were elevated in a number of patients with the peripheral nerve disorders Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP) [20].

A recent meta-analysis of 16 studies comparing CSF NFL in a total of 930 ALS patients and 593 healthy controls showed a ratio of 9.64 between ALS and controls, while the 11 studies on 1239 ALS patients versus 806 patients with ALS mimics

resulted in a ratio of 3.35 [21]. In summary, there is now sufficient evidence in support of the introduction of neurofilament measurement in the CSF for ALS diagnosis in the clinical scenario.

16.2.3 Blood Neurofilaments for the Diagnosis of ALS

Neurofilaments are raised also in the blood (serum and plasma) of ALS patients as a consequence of their passage from the CSF. Due to their consequently lower blood concentrations as compared to CSF, they can only be measured by means of more sensitive methods than normal ELISAs, i.e., with optimized ELISA protocols using biotin and streptavidin (for pNFH), electrochemiluminescence (ECL) assays (for NFL), and, most importantly, the so-called digital ELISA, or single molecule array (SiMoA) technology (for NFL and more recently also for pNFH). De Schaepdryver et al. analyzed CSF and serum pNFH in 85 ALS patients, 31 ALS-mimic patients, and 215 disease controls. There was good correlation between pNFH levels in the two biological fluids. Serum pNFH discriminated between ALS and ALS mimics in serum with an AUC of 0.812, 71.8% sensitivity, and 85.2% specificity although the diagnostic performance for serum was significantly lower than that for CSF (AUC, 0.971) [22]. A recent study has demonstrated higher levels of serum pNFH in ALS in comparison to MNDs with pure UMN or pure LMN phenotypes [23].

Coming to NFL, there is a strong correlation between CSF and serum levels [24]. In the study by Verde et al., at a cut-off of 62 pg/mL, serum NFL (measured with the Simoa technology) discriminated between 124 ALS patients and 44 disease controls (patients with neurological conditions in the differential diagnosis of ALS) with an AUC of 0.873, sensitivity of 85.5%, and specificity of 77.3%. Like CSF neurofilaments, also serum NFL has a good diagnostic performance for ALS in patients with recent onset (i.e., within 6 months) [19]. While serum NFL does not precisely differentiate between ALS and GBS, CIDP, or the LMN-only MND progressive muscular atrophy (PMA), it is higher in ALS than in PLS and discriminates well between the two diseases, with one study reporting an AUC of 0.89, 80.5% sensitivity, and 90.9% specificity [24]. Serum NFL slightly increases with increasing age in controls without neurodegenerative diseases [25] and possibly also in ALS itself [26]. Notably, patients with Creutzfeldt-Jakob disease display high levels of serum NFL, presumably due to massive neuronal degeneration, making the biomarker not suitable for the rare need of a differential diagnosis between ALS and CJD [25].

According to the above-cited meta-analysis of NFL studies in ALS, the 10 studies comparing NFL in serum of 693 ALS patients versus 413 cognitively healthy controls resulted in a ratio of 9.80, while the four studies performed on ALS versus ALS mimics showed a ratio of 8.15 [21]. In summary, although blood neurofilaments have a slightly lower diagnostic performance than their CSF counterparts,

accumulating evidence supports their use as an additional diagnostic investigation for ALS in clinical practice, especially in cases where their CSF measurement is not feasible, for example, due to contraindications to lumbar puncture.

16.2.4 Prognostic Value of Neurofilaments

Both CSF neurofilaments show a moderate negative correlation with disease duration at sampling, which is presumably due to the moderate positive correlation with disease progression rate, as patients with more aggressive disease course come earlier to medical attention [16]. Both neurofilaments are associated with survival, while some studies have reported a very weak association of both neurofilaments with the absolute score on the functional scale ALSFRS-R, which has uncertain significance [16]. CSF NFL seems to be associated longitudinally with future ALSFRS-R score and disease stage according to the MiToS (Milano-Torino) staging system [27].

pNFH in blood has also prognostic significance, with both plasma and serum levels showing an inverse association with survival [23, 28] and serum levels correlating positively with disease progression rate and negatively with disease duration at sampling [22]. Serum NFL correlates positively with disease progression rate and negatively with disease duration at sampling [24] but not with ALSFRS-R score at sampling [25], and is associated with survival, with one study reporting a hazard ratio (HR) of 2.4 for patients having NFL levels above the median compared to the rest of the cohort [25]. Indeed, serum NFL at diagnosis is the best predictor of survival among a list of prognostic factors including site of onset, weight loss, ALSFRS-R score, and respiratory parameters [29]. Importantly, a recent study has shown that serum NFL (but not pNFH) at baseline predicts future slope of decline in the ALSFRS-R functional scale score [26]. Nevertheless, neither CSF nor serum neurofilaments (pNFH in CSF, NFL in both fluids) at baseline seem to differ between patients evolving and those not evolving to higher El Escorial diagnostic categories at follow-up [19].

The above-cited associations between neurofilament levels and indicators of disease progression are important not only for individual patient prognosis but also for a possible future use of neurofilaments as biomarkers of target engagement and drug response in therapeutic trials. As an example from a related disease, it has recently been demonstrated that CSF NFL returns to normal levels in children with spinal muscular atrophy (SMA) after treatment with the antisense oligonucleotide (ASO) nusinersen, mirroring clinically measured motor improvement [30]. A similar observation has been made for another experimental drug in an animal model of ALS, where an ASO against mutant *SOD1* administered to m*SOD1* mice was demonstrated to delay disease onset and mitigate disease progression as well as to reduce the rise of serum pNFH levels [31]. A recent study has shown that the use of serum NFL instead of change in ALSFRS-R slope as an outcome measure in ALS therapeutic trials would allow large sample size savings; the advantage is less clear for serum pNFH [26].

16.2.5 Relationship of Neurofilaments with Disease Characteristics

CSF neurofilament levels (both pNFH and NFL) are not associated with site of onset of ALS [20] and the same is true for their serum counterparts [22, 25]. pNFH in CSF, but not in blood, and possibly also NFL in CSF, seem to be associated with the number of regions displaying signs of both UMN and LMN dysfunction (the former detected clinically, the latter represented by clinical or electromyographical signs) [20, 22].

While some authors have reported an association between CSF NFL and the number of regions showing EMG signs of LMN degeneration [32], other data suggest that NFL elevation in serum is driven—in addition to the disease progression rate—by the number of regions affected by UMN degeneration (as detected clinically) [24]. Similarly to an early observation indicating higher CSF pNFH levels in cases of ALS with predominant UMN signs (UMN-ALS) [33], also CSF NFL has been reported to be higher in classic ALS, progressive bulbar palsy, and UMN-ALS in comparison to the LMN-predominant variants flail limb (flail arm or flail leg) syndrome and progressive muscular atrophy (PMA) [27]. In agreement with this, CSF NFL has been shown to correlate with the UMN Penn score mirroring clinical UMN signs [34]. The relationship of NFL to UMN degeneration has also been analyzed from the point of view of neuroimaging, with some authors reporting a negative correlation between CSF NFL and the fractional anisotropy (FA) of the corticospinal tract and a positive correlation with corticospinal tract radial diffusivity (RD) on magnetic resonance (MR) diffusion tensor imaging (DTI) [34] and another investigation extending this observation to serum NFL [35], but other studies have not found such associations [16]. A recent study has demonstrated an inverse correlation of serum pNFH with the mean MEP (motor evoked potential)/cMAP (compound muscle action potential) ratio at the four limbs, considered as a proxy of UMN dysfunction or loss [23].

Neither pNFH in CSF nor NFL in CSF or serum differ between ALS patients in different categories according to the revised El Escorial diagnostic criteria [19]. Similarly, no association has been found between serum NFL and neuroradiological ALS stages identified by means of DTI of relevant white matter tracts of the brain mirroring ALS neuropathological staging [5, 25]. However, a recent study has shown significantly higher serum pNFH levels in MND patients in King's stages 3 and 4 compared to those in stages 1 and 2 [23]. Finally, neither CSF or serum NFL nor serum pNFH correlate with cognitive impairment in ALS [23, 27, 36], and blood NFL does not differ between patients taking and those not taking riluzole at sampling [25, 37].

16.2.6 Longitudinal Studies of Neurofilaments

Several studies have investigated changes of neurofilament levels over time. In the cohort of Steinacker et al., 11 patients underwent a repeat lumbar puncture in the follow-up, showing that pNFH was stable over time while NFL tended to decrease

[16]. In the study by Poesen et al., 17 patients underwent longitudinal CSF sampling, confirming stability of pNFH, whereas in a subset of ALS patients with fast and intermediate progression NFL increased over time [20]. The more numerous investigations performed on blood neurofilament levels are more informative due to the higher numbers of samples allowed by the less invasive nature of blood draw compared to lumbar puncture. Lu et al. reported stable NFL levels in plasma in the three categories of patients with fast, intermediate, and slow progression; in serum (sampled in a different cohort of patients) NFL was stable in slow and intermediate progressors and showed a slight increase in fast progressors, while in the CSF (sampled in the same cohort as serum) it was stable in intermediate progressors and had a slight increase in both slow and fast progressors [37]. Most other studies agree on a general stability of serum NFL over time [25, 26, 36], with one study showing an increase in the first 20 months of disease in some patients [24]. The stability of blood NFL over time is particularly advantageous when considering this protein as a possible future pharmacodynamic biomarker in drug trials because only a marker which is per se stable over time in the natural history of the disease can be exploited to reliably mirror putative drug effects as reflected by modifications of its levels, with large sample size savings in comparison to the use of clinical parameters [26]; this obviously pertains more to blood than to CSF due to the above-mentioned less invasive sampling allowing several measurements over the disease course.

Longitudinal kinetics of blood pNFH have been studied less extensively due to the more challenging and less widely used measurement of this marker in blood compared to NFL. In the whole ALS population an initial increase and then a reduction in serum has been reported [38], while a recent study has found no consistent longitudinal variations [26]. An investigation on plasma from patients subdivided according to rate of progression showed decrease in fast progressors, stability for 6 months followed by increase in slow progressors, and initial increase followed by later decrease in intermediate progressors [39].

16.2.7 Antibodies Against Neurofilaments

Some studies have focused on the immune response to neurofilaments in the blood of ALS patients. Following an early observation on serum by Fialová et al. [40], Puentes et al. reported higher levels of antibodies directed against NFL (but probably reacting against neurofilaments in general) in the plasma of ALS patients compared to controls [41]. The difference was significant enough to enable diagnostic discrimination between the two categories with an AUC of 0.78, 71% sensitivity, and 77% specificity. Anti-NFL antibody levels also negatively correlated with ALSFRS-R score at sampling, were higher in the advanced and intermediate stages of ALS compared with the early stage, and were inversely associated with survival. In the whole cohort, antibody levels did not significantly differ between patients treated and those not treated with riluzole, but among young patients with a short diagnostic delay, they were higher in those taking the drug compared with those not treated [41]. Finally, immunoblot of plasma of ALS patients has demonstrated IgG

antibodies reacting against NFH as well as aggregates and fragments thereof [39]. Altogether, these findings suggest that in ALS an immune reaction against neurofilaments develops, which seems to be dependent on disease stage. These antibodies could be neutral bystanders, but alternatively they could have a role in disease pathophysiology. In either case, their presence should be taken into account when considering analytical aspects of neurofilament measurements, whose results could be influenced by antibody levels.

16.3 Neuroinflammatory Biomarkers

16.3.1 The Involvement of Neuroinflammation in ALS

Neuroinflammatory mechanisms are thought to play a role in ALS pathogenesis [42]. This is supported by several lines of evidence. Early investigations reported infiltration of T-lymphocytes in the spinal cord in a high proportion of ALS patients, both in proximity of degenerating corticospinal tracts and in ventral horns, while infiltrates were rare among controls [43]. Furthermore, the spinal cord in ALS also contains activated microglia/macrophages as wells as dendritic cells, whose transcripts are more highly expressed in patients with faster disease progression [44]. Widespread microglial activation was also demonstrated with PET imaging in the brain of ALS patients [45]. Certain genes causing familial forms of ALS have a role in immune function: loss of *C9orf72* gene in mice causes morphologic alterations of immune system organs and dysfunction of microglia and macrophages [46], while the TBK1 kinase, whose gene is also mutated in familial forms of ALS and FTD, is involved in autophagy and innate immunity, playing a crucial role in regulation of interferon production [47]. Most importantly, experimental studies on ALS animal models have demonstrated that non-neuronal cells are essential components of the non-cell-autonomous process of ALS pathogenesis: whereas disease onset and early progression are determined by motor neurons, later progression is mostly driven by neighboring microglia [48]. The role of microglia is actually not uniform over the course of ALS pathogenesis: the early phases are characterized by a protective phenotype, whereas in later disease course a pro-inflammatory phenotype predominates, promoting damage to motor neurons [49]. Furthermore, astrocytes contribute to disease pathogenesis by releasing factors which are toxic to motor neurons [50].

16.3.2 Limited Role of Cytokines and Other Mediators as Biomarkers

The involvement of neuroinflammation in ALS pathogenesis is reflected by alterations of inflammatory mediators in the CSF of patients. Several investigations have reported alterations in the CSF levels of several cytokines in ALS patients compared to controls. As an example, Mitchell et al. proposed a panel composed of the five

molecules among a set of cytokines and growth factors which showed the highest concentration differences in ALS patients compared to neurological disease controls (i.e., increased IL-6, GM-CSF, IL-2 and IL-15 and decreased IL-10), reporting accuracy of 89.2% in discriminating between the two conditions [51]. Several other investigations on ALS patients have shown elevated CSF or blood levels of the chemokine macrophage chemoattractant protein-1 (MCP-1), which is mainly secreted by spinal cord astrocytes [52, 53]. However, the use of inflammatory molecules as biomarkers in ALS has two important limitations: (1) most data from the numerous investigations are inconsistent; and (2) the differences in concentrations between ALS and other categories are not marked enough, at least on an individual biomarker scale, to enable clinically meaningful discriminations [54]. However, an exception to this conclusion seems to be represented by the class of chitinases, which have been increasingly studied as potential ALS biomarkers in the last years [55].

16.3.3 Chitinases and Chitotriosidase

Chitinases are a family of glycosyl hydrolases which degrade the polysaccharide chitin to N-acetylglucosamine, which is a source of carbon and nitrogen. They are expressed in a wide range of organisms including humans and are evolutionarily conserved. Humans possess several members of this family, among which only two retain chitinase action (chitotriosidase [Chit1] and acidic mammalian chitinase [AMCase]), while the others are devoid of this function and are called chitinase-like proteins (e.g., chitinase-like protein 3L1 [CHI3L1, also known as YKL-40] and chitinase-like protein 3L2 [CHI3L2]) [56]. Chit1 is the main human chitinase. It is mostly produced by activated macrophages and related cell types (e.g., microglia) but also by neutrophils and epithelial cells. The original function of Chit1 is thought to be degradation of chitin produced by fungi and arthropods; however, additional still undefined functions of the protein cannot be ruled out, and it is currently not known whether an endogenous receptor for secreted Chit1 or an endogenous substrate exist [57]. Of note, a common 24-bp duplication in the *CHIT1* gene alters its splicing, producing a defective form of the protein which results in low concentration and activity in the blood [58]. Chit1 has been studied as a biomarker especially in the lysosomal storage disorder Gaucher's disease, where its enzymatic activity is markedly increased in plasma [59]; moreover, Chit1 may have a role as a biomarker of sarcoidosis, in which its serum levels are elevated and reflect disease severity [60].

16.3.4 Chit1

In the field of neurodegenerative diseases, Chit1 activity was originally shown to be higher in the CSF of patients with Alzheimer's disease compared to healthy controls and patients with stable mild cognitive impairment (MCI) [61]. The first study of

Chit1 in ALS is that of Varghese and colleagues, who in 2013 demonstrated with a liquid chromatography-tanden mass spectrometry (LC-MS/MS) study that Chit1 levels in the CSF were ten-fold higher in sALS patients compared with neurologically healthy controls, which was confirmed by an ELISA assay and by investigation of enzymatic activity. Importantly, Chit1 was expressed in cultured microglia [62]. Pagliardini et al. examined Chit1 activity in dried blood spots and found it to be higher in ALS patients in comparison to healthy controls, a finding which was not related to the common 24-bp duplication polymorphism of *CHIT1* gene. Moreover, Chit1 activity was higher in ALS patients exhibiting faster disease progression [63]. Similar results were replicated also for CSF, with higher Chit1 activity in ALS compared with healthy controls [64].

The largest study of Chit1 as ALS biomarker is that of Steinacker et al. conducted on a total of 316 individuals. Chit1 levels in the CSF were higher in ALS patients compared to healthy controls and to patients with ALS mimics, AD, PD, and FTD, but not compared to patients with CJD [55]. In the differentiation between ALS and ALS mimics, CSF Chit1 had 82% sensitivity and 51% specificity, thus showing a less satisfactory diagnostic performance than both pNFH and NFL. Importantly, the elevation of Chit1 levels in the CSF of ALS patients has been demonstrated also with use of unbiased proteomic techniques [65]. The allele distribution of the 24-bp duplication polymorphism does not significantly differ between ALS and other diagnostic categories, though lowering both CSF and serum levels of the protein [66]. CSF Chit1 correlated moderately with disease progression rate and, accordingly, was inversely associated with disease duration at sampling and with ALSFRS-R score [55]. The latter association is in agreement with the finding of an association of CSF Chit1 levels with the anatomical extent of disease burden reported by another study, showing higher CSF Chit1 levels in patients with signs of UMN and LMN dysfunction in three body regions compared to those with signs in only one region [67]. A negative association with survival has been reported inconsistently, with higher levels of Chit1 predicting shorter survival only when considering the biomarker in isolation or, conversely, only when taking multiple variables into account [67–69]. Chit1 levels in the CSF correlate moderately with levels of both pNFH and NFL [55]. Notably, there is a high correlation between Chit1 levels and enzyme activity in the CSF [69]. Although a weak correlation between serum and CSF Chit1 levels has been reported [69], serum Chit1 levels do not significantly differ between ALS and other diagnostic categories, do not show consistent changes longitudinally, and do not correlate with disease progression rate [55]. Interestingly, immunohistochemistry demonstrated Chit1 expression in the corticospinal tract of autopsied ALS patients but not of CJD patients, in spite of the high CSF Chit1 levels in the latter condition (presumably due to massive neurodegeneration), while only sparse staining was noted in AD patients and healthy controls, thus pointing to an ALS-specific pathophysiologic role of Chit1 in the corticospinal tract. Moreover, immunohistochemistry of spinal cord sections demonstrated colocalization of Chit1 with markers of microglia (Iba-1) and macrophages (CD68) [55].

16.3.5 The Other Chitinases

In their proteomic study, Thompson et al. investigated the CSF levels of 3 chitinases (Chit1, CHI3L1 [YKL-40] and CHI3L2) using LC-MS/MS with label-free quantification. Interestingly, while all three chitinases were higher in ALS compared to neurological disease controls and healthy controls, the comparison between ALS and PLS demonstrated significantly higher levels of only Chit1 and CHI3L2 in ALS, whereas CHI3L1 (YKL-40) did not significantly differ between the two MNDs. In ALS, Chit1 correlated moderately with disease progression rate and—accordingly–with CSF pNFH, while for the other two molecules both correlations were weaker [68]. Similar findings were reported by the same group using ELISA assays, confirming differential levels of Chit1 and CHI3L2, but not of CHI3L1 (YKL-40), between ALS and PLS. The correlation between CSF chitinases and CSF pNFH followed a decreasing gradient with the highest value for Chit1, the lowest value for CHI3L1 (YKL-40), and an intermediate value for CHI3L2 [69]. Accordingly, Chit1 correlated with both other chitinases, and in another study on CSF Chit1 and CHI3L1 (YKL-40), both correlated with CSF NFL [66].

However, the diagnostic performance of CSF chitinases in discriminating between ALS and ALS mimics (AUCs: Chit1, 0.84; CHI3L1 [YKL-40], 0.73; CHI3L2, 0.88) is lower than those of both pNFH (AUC, 0.91) and NFL (AUC, 0.92) [32, 69]. The same is valid for the differentiation between ALS and PLS, where all three chitinases, especially CHI3L1 (YKL-40), have lower discriminative power than pNFH (AUCs: Chit1, 0.74; CHI3L1 [YKL-40], 0.58; CHI3L2, 0.78; pNFH, 0.88) [69]. Importantly, all 3 chitinases are longitudinally stable in the CSF [69]. An association of higher CHI3L1 levels with shorter survival has been inconsistently reported [67]. Interestingly, CSF levels of CHI3L1 (YKL-40), but not those of the other two chitinases, seem to correlate weakly with cognitive dysfunction in ALS as measured with the ALS-specific ECAS cognitive battery (Edinburgh Cognitive and Behavioural ALS Screen) [69]. A weak correlation between CSF and serum levels for CHI3L1 (YKL-40) has been found in one study [66]; however, like Chit1, also CHI3L1 (YKL-40) and CHI3L2 levels do not differ in serum or plasma between ALS and other diagnostic categories [69, 70]. A recent study has investigated the expression of CHI3L1 in ALS autopsy samples: this chitinase is found in the white matter of motor cortex and spinal cord and colocalizes with glial fibrillary acidic protein (GFAP), indicating that CHI3L1 in ALS brain and spinal cord white matter is expressed by activated astrocytes [70].

Finally, recent investigations have focused on the differential role of chitinases and other neuroinflammatory molecules in ALS and FTD. A study mostly conducted on genetic forms of both diseases, but with similar findings also in their sporadic counterparts, has shown an elevation of Chit1 and CHI3L1 (YKL-40), but not of the astrocyte marker GFAP, in the CSF of ALS patients, while FTD is characterized by increased CSF levels of CHI3L1 and GFAP, but not Chit1 [66]. A study using iTRAQ (isobaric tags for relative and absolute quantitation) LC-MS/MS has demonstrated significantly higher Chit1 levels in the CSF of ALS patients carrying the hexanucleotide repeat expansion in *C9orf72* gene compared with FTD patients

with the same mutation [71]. Furthermore, CSF Chit1 is higher in FTD patients with documented or presumed TDP-43 pathology (FTLD-TDP) compared with those with tau pathology (FTLD-tau), and, among FTD-TDP patients, those with additional ALS display higher levels than those without ALS; similarly, CSF CHI3L1 (YKL-40) is higher in ALS-FTD patients compared to FTD patients without ALS [72]. Taken together, these studies suggest that, within the spectrum of the related diseases ALS and FTD, differences in the CSF neuroinflammatory profiles, conceivably reflecting differences in underlying pathological processes, could play a role in driving clinicopathological expression towards either of the two entities starting from a common basis of susceptibility to TDP-43 proteinopathy.

16.3.6 Perspectives for the Use of Chitinases as ALS Biomarkers

In conclusion, there is sufficient evidence in support of a role of chitinases, and especially Chit1, as biomarkers for ALS. Indeed, it is unlikely that they will acquire a primary position in the diagnostic workflow, in that their performance is lower than that of neurofilaments as pertains to the differentiation of ALS from other conditions [69]; the same is true for prognostic value, as neurofilaments are more strongly associated with survival and progression rate [55]. Nevertheless, chitinases could complement the use of neurofilaments in diagnosis and prognostic stratification. More importantly, however, the alterations of chitinase levels in ALS point to a role of neuroinflammation in pathogenesis. As such, these neuroinflammatory biomarkers may not only help to elucidate disease mechanisms, but also play a role in stratification of patients in therapeutic trials, especially those targeting neuroinflammatory processes. In this context, CSF chitinases—thanks to their longitudinally stable levels [69]—could also be used as biomarkers of target engagement and drug effect. Similarly, in a future scenario, they could prove useful in clinical practice as a screening tool for directing patients towards specific neuroinflammation-targeted ALS therapies and then for monitoring treatment response.

Key Points

- ALS is a TDP-43 proteinopathy characterized by degeneration of upper (UMNs) and lower motor neurons (LMNs) causing progressive paralysis with death after 3–5 years.
- ALS neurochemical biomarkers still do not have any established role in current clinical practice, but they are needed to facilitate early and differential diagnosis.
- The most important ALS neurochemical biomarkers are neurofilament phosphorylated heavy chain (pNFH) and light chain (NFL), which are released in the CSF from degenerating motor neuron axons.
- Both pNFH and NFL in the CSF have a good diagnostic performance in discriminating ALS from other conditions and provide prognostic information in ALS.

- Progress in analytical techniques allows measurement of neurofilaments also in peripheral blood, providing diagnostic and prognostic support with less invasive sampling and enabling longitudinal measurements.
- Neurofilaments may be used as pharmacodynamic biomarkers for therapeutic trials, confirming target engagement and quantifying treatment effect.
- The chitinase Chit1 is a promising CSF neuroinflammatory biomarker for ALS, reflecting microglial neuroinflammation.
- Chit1 has lower diagnostic and prognostic performance than neurofilaments, but it may prove useful as pharmacodynamic biomarker in therapeutic trials targeting neuroinflammation.

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Paraneoplastic Diseases of the Peripheral Nervous System

17

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17.1 Introduction

Peripheral nervous system involvement is a frequent condition in patients with paraneoplastic neurological syndromes (PNS), yet its clinical manifestations are highly heterogeneous. The peripheral nervous system can be variously involved, but the most frequently affected sites are the dorsal root ganglia and presynaptic nerve endings of the neuromuscular junction, whereas the extent of axonal and myelin damage of peripheral nerves is still unresolved. Several of the autoimmune neuropathies have a subacute and rapid development. The discussion of the therapeutic window of possible interventions is ongoing [1, 2].

While peripheral nervous system damage can be induced by various mechanisms in patients with systemic malignancy, in patients with neuropathy, it is usually of autoimmune origin and only rarely associated with direct tumour infiltration or at a later stage caused by chemotherapy. Chemotherapy-induced neuropathy, although frequent, has a different time profile and appears as conventional neurotoxic neuropathies [3].

Metabolic-related causes are uncommon. PNS arise in less than 1% of patients with malignancy, preceding the diagnosis of cancer by months or even years in the majority of cases. Specific serological markers can be used to screen for classical paraneoplastic syndromes, as defined by Graus et al. [4, 5].

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In this chapter, the issue of neuropathies and disorders of the neuromuscular junction will be discussed. Specific cancer-associated muscular syndromes will be discussed in Chaps. 6 and 7.

Subacute sensory neuronopathy (SSN) and Lambert-Eaton myasthenic syndrome (LEMS) are classified as classical paraneoplastic syndromes. Other diseases like neuromyotonia or inflammatory neuropathies like Guillain–Barré syndrome (GBS) or chronic inflammatory demyelinating polyneuropathy (CIDP) associate with tumours in a minority of cases.

Onconeural antibodies directed against neural antigens expressed by the tumour may occur in most affected patients, suggesting an underlying autoimmune process [6]. Conversely endplate disorders as LEMS and neuromyotonia are caused by surface antibodies. The emerging spectrum of surface antibody-associated neuromuscular disorders has shown, that, contrary to onconeuronal antibodies, there is a large overlapping field with other autoimmune diseases.

The disorders are presented here below according to peripheral nervous system site (Table 17.1).

The clinical referral to neurological consultation is often neuropathy of unknown cause. Apart from the fact that in the age group of 50 years and more, 30–40% of neuropathies remain idiopathic, usually a screen for cancer is not warranted in sensory motor neuropathy. However if sensory ataxia, pain, and rapid onset appear, a sensory neuronopathy seems likely and can be associated with a PNS. However, also this is not specific as paraneoplastic neuropathy can be found in a range of other conditions (e.g., idiopathic Sjögren syndrome).

Table 17.1 Paraneoplastic diseases of the peripheral nervous system: a summary

Neuropathies
Subacute sensory neuronopathy
Monoclonal gammopathy and neuropathy
Paraneoplastic vasculitic neuropathy
Paraneoplastic dysautonomic neuropathy
Disputed and uncharacteristic:
Sensory neuropathy
Sensorimotor neuropathy
Inflammatory neuropathies:
Guillain-Barré syndrome (sporadic cases)
Chronic inflammatory demyelinating polyneuropathy associated with lymphoma
Motor neuron disease
Neuromuscular junction
Lambert-Eaton myasthenic syndrome
Myasthenia gravis
Neuromyotonia
Myopathies: see Chaps. 6 and 7
Cachexia: see Chap. 10

17.1.1 Subacute Sensory Neuronopathy

SSN, firstly described by Denny-Brown in 1948 [7], results from lymphocytic-inflammation with destruction of sensory neurons in the dorsal root ganglia. It is characterised by subacute, rapidly progressive onset, with chiefly multifocal or asymmetrical sensory loss, symptoms of paraesthesia and pain and, typically, asymmetric upper limb involvement, extending in some cases to the face, chest, or abdomen. Sensory loss, most markedly affecting deep sensation, leads in many cases to severe sensory ataxia of the four limbs. Sensory perception is markedly reduced and often slight touch is associated with allodynic pain. Fine motor tasks as buttoning, writing even holding a cup for drinking may be impossible.

Although very disabling, subacute sensory neuronopathy has been reported to have an indolent clinical course [8] often described as plateau phase. The prognostic aspects in regard to recovery and rehabilitation are poor.

SSN is the hallmark characteristic in over 50% of patients with paraneoplastic encephalomyelitis (PEM). Many patients with SSN also present signs and symptoms suggestive of multifocal limbic, cerebellar, brainstem, or spinal cord involvement, determining the picture of paraneoplastic encephalomyelitis [8–10].

Paraneoplastic SSN is characterised by a marked loss of primary, most notably large-diameter sensory neurons in the dorsal root ganglia, following a diffuse but patchy, asymmetric pattern. Signs of non-specific degenerative changes are present in the remaining neurons. Infiltration by T and B lymphocytes, plasma cells, and macrophages varies considerably and often shows perivascular distribution. Myelinated fibres are severely depleted in the dorsal columns, posterior nerve roots, and peripheral sensory nerves, believed to be secondary to the loss of dorsal root [11]. Sural nerve biopsy reveals non-specific axonal degeneration and a decrease in myelinated fibres [12, 13] and is rarely necessary.

Cerebrospinal fluid is usually altered (with non-specifically raised protein, mild mononuclear pleocytosis, elevated IgG index, and/or oligoclonal bands) but is reported to be normal in at least 10% of patients.

At electrophysiology exam, sensory nerve potentials are characteristically absent or severely diminished in amplitude, with normal or only slightly reduced sensory nerve conduction velocities, when a response can be elicited. Some uncharacteristic electrophysiological abnormalities can be seen in motor nerve conduction and are observed in the majority of patients, with or without symptoms of mixed sensorimotor polyneuropathy, but motor nerve is almost always less impaired than sensory nerve conduction. In patients with a motor, an additional motor neuropathy speculatively additional anterior horn cell involvement has been suspected.

The differential diagnosis of paraneoplastic sensory neuronopathy [14] includes dorsal root ganglionitis associated with Sjogren's syndrome [15], sensory neuropathy associated with anti-disialosyl ganglioside antibodies and idiopathic forms.

In the presence of a known cancer diagnosis, the differential diagnosis includes cisplatin- or paclitaxel-induced sensory neuropathy but is only relevant after sufficient treatment exposure. In addition, SSN usually develops at the time of cancer presentation and not during its course.

Table 17.2 Onconeural antibodies in paraneoplastic neuropathies (SSN)

Onconeuronal antibody-associated tumour(s) antibody	
Antibody	Associated to cancer
Anti-Hu (ANNA-1)	Small-cell lung cancer
Anti-CV2 (CRMP-5)	Small-cell lung cancer, other carcinomas
Anti-Ma2	Lung, testis, and other carcinomas

Although SSN can be associated with various tumours, small-cell lung cancer accounts for 70–80% of cases [8]. Most patients harbour anti-Hu antibodies, which show 99% specificity and 82% sensitivity for the diagnosis of cancer in patients with suspected SSN [6]. Anti-Hu antibodies stain the nuclei and, to a lesser degree, the cytoplasm of all neurons in the dorsal root ganglia, autonomic ganglia, and central nervous system. They react with a group of closely related 35–40 kD RNA-binding proteins, several of which have been cloned.

Expression Hu auto-antigens is frequent but not universal among small-cell lung carcinomas, including tumours of patients with SSN/PEM and anti-Hu antibodies and tumours of patients with no neurological symptoms. A small number of patients with SSN/PEM associated with small-cell lung carcinoma or with another malignancy either have no detectable anti-neuronal autoantibodies or harbour antibodies with patterns of immunoreactivity that differ from anti-Hu antibodies (Table 17.2). These include anti-CV2 (CRMP-5) antibodies targeted against a group of proteins expressed by neurons and oligodendrocytes, anti-amphiphysin antibodies and anti-Ma antibodies.

Anti-Hu-antibody-negative patients do not reliably differ from the spectrum of signs and symptoms seen in patients with anti-Hu antibodies. Anti-Hu or other anti-neuronal antibodies in patients with sensory neuronopathy are a robust (but not absolute) marker of an underlying tumour. Nevertheless, a paraneoplastic condition and an occult neoplasm may be present even in the absence of anti-neuronal antibodies.

17.1.2 Monoclonal Gammopathy and Neuropathy

Neuropathies associated with monoclonal gammopathy in some cases may be of paraneoplastic origin. Indeed, monoclonal gammopathy may underscore or transform into a haematological malignancy on whose treatment neurological improvement often depends [16].

In particular, the neuropathy associated with monoclonal gammopathy requires further investigation in the presence of IgM gammopathy to exclude Waldenström's macroglobulinaemia or other lymphomas [17].

Peripheral neuropathy causes complications in as many as 20% of patients with Waldenström's macroglobulinaemia [18], and the IgM antibodies may be directed at the myelin sheath [19]. Solitary plasmacytoma, multiple myeloma, or polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes

(POEMS) should be considered in patients with IgG or IgA monoclonal gammopathy. Peripheral neuropathy at the time of diagnosis is uncommon in patients with multiple myeloma, and neuropathies are often secondary to the use of neurotoxic drugs (e.g., thalidomide and bortezomib).

POEMS (or Crow-Fukase syndrome) is a rare plasma cell condition with multiorgan involvement. Its aetiology is not yet known, but there is an increasing body of evidence that vascular endothelial growth factor (VEGF) may play a role. Diagnosis continues to be based on clinical findings [20] since there are no specific tests or pathognomonic signs. POEMS syndrome targets in particular peripheral nerves, and neuropathic symptoms dominate the clinical picture [21]. It is frequently misdiagnosed as CIDP due to its prominent features of demyelination and axonal degeneration.

The associated monoclonal gammopathy is generally IgG or IgA, often carrying a lambda light chain. Correct diagnosis is critically dependent on the concurrent presence of systemic symptoms/signs (including organomegaly, endocrinopathy, pleural effusions, ascites, peripheral oedema, Castleman's disease, sclerotic bone lesions, thrombocytosis, skin changes, papilloedema) and increased levels of serum VEGF. Early treatment is crucial [22]. First-line therapy in patients with dominant sclerotic plasmacytoma includes radiation of the lesion. Systemic therapy is indicated for patients with diffuse disease [23–25] and also other haematological therapies as bone marrow transplant are used. Clinical response to treatment is associated with VEGF levels [21].

Immunoglobulin Light Chain (AL) Amyloidosis is a plasma cell disorder determined by deposition of monoclonal light chains in several tissues. Peripheral nerves are often involved, together with kidney, heart, lung, and liver. Fatigue and weight loss are frequent onset symptoms. Peripheral nervous system involvement consists in an axonal symmetric length-dependent neuropathy with loss of temperature sensation, neuropathic pain, and dysautonomia. Diagnosis is performed with bone marrow biopsy combined with abdominal subcutaneous fat aspiration [20].

17.1.3 Paraneoplastic Vasculitic Neuropathy

Peripheral nerve microvasculitis may be rarely associated with lymphomas or carcinoma of the lung, prostate, uterus, kidney, or stomach [26, 27].

Neurological symptoms usually precede diagnosis of the tumour. The disorder usually presents as mononeuritis multiplex or asymmetric distal sensorimotor neuropathy. Pain is frequently reported. Patients' sedimentation rate is usually high, but cutaneous vasculitis or other systemic symptoms are rarely present. Clinical involvement is asymmetric as shown in nerve conduction studies by varying levels of motor and sensory axonal degeneration. Sural nerve biopsy or autopsy demonstrates focal mononuclear cell infiltration of epineurial vessel walls and active nerve fibre degeneration. Additionally, arteriolar fibrinoid necrosis may be present with obliteration of lumina. Patients with peripheral nerve vasculitis and small-cell lung cancer may also present clinical and pathological features of SSN/PEM. Patients with small-cell

lung cancer can harbour anti-Hu antibodies, with or without overt central nervous system involvement [28].

Vasculitic neuropathies can also occur as a rare complication of immune check point inhibitor therapy [29].

17.1.4 Paraneoplastic Dysautonomic Neuropathy

Dysautonomia may be considered to be an isolated paraneoplastic peripheral neuropathy in a minority of patients. Chronic pseudo-obstruction is the most common syndrome. Symptoms may include severe, progressive gastrointestinal dysmotility, as gastroparesis, chronic intestinal pseudo-obstruction, and severe constipation/obstipation, preceding detection of the small-cell lung carcinoma by several months [8, 9].

The majority of patients do, however, develop dysautonomia alongside another paraneoplastic syndrome (mostly sensory neuronopathy), and the association with lung tumours, especially small-cell lung carcinoma, and Hu antibodies is robust [30, 31]. The syndrome responds rarely to tumour treatment, and no case studies have been conducted with immunotherapy. A recent review suggest that autonomic syndromes may be more frequent in cancer patients as currently assumed [32].

17.1.5 Other Paraneoplastic Neuropathies

Cases of sensory and sensorimotor neuropathies associated to malignancy, in the absence of onconeural antibodies, have been reported. Graus et al. [5] classifies these as possible PNS, if a tumour is detected within 3 years. To classify these cases as paraneoplastic, further investigation is needed: firstly, to rule out any pre-existing neuropathies or carcinomatous neuropathy associated with severe illness and/or weight loss and, secondly, bearing in mind that some tumours, as multiple myeloma, may give rise to several types of peripheral neuropathy (see above).

In addition, several novel observations and antibodies are described [33, 34]. The robustness and reliability of new observations will decide on the future use and application.

Some controversy surrounds the possible relationship between inflammatory neuropathy-like GBS and cancer, and the existence of a paraneoplastic form of GBS has not been confirmed. The cases with malignancies are usually clinically similar to the other cases of GBS [35], and 18 patients in the PNS Euronetwork database (unpublished reports) have been diagnosed with GBS or CIDP and a tumour. Several patients had both Hu antibodies and signs of demyelination on electromyography. These cases are most likely rare variants of Hu antibody-associated neuropathies.

Lymphoproliferative malignancies can favour the occurrence of inflammatory neuropathies, like GBS (more associated with Hodgkin's disease) or CIDP (more common in Non-Hodgkin Lymphoma) [36].

17.1.6 Motor Neuron Disease

Amyotrophic lateral sclerosis is not considered a paraneoplastic disease although association with onconeural antibodies and cancer in motor neuron disease (MND) has been reported [37]. A rapidly progressive form of MND could be raised the suspicion of an associated cancer [38]. Motor neuron involvement is recognised also in the context of anti-Ma2 paraneoplastic syndrome [39].

17.2 Syndromes of the Neuromuscular Junction

17.2.1 Presynaptic Disorders

17.2.1.1 Acquired Neuromyotonia

Neuromyotonia (NMT) is a generalised peripheral nerve hyperexcitability disorder. The characteristic clinical picture is of muscle stiffness, twitching (fasciculations) and/or rippling (myokymia), painful cramps, impaired muscle contraction or pseudomyotonia, and muscle weakness. Muscle hypertrophy can develop. The limb or limb and trunk muscles are most frequently affected [40]. One-third of patients also have sensory symptoms and approximately 50% develop hyperhidrosis. Central nervous system features (as hallucinosis, insomnia, chorea) could be present and, in its florid form, may be referred to as Morvan's syndrome. EMG reveals neuromyotonic and or myokymic discharges.

Neuromyotonia is paraneoplastic in around 25% of cases (5–10% in the PNS Euronetwork database) and can precede the discovery of a tumour by up to 4 years. The most frequently associated tumours are thymoma with or without myasthenia gravis, small-cell lung cancer, and haematological malignancies. Anti-voltage-gated potassium channel (anti-VGKC) antibodies are usually associated with these syndromes and are found in about 35% of all patients with peripheral nerve hyperexcitability and in as many as 80% in the presence of thymoma. They do not, however, differentiate the paraneoplastic from the non-paraneoplastic form. In 2010, true targets of anti-VGKC's antibodies were identified in channel-associated proteins, namely leucine-rich glioma inactivated 1 (LGI1) and contactin-associated protein-like 2 (CASPR2). These antibodies act by modulating degradation or expression of VGKCs in the presynaptic surface [41].

Anti-LGI1 antibodies are usually found in patients with limbic encephalitis. Differently, anti-CASPR2 antibodies are described in a clinical spectrum ranging from pure peripheral involvement, for example, neuromyotonia, to central nervous system manifestations, for example, limbic encephalitis. Morvan's syndrome, in which peripheral hyperexcitability coexist with dysautonomia, psychiatric disturbances, and sleep disfunction, is strongly associated with anti-CASPR2 antibodies although seronegative cases are reported [42]. In 20% of patients with anti-CASPR antibodies, a tumour, mostly thymoma, can be detected [43].

Notably, neuromyotonia and Morvan's syndrome have also been reported in children "double positive" for anti-LGI1 and anti-CASPR2 antibodies [44, 45].

17.2.1.2 Lambert-Eaton Myasthenic Syndrome

LEMS is a presynaptic disorder of the cholinergic neuromuscular and autonomic synapses. It is paraneoplastic in 60% of cases and usually associated with small-cell lung cancer [46]. Muscle weakness is the predominant feature in the proximal lower limbs and can extend to other skeletal muscles, rarely including the eye muscles. Respiratory failure is uncommon and tendon reflexes are depressed or abolished. Autonomic dysfunction is characterised by mouth or eye dryness, blurred vision, impotence, constipation, impaired sweating, or orthostatic hypotension [47]. Paraneoplastic forms usually have a more rapid progression of symptoms [48]. Repetitive nerve stimulation usually shows low compound muscle action potentials after nerve stimulation, with decrement at low-frequency stimulation and increment of over 100% after high-frequency stimulation or brief maximal effort. In a minority of patients, LEMS is associated with paraneoplastic cerebellar degeneration [49].

This syndrome depends on antibodies directed to P/Q-type voltage-gated calcium channels (VGCC). 10–15% of patients are negative for anti-VGCC antibodies; these are patients with similar clinical phenotype but with a lower incidence of associated small-cell lung cancer.

A recently described antibody marker, anti-SOX1, has been shown to be associated with paraneoplastic LEMS (65% of patients), rather than non-tumoural LEMS in which usually it is not detected [50].

17.2.2 Post-synaptic Disorders

17.2.2.1 Myasthenia Gravis

Myasthenia gravis (MG) is the most common autoimmune neuromuscular disease, characterised by muscle weakness especially after exercise. It is determined by autoantibodies against antigens placed in the postsynaptic membrane of the neuromuscular junction (sarcolemma).

In 85% of MG patients, antibodies against acetylcholine receptors (AchRs) are found, especially of the subclass IgG1 and IgG3. Another portion of patients has antibodies against MuSK and LRP4, which is protein involved in the clustering of AchRs. MuSK antibodies are mainly against the IgG4 subclass and determine a reduction of AchR density.

MG is considered a paraneoplastic disease in 10–15% of cases, in which a thymoma is found. Usually, paraneoplastic MG is found in AchRs-positive patients with higher anti-AchR antibody titre and severe disease [41]. Up to 40% of patients affected with thymoma can develop MG [51].

Usually, MG is not considered to be paraneoplastic in other tumours.

17.3 Differential Diagnosis

In investigating neuropathies of unknown origin, often a paraneoplastic cause is suspected.

Several other types of neuropathies are often considered to be paraneoplastic in the search of a possible cause. It is important to emphasise that 30–40% of

neuropathies in persons aged over 50 years are cryptogenic [52]. Extensive workup rarely reveals additional information regarding the diagnosis. From the clinical point SSN, LEMS, NMT need to be carefully checked for paraneoplastic neurological causes.

17.3.1 Neoplastic Neuropathies

Depending on the cancer type, several different types of peripheral nerve involvement exist, all of which are rare and are often focal and rarely resemble a symmetric neuropathy.

1. In rare cases, meningeal spread can mimic peripheral neuropathy. Severe meningeal carcinomatosis can affect multiple roots and resemble neuropathy. Rarely, isolated infiltration of the cauda equina can produce flaccid paraparesis, which is usually associated with pain, some asymmetry, and additional cranial nerve and central nervous system involvement. Neuropathies caused by immune therapies may mimic meningeal carcinomatosis [53].
2. Infiltration of peripheral nerves by cancer can be widespread and occur in a neuropathy-like lesion, can affect individual parts of peripheral nerves and the plexus, and can also spread along peripheral nerves. Anastomosis between cranial nerves can also be the target of cancer infiltration [54].
3. Symmetric involvement of peripheral nerves mimicking polyneuropathy is almost exclusively observed in lymphoma and leukaemia. In lymphoma in particular, the term neurolymphomatosis is used, in addition to the intravascular type. Several reports have suggested that neurolymphomatosis can mimic CIDP [55].

17.3.2 Chemotherapy-Induced Peripheral Neuropathy (CIPN)

In patients being treated for cancer, neuropathies are usually caused by therapy. In addition to the cumulative toxicities observed with vinca alkaloids, taxanes, platinum drugs, bortezomib, and thalidomide [56], several other paradigms need to be considered.

17.3.2.1 Acute Effects of Chemotherapy

Effects are observed with oxaliplatin [57], and taxanes can in some cases produce acute effects. In addition to acute effects, oxaliplatin also develops cumulative toxicity. A new class of drugs, delivering neurotoxic agents by targeted antibodies, as brentuximab or ado trastuzumab emtansine, are under evaluation.

17.3.2.2 Late Effects

There are increasing reports of the late effects of chemotherapy [58]. These manifest in persisting neuropathic symptoms as pain and Raynaud's syndrome.

17.3.2.3 Autoimmune Effects

Autoimmune diseases and inflammatory neuropathies have appeared in therapies with immune checkpoint inhibitors [59, 60].

17.3.2.4 Mimics

In addition to the differential diagnosis, the clinician must also be aware of local cauda compression, acute myopathies, and electrolyte disorders.

17.4 Conclusions

Peripheral nerves are a target for paraneoplastic diseases. In neuropathies, there is a clear predominance of SSN with well-established clinical features and oncological and immunological associations.

However, the association between onconeural antibodies and a specific clinical picture and outcome remains to be demonstrated. In addition, no randomised clinical trials have been conducted on the treatment of SSN, and it is general (but not evidence based) opinion that the best treatment opportunity for these cases is through early tumour detection [61].

Paraneoplastic vasculitic and dysautonomia are rare and documented. They may fail to respond to immunotherapy and rarely improve with cancer treatment.

The occurrence of other neuropathies as sensory, sensorimotor, and also inflammatory neuropathies still not resolved, except some small series in lymphoma.

In the presence of a neuropathy associated with a monoclonal gammopathy, a combined neurological and haematological approach is recommended to exclude any underlying malignancies. IgM monoclonal gammopathy of undetermined significance (MGUS) neuropathies are mainly demyelinating, more sensory than motor in character, slowly progressive, and sometimes associated with anti-MAG antibodies. The underlying tumour may be Waldenström's macroglobulinaemia. The neuropathies associated with IgG or IgA MGUS present with a CIDP- like course, but multiple myeloma, solitary plasmacytoma, and POEMS should be ruled out. Treatment depends on both the severity of the neuropathy and the haematological condition.

Acquired NMT is a generalised peripheral nerve hyperexcitability disorder. The characteristic clinical picture is of muscle stiffness, twitching (fasciculations) and/or rippling (clinical myokymia), painful cramps, impaired muscle contraction and muscle weakness. The limb or limb and trunk muscles are most frequently affected, a specific form "limb myotonia" has been described. Association with anti-CASPR2 antibodies has been described. Not all cases of myotonia are paraneoplastic.

LEMS is an autoimmune disorder of paraneoplastic and non-paraneoplastic origin. LEMS patients should thus be screened not only for VGCC antibodies but also for onconeural and SOX1 gene (protein coding) to search for a paraneoplastic origin.

MG is a paraneoplastic disease in 10–15% of cases in thymoma. Usually, AchRs-positive patients with severe disease are detected.

The relation of cancer and the muscular system (muscle) is discussed in Chaps. 6 and 7.

The relation of cancer and the neuromuscular system is important in clinical practice, and is within the primary responsibility of a neurologist to identify neuromuscular disorders of paraneoplastic origin, not only to detect cancer, but also being able to treat the often debilitating neurological diseases.

The meta level is the relation of the tumours and the neuromuscular system, which seems to be diversified into several mechanisms as onconeuronal antibodies, surface antibodies, antibodies within the paraproteinaemias, antibodies against muscle tissue and no detectable antibodies, as in cachexia, and in the often indolent late-stage neuropathies of cancer patients.

The primary clinical tool is the complex neurological investigation, aided by electrophysiology and increasingly imaging.

Further studies are warranted (a) to better characterise the relationship between sensorimotor neuropathies and malignancies after ruling out all potential confounding conditions related to treatment toxicity and (b) to better characterise the oncological profile of patients with paraneoplastic neuropathies in addition to the consolidated relationship with small-cell lung cancer.

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Diabetic Neuropathy

18

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18.1 Definition

Diabetic peripheral neuropathy (DPN) is defined as the presence of peripheral nerve dysfunction in people with diabetes mellitus (DM) after the exclusion of other causes of neuropathy [1].

18.2 Epidemiology

DM is by far the most common cause of neuropathy. Prevalence estimates may vary depending on differences in inclusion criteria; approximately 54% of patients with type 1 DM (T1DM) and 45% with type 2 DM (T2DM) disclose symptoms and/or signs of neuropathy [2]; DPN is present in up to 10% of patients at the time of diagnosis and up to 50% after 20 years history of diabetes.

Duration and severity of hyperglycaemia represent modifiable risk factors for the development of neuropathy; indeed, enhanced glycaemic control was shown to

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reduce the prevalence of clinical neuropathy by 60–70% in T1DM [3] and by 7% in T2DM [4].

Diabetes is a clear risk factor for nondiabetic neuropathies; for example, the incidence of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is 11-folds higher in diabetic patients than in nondiabetic individuals.

The link between prediabetes (impaired fasting glucose or impaired glucose tolerance (IGT) [5] needs further scientific proofs; indeed, the prevalence of "idiopathic peripheral neuropathy" was found to be three times higher in IGT than in normal age-matched controls [6]. Additional studies are ongoing in order to identify modifiable risk factors within the metabolic syndrome that may influence the development and progression of DPN [5].

18.3 Clinical Subtypes

DPN encompasses all patterns of neuropathy (Table 18.1) with significant overlaps and combinations between different clinical subtypes.

Distal symmetrical polyneuropathy (DSP). DSP is the most common syndrome [7]. It may become symptomatic several years after the onset of T1DM, or it may reveal an underlying T2DM. DSP may be entirely asymptomatic despite of minimal signs and clear electrophysiological changes.

DSP is a length-dependent neuropathy dominated by sensory disturbances with stocking-glove distribution: early symptoms begin in the toes and extend over the feet; in more progressed cases, the sensory loss extends above the knees and involve the fingers, hands, and forearms; eventually, sensation over the anterior chest and abdomen may become also affected due to involvement of the shorter sensory axons in intercostal nerves. Motor impairment is usually minor and is mainly distributed in the distal lower limb muscles.

The syndromic spectrum of DSP varies accordingly the prevalent involvement of the large or small fibres. In the large-fibre variant, the neuropathy manifests with painless paraesthesias, impairment of light touch sensation, sensibility to pressure and vibration and joint position sense and with diminished stretch reflexes. Large-fibre DSP may result in increased instability with sensory ataxia and positive Romberg's sign; however, the severest diabetic pseudotabes picture (loss of joint sensations, severe lancinating pain and pupillary abnormalities) is uncommon nowadays.

Small-fibre neuropathy (SFN) is increasingly recognized as an early manifestation of DSP. New SFN cases are often associated with prediabetes. Selective SFN is a "pseudo-syringomyelic" neuropathy which spares the large-fibre functions. SFN manifests with spontaneous pain and diminished thermal and pain perception. Severest SFN results into painless burns, persistent foot ulcers and neuropathic osteoarthropathy and is often accompanied by prominent autonomic neuropathy.

Diabetic autonomic neuropathy (DAN) is usually associated with predominant SFN; it may range from subclinical functional impairment of cardiovascular reflexes and sudomotor function to overt symptoms and severe life-threatening

Table 18.1 Clinical patterns of diabetic neuropathy and differential diagnoses

Diabetic neuropathy	Differential diagnosis[a]
Symmetrical neuropathies	
Distal symmetrical polyneuropathy	Vitamin B12 deficiency. Alcohol. Chemotherapy. CIDP. Monoclonal gammopathies
Small-fibre neuropathy	Idiopathic. Connective tissue diseases. Vitamin B12 deficiency. Monoclonal gammopathies. HIV infection. HCV infection. Celiac disease. Chemotherapy
Diabetic autonomic neuropathy	Hereditary or haematological amyloidosis. Sjögren syndrome. Immune-mediated autonomic neuropathies (anti-ganglionic AchR antibodies). Paraneoplastic neuropathies (anti-Hu antibodies; anti-PAC2 antibodies). HSAN
Symmetrical, episodic painful neuropathies	
Treatment-induced neuropathy	
Diabetic neuropathic cachexia	
Asymmetrical focal/multifocal neuropathies	
Limb mononeuropathies	Other compression/entrapment neuropathies
Mononeuritis multiplex	Systemic or nonsystemic vasculitides. Peripheral neurolymphomatosis
Proximal motor (or radiculoplexopathy, or diabetic amyotrophy, or Bruns-Garland syndrome)	Systemic or nonsystemic vasculitides. Neoplastic or infectious polyradiculoneuropathies. Radiation-induced plexopathy. Compressive radiculopathies. Spinal stenosis. Idiopathic plexopathies
Truncal neuropathy	Intrathoracic, intra-abdominal or intraspinal diseases. Herpes zoster
Cranial neuropathies	Compressive cranial neuropathies

CIDP chronic inflammatory demyelinating polyradiculoneuropathy, *Anti-PAC2* anti-type 2 Purkinje cell antibody, *HSAN* hereditary sensory autonomic neuropathy

[a] Note that up to 10% of cases of peripheral neuropathy in diabetic patients is not related directly to DM. Since the diagnosis of diabetic peripheral neuropathy requires the exclusion of other causes, clinically convergent neuropathies should be considered and ruled out. CIDP and neuropathies related to vitamin B12 deficiency, hypothyroidism and uraemia also occur more frequently in diabetic patients than in nondiabetic; metformin is associated with higher risk of vitamin B12 deficiency with hyperhomocysteinaemia

complications. The incidence of DAN increases with patients' age, disease duration and poor glycaemic control, but subclinical autonomic changes may occur within a year of diagnosis of T2DM and within 2 years in T1DM. Cardiovascular autonomic neuropathy (CAN) manifests with early resting tachycardia (due to early involvement of longest parasympathetic fibres), exercise intolerance and postural hypotension (with possible postural syncopes) which may be aggravated by diarrhoea or by tricyclic antidepressant used for pain treatment. Gastrointestinal dysfunction includes asymptomatic or symptomatic gastroparesis (which may concur to poor glycaemic control inducing insulin-related hypoglycaemia), constipation or, rarely, explosive diarrhoea. Intensive insulin treatment may be complicated also by blunted

sympathetic response to hypoglycaemia with unawareness of hypoglycaemia. Genitourinary dysfunction with impotence (erectile failure and retrograde ejaculation) is often the first manifestation of DAN in diabetic men (prevalence between 27 and 75%); concurrent causative factors include vasculopathies and hormonal alterations. Urinary dysfunction due to bladder atony and impaired bladder sensation may lead to urine retention, dysuria, nocturia, incomplete bladder emptying and urgency up to overflow incontinence. Sudomotor abnormalities cause distal anhidrosis with skin's dryness, compensatory facial and truncal sweating and heat intolerance; gustatory sweating refers to profuse sweating in face and forehead immediately after food intake. Pupillary abnormalities such as sluggishly reactive pupils to light or Argyll Robertson-like pupils may be detected in up to 20% of diabetic patients. Finally, reduced peripheral and central chemosensitivity to hypoxia and altered bronchomotor tone in lung may concur to explain the higher prevalence of sleep apnoea syndrome in DM.

Episodic painful neuropathies are rare. *Treatment-induced neuropathy* develops with acute burning pain in the distal lower limbs in T1DM or T2DM patients after first achievement of tight glucose control by insulin ("insulin neuritis"), oral hypoglycaemic medications or severe dietary restriction. Pain has marked nocturnal exacerbations, may persist for weeks/months before spontaneous resolution, and may affect areas such as the trunk and abdomen. Treatment-induced neuropathy is a usually length-dependent SFN often accompanied by autonomic dysfunction in the absence of relevant neurological signs besides hyperalgesia and allodynia. Proposed pathogenetic mechanisms include endoneurial ischaemia, hypoglycaemic microvascular neuronal damage and regenerating nerve firing. *Diabetic neuropathic cachexia* is an acute, severe, symmetrical, painful neuropathy with allodynia, associated with marked unintentional weight loss, depression, insomnia and impotence in males. The disorder may affect patients with T1DM or T2DM irrespectively of the duration of the disease. The condition is reversible over weeks to months after adequate diabetic control.

Limb mononeuropathy. Compression or entrapment neuropathies of the median, ulnar or peroneal nerves have an increased frequency in DM. Approximately a quarter of patients have electrophysiological signs of carpal tunnel syndrome (CTS) and 5–10% are symptomatic for CTS. Rarer mononeuropathies with abrupt painful onset followed by weakness and atrophy are caused by nerve infarction from occlusion of *vasa nervorum*; the sequential involvement of two or more nerves is known as *mononeuritis multiplex*.

Lumbosacral radiculoplexopathy (LSRP). LSRP (alias diabetic amyotrophy or Bruns-Garland syndrome) affects the lumbosacral or more rarely the cervical plexus. LSRP is unrelated to the duration of disease or degree of glucose control, and it may develop prior to diagnosis of T2DM in middle- or old-aged men with concomitant weight loss. Onset is acute or subacute, with burning or lancinating pain in the back, hip or thigh, spreading to involve the entire limb; after days or weeks pain is followed by difficulty in walking and climbing stairs with wasting and weakness of the quadriceps, iliopsoas, gluteus and, to a lesser extent, hamstring and anterior tibialis muscles; knee and ankle jerks are usually lost. In some cases, the

opposite leg may become affected after days or months. Progression may be steady or stepwise and may continue for months before the disease stabilizes. Up to 60% of cases overlap with DSP and disclose a more gradual onset.

Truncal radiculopathy is usually unilateral, affects spinal roots from T4 through T12 and manifests with pain and dysaesthesias possibly associated with bulging of abdominal muscles and focal anhidrosis.

Cranial neuropathies. Acute unilateral oculomotor palsies affecting the third or sixth nerves are common; facial Bell's palsy also appears more common in diabetics than in nondiabetic individuals. The third nerve involvement is likely ischaemic since pathological cases disclosed a centrofascicular lesion in the intracavernous portion sparing the peripheral parasympathetic fibres [8]. Onset is abrupt and may be heralded or accompanied by transient pain in the frontal head or behind the affected eye; progression develops over 1–2 days and results into a nearly or fully complete dysfunction with diplopia, dysconjugate gaze and ptosis; pupillary reaction to light is typically (but not always) spared; spontaneous recover ensues within 2–3 months but relapses on the opposite sites can occur.

Six-month prognosis does not differ significantly from Bell's palsy in individuals without diabetes; treatment with steroids should be cautious.

Young patients with poorly controlled T1DM may develop a mild form of anterior ischaemic optic neuropathy.

18.4 Complications

Neuropathic pain occurs in up to 21% of diabetic patients without a clear relation to age, diabetes duration, metabolic control or severity of neuropathy. Pain may develop in the absence of relevant clinically and neurophysiological findings and may be highly disabling leading to sleep disturbances, anxiety and significant functional limitations [9]. Pain is spontaneous, intermittent or continuous, and usually worse at night making uncomfortable the touch of feet against bedclothes. Pain is symmetrically localized in toes and may progress with a stocking-and-glove distribution; it is usually burning, electric or stabbing (but it may be aching and deep in the feet, especially on weight bearing) and associated to dysaesthesias, hyperalgesia and allodynia to light touch.

Acrodystrophic changes are a major complication of DSP-SFN. Calluses or phlyctenular lesions in feet usually precede the development of chronic ulcers which may develop in 4–10% of patients (due to sensory loss, unnoticed traumatic tissue damage and vascular insufficiency) and may be complicated by chronic osteomyelitis. In some cases, idiopathic bullae (*bullosis diabeticorum*) of hands may develop before plantar ulcers. *Neuropathic arthropathy* (diabetic Charcot foot) involves mainly the small joints with painless fractures of metatarsal bones, disruption or articular surfaces and disorganization of metatarsophalangeal joints.

Falls. Sensory ataxia and postural hypotension are major factors for increased risk of falls in diabetic patients together with retinopathy and vestibular dysfunction.

Cardiac mortality. Increased risk of mortality results from complications of CAN such as increased occurrence of asymptomatic ischaemia and impaired hypoglycaemic awareness, as well as from other diabetic complications such as coronary artery disease, stroke and diabetic nephropathy.

18.5 Pathology and Pathophysiology

The pathological process differs in different clinical subtypes.

DSP. Sural nerve biopsy (Fig. 18.1) discloses variable loss of myelinated fibres and unmyelinated fibres and axonal degeneration; there may be some degree of primary demyelination, proliferation of Schwann cells (SC) and onion-bulb formation. Endoneurial capillaries show signs of microangiopathy with hyperplastic endothelial cells and thickening of the capillary wall and basal lamina. Dying-back

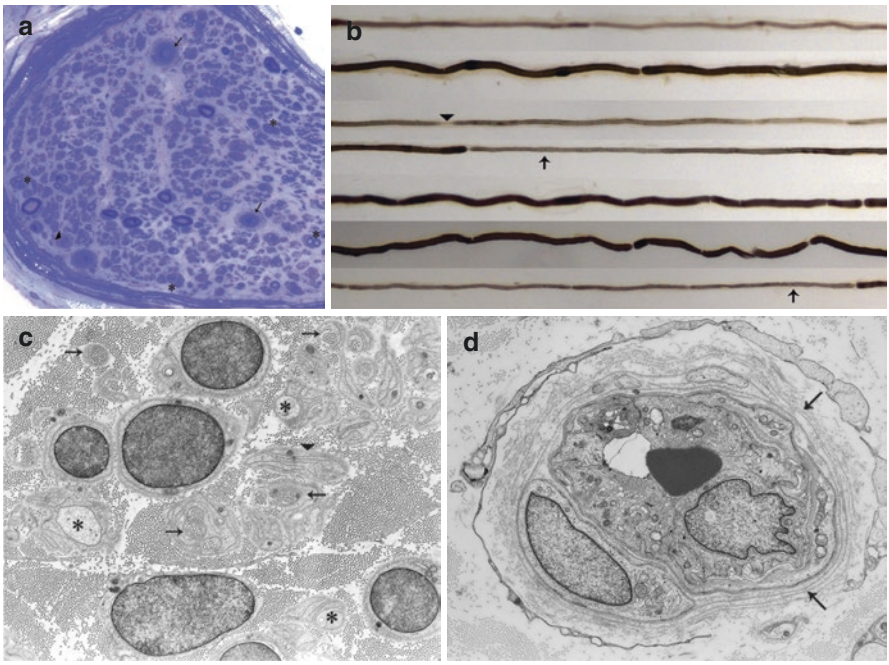

Fig. 18.1 Sural nerve biopsy from a patient with diabetic DSP. (**a**) Semithin section (original magnification 20×) showing loss of myelinated fibres, two endoneurial capillaries with thickened walls (*arrows*), some regenerative clusters made by small fibres (*asterisks*) and a small onion bulb indicative of repeated demyelination (*arrowhead*). (**b**) Teased fibres (original magnification 20×) showing contiguous portions of myelinated fibres with paranodal demyelination (*arrowhead*) and segmental remyelination (*arrows*). (**c**) Electron micrograph (transverse section, original magnification 4400×) showing severe loss of unmyelinated fibres with collagen pockets (*arrows*) and denervated Schwann cell processes (*arrowhead*); few unmyelinated fibres are identifiable (*asterisks*). (**d**) Electron micrograph (transverse section, original magnification 3000×) showing an endoneurial capillary with multiple layers of reduplicated basal lamina (*arrows*)

centripetal axonal degeneration may be evident with regeneration subsequent to degeneration of the distal axons; fibre degeneration predominates distally correlating with diffuse abnormalities of nerve conduction velocities (NCV) with a proximo-distal gradient; this distal "dying-back" pattern is consistent with a metabolic disturbance. Loss of nerve fibres may be multifocal suggesting that microangiopathy also plays a causative role.

SFN. Skin biopsy reveals a selective reduction of thinly myelinated Aδ- and unmyelinated C-fibres. The morphometrical analysis of intraepidermal nerve fibres (IENF), a widely validated diagnostic method, demonstrates a reduction of unmyelinated sensory endings which represent the most distal nociceptors [10]. The loss of IENF occurs usually in a length-dependent manner as shown in skin from distal leg and thigh and may be associated with morphological abnormalities such as axon swellings, varicosities, branching and sprouting (Fig. 18.2). These abnormalities may be present early in the course of diabetic neuropathy and are considered pre-degenerative changes [11]. New promising methods of analysis will provide an accurate quantitation of other nerve fibre subtypes such as autonomic dermal fibres to sweat glands,

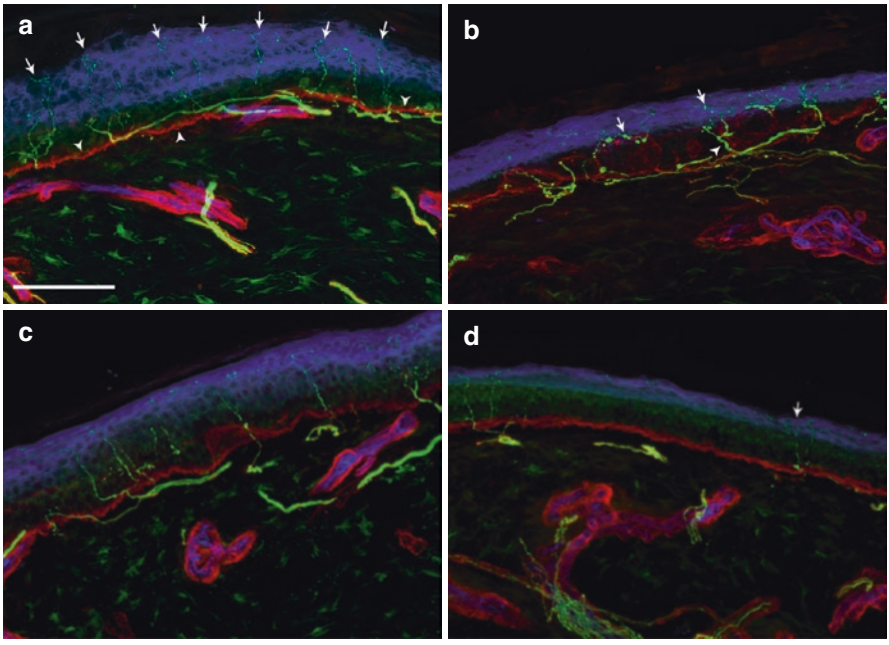

Fig. 18.2 Confocal images showing a length-dependent loss of intraepidermal nerve fibres in a diabetic patient (**b**, **d**) compared to a healthy control (**a**, **c**). Intraepidermal nerve fibres (IENF *arrows* in **a**) are regularly distributed with higher density in the proximal site (thigh, **a**) compared to the distal site (leg, **c**). In the diabetic patient, there is a loss of IENF more severe in the distal site (**d** compared to **b**) with evidence of morphological abnormalities such as axon swellings (*arrowhead* in **b**), varicosities (*arrows* in **b**) and branching (*arrow* in **d**). Nerve fibres are in *green* (protein gene product 9.5); basal membranes and vessels are in *red* (collagen IV); epidermis and endothelia are in *blue* (*Ulex europaeus*, agglutinin). Scale bar = 100 μm

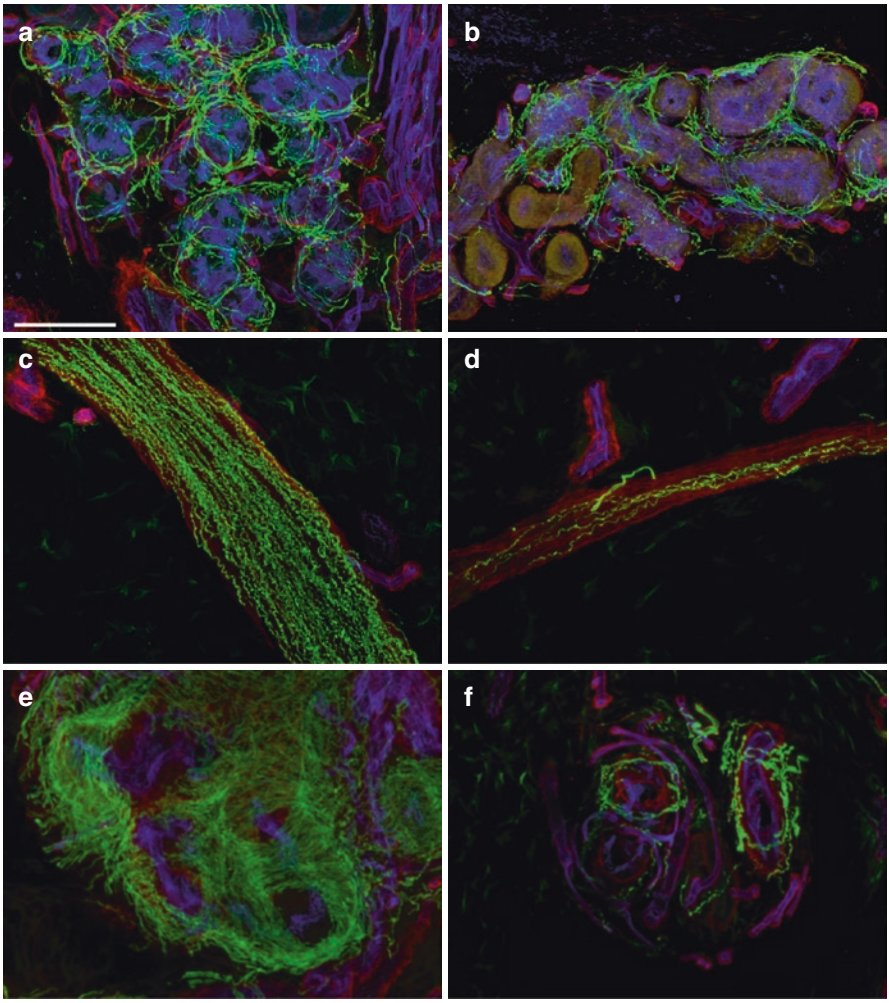

Fig. 18.3 Confocal images showing cutaneous autonomic innervation to sweat gland (**a**, **b**), arrector pili muscle (**c**, **d**) and arteriovenous anastomosis (**e**, **f**) in a healthy control (**a**, **c**, **e**) and in a diabetic patient (**b**, **d**, **f**). In the diabetic patient, there is a loss of sudomotor (**b** compared to **a**), pilomotor (**d** compared to **c**) and vasomotor (**f** compared to **e**) nerve fibres. Nerve fibres are in *green* (protein gene product 9.5); basal membranes and vessels are in *red* (collagen IV); epithelia and endothelia are in *blue* (*Ulex europaeus*, agglutinin). Scale bar = 100 μm

arrector pilorum muscles and vessels (Fig. 18.3); in diabetic neuropathy, the depletion of autonomic fibres correlates with sweating impairment [12]. SFN can also be detected at the corneal level by in vivo corneal confocal microscopy (CCM) which analyses the density of both Aδ- and C-fibres of trigeminal origin; despite of the shortness of corneal nerves, CCM is able to detect the length-dependent diabetic SFN even at early stages, and it may represent a reliable non-invasive marker of SFN and a possible surrogate endpoint for nerve fibre regeneration [13].

With regard to *diabetic pain*, loss of IENF itself is neither sufficient nor necessary to result into neuropathic pain and additional/alternative functional factors may play an important role [14]. Spontaneous and paroxysmal pain in the absence of any external stimulus is caused by ectopic impulse generation within the nociceptive pathways due to altered functions of ion channels. Hyperglycaemia and activation of the polyol pathway can induce hyperexcitability through reduced pump activity of NA+/K+ ATPase, altered distribution and functioning of the $K_V1.2$ potassium channel subunit and increased of persistent sodium currents due to upregulation of $Na_V1.7$ and $Na_V1.8$ in nociceptive C-fibres. Upregulation of the transient receptor potential V1 associated with uninjured C-fibres may also be involved causing spontaneous nerve activity induced by normal body temperature [14]. Following the genetic characterization of mutations in the SCN9A ($Na_V1.7$), SCN10A ($Na_V1.8$), SCN11A ($Na_V1.9$) in a wide spectrum of familial painful neuropathies/disorders as well as in idiopathic SFN, it may be hypothesized that several genetic factors may modulate the expression of neuropathic pain in the diabetic population.

18.5.1 Multifocal Neuropathies

In patients with painful proximal neuropathies (LSRP), biopsy of the affected nerves (e.g. the intermediate cutaneous nerve of the thigh) indicated vasculitis of perineurial and endoneurial vessels as the main pathological process.

18.6 Risk Factors and Pathogenesis

DPN results from metabolic dysfunctions, ischaemic factors and inflammation; metabolic dysfunctions prevail in length-dependent DSP/SFN, whereas altered microcirculation and superimposed inflammation prevail in focal neuropathies. The complexity of pathogenesis limits suitable animal models that recapitulate both chronic and/or acute damages leading to DPN [15]. The best model should represent major pathogenetic pathways, should be sensitive to antidiabetics and to antineuropathic drugs and should be suitable for pathogenetic investigations and therapeutical screenings. Better models include the C57BL/Ks (db/db) mice, streptozotocin-induced C57BL6/J and ddY mice, spontaneously diabetic WBN/Kob rats, nonobese diabetic mice, spontaneously induced Ins2 Akita mice and leptin-deficient (ob/ob) mice; high-fat diet-fed female C57BL6/J mice might be suitable models for prediabetic or obesity-related diabetic neuropathy.

Metabolic players include hyperglycaemia, dyslipidaemia and changes in insulin signalling [16].

In either T1DM and T2DM, chronic hyperglycaemia increases oxidative stress by glucose auto-oxidation, production of advanced glycosylation end products (AGEs) and activation of the polyol pathway, protein kinase C (PKC) and poly (ADP-ribose) polymerase (PARP). Enhanced glycolysis causes an overload of the mitochondrial electron transport chain, generating reactive oxygen species (ROS)

which lead to DNA damage and stress of the endoplasmic reticulum (ER). AGEs form through the attachment of reactive carbohydrate groups to proteins, lipids and DNA; AGE peptides bind and activate the cell surface receptor (RAGE) on monocytes and endothelial cells, thus increasing the production of cytokines and adhesion molecules and initiating inflammatory signalling cascades; furthermore, AGE receptor ligation can activate the transcription of the pleiotropic factor NF-κB and contribute to production of various pro-inflammatory mediators. Enhanced flux of the polyol pathways by the excess glucose, which is reduced to sorbitol by aldose reductase (AR) and then to fructose by sorbitol dehydrogenase, causes depletion of the NADPH needed for regeneration of the antioxidant glutathione. Glycolytic intermediates derived from excess glucose, which is shunted into the hexosamine pathway, also contribute to the inflammatory injury. Enhanced activity of PKC isoforms promotes inflammation through the activation of various signalling mechanisms such as mitogen-activated protein kinases (MAPK) and NF-κB.

T2DM-associated dyslipidaemia contributes to the pathogenesis of DPN through various mechanisms: free fatty acids may be toxic to Schwann cells (SC) and promote inflammation; modified (oxidized or glycated) plasma lipoproteins (especially LDL) can bind to extracellular receptors, such as oxidized LDL receptor 1 (LOX1), toll-like-receptor 4 (TLR4) and RAGE, and can activate several signalling cascades leading to oxidative stress.

Since insulin functions as a neurotrophic factor to peripheral neurons, insulin deficiency in T1DM and insulin-resistance in T2DM may concur to the pathogenesis by compromising nerve viability and repair.

All the metabolic alterations described above converge producing complex mitochondrial dysfunction, DNA damage and ER stress, in neurons (axons and nerve terminals), SC and endothelial cells of the nerve microvasculature that, in turn, promote macrophage activation and inflammation [17].

It should be noted that all metabolic pathways and molecular players involved in DPN (Na+ and K+ channels, mitochondria, insulin receptors and insulin-independent glucose transporters which facilitate the diffusion of glucose into neurons and SC) are particularly enriched at Ranvier's node and paranodal region where the greatest cross talk between SC and axons occurs that could contribute to the mixed pattern of axonopathy and schwannopathy [18].

18.7 Diagnosis and Laboratory Investigations

18.7.1 Diabetes and Prediabetes

DM is defined by a 2-h plasma glucose ≥200 mg/dL during an oral glucose tolerance test, fasting glucose ≥126 mg/dL or glycosylated haemoglobin (HbA1c) ≥6.5%. Prediabetes is a fluctuating and reversible state which identifies patients who are at an elevated risk for developing DM; it is defined by impaired fasting glucose (fasting plasma glucose between 100 and 125 mg/dL) or IGT (2-h glucose value in an oral glucose tolerance test of 140–199 mg/dL). An HbA1c range of 5.7–6.4% can also be used to identify prediabetes [19].

18.7.2 DSP and SFN

According to the American Diabetes Association, all patients should be screened clinically for DSP at the diagnosis of T2DM and 5 years after the diagnosis of T1DM and at least annually thereafter [1]. The examination should include ankle reflexes as well as pinprick, temperature and vibration perception (using the 128 Hz graduated Rydel-Seiffer tuning fork) and pressure sensation (using the 10-g Semmes-Weinstein monofilament). Sensations should be assessed at each foot at the hallux and metatarsal heads 1, 3 and 5. With regard to symptoms, a number of validated questionnaires may help practitioners to diagnose correctly neuropathic pain [20].

There is no uniform consensus regarding the diagnostic criteria. According to Boulton et al. [1], the diagnosis of DPN should include at least two of the following abnormalities: symptoms and/or signs of neuropathy, nerve conduction (NC) changes or altered quantitative sensory testing (QST). According to Tesfaye et al. [21], the diagnosis of DSP can only be "possible" or "probable" when NC changes are lacking. NC and quantitative tests are required for clinical trials or epidemiological studies, but are usually not mandatory in the clinical routine unless the diabetic origin is uncertain, the presentation atypical or a coexisting CIDP suspected.

Conventional neurophysiology may be normal if only small-diameter fibres are damaged. Usually, bilateral and symmetrical abnormalities of NC studies and electromyography (EMG) are first detected in the sensory nerves of feet with decreased amplitudes or absence of the sensory nerve action potentials (SNAP) and, less frequently, of sensory NC velocity (SNCV); subsequently, compound motor action potentials (CMAP) in feet decrease together with mildly prolonged distal motor latencies (DML) and F-waves and with mildly slowed motor NC velocities (MNCV). Initially, needle EMG discloses abnormal spontaneous activity and enlarged polyphasic motor units with decreased recruitment. NC changes in the feet are frequently associated with clinically silent signs of compressive neuropathy of the ulnar and median nerves.

Although several new functional tests have been developed for analysing SFN, their cost-effectiveness and limited standardization should be fully weighed up in the everyday practice [10].

QST is a psychophysical examination of sensory functions which are tested by different thresholds and suprathreshold stimuli (mechanical, pressure, vibration, cold, warm, heat, cold, pain, heat pain) and may be a useful tool in diagnosing SFNs. Besides difficulties in standardizing methods and equipment (there are over 15 devices available commercially), QST needs a full cooperation of patients and has a relatively poor ability to localize the peripheral or central sites of the somatosensory dysfunction [22]. New nociceptive evoked potentials have mainly a clinical-research role; they include the laser-evoked potentials (LEP) or contact heat-evoked potentials, which activate the Aδ- and C-fibres, and the pain-related evoked potentials (PREP), which activate the Aδ-fibres. Although LEP are independent of patient's attention, they cannot discriminate between the peripheral or central pathways related to pain and temperature.

Skin biopsy is a minimally invasive method that provides a reliable and objective measure of small-fibre involvement [23]. The reduction of IENF is a widely recognized diagnostic criterion of definite SFN in diabetic patients with normal SNCV and length-dependent symptoms/signs of small-fibre damage [21]. Skin biopsy, repeated overtime, allows to study the natural course of the neuropathic process and eventually the regenerative response to therapies. Using this tool, an impaired ability of epidermal axons to regenerate has been demonstrated in diabetic patients after chemical axotomy induced by topical application of capsaicin [24]. Interestingly, an increased IENF density has been found in patients with impaired glucose tolerance [25] and with diabetes [26] after changes of lifestyle and supervised exercise intervention.

CCM is still limited to specialized centres and has mainly a research application.

18.7.3 DAN

Screenings for autonomic dysfunction are based on careful examination and history as well as on testing of cardiovascular reflexes especially in at-risk patients with poor glycaemic control, cardiovascular risk factors and macro-/microangiopathic complications. Testing includes postural blood pressure and measure of heart rate variability (HRV) to deep breathing, standing and Valsalva manoeuvre. Bladder function should be investigated by a urodynamic study in patients with recurrent urinary tract infections or incontinence. Erectile dysfunction not responding to phosphodiesterase-5 inhibitors should be examined to assess the penile, pelvic and spinal nerve function. In sudomotor dysfunction, the quantitative sudomotor axon reflex test (QSART) may detect distal SFN with a sensitivity of 75%.

18.7.4 Asymmetrical Focal/Multifocal Neuropathies

NC studies disclose a multifocal axonal pattern of involvement. In LSRP, EMG discloses low femoral-nerve CMAP, prominent fibrillation potentials in thoracic and lumbar paraspinal muscles and active denervation in affected muscles. ESRs and cerebrospinal fluid (CSF) proteins are usually increased. MRI of lumbar spine and lumbosacral plexus may rule out structural radiculoplexopathies and demonstrate signs of inflammation.

18.7.5 Other or Additional Causes of Neuropathy

The diagnosis of DPN involves the exclusion of nondiabetic causes. Investigations should be guided by clinical findings. Coexistence of DPN and CIDP should be investigated by NC studies, MRI of spine, roots and plexuses, nerve ultrasound, CSF analysis and, in selected cases, sural nerve biopsy following the diagnostic guidelines for CIDP [27].

18.8 Therapy

18.8.1 Disease-State Modifiers

Enhanced glucose control is the only disease-modifying therapy for DPN. This statement has a robust evidence in several randomized control trials (RCT) conducted in T1DM with more tight insulin dosing [3, 28, 29], but is weak in T2DM: recent meta-analysis of RCT [30] and a Cochrane review [31] found no significant improvement of T2DM DPN under intensive glycaemic control; nevertheless, besides real differences of T1DM/T2DM trajectories, studies in T2DM were not designed specifically to assess the neuropathy carefully. The effects of pancreas or islet-cell transplantation on DSP in T1DM received limited studies; pancreas transplantation was not accompanied by significant changes in electrophysiology, QST and IEFND (although there was significant corneal regeneration in 15 patients 6 months after the procedure [32]); islet-cell transplantation demonstrated some improvement in neurophysiological end points but not in skin biopsy findings [33].

α-Lipoic acid (ALA) had been found to be well tolerated and capable of providing a clinically meaningful improvement of both positive symptoms and neuropathic deficits when administered at 600 mg/day i.v. [34]; nevertheless, the combination of parenteral (600 mg daily for 3 weeks) and oral therapy (600 mg 3 times daily for 6 months) administered over 7 months [35], as well as a 4-year placebo-controlled, randomized, double-blind trial [36], reported no clinical or neurophysiological improvements; current guidelines by the American Academy of Neurology (AAN) and European Federation of Neurological Sciences (EFNS) do not support the use of ALA [37, 38].

Modifiers of the polyol pathway such as aldose reductase inhibitors (ARI) have been investigated thoroughly; only epalrestat is commercially available in India and Japan. A Cochrane meta-analysis which included 32 trials found no overall significant difference between the treated and control groups although one subgroup (4 trials with tolrestat) favoured treatment; furthermore, three ARIs had dose-limiting adverse effects that caused withdrawal of their use in humans: liver toxicity with tolrestat, elevation of creatinine with zenarestat and severe hypersensitivity with sorbinil [39].

Other modifier options are under investigation including angiotensin-converting enzyme (ACE) inhibitors, protein kinase C β-inhibitor, medical foods such as folate and B vitamins, insulin C-peptide and lipid-lowering diets or drugs (statins, fenofibrate) [9].

18.8.2 Symptomatic Treatment of Pain

Pain management is essential for improving the quality of life of DPN patients. Several consensus guidelines are available providing the best-evidence practice [37, 38]. Although algorithms may differ in choosing the first-line and the second-line agents, most guidelines include tricyclic agents (TCAs), serotonin-norepinephrine

reuptake inhibitors (SNRIs) and epileptic drugs (AED) within first-line options and opioids within second-line options. The choice of the first drug depends mainly on the side-effect profile related to comorbidities of each patient; the preferred drug should be titrated to the maximum tolerated dose before switching to another first-line drug, to a combination therapy or to a second-line agent; most agents require a 4- to 6-week trial to assess efficacy.

Acetaminophen (paracetamol) does not attenuate neuropathic pain but may be helpful if neuropathic pain is exacerbated by nociception. Nonsteroidal anti-inflammatory drugs (NSAIDs) are often prescribed for short-term analgesia; however, due to potential harmful side effects (e.g. haemorrhage, exacerbation of renal dysfunction), their use has not been studied extensively so that they are not included in common guidelines.

TCA antidepressant (amitriptyline, imipramine, desipramine) were the first medications with proved effect on neuropathic pain in placebo-controlled trials. They have multimodal actions including blocking of serotonin and noradrenaline reuptake, as well as blocking of histaminic, cholinergic and α1-adrenergic receptors and of sodium channels [9]. The heterogeneity of sites of actions explains their multiple side effects (orthostatic hypotension, slow cardiac conduction, cardiac arrhythmias, increased heart rate, drowsiness, weight gain, constipation, dizziness, blurred vision, urinary retention and precipitation of narrow-angle glaucoma). Although side effects may limit the use of TCA, they may be partially overcome by slow titration: the starting dose for amitriptyline is 10–25 mg at night that may be increased by 10–25 mg at night every 3–7 days up to 75 mg.

Serotonin-norepinephrine reuptake inhibitors (SNRI) are also supported by major guidelines [37, 39]. Recommended doses are 60–120 mg daily for duloxetine and 75–225 mg daily for venlafaxine; common minor sides effects include nausea, somnolence and constipation observed usually with higher dosages and can be limited by slow titration starting (e.g. starting dose for duloxetine = 30 mg/day).

The AED gabapentin and the higher potent gabapentin, i.e. pregabalin binds to the α2-δ subunit of calcium channels and inhibit the release of neurotransmitters. Recommended daily doses are 300–600 mg (divided into two doses) for pregabalin and 900–3600 mg (divided into three doses) for gabapentin. Common side effects include somnolence and dizziness; cerebral oedema due to abrupt discontinuation and ankle oedema have been also reported with pregabalin [9]. Among AED, lamotrigine, carbamazepine and oxcarbazepine are not recommended [37, 39].

Opioids are recommended as second-line agents if all the three classes of first-line drugs or their combinations have failed. The use of opioids should be carefully considered taking into account side effects (dose-related respiratory depression, confusion, delirium, bradycardia/tachycardia, orthostatic hypotension, urinary retention, constipation), the long-term adverse effects of dependency and abuse, changes in immunological functioning, suppression of the pituitary axis and risk of aberrant opioid use [9]; such a risk should be evaluated through the use of a validate screening methods such as the Opioid Risk Tool or the Diagnosis, Intractability, Risk, Efficacy score [40]. EFNS recommended controlled-release oxycodone (daily dose 10–60 mg daily) and tramadol (200–400 mg a day or tramadol 37.5 mg with 325 mg

acetaminophen) [38]; AAN recommended controlled-release oxycodone (mean 37.5 mg a day, up to 120 mg a day), morphine (up to 120 mg a day) and tramadol (210 mg a day) [37]. Among new opioid analgesic available to treat severe chronic pain, tapentadol extended release (ER) controls both nociceptive and neuropathic pain by acting through an opioid spinal-supraspinal synergy, as well as through an intrinsic spinally mediated μ-opioid receptor agonist-norepinephrine reuptake inhibitor effect (MOR-INR) [41]. The starting dose is 50 mg twice daily which may be slowly titrated up to 500 mg a day; side effects include commonly headache, drowsiness, dizziness, nausea, constipation and, uncommonly, syncopes and instability.

Topical treatments with capsaicin cream and lidocaine 5% patches may be useful in decreasing focal pain and were included within potential therapeutic options by the AAN guidelines [37]. Botulinum toxin type A, which may provide a relief of neuropathic pain through a modulation on afferent sensory fibre firing, is considered probably effective by EFNS [38] but it is not included by AAN [37].

Among non-pharmacological approaches in managing pain of diabetic neuropathy, transcutaneous electric nerve stimulation can also be considered [42]. There are no sufficient evidences supporting or disclaiming the use of acupuncture in DPN [43].

18.8.3 Treatment of DAN

There is no treatment that can effectively stop or reverse clinically evident DAN. Apart from a preventive role of intensive glycaemic control in T1DM, of multifactorial cardiovascular risk reduction and of lifestyle intervention, definite recommendations cannot be given [44]. Symptomatic orthostatic hypotension may be treated by physical counter manoeuvres and by therapy with the α1-adrenergic agonist midodrine and/or mineralocorticoid fludrocortisone. Symptomatic gastroparesis may be approached with low-fat/fibre diets and prokinetic drugs. Treatment of diarrhoea and constipation is symptomatic; bacterial overgrowth may occur in up to 40% of diabetic patients and should be treated with antibiotics.

18.8.4 Treatment of LSRP

Although some case reports suggest that patients with LSRP may benefit from intravenous methylprednisolone or immunoglobulins in controlling pain and positive neuropathic symptoms, there is no evidence from any trial supporting the use of immunotherapies [45].

Key Points

- Diabetic neuropathies encompass the whole spectrum of peripheral neuropathies and may develop at any time along the natural history of T1DM or T2DM.
- Distal symmetrical polyneuropathy (DSP) is the most common clinical form; all diabetic patients should be screened for DSP at the time of diagnosis and annually thereafter.

- Painful small-fibre neuropathy (SFN) is increasingly recognized as an early manifestation of DSP. SFN may have no abnormalities on nerve conduction studies, and they may be confirmed by skin biopsy, which measures the intraepidermal nerve fibre density, and by sudomotor testing.
- SFN is increasingly recognized in individuals with prediabetes. An oral glucose tolerance test and/or a measurement of HbA1c values should be performed in a patient with otherwise idiopathic neuropathies.
- Cardiac autonomic neuropathy is usually associated with SFN and may lead to life-threatening complications.
- Enhanced glucose control is the only disease-modifying therapy for DSP.
- Neuropathic pain is the most common complication of diabetic neuropathy. First-line agents for the treatment of pain include amitriptyline, venlafaxine or duloxetine, gabapentin or pregabalin or a combination of them. The choice of the first drug depends on the side-effect profiles and comorbidities of each patient.

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Infectious Neuropathies

19

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19.1 Introduction

Infectious agents are common causes of *neuropathy* in endemic areas while they are rarely observed in other regions. However, in the current era characterized by quick and easy migration processes, the knowledge of the main infectious neuropathies is required. In course of infectious diseases, the peripheral nervous system (PNS) may be affected by direct infiltration of peripheral nerves, indirect damage induced by immune and inflammatory responses or by therapy-induced toxicity.

19.2 Hepatitis C Virus (HCV)-Related Neuropathies

PNS is frequently affected in the course of *HCV infection*. Peripheral nerves involvement can be due to a direct viral damage, the presence of *cryoglobulinemia* (CG), and the multiple comorbidities that affect HCV-infected patients. CG is the most frequent extrahepatic manifestation of HCV infection, detectable in up to 50% of patients, and is the most important risk factor for the peripheral neuropathy (PN). Cryoglobulins (CGs) are cold-precipitable immunoglobulins, which deposit in small and medium size vessels and cause ischemic damages, lymphocytic microvasculitis, and/or necrotizing arteritis, with polymorphonuclear cell infiltration. Three types of CG are recognized, type I is a monoclonal immunoglobulin (Ig), type II is a mixture of monoclonal and polyclonal Ig, while type III is composed of polyclonal Ig. "Mixed CG" (MC) is defined by either type II or III CG and in up to 95%

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of cases is associated with chronic HCV/HIV infection. About 17–60% of patients with CG develop peripheral neuropathy, often at disease onset [1, 2]. Neuropathy is rarely seen in patients with HCV infection and type I CG. In our experience, axonal polyneuropathy is the main presented form, pathologically characterized by perivascular infiltrates, endoneurial purpura, and microangiopathy, overall suggesting an ischemic pathogenesis linked to endoneurial microcirculation obstruction [3]. Conversely, in patients with HCV-associated MC, the PNS involvement is frequently observed and is more severe when higher cryocrit is present. *Pathological features* are consistent with a small vessel vasculitic or necrotizing arteritis of medium-sized vessel with consequent ischemic nerve damages (Fig. 19.1) [4]. In the less frequently reported cases of HCV-associated neuropathy without CG, the vascular and perivascular inflammation can be due to a direct HCV damage or HCV-induced autoimmune mechanisms. HCV active replication has never been demonstrated in the PNS and the observation of nonreplicative HCV-RNA in epineurial cells, in close relationship with mononuclear inflammation, is in favor of an immune-mediated pathogenesis [5, 6]. Russi et al. demonstrated the presence of HCV genomic RNA sequences and HCV-E2 viral antigen in sural nerve biopsies from patients with peripheral neuropathy and chronic HCV infection, with and without cryoglobulinemia. These data further support an immune-mediated mechanism in nerve damage triggered by viral infection [7].

Accordingly to previously reported studies, the most common form of neuropathy observed in HCV-infected patients is a length-dependent symmetrical sensory or sensorimotor axonal polyneuropathy, clinically characterized by distal sensory loss and weakness. Other reports describe sensory neuropathy as the most prevalent neuropathic form of HCV infection [8]. Sensory neuropathy can be distinguished in symmetric or asymmetric forms, the latter variants including *large-fiber sensory neuropathy* (LFSN) and *small-fiber sensory polyneuropathy* (SFSN). LFSN usually manifests with sensory loss, paresthesias, numbness, and cramps while SFSN is characterized by burning feet, tingling, restless leg syndrome and sometimes with a

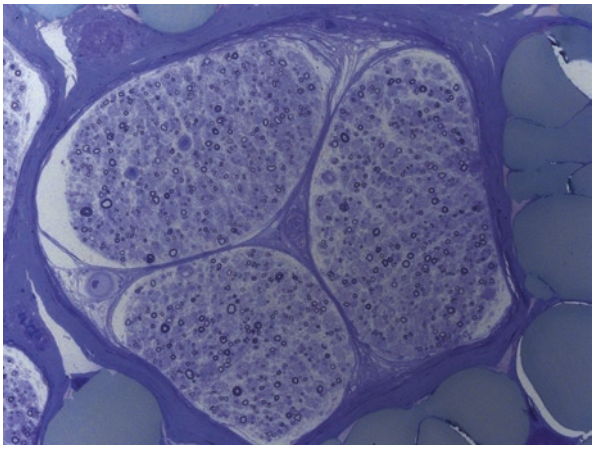

Fig. 19.1 Sural nerve biopsy in a patient with HIV-associated CIDP. Some onion bulb formations are evident in toluidine blue stain (original magnification ×100)

non-length-dependent pattern suggestive of ganglionopathy [9]. In some cases, the damage of both small and large fibers may coexist. *Mononeuropathies* and *mononeuropathy multiplex* (MM) characterized by stocking-glove asymmetric neuropathy or overlapping forms have also been reported. In a prospective clinical, neurophysiological, and skin-biopsy study, Biasiotta and colleagues described 47 subjects with PN and 29 with neuropathic pain in a series of 69 patients with HCV-related CG. The authors describe a prevalence of predominantly sensory distal polyneuropathy and report the relevance of nociceptive pathway damages. They also note an association between neuropathy, age and HCV infection rather than CG [10]. Unusual forms of HCV-associated neuropathy are pure motor polyneuropathies [11] and autonomic neuropathy [12]. Cranial nerves damages have been anecdotally described as an involvement of the abducens, facial, and motor trigeminal nerves. Intriguingly, the spectrum of PN in course of HCV infection is not limited to axonal forms but encompasses a number of demyelinating conditions. The association between *Guillain-Barré syndrome* (GBS) and non-A, non-B hepatitis has been described before the discovery of HCV [13], and, more recently, in patients with chronic HCV infection [14]. *Acute inflammatory demyelinating polyneuropathy* (AIDP), the demyelinating variant of GBS, has been described in a single case with subclinical HCV infection during the pre-convalescent phase [15]. As for chronic forms of neuropathy, few reports describe an association between HCV infection and *chronic inflammatory demyelinating polyneuropathy*-CIDP [16]. This form can also be seen as an uncommon side effect in patients treated with *IFNα* which could have immunomodulating effects as a reduction of proinflammatory cytokines, and, at the same time, could play a major role in favoring immune-mediated mechanisms [17]. A single case of the Lewis-Sumner syndrome, an asymmetric form of CIDP, has been described in course of HCV infection; these patients improved after high-dose intravenous immunoglobulins (IVIG) and methylprednisolone treatment, relapsed after administration of INFα and ribavirin and finally recovered after the discontinuation of INF coupled with IV methylprednisolone [18]. We reported an intriguing association between HCV infection and neuropathy with *anti-MAG* (myelin-associated glycoprotein) antibodies, which is usually reported in association with hematological disorders or, more rarely, with primary amyloidosis, cryoglobulinemic vasculitis, Charcot-Marie-Tooth type 1 or amyotrophic lateral sclerosis and HCV infection [19]. This neuropathy is usually characterized by a distal demyelinating disorder that involves large fibers and manifests as sensory ataxia, mild motor involvement, and hand intention tremor. Significant weakness or small-fiber neuropathy are encountered in few atypical cases. We studied a cohort of 59 consecutive patients with neuropathy and chronic HCV infection who had undergone nerve biopsy. We detected CG in 39 patients (18 cases with axonal polyneuropathy, 11 with overlapping MM, and 10 with MM). In 14 patients, CG has not been detected, but they resulted positive for rheumatoid factor (RF); 10 of them had an axonal polyneuropathy, 1 an overlapping form, and 3 cases a MM. Surprisingly, in 3 of the 6 patients without CG or RF, and IgM monoclonal gammopathy with anti-MAG activity was detected. Nerve biopsy showed loss of fiber and ongoing segmental demyelination with onion bulb formation, mild

perivascular infiltrates of lymphocytes and monocytes at the epineurial level in one case and endoneurial edema and microangiopathy in the other one. IgM and complement deposition have been observed on myelin sheaths. The high association between anti-MAG neuropathy and HCV infection could be the result of the production of monoclonal and polyclonal immunoglobulins triggered by HCV-induced B-cell expansion. These data are supported by the description of a significant association between anti-GM1 gangliosides and anti-sulfatide antibodies and HCV-related PN. As for the treatment of HCV-related neuropathy, *antiviral therapy* is the first therapeutic choice but studies with rituximab have also been performed. After the introduction of direct-acting antiviral agents, sustained virologic response has been achieved in the majority of patients with cryoglobulinemic vasculitis [20]. According to Cochrane review, high quality studies are lacking, and there are insufficient data to make an evidence-based decision [21].

19.3 Human Immunodeficiency Virus (HIV)-Related Neuropathies

PN is considered the most common neurological complication of *HIV infection*, affecting actually 21% of patients, even after the introduction of *combination antiretroviral therapy* (cART) [22]. The increase life expectancy in linked to a prolonged exposure to neurotoxic antiretroviral therapies and their potential complications. Various types of PN have been reported in association with HIV infection, including distal symmetrical polyneuropathy, *toxic neuropathy* induced by antiretroviral drugs, demyelinating neuropathies, mononeuropathy multiplex, diffuse infiltrative lymphocytosis (DILS), and progressive polyradiculopathy [23]. The diagnosis and appropriate treatment of PN in course of HIV infection represent a challenge also for expert neurologist and for consultant in infectious diseases. The difficulty lies in the overlap between the different forms of PN and the frequently unusual clinical presentations.

19.3.1 Distal Symmetrical Polyneuropathy (DSP)

DSP is the most common form of neuropathy in course of HIV infection. Its prevalence in cART era ranges from 20 to 60% [24]. According to some studies, the most important risk factors for DSP are age, height, and stavudine exposure while it seems not associated with decreased *CD4 count* or increased viral load [25]. On the contrary, other studies identify the lower CD4 nadir reached during HIV infection as the leading risk factor for DSP [26]. The clinical presentation of DSP is characterized by a distal symmetric predominantly *sensory neuropathy* with painful feet and hyperpathia. Neurological examination reveals decreased or absent ankle tendon reflexes with impaired pinprick and vibration sensations. Distal muscle weakness is usually mild or absent. According to recent studies, *autonomic dysfunction* including orthostatic hypotension, gastroparesis, diarrhea, constipation, urinary

incontinence, sexual dysfunction, sweating, and pupillary abnormalities are commonly associated with DSP in HIV subjects [27]. Electrophysiological studies may be useful to confirm a length-dependent axonal polyneuropathy with small or absent sural sensory nerve action potentials. It also allows to distinguish between DSP and PNS demyelinating conditions as CIDP. Total Neuropathy Score (TNS) [28] and Brief Peripheral Neuropathy Screen (BPNS) [28] are the main clinical tools used to assess DSP. TNS has been validated in diabetic neuropathy and analyzes both the grading of sensory, motor, autonomic symptoms and signs, and nerve conduction studies. BPNS is a quick and easy clinical score that includes questions about neuropathic symptoms, examination of vibration at great toe and ankle reflexes. The *neuropathological features* of DSP at sural nerve are usually characterized by loss of myelinated and unmyelinated fibers with variable extent of axonal degeneration and macrophage infiltration. Demyelinating features are more rarely observed and are considered secondary to axonal damage. The direct detection of HIV in nerve fascicles has been rarely observed indicating the variable nature of this form [29]. Autoptic series show that fiber loss and axonal degeneration prevail in the distal regions of peripheral nerves rather than in the proximal ones, confirming the length-dependent nature of this polyneuropathy. Distal *skin biopsies* may show a loss of the epidermal nerve fiber that correlate with neuropathic pain scores [30]. The differential diagnosis of DSP always requires the exclusion of other causes of neuropathy such as alcoholism, diabetes mellitus, vitamin B12 deficiency, monoclonal gammopathy, and uremia. Moreover, in course of *antiretroviral therapy*, the patient may present with a clinical picture similar to DSP. Finally, the introduction of cART can induce per se DSP through the toxicity due to antiretroviral transcription reverse inhibitors as didanosine, zalcitabine, and stavudine. DSP associated with antiretroviral therapy has usually a faster onset and a more rapid course compared to HIV-DSP. Actually, the exact cause of axonal damage of small myelinated and unmyelinated nerve fibers in patients with HIV-DSP has not been established. A direct neuronal damage induced by HIV infection is unlikely since neurons do not express CD4 receptor that is required for the entry of the virus into the cells. Soluble HIV viral gene products as gp120 and *viral protein R* (Vpr) were used in experimental models in order to assay neurotoxicity. Gp120 is a coat glycoprotein that mediates the binding and transmission of HIV into cells by interaction with CD4 receptor via C-C chemokine receptor type 5 (CCR5). Vpr is a protein with cytotoxic effects that modulates HIV infectivity and increases oxidative stress**.** Recent in vitro studies suggest a primary role of *macrophages activation* in the induction of sensory neurons damage due to an indirect action of HIV protein gp120 that stimulates macrophages by CCR5 binding. These data are confirmed by the inhibitory effect of gp120-induced tumor necrosis factor gene expression, obtained with maraviroc, a CCR5 antagonist [31]. Another study demonstrates the association between large mitochondrial deletions and sural nerve amplitude and intra-epidermal nerve fiber density in HIV-DSP patients, suggesting a role of these mutations in the pathogenesis of neuropathy [32]. Since pain is the major symptom in patients with HIV-DSP, symptomatic pain management is the main target of current treatment. *Treatments* include the use of different classes of drugs: non-steroidal inflammatory drugs,

topical agents (lidocaine and capsaicin), tricyclic antidepressants (amitriptyline, duloxetine), anticonvulsant agents (gabapentin, pregabalin, lamotrigine), and opioids (oxycodone, morphine, and fentanyl patch). In clinical practice, a combination of different drugs with distinct specific mechanisms of action is frequently needed to achieve relief from neuropathic pain. A meta-analysis of seven randomized double-blind studies demonstrated the efficacy of high-dose (8%) capsaicin patch in single application: 41% of patients with HIV-DSP obtained 30% relief of neuropathic pain, and 7% had complete analgesia starting within few days after treatment and lasting after an average 5 months [33]. *Pregabalin* resulted to be effective in a small placebo-controlled trial but failed to show relief of pain according to a randomized double-blind placebo-controlled trial [34]. As shown in a placebo-controlled trial performed by Abrams and colleagues, smoked *cannabis* produced a significant reduction of pain in DSP [35]. Among the disease-modifying drugs, human recombinant nerve growth factor (hrNGF) seems to be more effective than placebo; however in a more recent open-label study, hrNGF did not cause an improvement in the severity of neuropathy [36]. Given the lack of concordance of the different studies, this drug has been withdrawn in patients with HIV-associated neuropathy. According to literature, other disease-modifying drugs, as acetyl-L-carnitine, prosaptide, and peptide T, did not show significant efficacy in DSP.

19.3.2 Demyelinating Neuropathy

Demyelinating neuropathies as *AIDP* and *CIDP* have been reported in acute and chronic forms of HIV infection. Due to the lack of large series and controlled studies, the incidence of AIDP and CIDP in the course of HIV infection is unknown. AIDP frequently occurs in the early stage of HIV infection, sometimes preceding the diagnosis of AIDS, when the immunosuppression is less pronounced. Even if CIDP may occur in early HIV infection, it frequently manifests in more advanced stages of the disease. Rare cases of *Miller Fisher syndrome* associated with HIV-seroconversion and a patient who developed Miller Fisher/AIDP overlap in the presence of serum anti-GQ1b antibodies have also been reported [37]. In these patients, clinical features, disease course, and neurophysiological findings appear similar to that of HIV-negative patients. In accordance, in AIDP cases the nadir of neurological signs is reached within 4 weeks, whereas in CIDP the neurological impairment progresses for more than 8 weeks and may be relapsing and remitting.

Neurophysiological analysis shows slow conduction velocities, increased distal motor and F-wave latencies, and partial conduction blocks, characteristics of demyelinating neuropathies. At *cerebrospinal fluid* (CSF) examination, high protein content is frequently observed, but at variance with non-HIV inflammatory polyneuropathies, a mild lymphocytic pleocytosis is frequently found. Brannagan et al. reviewed ten cases with HIV-AIDP and observed a CSF with blood cell count of less than 10/mm^3 in 7 cases, concluding that the absence of pleocytosis in AIDP patients does not exclude HIV infection [38]. Like in non-HIV patients, *pathological examination* of peripheral nerve in CIDP cases shows segmental degeneration

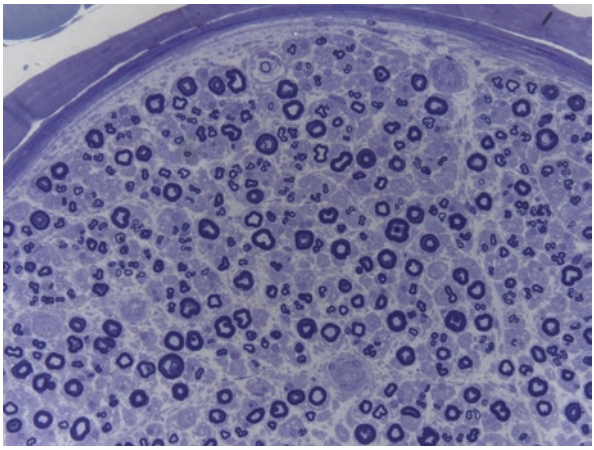

Fig. 19.2 Sural nerve fascicles showing asymmetric loss of myelinated fibers in a patient with cryoglobulinemic neuropathy and HCV infection (toluidine blue, original magnification ×50)

and onion bulb formation, infiltration of mononuclear cell of nerve fascicles, and endoneurial edema (Fig. 19.2). *Treatment* of AIDP includes IVIG and plasmapheresis. Clinical signs of CIDP improve with *steroids*, IVIG or plasmapheresis, but IVIG are considered the treatment with fewer complications. Finally, some data suggest that patients with clinical picture of AIDP and CD4 count less than 50 cell/μL should be treated presumptively *for cytomegalovirus (CMV)* infection, but some cases reported by Brannagan et al. do not support this recommendation [38].

19.3.3 Mononeuropathy Multiplex (MM)

MM, characterized by asymmetrical sensorimotor involvement of single nerves, is a rare complication occurring in early and late stages of HIV infection. The initial asymmetrical involvement of peripheral nerves may progress, during the evolution of the disease, in a clinical picture simulating a distal symmetrical neuropathy. If it occurs at the onset of HIV infection, MM is the result of self-limited immune-mediated vasculitis. Vasculitis is a rare event in HIV infection and occur only in 0.3–1.0% of patients with AIDS either as isolated process involving peripheral nerves or as a manifestation of a systemic disease. In post-cART era, vasculitis of PNS may present as distal symmetrical polyneuropathy [39]. *Pathological features* of nerve biopsy show focal loss of fibers, variable axonal degenerations, and perivascular epineurial inflammatory cell infiltration with fibrinoid necrosis. In the later stages of the infection, when fewer than 50 CD4 cells/μL are present, MM can be associated with CMV infection or, more rarely, with *varicella zoster*, *HCV*, or *lymphomatous infiltration* of nerve. CMV is an opportunistic agent that predominantly affects HIV-infected patients with fewer than 50 CD4 cells/μL. Clinical picture of CMV infection includes a multiorgan involvement with retinitis, pneumonia, gastrointestinal system involvement, epididymitis, pancreatitis, cervicitis, hepatitis, encephalitis, and MM or polyradiculopathy. Nerve biopsy shows lympho-monocytes cell infiltration of nerve fascicles and the presence of cytomegalic cells filled with CMV particles.

19.3.4 Progressive Polyradiculopathy (PLP)

Progressive lumbosacral polyradiculopathy (PLP) starting with back and leg pain and evolving into paraparesis and sensory and sphincter dysfunction has been frequently observed in HIV-infected subjects pre-cART era [40]. PLP usually occurs in late stages of HIV infection in concomitance with low count of CD4 lymphocytes. Usually PLP is related to *CMV* infection, but it can be caused by different conditions including mycobacterial, syphilis, cryptococcus, herpes simplex infection, and lymphomatous infiltration [23]. The diagnosis includes *CSF* examination with polymerase chain reaction amplification to detect viral agents, mycobacterial and cryptococcus antigen. In our experience, cytofluorimetric analysis of CSF is helpful and should be recommended in the suspect of lymphomatous meningoradiculitis. *Electrophysiological study* shows denervation in paraspinal muscles followed by denervation potential in the legs in the course of disease progression. Contrast-enhanced MRI can reveal enhancement of nerve roots [41]. Treatment for CMV infection should be started early in clinical suspicion of PLP and include ganciclovir, valganciclovir, foscarnet, cidofovir, and fomivirsen.

19.3.5 Diffuse Infiltrative Lymphocytosis Syndrome (DILS)

Diffuse infiltrative lymphocytosis syndrome (DILS) is a rare multisystem syndrome described in HIV-infected patients and characterized by persistent blood polyclonal CD8 T-cell lymphocytosis and organ infiltration [42]. This syndrome may affect salivary glands, lymph nodes, lungs, liver, kidneys, digestive tract, and PNS. *Polyneuropathy*, *aseptic meningitis*, and *facial nerve palsy* are the neurological abnormalities most frequently reported. DILS neuropathy usually presents as a painful and symmetrical neuropathy. Electrophysiological examination shows signs of axonal neuropathy. Nerve biopsy is characterized by angiocentric CD8 T-cell infiltration without vessel wall necrosis, and abundant expression of HIV p24 protein in macrophages [43]. In these patients, chronic HCV infection and *immune reconstitution inflammatory syndrome (IRIS)* are the principal differential diagnosis to be considered. *Treatment* of DILS consist mainly in *cART* but *steroids* may also be added when organ infiltration persist.

19.3.6 IRIS and Peripheral Nervous System

IRIS is an aberrant immune response due to the restoration of the immune system that occurs in the cART era. IRIS is defined as an unmasking or paradoxical worsening of a pre-existing infection in the presence of rapid decrease of viral load and recovery of *T-cell* immunity. In the course of IRIS, peripheral nerve involvement can occur in subjects previously affected by *Mycobacterium leprae* infection, inflammatory demyelinating radiculopathy, and cryptococcal radiculoplexopathy. IRIS-associated *AIDP* has also been described. Treatment is controversial and includes the use of *anti-inflammatory*, *corticosteroids*, *IVIG*, and *plasmapheresis*.

19.4 Leprosy

Mycobacterium leprae (ML) is an obligatory intracellular agent with tropism for macrophages and Schwann cell, which infects the skin and peripheral nerves resulting in chronic inflammation and neuropathy. The prevalence of leprosy is declining but, according to WHO data, it remains a common cause of neuropathy in 17 highly endemic countries. About 81% of all new cases occurs in three countries: Brazil, India, and Indonesia [44]; however, it is a worldwide problem since new cases have also been reported in travellers from endemic areas. Transmission of ML occurs via nasal mucosa and is followed by hematogenous spread. According to the classification of Ridley and Jopling, leprosy is classified into different subtypes: tuberculoid (T), borderline tuberculoid (BT), borderline (B), borderline lepromatous (BL), and lepromatous (L). A further form was later defined as indeterminate (I) [45]. The classification is based on the balance between bacterial particles and *immune reaction*. Patients with L-form are anergic to the bacillus and examined tissues are rich in mycobacteria. On the other hand, in T-form a strong immune reaction with paucity of mycobacteria particles is usually observed. In these latter cases, the immune response is able to limit bacterial growth, but skin lesions, and nerve damage are frequently observed. Clinical condition correlates with the entity of activation of cell-mediated immune response to ML. To simplify, WHO recommended a dichotomic classification into *paucibacillary* (PB) and *multibacillary* (MB) category. PB group includes I, T, BT, B, and BL forms, whereas MB includes BT, B, BL, and L subtypes. The two classifications are considered complementary, but the Ridley and Jopling one fit better with patients' clinical condition and prognosis. The suspect of ML infection is established when *multifocal neuropathy* is associated with hypo-pigmented, hypoesthetic or reddish *skin lesions*, even if some patients may present signs of neuropathy in the absence of the characteristic skin lesions. Indeed, 3 to 10% of patients present the *pure neuritic form* (PNL) that manifests as PN without any skin lesion. At onset *sensory symptoms* are the most common ones; small fibers are affected early, whereas large fibers are involved later. Clinical characteristics of PN in course of leprosy include *mononeuritis*, MM, and polyneuropathy. Mononeuritis is the most common presentation and usually affects nerves of the upper limbs as ulnar, median, posterior auricular, and superficial radial. Lower limbs can also be affected with the involvement of common peroneal, superficial peroneal, and posterior tibial. Rarely, also cranial nerves, primarily facial and trigeminal ones, can be damaged [46]. Typically, in the course of ML infection nerves are enlarged and painful on palpation and electrophysiological examination shows axonal changes. The use of imaging techniques as *nerve sonography* and MRI may be useful in the diagnosis. Analyzing high-resolution sonography, Visser and coll. showed that the epineurium of the ulnar nerve is often strikingly thickened in these patients, especially in those with ulnar involvement [47]. Symmetric *polyneuropathy* is rarely reported in leprosy while regional *autonomic* dysfunctions are frequently observed. Although the diagnosis of leprosy is mainly clinical, *nerve biopsy* can be helpful, especially in atypical cases or in patients

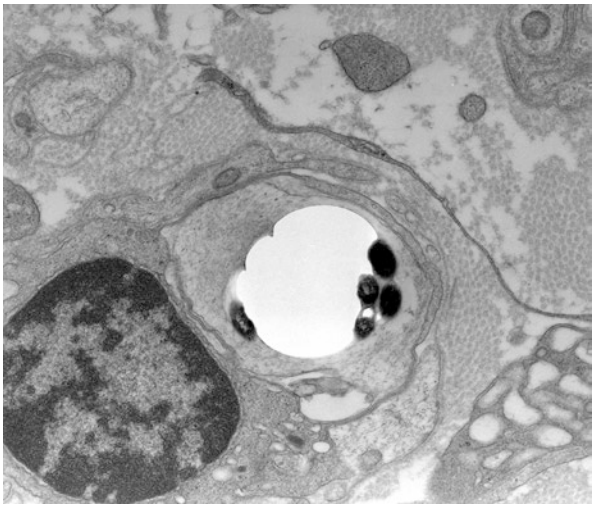

Fig. 19.3 Electron micrograph of sural nerve biopsy in pure neuritic form of leprosy showing a Schwann cell containing *Mycobacterium leprae* particles (original magnification ×12000)

with pure neuritic forms, not only as diagnostic procedure but also in personalizing therapy [48]. The definite diagnosis is based on skin smear or biopsy demonstrating granulomatous inflammation or foamy macrophages with acid-fast bacilli (Fig. 19.3).

19.5 *Borrelia burgdorferi*-Related Neuropathies

The tick-borne spirochete *Borrelia burgdorferi* is responsible of a vector-borne disease, known as Lyme borreliosis, transmitted by the *Ixodes* complex. This zoonosis, more diffuse in temperate regions and rural areas, causes a multisystem disease that affect humans as incidental hosts. Skin and the nervous system are the main involved organs. Nervous system involvement can occur through the hematogenous or transneural spread along peripheral nerves, few weeks after a tick bite or in the late and chronic disease [49]. Subacute painful *meningoradiculitis*, which consists in painful migrant burning radiculitis, peripheral motor deficit, and *CSF* inflammation, alone or in combination, are the prevalent manifestation of early neuroborreliosis. Motor damage consists frequently of bilateral and asymmetrical peripheral facial nerve palsy. More rarely, third or fourth cranial nerve involvement is present, sometimes only observed at MRI [49]. Isolated or concomitant limb paresis often bilateral, asymmetrical, and predominantly proximal can occur. These symptoms are consistent with root or plexus lesions and, more rarely, with a distal *mononeuropathy*. Distal *nerve pathology* has been demonstrated in course of Lyme borreliosis and confirmed by sural nerve findings of small lymphocytic infiltration around endoneurial vessels, perineurial fibrosis, and Wallerian degeneration [50]. However, nerve involvement in the absence of radicular symptoms or CSF inflammation has been rarely described [49]. *Brachial neuritis*, *Guillain-Barré*, and *CIDP*-like syndrome have also been reported [51, 52]. On the other hand, patients with chronic

dermatoborreliosis can develop a distal mainly *sensory neuropathy* in the absence of CSF inflammation. It consists of a mild distal axonal neuropathy probably due to a cutaneous neuritis. Sural *nerve biopsies* show lymphocytic perivasculitis and Wallerian degeneration. On the basis of these data, the opportunity to perform the screening for Borrelia in patients with PN of unknown etiology, in the absence of the above-described symptoms, is still very controversial. Since there is a high percentage of positive *anti-Borrelia IgG* blood test in the general population, the association between polyneuropathy and this infectious agent must be demonstrated by the concomitance of specific markers of active *Lyme borreliosis* as *CFS* pleocytosis, increased protein concentration, intrathecal IgM and IgG synthesis, and PCR positivity in CSF or blood. The data previously reported are mainly referred to European experience since in American studies the involvement of peripheral nerve has been rarely reported. In early neuroborreliosis radiculopathy, cranial neuropathy and MM have been described, while late symptoms as a distal polyneuropathy with mild diffuse stocking glove process are rarely detected [49]. Biopsy of sural nerve shows a prevalent axonal damage with perivascular infiltration [53, 54]. The acute neurological involvement in course of borreliosis presents usually with a benign course, but antibiotics as penicillin, cephalosporin, ceftriaxone, or oral doxycycline accelerates clinical recovery and prevents the development of new neurological deficit. Also chronic symptoms frequently improve with antibiotic treatment. Of note, some patients report long lasting and relapsing, recurrent and persistent nonspecific symptoms with negative active *Borrelia* serology. Patients do not improve after antibiotic treatment leading the idea that the pathogenesis of this condition, known as "*chronic arthropod-borne neuropathy*" could be linked to toxins, immunological, autoimmune, or psychological illness rather than the infectious agent [49].

19.6 *COVID-19* Pandemic and PNS Involvement

The new coronavirus disease-19 (COVID-19) pandemic, caused by *severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)* infection, started in December 2019 and rapidly spread all over the world. Since the beginning of the pandemic, many cases of patients with neurological symptoms and diseases have been described in patients affected with COVID-19. Furthermore, an increasing number of studies describing the involvement of the peripheral nervous system (PNS) in COVID-19 have been reported in the literature.

19.6.1 Critical Illness Polyneuropathy

The prolonged *Intensive Care Unit* (ICU) stay caused by severe respiratory impairment, characteristic of COVID-19, exposes the patients to the risk of care-related PNS complications as critical illness polyneuropathy (*CIP*). According to the different studies CIP affects 30–50% of severely critically ill patients and represents the most frequent form of acute neuropathy in ICU [55]. Several studies reported CIP

in COVID-19 affected patients. Among 12 patients affected with COVID-19 and suspected critical illness syndrome, Cabanes-Martinez et al. identified by nerve conduction studies (NCS) and electromyography (EMG) 4 patients with CIP [56]. All the patients suffered from a severe acute respiratory syndrome with long ICU stays (more than 12 days) and presented general weakness and difficulty to wean from the ventilator, which are typical manifestations of CIP or *critical illness myopathy* (CIM). The authors were not able to find any distinctive clinical and electrophysiological features of CIP between ICU patients affected by SARS-CoV-2 infection and ICU patients not affected by SARS-CoV-2 infection. NCS and EMG studies play an essential role in the diagnosis of the patients with general weakness in ICU in order to distinguish the predominance of PNS or muscular involvement and to identify the axonal or demyelinating pattern of nerve involvement. The lack of extensive studies in CIP patients is partly due to the severity and infectious risk of COVID-19 patients in ICU, which make it difficult to perform appropriate neurophysiological studies in large numbers of patients. Furthermore in exceptional situations as a pandemic the medical resources may be limited. The prospective study of Frithiof et al. [57] included 111 patients, 11 of whom developed CIN or critical illness myopathy (CIM). Subjects with CIN/CIM presented more severe disease, longer ICU stay and underwent invasive ventilation for more than 2 weeks. In particular, CIN was more frequent among COVID-19 patients compared with a non-COVID-19 cohort. The authors emphasize the importance of serum biomarkers such as *neurofilament light chain (NfL)* and *glial fibrillary acidic protein (GFAP)* whose levels were higher in the CIN/CIM group. These data support the role of NfL and GFAP levels in diagnosing and predicting axonal damage in CIN.

19.6.2 Guillain Barré Syndrome (GBS)

Almost every known variant of GBS has been described in the course of COVID-19, but the most frequently reported is the classic sensory-motor demyelinating form.

The first series of patients with GBS after SARS-CoV-2 infection was reported by Toscano et al. Subsequently, many case reports and cases series of GBS syndrome in ongoing COVID-19 have been described so far, but the direct influence of SARS-CoV-2 infection in the development of GBS is still debated. Case series of Northern Italy and Spain reported an increased incidence of GBS during COVID outbreak [58–60] supporting a possible role of SARS-CoV-2 infection in pathogenesis of GBS. The interesting study of Keddie et al. examined, in the first 6 months of SARS-CoV-2 pandemic in the UK, the epidemiological data of cases with confirmed COVID-19, the incidence of hospitalized patients with GBS and a large cohort of the GBS cases presenting with and without COVID-19 [61]. Finally the authors, also using a proteomic approach, found no association between GBS and COVID-19. Following the systematic review of Adalawi et al., most cases GBS in COVID-19 are sensorimotor demyelinating subtype with frequent facial palsy [62]. *Anti-ganglioside antibodies* were analyzed in a small number of cases of GBS/COVID-19 and in the majority of cases resulted negative, in the positive ones the

antibodies were directed against different gangliosides, mainly GM1, GD1a, GD1b, and GM2. The latency between infection and onset of neurologic symptoms, as well as the absence of SARS-CoV-2 detected by PCR in CSF, suggest a *post-infectious*, rather than a direct infectious mechanism. Uncini et al. examined the clinical and neurophysiological features of 24 patients with acute inflammatory demyelinating polyneuropathy (AIDP) and COVID-19 compared with 48 control AIDP without SARS-CoV-2 infection [63]. In COVID-AIDP patients, the authors frequently found increased dCMAP durations and absent F waves. These findings may be partially due to muscle fiber conduction slowing for the *hyperinflammatory state* of COVID-19, involving also the skeletal muscle, as in CIM.

19.6.3 Chronic Inflammatory Demyelinating Polyneuropathy (CIDP)

A clinical worsening of rare cases of CIDP may occur during COVID-19 [64, 65]. Although a causal association between SARS-CoV-2 and exacerbations in CIDP has been not previously reported, it has been assumed that SARS-CoV-2 infection may trigger a proinflammatory state that may be able to amplify autoimmune processes as CIDP.

19.6.4 Nerve Injuries

The guidelines for acute respiratory treatment of COVID-19 recommend a prolonged prone position of patients, who are therefore exposed to peripheral nerve injuries involving in the majority of cases the upper limbs. General anesthesia and malpositioning increase the risk of nerve injury. In an Italian experience, 7 cases of compression injuries of 135 patients who underwent invasive ventilation in prone positioning have been observed [66]. According also with other studies, the most frequent sites of injuries are the ulnar nerve, radial nerve, sciatic nerve, brachial plexus, and median nerve [67]. In conclusion, peripheral nerve injuries following prone position are common in severe COVID-19 and the patients require greater care in positioning due to increased susceptibility to nerve compressions.

Key Points

- Neuropathy remains the most common neurological complication of HIV infection.
- Different forms of neuropathy may occur during HCV chronic infection, frequently associated with cryoglobulinemia.
- Lepromatous neuropathy may present in pure neuritic form, requiring diagnostic nerve biopsy.
- The association of neuropathy with anti-*Borrelia* IgG antibodies in serum must be confirmed with blood and CSF demonstration of infectious activity.
- The SARS-CoV-2 pandemic exposes patients to increased risk of neuropathy, in particular critical illness neuropathies and compression neuropathies.

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Toxic Neuropathies

20

Guido Cavaletti and Paola Marmiroli

20.1 Introduction

The peripheral nervous system (PNS) is a uniquely positioned and complex network allowing the central nervous system (CNS) to communicate with all other organ systems throughout the body. Nerves of the PNS carry sensory information regarding internal and external environmental conditions as well as efferent motor signals to control the activities of visceral organs and musculoskeletal system. This complexity requires all these elements are investigated when seeking to evaluate the adverse effects of potential neurotoxic agents. However, information regarding practical means for conducting well-designed, comprehensive PNS toxicity studies and risk assessments (particularly for early detection during drugs development, or in the presence of environmental hazard) is often difficult to find in the scientific literature.

Humans have historically served the role of sentinel species for many neurotoxic agents resulting from occupational and environmental exposures. Subsequently, considerable effort is being directed at finding efficient and reliable in vitro and in vivo methods to identify potential PNS neurotoxicants during product/drug development. However, considerable benefit can be gained by mechanistic investigations (e.g., formulating structure–activity relationships), which then can be useful for predicting the neurotoxic effects of other agents acting through a similar mechanism.

There is growing recognition of the need for more efficient methods to assess the risks of the increasing and large number and types of chemicals to which humans are exposed. Modes of Action (MOA) and Adverse Outcome Pathways (AOPs) describe mechanistic knowledge at varying levels of biological organization. While conceptually similar, MOA includes chemical-related key events (e.g.,

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metabolism), whereas AOPs are restricted to the biological cascade of key events resulting from perturbation by an event (e.g., exposure to a toxic substance). Thus, AOPs describe a sequence of measurable key events originating from a molecular initiating event resulting in cellular, structural, and functional changes and ultimately measurable adverse effects relevant to the human organism and the human population. Based on this simple description, MOA and AOP are conceptually similar, representing the subdivision of the pathway between exposure and effect in either individuals or populations into a series of hypothesized key events at different levels of biological organization (e.g., molecular, subcellular, cellular, tissue). In summary, they represent pragmatic simplifications of complex biological pathways that might be extremely useful in the investigation of a multifaceted problem such as PNS neurotoxicity. In fact, recent international developments are anticipated to contribute to increasing collective confidence in applying AOPs for both regulatory risk assessment and research in several fields, including neurotoxicity. These include an update of the World Health Organization/International Programme on Chemical Safety (IPCS) mode of action/human relevance (MOA/HR) framework [1].

20.2 General Aspects of PNS Neurotoxicity

The high metabolic rate and the limited capacity of the nervous system to recover from damage explain why toxic injury may be particularly frequent and clinically relevant in different settings. The peripheral nervous system (PNS), despite repair is more effective, is more vulnerable than the central nervous system to toxic insults because the blood–nerve barrier is by far less stringent than the blood–brain barrier. Moreover, virtually no effective barrier exists at the dorsal root ganglia (DRG) level. As a result, several toxic agents can reach the peripheral nerves and, even more easily, the DRG neurons.

This specific vulnerability of the DRG contributes to explain why most of the clinical features of toxic neuropathies are represented by sensory disturbances although clinically relevant motor and autonomic impairment can also be present in some case.

Several compounds have been reported to be toxic on the PNS, but scientific and epidemiological data indicate that causal relationship is clearly established only for some of them. In fact, temporal relationship between the exposure to a given compound and the onset of PNS impairment is frequently the only evidence supporting causality. Criteria have therefore been proposed to strengthen the validity of this association, including (1) evident dose relationship, (2) proximity of symptoms to compound exposure, (3) stabilization or improvement after withdrawal from substance exposure, (4) possibility to reproduce the clinical features in animal models, and (5) similar clinical features in different subjects [2]. However, these criteria can be unable to capture a real relationship in specific instances, such as prolonged exposure to very low doses of toxic compounds that need to reach a threshold in

Table 20.1 Medications associated with peripheral neurotoxicity presenting as distal symmetrical polyneuropathy

Anticancer drugs	Cardiovascular agents	Antibiotics/Antivirals	Miscellaneous agents
Alemtuzumab	**Amiodarone**	**Chloroquine**	**Colchicine**
Bortezomib	**Perhexiline**	**Dapsone**	**Dichloroacetate**
Brentuximab vedotin	Clofibrate	**Isoniazid**	**Disulfiram**
Epothilones	Flecainide	**Linezolid**	**Etanercept**
Platinum compounds (Cisplatin, Carboplatin, and Oxaliplatin)	Hydralazine	**Metronidazole**	**Infliximab**
	Procainamide	**Nucleoside analogs**	**Pyridoxine excess**
Suramin	Propafenone	**Nucleoside reverse transcriptase inhibitors (NRTI)**	Acitretin
Taxanes	Statins		Allopurinol
Thalidomide			Almitrine
Vinca alkaloids		Chloramphenicol	Botulinum toxin
Conjugated monomethyl auristatin E		Ethambutol	Cyclosporin A
		Fluoroquinolones	Gold salts
Immune checkpoint inhibitors		Griseofulvin	Interferons: alfa2a, alfa2b
		Nitrofurantoin	Leflunomide
5-Azacitidine		Podophyllin resin	Lithium
5-Fluorouracil			Nitrous oxide
Clioquinol			Penicillamine
Cytarabine			Phenytoin
Etoposide (VP-16)			Sulphasalazine
Gemcitabine			Tacrolimus
Hexamethylmelamine			Zimeldine
Ifosfamide			
Misonidazole			
Teniposide (VM-26)			

Bold = drugs with established peripheral neurotoxicity

order to determine PNS injury, or when worsening occurs after exposure withdrawal (the "coasting" effect) and improvement is prevented by severe axonal damage. Moreover, animal models of PNS damage [3–5] have never been reported for agents that are definitely neurotoxic (e.g., thalidomide), probably due to species-specific characteristics of their toxicity .

Among the substances able to damage the PNS, several medications have been identified, with a particular relevance for anticancer drugs (Table 20.1). However, alcohol is a frequent cause of neuropathy (despite the pathogenesis of alcohol-related neuropathy is still debated and a role of nutritional deficiency cannot be ruled out) and environmental as well as industrial toxics can target the PNS. While these toxic agents are generally responsible for distal, symmetrical polyneuropathies, local toxicity can induce mononeuropathies that are easily recognizable in most cases, but that are not for this reason less troublesome in the most severe cases. Local anesthetic drugs are the most frequent cause of toxic mononeuropathies.

20.3 Drug-Induced Peripheral Neurotoxicity

In the case of drug-induced PNS damage, the term "neurotoxicity" is more appropriated than the commonly used "neuropathy" because DRG neuronal and glial targeting is frequently a major event beyond peripheral nerve fibers damage. Among the drugs with a well-established neurotoxicity, antineoplastic, antiarrhythmic, and antiretroviral agents have been described.

20.3.1 Antineoplastic Drugs

Several classes of effective antineoplastic drugs are severely neurotoxic, and some of these compounds are still used in the treatment of the so-called big killers, i.e., lung, breast, and gastro-intestinal cancers. This widespread clinical use leads to millions of subjects to be potentially exposed worldwide to the risk of developing Chemotherapy-Induced Peripheral Neurotoxicity (CIPN) [6–8]. Platinum drugs, antitubulins, proteasome inhibitors, and anti-angiogenic anticancer drugs have all been reported to cause severe CIPN. However, even the "targeted therapies" (i.e., new drugs able to specifically damage cancer targets) are not free from off-target side effects, including peripheral neurotoxicity [6].

The fundamental mechanisms of CIPN are only scarcely known, but it is now widely accepted the concept that these mechanisms do not perfectly overlap with those exploited by these drugs to exert their anticancer activity. Except for vinca alkaloids and thalidomide, clear DRG damage has been demonstrated at the pathological level in relevant animal models [9, 10]. Platinum drugs are able to interact with nuclear DNA of highly replicating cancer cells, thus limiting their capacity to complete mitosis and inducing apoptotic cell death. This mechanism is unlikely to be relevant in non-replicating neurons although using very high doses of cisplatin apoptosis can be induced also in neurons and an indirect effect on glial cells cannot be completely ruled out by the available preclinical data. Therefore, a number of studies have been performed in order to identify alternative pathogenetic mechanism of the toxicity of platinum drugs, including increased oxidative stress and mitochondrial damage [11]. Besides their known effect as microtubulin stabilizers, mitochondrial damage has also been suggested to explain the neurotoxicity of taxanes and that of bortezomib, the first proteasome inhibitor entered in clinical practice. However, an effect on tubulin has also been demonstrated for bortezomib, and this might be relevant to explain its peripheral neurotoxicity [12]. Vinca alkaloids capacity to disrupt the cytoskeleton is likely to be the main mechanism of axonal damage, while epothilones probably share the same mode of action of taxanes, at least on microtubules. So far, no explanation has been provided for the peripheral neurotoxicity of thalidomide. Selective accumulation of antineoplastic drugs into DRG neurons could result from the presence on their plasma membrane of transporters able to carry them into the cell cytoplasm [13], thus mimicking a mechanism already described to explain cisplatin nephrotoxicity [14, 15].

Platinum drugs induce a chronic, sensory neuropathy, which might lead to disabling ataxia in the most severely affected subjects. Only oxaliplatin can also present an acute neurotoxicity, with cold-induced paresthesias and cramps. Sensory symptoms are also largely predominant with all the other antineoplastic drugs, although the clinical spectrum is different. Neuropathic pain is prominent in bortezomib-treated patients and a painful syndrome is also frequent after taxanes administration, where distal motor impairment can be present. Autonomic dysfunction is the most worrisome toxic effect in patients treated with vinca alkaloids, particularly vincristine. Motor impairment is very rare after thalidomide administration. In all the cases of CIPN with a regular clinical course, the use of neurophysiological assessment is relatively non-informative and axonopathy is the common feature [6].

The neurotoxicity of "targeted-drugs" is less well known although it is now clear that peripheral neurotoxicity can also result from the use of these drugs and can be severe. Interference with the immune system has been advocated as a possible pathogenetic event. Cases of acute inflammatory polyradiculoneuropathy resembling Guillain-Barrè syndrome have been reported in alemtuzumab-treated patients [16], and it has been hypothesized that alemtuzumab may trigger an autoimmune cascade resulting from indiscriminate dysregulation of regulatory T cells or through a molecular mimicry. The administration of brentuximab vedotin can be associated with a dose-dependent peripheral neuropathy, probably associated with unconjugated microtubule inhibitor monomethyl auristatin E (i.e., the active part of the molecule acting as the classical chemotherapy drugs). Despite generally peripheral nerve sensorimotor neuropathy is mild to moderate, dramatic motor neuropathy has also been associated with brentuximab vedotin use [17], and this may also occur with other "vedotins".

The use of immune checkpoint inhibitors (ICI) is now entered in oncology clinical practice, and they have the potential to transform the treatment landscape of numerous cancers, producing long-term responses in a substantial fraction of patients and improving their outcome. Approved ICI target programmed death-1 receptor (PD-1, nivolumab, pembrolizumab, cemiplimab), its ligand (PD-L1; atezolizumab, avelumab, durvalumab), and cytotoxic T lymphocyte antigen-4 (CTLA-4; ipilimumab), but it is very likely that several new agents will soon be available. Toxic effects from ICI are related to autoimmune-like phenomenon, which can be severe and even fatal. Among neurologic toxicities of ICI, encephalitis, aseptic meningitis, myasthenia gravis, and peripheral neuropathies (alone or, frequently, in combination) may occur in 1–5% of treated patients. In an observational, retrospective, pharmacovigilance study based on adverse drug reactions reported in VigiBase, the World Health Organization database of global, deidentified individual case safety reports, which includes reports from more than 130 countries, Guillain-Barre syndrome comprised 22% of reported cases of peripheral neuropathies (Table 20.2) [18]. The incidence of peripheral neuropathies is variable among the different ICI subclasses, particularly regarding Guillain-Barrè syndrome which was more frequently reported with anti-CTLA4 monotherapy than with anti-PD-1/PD-L1, and with combination PD-1/PD-L1 + CTLA-4 blockade compared with monotherapy. However, the real

Table 20.2 Neurologic adverse events involving the peripheral nervous system reported with immune checkpoint inhibitors versus those reported in the full VigiBase database, from November 14, 1967, to September 28, 2018 (Adapted from [18])

	Overall ICIS	Full database	IC/IC_{025}
Total number of ICSRS available	48,653	18,518,994	
Number of ICSRS by IRAE subgroups			
Peripheral neuropathy	564 (1.16%)	123,463 (0.67%)	0.80/0.68[a]
– Guillain-Barre syndrome	122 (0.25%)	9508 (0.05%)	2.27/2.00[a]
– Chronic polyneuropathies	23 (0.05%)	6428 (0.03%)	0.43/−0.22
– Mononeuropathies	42 (0.09%)	17,075 (0.09%)	−0.09/−0.58
Cranial nerve disorders (excluding neoplasms)	226 (0.46%)	112,639 (0.61%)	−0.39/−0.58
Spinal cord and nerve root disorders	27 (0.06%)	11,875 (0.06%)	−0.21/−0.80
Neuromuscular junction dysfunction (myasthenia gravis)	228 (0.47%)	7455 (0.04%)	3.51/3.31[a]

ICIs Immune Checkpoint Inhibitors, *ICSRs* Individual Case Safety Reports reported for treatment with nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, ipilimumab, or tremelimumab, *IC* Information Component, IC_{025} Lower end of a 95% credibility interval for the Information Component: a positive IC_{025} value (>0) is the traditional threshold used in statistical signal detection with the VigiBase database

[a] Statistically significant differences

spectrum of ICI-associated peripheral neurotoxicity still needs to be properly investigated, and this will be one of the relevant aspects to be clarified in future studies with these agents.

20.3.2 Antiarrhythmic Drugs

The use of several antiarrythmic drugs such as propafenone, flecanide, and procainamide is reported to be associated with the onset of peripheral neuropathy, but the number of cases described is very low. Amiodarone, a highly effective drug used in the treatment of atrial fibrillation and ventricular arrhythmias, has several neurological and non-neurological toxicities, including tremor, ataxia, encephalopathy, parkinsonism, optic nerve damage, and myopathy. Besides these toxicities, peripheral nerve damage has also been described as one of the commonest neurological toxic effects of amiodarone. Since amiodarone neurotoxicity is time- and dose-related, its real incidence was probably over-estimated in the earliest reports due to the high dose of the drug received by the treated subjects, while maintenance dose nowadays is much lower and in a retrospective study the overall incidence was 2.8% with only a few cases of confirmed peripheral nerve damage [19]. The typical pathological features of amiodarone-induced neuropathy are lamellated inclusions particularly evident in the cytoplasm of Schwann cells, associated with secondary myelin changes. It is likely that these alterations (similar to those observed in

perhexiline or chloroquine neuropathy) are due to drug-induced inhibition of lysosomal phospholipases, leading to the formation of these lysosomal bodies in many cells types. A peculiar aspect of amiodarone is its very long elimination half-life (around 58 days as an average), so that a complete wash-out requires months after drug withdrawal. Amiodarone-induced neuropathy is a distal, sensorimotor neuropathy frequently associated with aching pain, a symptom which may help in the differential diagnosis with neuropathy due to hypothyroidism in patients suffering from amiodarone-induced thyroid gland dysfunction.

20.3.3 Antiretroviral Drugs

Mitochondrial DNA replication requires the function of DNA polymerase γ, and this enzyme can be inhibited by some of the Nucleotide Reverse Transcriptase Inhibitors (NRTI) which are used as component of the Highly Active Antiretroviral Therapy (HAART) for HIV-infected patients. Although replacement of dideoxyinosine, zalcitabine, stavudine, and lamivudine (the NRTI which are commonly associated with peripheral neuropathy) is generally implemented in the USA and Europe, they are still used for patients' intolerant or non-eligible for other treatments and are also a fundamental treatment in most low-income countries. Patients with NRTI-induced peripheral neuropathy present with a distal, symmetrical painful sensory neuropathy, with mild or absent motor impairment, due to axonal damage and associated with mitochondrial evident alterations at the ultrastructural examination [20]. The cause of the specific selectivity of axons with preservation of Schwann cells is unknown, but it has been reproduced in vitro, where inhibition of mitochondrial transmembrane potential has been demonstrated in neurons, but not in co-cultured Schwann cells, exposed to NRTI. Since NRTI- and HIV-related neuropathies are both severely painful, the differential diagnosis may be problematic and onset (which is more subtle in the infectious form) is the main clinical clue to discriminate between them.

20.4 Alcohol-Related Peripheral Neuropathy

According to the World Health Organization (http://www.who.int/substance_abuse/facts/en/), on average every person in the world aged 15 years or older drinks 6.2 liters of pure alcohol per year, but since less than half the population (38.3%) actually drinks alcohol, this means that those who do consume on average 17 liters of pure alcohol annually. The relationship between chronic alcoholism and peripheral neuropathy is frequent (25–66% of exposed subjects) [21], and it was first reported in 1787 by Lettersom [22], who described the event as due to direct toxicity of ethanol. This pathogenetic hypothesis resisted substantially unchallenged until 1928, when a possible alternative explanation for alcohol-related neuropathy was suggested based on the similarities observed with beriberi neuropathy caused by thiamine deficiency. Over the following years, clinical as well as animal studies

strengthened the "thiamine hypothesis," but the lack of consistent positive results after thiamine supplementation raised some concern on the real extent of involvement of the vitamin in the onset of peripheral nerve damage in chronic alcohol misusers. More recent studies compared the features of peripheral neuropathy in patients with demonstrated vitamin deficiency, and they concluded that the clinical picture and course as well as measured vitamin levels (including thiamine) were sufficiently dissimilar to suggest that the pathogenesis should be different [23]. The typical clinical features of alcohol-related neuropathy are represented by slowly progressive, distal, symmetrical neuropathy, predominantly involving sensation perception and autonomic function, in most cases with an important painful component. The neurophysiological correlates of these clinical features are represented by slowing in motor and sensory nerve conduction with marked reduction of potential amplitudes, which are consistent with the pathology results evidencing axonopathy.

20.5 Environmental and Industrial Toxics

20.5.1 Organic Solvents

Organic solvents, able to induce peripheral nerve damage, comprise a wide range of compounds, including n-hexane, styrene, toluene, tricholoroethylene, and methyl-n-butyl ketone. Axonal swelling with accumulation of neurofilaments has been reported as the hallmark of hexacarbon-induced PNS damage although demyelinating features can also be detected by the neurophysiological examination. Chronic, subacute sensorimotor neuropathy is the most typical clinical presentation, but autonomic dysfunction has also been described, as well as the association with encephalopathy and myelopathy. Occasionally, also cases of recreational exposure have been reported in "glue sniffers" [24]. Rapid onset of peripheral neuropathy has been more commonly reported in glue sniffers than in subjects exposed to chronic industrial intoxication, and coasting can occur after the removal from toxic exposure.

20.5.2 Industrial Chemicals

Carbon disulfide and acrylamide are among the most extensively studied industrial chemicals able to induce PNS damage. It has been estimated than only in the USA more than 30 million kilograms of carbon disulfide are released in the atmosphere each year during production of cellophane and rayon. Exposure to carbon disulfide induces an axonopathy affecting preferentially the largest myelinated fibers, with accumulation of neurofilaments at the paranodal zone [25]. The clinical features of carbon disulfide-induced neuropathy are those of a distal, sensory neuropathy with limb weakness.

The water-soluble vinyl monomer acrylamide is widely used in chemical industries and is a by-product in some food preparations. Acrylamide is severely toxic on the cerebellar Purkinje cells, and it causes axonal degeneration in sensory and motor

nerves. In animal models extensive swelling of the neuromuscular junction with accumulation of neurofilaments, tubulovesicular profiles, and degenerated mitochondria have been described [4]. Axonal loss and denervation are confirmed by neurophysiological examination. At onset distal sensory impairment is present in hands and feet, then autonomic dysfunction may appear and only mild motor impairment is generally present during the course of the neuropathy, which is slow.

20.5.3 Heavy Metals

Several heavy metals including lead, manganese, organic tin, mercury, arsenic, and thallium can cause peripheral neuropathies.

Chronic arsenic poisoning mostly results from consumption of contaminated ground water, particularly in Asia, secondary to natural sources and anthropogenic activities, for example, mining or pesticides use [26]. In Western countries, cases of arsenic neuropathy have been reported to be occasionally associated with the assumption of contaminated dietary supplements or seafood and acute neuropathy mimicking Guillain-Barrè syndrome has been reported after use of herbal medicines [27]. Peripheral neuropathy is not among the most frequent toxicities of arsenic being present in approximatively 5–10% of chronically intoxicated people. The usual clinical presentation is represented by distal sensory loss with mild weakness secondary to predominantly axonal damage. Cerebrospinal fluid examination can show mild hyperproteinorrachia, and skin changes are present in chronically exposed subjects, thus providing useful clues to the diagnosis which should be confirmed with specific urine search.

Environmental and industrial exposures are also the main reasons for mercury intoxication. The outbreak of methylmercury poisoning in Minamata Bay (Japan) in the 1950s allowed to clearly establish the hallmark lesions in the anterior portion of the calcarine cortex and depletion predominantly of granular cells in the cerebellar cortex, but these patients had also evidence for a sensory neuropathy affecting the distal extremities. Pathological studies evidenced endoneurial fibrosis, fiber loss, Büngner's bands, and regenerated myelin sheaths. Subacute mercury-induced neuropathy is predominantly motor, while chronic neuropathy is sensorimotor. The real extent of peripheral nerve damage can be masked by the concomitant presence of signs of central nervous system damage [28]. Urine examination can confirm the diagnosis in suspected cases. The claim that the use of mercury-containing dental amalgams could be associated with increased risk of neurological toxicities, including peripheral neuropathy, has never been supported by firm scientific evidence [29].

Lead is one of the major pollutants accumulated in our environment over the centuries. It has been calculated that there has been a 100-fold increase in the amount of lead accumulated in the human skeleton bones over the last 5000 years [30]. The use of lead is surpassed only by ferrous metals (e.g., use of lead-based fuels, manufacture of batteries, crushing and smelting of lead-containing residues, technology industry). Despite strict regulations at least in developed countries, subacute intoxication is in the vast majority of cases due to unprotected industrial

exposure and produces motor impairment of variable severity, frequently asymmetrical and described to involve the hands and finger extension before spreading to other districts, including cranial nerves. Chronic, low-intensity exposure is generally associated with sensorimotor impairment. Association with gastro-intestinal symptoms (e.g., constipation, abdominal pain) is fairly common and may direct the diagnosis. Axonal damage is the pathological hallmark of lead-induced neuropathy in humans although demyelination has been observed in animal models. It has been proposed that lead can induce peripheral nerve damage through interference with porphyrin metabolism and mitochondrial toxicity [30].

20.6 Toxicity of Local Anesthetic Drugs

Long-term PNS injury is a known complication after peripheral nerve blocks [31]. These lesions can be severe and debilitating although they are relatively rare with an incidence ranging between 2.4 and 4 per 10,000 blocks [32]. Evidence from human and animal studies indicates that multiple factors contribute to the risk of PNS damage [33]. Amon them, type of nerve block, presence of pre-existing neuropathy, occurrence of intraneural injection and needle trauma, pressure injury, nerve ischemia, and local anesthetic neurotoxicity. Using animal models, it has been suggested that intraneurally injected local anesthetics may produce histologic changes without any functional neuropathy [34]. Similarly, although there is long-lasting evidence regarding a more severe nerve damage after intrafascicular injection compared with topical application [35], it is still unsettled whether this damage is secondary to mechanical injury or direct neurotoxicity of local anesthetics because intrafascicularly injected saline produced similar nerve damage in an animal model [35]. Regarding their potential neurotoxicity, all local anesthetics carry more or less the same risk [36], with their effect probably due to prolonged increases in cytosolic Ca2+, leading to depletion of adenosine triphosphate, mitochondrial injury, membrane dysfunction, and, eventually, cell death [37]. Small-diameter axons (carrying pain and temperature sensation) are more susceptible to the effect, but also to the neurotoxicity, of local anesthetics than large-diameter axons (carrying motor and proprioception impulses). However, apart from direct effect on nerve fibers, local anesthetics can also have an effect on nerve blood vessels inducing vasoconstriction (this seems not to be the case for bupivacaine), that might eventually lead to nerve ischemia and secondary fiber function loss. An additional issue in the interpretation of the effects of local anesthetics on peripheral nerve function is the understanding of the possible role of adjuvant drugs, such as opioids, clonidine, dexamethasone, neostigmine, ketamine, and midazolam. Despite preclinical models suggest that local anesthetics neurotoxic effects are time- and concentration-dependent [38], this has not clearly been established in humans.

Key Points

- The peripheral nervous system is frequently targeted by toxic agents.
- Several widely used medications are toxic on the peripheral nervous system.

- Environmental and industrial exposure to neurotoxic agents is still relatively frequent, and it can be difficult to be detected.
- Recreational abuse substances besides alcohol are neurotoxic.
- Sensory impairment is generally predominant over motor or autonomic damage.
- The clinical course is highly variable and "coasting" can occur.
- Distal, symmetrical polyneuropathy is the most frequent clinical feature, but mononeuropathies can occur after local delivery of neurotoxic agents.

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