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TITLE PAGE

Neuromuscular Adverse Events Associated with Anti-PD-1 Monoclonal Antibodies: Systematic Review

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We also submit a *Supplementary File*, including study protocol, literature search flow chart, and the PRISMA checklist.

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ABSTRACT

Neuromuscular adverse events following cancer treatment with anti-programmed cell death protein 1 (PD-1) monoclonal antibodies are relatively rare, yet potentially fatal. Using the PRISMA approach, we performed a systematic review to characterize the clinical presentation, diagnostic workup, and management of neuromuscular disorders (NMDs) in patients treated with nivolumab or pembrolizumab. Sixty-three publications on 85 patients (mean age 66,9 years (range 34-86); male/female 2.6:1; 59% metastatic melanoma) were identified from selected indexing databases until June 2018. Forty-eight patients had received nivolumab and 39 pembrolizumab. The mean number of PD-1 inhibitor treatment cycles prior to onset of symptoms was 3,6 (range 1-28). Symptoms included oculomotor (47%); respiratory (43%), bulbar (35%), and proximal weakness (35%); as well as muscle pain (28%). Diagnoses were categorized as myasthenia gravis (27%), neuropathy (23%), myopathy (34%) and a combination of these (16%). After critical review of the data, however, evidence did not support the stated NMD diagnosis in 13% of cases, while up to 14% of patients had signs of additional NMDs. PD-1 inhibitor associated myasthenia was associated with cardiac complications in almost 30% of patients and with a more rapid clinical progression compared with idiopathic

myasthenia. Mortality was high despite adequate treatment strategies including corticosteroid, IV immunoglobulins and plasmapheresis. In conclusion, clinical presentation of NMDs associated with PD-1 inhibitors is often atypical, with significant overlap between myasthenia gravis and myopathy; and cardiac/respiratory complications are common, leading to more severe disease courses than idiopathic myasthenia.

INTRODUCTION

Neurological adverse events following cancer treatment with immune checkpoint inhibitor antibodies are relatively rare, yet potentially fatal complications¹⁻³.

Nivolumab and pembrolizumab, two human IgG4 anti-Programmed cell death protein 1 (PD-1) monoclonal antibodies, were approved for treatment of unresectable melanoma by the US Food and Drug Administration⁴ in 2014 and by the European Medicines Agency in 2015^{5,6}. Both drugs have since then been approved for treatment of other types of cancer, including non-small cell lung cancer. Nivolumab and pembrolizumab stimulate T-cells to respond against cancer cells by binding to PD-1, an inhibitory T-cell surface receptor^{7,8}.

Immune checkpoint receptors play an important role in balancing immune reactivity between a relevant immune response to pathogens while preserving self-tolerability⁷. Herein lies a potential risk for off-target effects related to reactivity against healthy tissue, i.e. immune-related adverse events such as myasthenia gravis¹. An increasing number of case reports on myasthenia gravis following nivolumab or pembrolizumab treatment have been published within the last few years. In a Japanese population, the incidence of myasthenia gravis was 0.12% in patients treated with nivolumab⁹. Of note, other authors found evidence of concurrent myositis and/or neuropathy, which indicates that side effects following

nivolumab and pembrolizumab also involve neuromuscular tissue distal and proximal from the neuromuscular junction¹⁰⁻¹⁶.

Diagnosing myasthenia gravis is often straightforward as symptoms are quite characteristic, including fluctuation of ocular or bulbar symptoms and/or weakness of neck and proximal limb muscles without sensory deficits^{17,18}. The documentation of autoantibodies against neuromuscular junction proteins such as the acetylcholine receptor (AChR) or muscle-specific kinase (MuSK) strongly supports the diagnosis. In case of negative antibody titers, neurophysiological work-up and a positive response to treatment may be needed. However, as outlined above, myasthenia gravis following treatment with immune checkpoint inhibitor antibodies is considered an immune-related adverse event, and hence its phenotype might differ from classical myasthenia gravis with respect to e.g. clinical presentation, neuromuscular junction antibodies, treatment response and prognosis. Further, the immunological response may not be limited to the neuromuscular junction; as stated above, other components of the neuromuscular system might also be affected to varying degrees. This could complicate and prolong the diagnostic process, and consequently delay therapy.

Due to the rapidly increasing use of anti-PD-1 monoclonal antibodies, neurologists and other clinicians are likely to encounter more and more oncological patients with neuromuscular symptoms suggestive of myasthenia gravis, myopathy and/or neuropathy. Yet, so far, no attempt has been made to summarize the literature on the salient features of this new neurological entity. Therefore, we performed a systematic review to characterize the symptoms, clinical findings and laboratory results of presumed anti-PD-1 monoclonal antibody treatment associated neuromuscular disease, focusing on how to distinguish these adverse events and we provide a case description for illustration (Box 1).

METHODS

We conducted a systematic review of the literature according to standard systematic review methodology (PRISMA¹⁹).

Standard Protocol Approvals, Registrations, and Patient Consents

The complete review protocol, as well as the PRISMA check-list, can be accessed in the *online supplementary file* and is registered with PROSPERO (<https://www.crd.york.ac.uk/prospero/>). A written *Consent-to-Disclose Form* was obtained from the patient's family.

Primary and secondary objectives

Using the PICO approach (Patients, Intervention, Comparison, Outcome²⁰), we have phrased the following primary research question:

- *In patients treated with an anti-PD-1 monoclonal antibody for disseminated cancer or melanoma, who develop neuromuscular symptoms or who have pre-existing neuromuscular symptoms that become more pronounced following anti-PD-1 monoclonal antibody therapy (P), do neurological examination and laboratory workup, including neurophysiology and antibody titers, (I) sufficiently discriminate between neuropathies and myopathies, including myositis, on one hand (C) and neuromuscular junction failure compatible with myasthenia gravis on the other (O)?*

We further phrased two secondary research questions:

- *Do patients treated with an anti-PD-1 monoclonal antibody for disseminated cancer or melanoma (P), who fulfill clinical and laboratory criteria for anti-PD-1 monoclonal antibody-associated myasthenia gravis (I), respond better, similar or worse to treatment with pyridostigmine and immunomodulation (C) than patients with myasthenia gravis in general (O)?*
- *Do patients treated with an anti-PD-1 monoclonal antibody for disseminated cancer or melanoma (P), who fulfill clinical and laboratory criteria for anti-PD-1 monoclonal antibody-associated myasthenia gravis (I), have a better, similar or worse prognosis with respect to their myasthenia (C) than patients with myasthenia gravis in general (O)?*

Search strategy

We evaluated all case reports, cross-sectional or longitudinal, retrospective or prospective observational studies as well as interventional trials reporting on patient history of neuromuscular symptoms following treatment with the anti-PD-1 monoclonal antibodies nivolumab and/or pembrolizumab.

We included only articles that allowed assessment of patient data at the single-subject level. We excluded articles that concerned patients already used in another article by the same authors (or the same institution). We included studies published in English and listed in Medline (PubMed), Cochrane Central Register of Controlled Trials (The Cochrane Library), and ClinicalTrials.gov up until June 30, 2018. The full search string can be found in the supplementary files.

All abstracts were evaluated. Eligible studies were identified on the basis of their full text. Non-English literature were included only if an English abstract was available and a reliable translation of the manuscript into English was possible. The

reference list of relevant articles was manually searched to identify additional articles.

The initial selection and further review were performed by SJC and AJ. Following identification of relevant studies, AJ and SJC independently extracted the relevant information from each study. Disagreement on whether to include a study or disagreement on data extraction was settled by DK. Data point are visualized in Figure 3.

Participants

We included adults (age ≥ 18 years) with anti-PD-1 monoclonal antibody therapy for disseminated cancer or melanoma, regardless of prior or concomitant therapy, presenting in health care facilities with neuromuscular symptoms, and who were diagnosed with a disorder of the peripheral nervous system. Patients were included irrespective of co-morbidities and previous history of neuromuscular diseases.

Target conditions

The target condition was defined as signs of neuromuscular disease compatible with myasthenia gravis, myopathy or neuropathy in patients treated with nivolumab or pembrolizumab monotherapy or one of these in combination with other immunological agents, e.g. the CTLA-4 inhibitor ipilimumab. We investigated the occurrence of neuromuscular disorders with regards to clinical and laboratory findings. Based on the reported findings, we sorted each patient case into the following categories: 1) diagnosis *likely*; 2) diagnosis *likely*, but signs of co-diagnosis; 3) diagnosis *possible*, but not corroborated; 4) diagnosis *less likely*, but signs of other NMD, based on the criteria listed in Table 1.

RESULTS

Systematic literature search

112 articles were identified in the database searches and additionally 2 articles from reference lists. Sixty-three articles^{2,10–16,21–68} with 85 individual patients met inclusion criteria; the first paper was published in 2014⁴⁹. A more detailed flowchart of the literature review as well as the full list of included references can be accessed from the supplementary file. Most articles were case reports ($n=59$, 94%), while a few were retrospective clinical database studies ($n=4$). No prospective studies reported on individual patient data.

Patient population

Of the 86 patients included (85 from the literature review plus a further case, which is presented as an illustrative case history in Box 1), 62 (72%) were male, mean age was 66,9 years (median of 68, range 34-86). The most common oncological diagnosis was metastatic melanoma ($n=51$, 59%), followed by non-small cell lung cancer ($n=15$, 17%), while the remaining group of patients had been diagnosed with a variety of other types of cancer, including thymoma, renal cell carcinoma, and urothelial carcinoma (see supplementary file for full list).

Forty-eight patients (56%) had been treated with nivolumab, of which 12 (25%) had received ipilimumab as an add-on treatment. Thirty-nine patients (45%) had been treated with pembrolizumab. One patient received sequential treatment with both nivolumab, pembrolizumab and ipilimumab, and is thus included in both PD-1 inhibitor groups.

The mean number of PD-1 inhibitor treatment cycles prior to onset of symptoms was 3,6 (median of 2, range 1-28), while the mean number of days following onset of treatment was 47,8 (median of 20, range 4-420). The patients presented with a

range of symptoms and findings upon physical examination including, in descending order, oculomotor symptoms, including diplopia, ophthalmoplegia and ptosis ($n=40$, 47%); respiratory symptoms ($n=37$, 43%), bulbar symptoms, including dysphagia and dysarthria ($n=30$, 35%), proximal weakness ($n=30$, 35%), muscle pain ($n=24$, 28%), unspecified or a combination of proximal and distal weakness ($n=22$, 26%), head drop ($n=14$, 16%), sensory symptoms ($n=13$, 15%), fatigability or fluctuation of symptoms ($n=7$, 8%), distal weakness ($n=4$, 5%). 16 (19%) experienced some form of cardiac involvement, e.g. myocarditis or conduction block.

Neurophysiological evaluation included electromyography (EMG) ($n=32$, 38%), electroneuronography (ENG) ($n=32$, 38%), repetitive nerve stimulation (RNS) ($n=22$, 26%) and single nerve fiber (SNF) ($n=7$, 8%). Forty-nine patients had been tested for AChR-ab, of which 20 (41%) had a positive titer. Fifty-one of 86 patients had their CPK levels measured, of which 90% were elevated. Seventy-eight (92%) of patients received treatment with corticosteroids, 41 intravenously and 37 orally. IVIG was commenced in 33 (39%) of patients, while 23 (27%) of patients underwent plasmapheresis at least once. Eighteen patients (21%) received pyridostigmine, while 12 (14%) received some other type of immunomodulatory treatment (infliximab, $n = 5$; rituximab, $n = 2$, mycophenylate, $n = 2$, NSAID, $n = 1$). Sixty-seven (79%) of patients experienced partial improvement or full recovery from their neuromuscular symptoms, while 29 (34%) were reported to have died at last follow-up, but most often the time to follow-up was not clearly stated.

Nivolumab versus pembrolizumab

Odds-ratios for selected parameters are shown in Table 2, and except for two parameters, demographics and characteristics of neuromuscular adverse events did not significantly differ; 1) the proportion of patients diagnosed with metastatic

melanoma was smaller for nivolumab than for pembrolizumab, OR 0.29 (95% CI 0.12;0.73, $p = 0.009$). 2) patients treated with nivolumab were more likely to have a positive AChR-ab titer compared with pembrolizumab, OR 6.55 (95% CI 1.66;20.83, $p = 0.008$).

Neuromuscular adverse events

Diagnoses

Table 3 shows selected data points related to symptoms and laboratory investigations based on localization in the peripheral nervous system. The diagnoses were categorized as *myasthenia gravis* ($n=23$, 27%; including ocular, bulbar or generalized myasthenia gravis), *neuropathy* ($n=20$, 23%; comprising almost exclusively Guillain-Barré syndrome, but also two cases of vasculitic neuropathy, one chronic inflammatory demyelinating polyneuropathy, and one Bell's palsy), or *myopathy* ($n=29$, 34%; mostly unspecified myositis and/or rhabdomyolysis). The category *combination* includes 14 patients, of which 11 (79%) were diagnosed with myasthenia gravis *and* myopathy, two (11%) with myopathy *and* neuropathy, and one patient (7%) diagnosed with myasthenia gravis, myopathy *and* neuropathy. Of the 23 patients diagnosed with only myasthenia gravis, eight (35%) had a history of preexisting myasthenia gravis, and thus these patients were diagnosed with either myasthenia gravis exacerbation or reactivation. Three of eight patients were treated with nivolumab, 5/8 were males, and this subgroup did not differ from the group of de novo myasthenia gravis in respect to symptomatology, laboratory work up, treatment or outcome.

Symptoms

Oculomotor symptoms were present in 91% ($n=21$) of patients diagnosed with myasthenia gravis, while this was the case for 9 (31%) with myopathy and even less frequent with neuropathy ($n=1$, 5%). Respiratory symptoms were also quite frequent; ($n=13$, 57%) of patients with myasthenia gravis and ($n=12$, 41%) myopathy had signs of respiratory distress, but less so patients with neuropathy ($n=3$, 15%). Weakness of axial and/or extremity muscles was present in all groups; 13 (57%) of myasthenia gravis, 11 (55%) of neuropathy and 22 (76%) of myopathy. As seen in Table 3, a large part of cases was classified as *unspecified weakness* making it difficult to identify a clear pattern of affected muscle groups. Despite being a hallmark of myasthenia gravis, fatigable weakness was only reported in 7 (30%) cases of pure myasthenia gravis and in none of the cases with >1 NMD diagnosis. Importantly, cardiac involvement was reported in 3 (13%) of myasthenia gravis cases, and in 11 (38%) and 5 (36%) of myopathy and combination cases, respectively, while not seen in any of the patients with neuropathy. Head-drop and respiratory symptoms were most prevalent among patients in the combination group.

Laboratory work-up

Neuromuscular junction antibodies

AChR-ab status was reported for 20/23 patients diagnosed with myasthenia gravis, of which 50% had a positive antibody titer. Ten patients had been tested for MuSK-ab as well, but all were negative. In the group of 29 patients with myopathy, 2/10 tested had a positive AChR-ab titer, while none of the seven patients tested was anti-MuSK positive.

In the group of patients diagnosed with more than one type of NMD, all 14 had been tested for AChR-ab, and the titer was positive in 8/12 patients with a co-diagnosis of myasthenia gravis. MuSK-ab testing was reported for 7/14 patients, and was positive

in one patient (with a titer of 0.02 nmol/L (normal range 0.00-0.01 nmol/L))⁵⁰, who had a history of ocular AChR-ab+ myasthenia gravis, and now was diagnosed with myositis and AChR-ab+ myasthenia gravis. Ca⁺-channel-ab were only tested in two patients, both of which were diagnosed with myasthenia gravis, and both were negative.

Creatine phosphokinase

CPK was elevated in 6 (26%) patients with myasthenia gravis with a mean of 2427 U/L (range 1156-6566, n = 5, one reported as 'slightly elevated'). CPK was only reported in one case of neuropathy, where levels were within normal range. 26 (90%) of patients with myopathy had elevated CPK, while 3 (10%) had normal levels; the mean CPK level was 7328 U/L (median 2450 U/L, range 72-43,130 U/L, n = 27). In the combination group, where all 14 patients had concurrent myositis, all patients had elevated CPK, with a mean of 7939 U/L (median 7740 U/L, range 478-27,703 U/L, n = 13).

Neurophysiological examination

Data on neurophysiological examination was less often reported; in the myasthenia gravis group single nerve fiber electromyography (SNF) was reported in 4/23 patients, who all had abnormal jitter (of which two were AChR-ab positive), while repetitive nerve stimulation (RNS) was performed in 8/23 patients, of which five were pathological. In patients with myopathy, RNS was performed in 8/29, with no abnormal results. In the combination group, SNF was performed in 2/14 patients, with one showing abnormal jitter, while RNS was normal in all six cases tested. EMG was more frequently reported: 6/23 patients with myasthenia gravis (abnormal in 2/6, pointing to neuropathic⁴³ and myopathic³⁶ changes); 6/20 with neuropathy

(3/6 with neuropathic changes); 12/29 with myopathy (four were normal, seven had indications of myopathy and two of neuropathy). Eight of 14 patients with more than one NMD diagnosis reported EMG examination (one was normal, remaining seven with signs of myopathy). One patient diagnosed with myasthenia gravis and rhabdomyolysis had denervation potentials in all tested muscles¹⁴.

ENG results were reported for 5/23 patients with myasthenia gravis, of which one had indications of axonal critical illness neuropathy⁴³. In the neuropathy group 17/20 reported on ENG; 2/17 were normal, while the remaining had abnormalities consistent with either axonal and/or demyelinating neuropathy and radiculopathy, affecting both motor and/or sensory nerves.

Histological evaluation

Biopsies were performed in 19/29 patients with myopathy, of which only one was reported as normal. Most often necrotic fibers and some degree of inflammation (often lymphocytic) was seen. In the combination group 6/14 had biopsy performed; three were inconclusive and three showed signs of inflammation.

Treatment and outcome

Corticosteroids were the most widely used treatment in all groups; 21 (91%) of myasthenia gravis, 18 (90%) of neuropathy, 25 (86%) of myopathy and 13 (93%) of patients with >1 NMD diagnosis (Table 3). IVIG and PEX were also frequently given, especially in patients with a combination of NMD (8 (57%) and 7 (50%), respectively), while less often in myasthenia gravis (8 (35%) in both cases), neuropathy (11 (55%) and 2 (10%), respectively) and myopathy (7 (24%) and 6 (21%), respectively). Pyridostigmine was commenced in 11 (48%) diagnosed with

myasthenia gravis and 9 (64%) with >1 diagnosis. Other immunomodulatory drugs such as infliximab and mycophenolate were used in a total of 11/86 patients.

Most patients had some degree of improvement early after treatment onset; 19 (95%) patients with neuropathy, 19 (83%) with myasthenia gravis, 20 (69%) with myopathy and 8 (57%) in the combination group. The time to follow-up was often not explicitly stated, and thus mortality rates should be interpreted with caution, but it is noteworthy that there were no deaths in the group with neuropathy.

Data were deemed insufficient for a more detailed meta-analysis.

Inconsistencies in the diagnosis of neuromuscular symptoms

Evaluating each case report in respect to the criteria formulated in Table 1, we found that for 67 (78%) of the patients included, clinical and laboratory findings were sufficient to deem the diagnosis *likely*, with no sign of other NMD-pathology (Table 4).

Myasthenia gravis

We found five cases had signs of other NMD; all had elevated CPK in the range 1156-6566 U/L, pointing to muscle involvement, and one had myopathic changes in the EMG³⁶, while EMG data were not reported for the remaining four patients^{25,32,40,47}. Three cases lacked specific clinical (signs of fatigable weakness) and laboratory findings corroborating the diagnosis; 2/3 had tested negative for AChR- and MuSK-ab^{48,51}, while antibody testing was not reported for the third one²⁹. SNF and CPK results were not reported for either of them, but one had a normal ENG (including RNS) and EMG. Finally, two cases lacked support for the diagnosis but showed signs of other NMD; again symptoms could be attributed to

myasthenia gravis, but fatigability was not mentioned, and in one case antibody results were not reported⁴³, while in the other AChR-ab results were negative². The first case had ENG findings that were interpreted as critical-illness neuropathy, while the latter had slightly elevated CPK, pointing to muscular involvement.

Myopathy

Three of 29 cases in the myopathy category showed signs of involvement of other neuromuscular structures. One patient treated with nivolumab for a thymoma was diagnosed with myositis and rhabdomyolysis based on a CPK of >40,000 and biopsies showing inflammation, but the patient also had a positive AChR-ab test²⁶. There were complaints of chest pain (and elevated cardiac enzymes), dyspnea and generalized myalgia, but not muscle weakness. Thus, it is unclear whether the positive AChR-ab test is related to the underlying thymoma⁶⁹ or the nivolumab treatment, and whether the AChR-antibodies were likely to be implicated in the pathology of this particular patient. In another case of myopathy⁴⁵, a patient with bulbar symptoms had positive AChR-ab of 0.10 nmol/L, anti-striated muscle-ab titer of 1:30270, CPK of 444 U/L. One patient⁴² with myopathy had elevated CPK, negative AChR-ab, normal EMG, in which ENG showed signs of length dependent peripheral neuropathy and a muscle biopsy showed necrotic fibers with no inflammation.

Neuropathy

Four of 20 cases did not present results supporting the diagnosis. For instance, patient 9 in ³⁰ was diagnosed with cranial polyneuropathy but symptoms were not described, and ENG/EMG were reported as normal. Three cases of patients diagnosed with meningo-radiculitis and polyradiculitis, respectively² and Bells's

palsy⁶⁶ also did not report on the diagnostic work-up, but the patient with meningo-radicularitis had muscle pain, which could indicate some degree of myopathy.

Combination of diagnoses

Two cases of myasthenia gravis-concurrent myositis^{11,60} did not report any symptoms or clinical findings suggestive of fatigable weakness, both patients had a negative AChR-ab test, while SNF and RNS were also not reported. CPK was moderately elevated and ENG showed signs of polyneuropathy in the former.

DISCUSSION

Diagnostic workup

This is the first systematic review on neuromuscular adverse events following treatment with monoclonal anti-PD-1-antibodies. Our aim was to investigate whether patients with neuromuscular symptoms after nivolumab or pembrolizumab treatment could meaningfully be classified into conventional diagnostic groups, i.e. NMJ/myasthenia gravis, myopathy and neuropathy (*primary research question*). Comprehensive diagnostic workup including neurophysiology, antibody titers, muscle enzymes and biopsies was only rarely available (and if so, only from singular case reports). Hence, the presented data are too heterogeneous to support a clear analysis on the sensitivity and specificity of clinical and laboratory findings. Data were compatible with the reported diagnosis (i.e. without signs of another NMD) in 78% of patients in general (Table 4); diagnostic accuracy was lowest in the group diagnosed with myasthenia gravis (57%) and highest in myopathy (90%). Nine percent of patients had findings suggesting co-diagnosis of another NMD (five with myasthenia gravis, three with myopathy), and in 8% the given diagnosis appears uncertain (three with myasthenia gravis, three with neuropathy, one with >1

diagnosis). In 5%, we found that the diagnosis given was not supported by the diagnostic procedures, which instead pointed to a different NMD.

These numbers clearly indicate that a broad and thorough diagnostic work-up is needed when patients treated with PD-1 inhibitors show signs of neuromuscular adverse events. In particular, clinicians should note that, based on our study, up to 25% of patients show signs of overlapping NMDs, especially myasthenia gravis and myopathy. This finding is consistent with data from a retrospective case-control study⁹. Findings also seem to differ from idiopathic NMDs in other regards, e.g. there is a lower proportion of AChR-ab positive myasthenia gravis⁷⁰ and a high prevalence of oculomotor and bulbar symptoms in patients with myopathy. The latter can easily prompt a diagnosis of myasthenia gravis, but if the element of fatigability is absent and diagnostic work-up does not support neuromuscular junction failure, other explanations must be sought, and neurologists specialized in NMDs should be consulted (indeed, oftentimes neurologists are not involved at all but patients are investigated and treated by their attending oncologists alone; in 32 (37%) of patient cases, authors were not affiliated with a neurological department). We also found a high prevalence of respiratory symptoms/complications in patients with myasthenia gravis and myopathy, likely contributing to the high mortality rate for these patients. This again shows the need for an accelerated and broad diagnostic workup to ensure that treatment is instituted quickly.

A high number of patients showed signs of cardiac involvement in the form of either elevated myocardial enzymes^{11–13,21,22,26,32,39,46,50,60,71,72}, ECG changes^{26,32,46}, abnormal echocardiography⁷¹, cardiac arrest¹⁴, or cardiac symptoms prior to death^{30,41,48,71}. The coexistence of myasthenia, myopathy and myocarditis was also noted by Suzuki et al⁷³. As all above mentioned cases involve patients with

myopathy and/or myasthenia gravis, clinicians should be aware of the propensity towards cardiac complications in these groups.

We identified a remarkably unequal gender distribution in the reviewed studies with 72% male patients. This is markedly different from the reported gender distribution for melanoma⁷⁴ and non-small cell lung cancer⁷⁵ which accounted for 76 % of the cancer types in this review. The reason for this skewing in gender distribution is unclear but one might speculate that, for unknown reasons, male oncological patients are more prone to neuromuscular side effects or female patients are less often treated with anti-PD-1 therapy. This is an important area for future research.

Treatment and outcome

The data available to date cannot sufficiently answer whether treatment responses and clinical outcomes differ between patients with idiopathic myasthenia gravis and patients with PD-1 inhibitor treatment related myasthenia gravis (*secondary research questions*). Corticosteroids were the most frequent treatment, followed by IVIG and PEX, reflecting the common approach when managing severe cases of myasthenia gravis and other NMDs. Although corticosteroid treatment for myasthenia gravis has been used since the 1970s⁷⁶, no randomized placebo-controlled trial (RCT) on idiopathic myasthenia gravis has been conducted⁷⁷.

Likewise, no RCTs exist with sufficient power for either IVIG or PEX to clarify the efficacy of these treatments on idiopathic myasthenia gravis⁷⁷⁻⁷⁹. Furthermore, only few authors of the papers included in this review gave a thorough description of the timing of these treatments, making it impossible to separate treatment effects.

To identify exact data on treatment response and clinical outcome of patients with neuromuscular symptoms following anti-PD-1 therapy, standardized prospective multicenter case-control studies are needed. Given the relatively low incidence of

neuromuscular adverse events compared to other autoimmune adverse events^{3,9,80,81} this could prove difficult. It should be noted that in the case series reviewed here 11 of 23 patients with isolated myasthenia gravis died, two due to cancer progression and one due to “unrelated cardiac issue”⁴⁸. Thus, for obvious reasons a high mortality seems to occur in this group of patients compared to myasthenia gravis in general⁸². In addition to an increased background mortality, however, neuromuscular decompensation might be more pronounced in patients with anti-PD-1 treatment, as observed in a retrospective case-control study⁹. Although one should keep in mind the high susceptibility of publication bias and unsystematic nature of these data, it is noteworthy that none of the 20 patients with neuropathy died during follow-up, while death rates were 41% (12/29 patients) for patients with a myopathy and 50% (7/14) for patients with a combination NMDs.

Based on reported findings we evaluated the given diagnosis and some cases were suspected to have been given an incorrect diagnosis of NMD (Table 4). A potential bias for this way of evaluating is the amount of information provided by the authors of the case reports; some provide normal/negative findings, while this may not be the case for others. We would expect findings supporting the diagnosis that the authors arrive at to be presented, but especially publications with several patient cases did not provide full diagnostic information. Thus, we cannot confidently conclude whether faulty diagnoses are because of inadequate workup or misinterpretation of the findings.

Future directions

Reviewing the current data raises important questions, not the least about the pathophysiology of anti-PD-1 associated myasthenia gravis and NMDs, e.g. why do

some patients experience symptoms after just one dose, while others go through several cycles before symptom onset? As to myasthenia gravis, does anti-PD-1 treatment initiate or unmask latent disease? Is there a paraneoplastic component? Are there any unidentified molecular targets in patients with atypical presentation and/or laboratory findings?

CONCLUSIONS

The list of cancers to be treated with PD-1 inhibitors is rapidly expanding, and neurologists and oncologist will see many more patients in the future with adverse events affecting the neuromuscular system, including myasthenia gravis, myopathy, and neuropathy. As shown in this review, clinical presentation is often atypical, with a significant overlap between myasthenia gravis and myopathy; and cardiac and respiratory complications are common, leading to more severe disease. This makes early recognition important because mortality is likely increased with treatment delay. We suggest implementing continuous screening for neuromuscular symptoms and signs, including basic investigations such as CPK and ECG, in patients started on PD-1 inhibitor treatment, in particular for patients with a previous diagnosis of neuromuscular disorders.

BOX 1

A 74-year old male with a history of hypothyroidism, hypertension and dyslipidemia was diagnosed with metastatic renal clear cell carcinoma in 2006. He was treated with pazopanib, everolimus and sunitinib, resulting in clinical remission, before commencing fourth line monotherapy with nivolumab 240 mg every 2 weeks in 2017 because of progressive metastatic disease.

The patient was admitted to the hospital in November 2017, 11 days after the 4th infusion of nivolumab with complaints of muscle weakness, muscle pain, hoarseness, intermittent double vision, difficulties swallowing and a left eye ptosis, which had developed within the previous three weeks. Neurological examination revealed a right eye abducens nerve palsy, left eye ptosis, hoarseness, dysphagia and proximal weakness of the upper and lower extremities. Deep tendon reflexes were brisk. Ice-pack test⁸³⁻⁸⁵ was negative, but a Jolly's test showed a slight increased ptosis on the left side. Blood test revealed elevated creatine phosphokinase (CPK) of 478 U/L and myoglobin 1110 µg/L. Brain and spine MRI was unremarkable, while lumbar puncture showed normal cell count, slightly elevated protein (0.82 g/L) and no malignant cells. Electromyography (EMG) revealed myopathic changes with reduced amplitudes of the motor unit potentials (MUPs), while on electroneuronography (ENG) normal nerve conductance velocities and amplitudes were noted. Repetitive nerve stimulation (RNS) was without abnormal decrement. Treatment with iv methylprednisolone 125 mg with a gradual tapering and infliximab was initiated for suspected myositis. The patient gradually improved. He was readmitted in December 2017 because of dyspnea and muscle weakness. These symptoms fluctuated and were worse in the evenings. Clinical examination now revealed left-sided abducens nerve palsy and ptosis, as well as dysphagia, proximal weakness, and normal sensation in the lower extremities. Electrocardiogram (ECG) showed a left branch block which had not been present four months previously. Troponin I was slightly elevated (50 ng/L, ref. <45 ng/L) but normalized few hours after admittance, and CK-MB was normal. CPK was normal (33 U/L; ref. 40-280 U/L) and myoglobin slightly increased (92 µg/L; ref. 24-77 µg/L). Test for acetylcholine receptor antibodies (AChR-ab) and anti-muscle-specific kinase antibodies (MuSK-ab) were negative. A repeated ice-pack test was

positive, i.e. following application of an ice-package on both eyes, ptosis was less pronounced in both eyes (**Figure 1**). Pyridostigmine was commenced but with no apparent effect. Treatment with iv immunoglobulin (IVIG) was planned but the patient suffered from acute respiratory insufficiency due to aspiration and died the following day. Post mortem muscle biopsies showed multifocal atrophy and fibrosis with a low degree of inflammatory cells with few CD3 and CD68 positive cells and very few CD8 positive cells. There were no CD20 positive cells (**Figure 2**). The clinical and histological data are best compatible with myasthenia gravis combined with myopathic changes, associated with anti-PD-1 therapy for metastatic renal clear cell carcinoma.

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Appendix 1. Authors

Name	Location	Role	Contribution
Annette Johansen, MD*	Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Denmark	Author	Design and conceptualized study; data collection; analyzed the data; drafted the manuscript for intellectual content
Søren Just Christensen, MD*	Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Denmark	Author	Design and conceptualized study; data collection; analyzed the data; drafted the manuscript for intellectual content
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Figure 1: Ice pack test. Picture of patient treated with nivolumab for metastatic renal cell carcinoma before (upper) and after (lower) ice pack test.

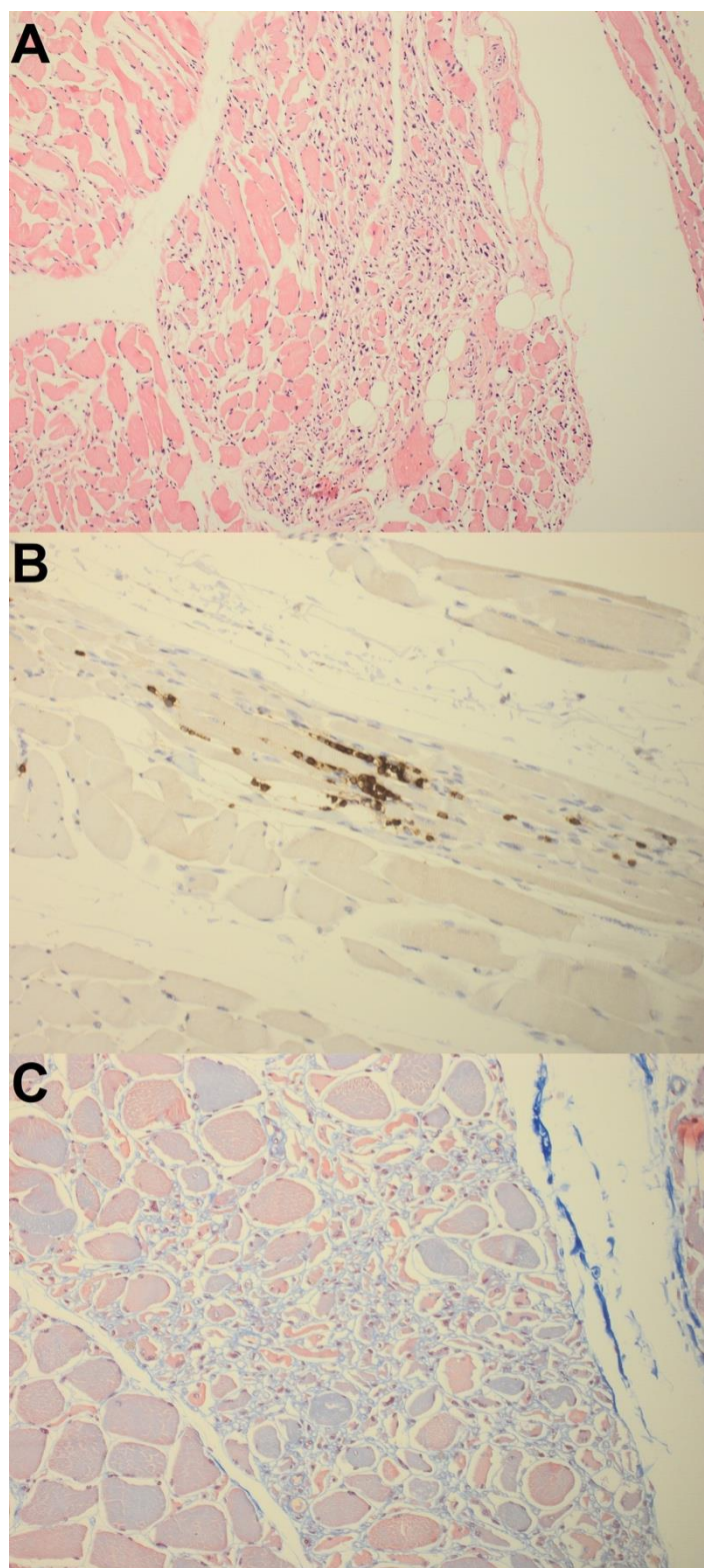


Figure 2. Muscle biopsy. A: Hematoxylin and eosin staining demonstrates multiple foci of atrophic fibers and fibrosis. B: CD3 staining shows a small interstitial infiltrate of CD3 positive lymphocytes, but inflammatory cells are scarce. C: Mason Trichrome staining highlights the fibrotic areas (blue color).

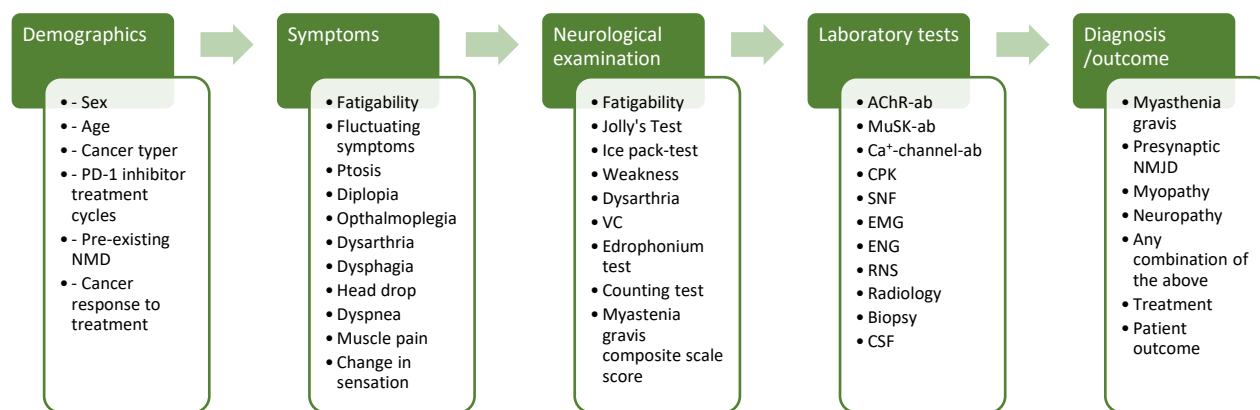


Figure 3: Main data extraction points. AChR-ab, acetylcholine receptor antibody; CPK, creatine phosphokinase; CSF, cerebrospinal fluid; EMG, electromyography; ENG, electroneuronography; MuSK-ab, muscle specific kinase antibody; NMD, neuromuscular disorder; NMJD, neuromuscular junction disorder; RNS, repetitive nerve stimulation; SNF, single nerve fiber; VC, vital capacity.

Table 1

	Myasthenia gravis	Neuropathy	Myopathy
Diagnosis likely	1) Symptoms of ocular, bulbar and/or proximal weakness <i>with</i> 2) fatigable and/or fluctuating aspect (incl. Jolly's-, Tensilon-, ice-pack test), <i>and/or</i> 3) supported by the presence of specific autoantibodies against neuromuscular junction components (e.g. AChR-, MuSK-, LRP4-ab) <i>and/or</i> 4) neurophysiological findings (SNF, RNS)	1) Symptoms including distal weakness and/or sensory loss 2) supported by the presence of specific autoantibodies against neuronal/myelin components <i>and/or</i> 3) increased protein in CSF, 4) ENG/EMG findings and/or 5) histopathology	1) Symptoms of proximal weakness, dysphagia and/or muscle pain 2) supported by the presence of specific autoantibodies against muscular components (e.g. myositis-specific- or myositis-associated-ab), 3) elevated CPK, 4) EMG findings <i>and/or</i> 5) histopathology
Diagnosis likely, but signs of co-diagnosis	If, in addition to the above stated, clinical or laboratory findings point to additional pathology of another neuromuscular structure.		
Diagnosis possible, but not corroborated	If symptoms are compatible with MG, <i>but</i> 1) do not have a fatigable aspect or	If symptoms are compatible with neuropathy, <i>but</i>	If symptoms are compatible with neuropathy, <i>but</i>

	2) no laboratory work-up supports the diagnosis	1) no laboratory work-up supports the diagnosis	1) laboratory work-up supports the diagnosis
Diagnosis less likely, but signs of other NMD	If symptoms are compatible with the diagnosis, <i>but</i> also 1) are compatible with another NMD <i>and</i> 2) laboratory work-up points to pathology of another neuromuscular structure		

Table 1: Diagnostic criteria for classification of neuromuscular symptoms following anti-PD-1

therapy. AchR-ab, acetylcholine receptor antibody; CPK, creatine phosphokinase; CSF, cerebrospinal fluid; EMG, electromyography; ENG, electroneuronography; LRP4-ab, Low-density lipoprotein receptor-related protein 4 antibody; MG, myasthenia gravis; MuSK-ab, muscle specific kinase antibody; NMD, neuromuscular disorder; RNS, repetitive nerve stimulation; SNF, Single nerve fiber electromyography.

Table 2

	Nivolumab		Pembrolizumab		OR	95% CI	P-value ^a
Demographics							
No. of patients (n; %)	48 ^b	55%	39 ^b	45%	-	-	-
Metastatic melanoma	22	46%	29	74%	0,29	0,12;0,73	0,009
Mean age (years; SD)	66,7	12,2	67,1	14,9	0,43	-5,49;6,36	0,88
Median (years; range)	68,0	35-85	68,0	34-86			
Males	36	75%	26	67%	1,50	0,59;3,82	0,48
Anti-PD-1 treatment							
Mean cycles prior to onset (n; SD) ^c	3,4	5,1	3,8	4,9	0,50	-1,7;2,7	0,66
Median (n; range)	2	1-28	2	1-23			
Time from 1 st dose to onset (days; SD) ^d	47	87,2	50	65,1	2,3	-37,7;42,4	0,91
Median (days; range)	14	4-420	28	5-322			
Diagnosis							
Myasthenia gravis (n; %)	10	21%	13	33%	0,53	0,22;1,37	0,23
Neuropathy	12	25%	9	23%	1,11	0,40;2,90	1,00
Myopathy	15	31%	14	36%	0,81	0,35;1,89	0,66
Combination	11	23%	3	8%	3,57	0,93;12,58	0,08
Symptoms							
Fatigability / diurnal fluctuation (n; %)	4	8%	4	10%	0,80	0,22;2,90	1,00
Oculomotor ^e	19	40%	22	56%	0,51	0,21;1,78	0,14
Bulbar (any) ^f	16	33%	15	38%	0,80	0,32;2,02	0,66
Head drop	6	13%	9	23%	0,48	0,15;1,51	0,26
Respiratory/dyspnea	23	48%	15	38%	1,47	0,63;3,62	0,39
Proximal weakness	14	29%	16	41%	0,59	0,25;1,50	0,27
Distal weakness	3	6%	2	5%	1,23	0,24;7,22	1,00
Unspecified weakness	13	27%	11	28%	0,95	0,37;2,51	1,00
Muscle pain	15	31%	10	26%	1,32	0,53;3,27	0,64
Sensory loss	9	19%	5	13%	1,57	0,46;4,57	0,56
Cardiac involvement ^g	12	25%	5	13%	2,27	0,77;6,25	0,18
Treatment and outcome							
IV corticosteroids (n; %)	24	50%	17	44%	1,29	0,56;3,07	0,67
PO corticosteroids	19	40%	18	46%	0,76	0,32;1,81	0,66

IVIG	19	40%	15	38%	1,05	0,44;2,60	1,00
Plasmapheresis	11	23%	13	33%	0,59	0,22;1,51	0,34
Pyridostigmine	11	23%	7	18%	1,36	0,51;3,38	0,61
Other immunomodulatory treatment	8	17%	5	13%	1,36	0,45;4,08	0,77
Improvement	36	75%	32	82%	0,66	0,25;1,96	0,60
Death at last follow-up	16	33%	14	36%	0,89	0,35;2,06	0,82

Table 2: Comparison of demographics, anti-PD-1 treatment, symptoms and outcome

for nivolumab and pembrolizumab. CI, confidence interval; IVIG, intravenous

immunoglobulin; OR, Odds ratio. ^a Fisher's exact test. ^b Including 1 patient who received

both nivolumab and pembrolizumab. ^c Data available from $n=45$ (nivolumab) and $n=35$

(pembrolizumab). ^d Data available from $n=39$ (nivolumab) and $n=26$ (pembrolizumab). ^e

Including ptosis, ophthalmoplegia, diplopia. ^f Including dysphagia and dysarthria. ^g Any

form of cardiac symptom or laboratory finding.

Table 3								
	Myasthenia Gravis		Neuropathy		Myopathy		Combination^a	
Demographics								
No. of patients (n; %)	23	27%	20	23%	29	34%	14	16%
Mean age (years; SD)	71,9	8,5	61,3	63,0	66,2	15,2	66,9	14,8
Age median (years; range)	71,0	57-86	62,0	38-85	70,0	35-86	69,0	34-85
Males	14	61%	16	80%	21	72%	11	79%
Previous history of NMD	8	35%	0	0%	2	7%	1	7%
Metastatic melanoma	12	52%	14	70%	19	66%	5	36%
Anti-PD1 treatment								
Nivolumab (n; %)	10	43%	12	60%	15	52%	11	79%
Pembrolizumab	13	57%	9	45%	14	48%	3	21%
Ipilimumab (add-on treatment)	3	13%	7	35%	5	17%	2	14%
Mean cycles prior to onset (n; SD) ^b	2,4	1	5,9	7	4,0	6	2,1	2
Median (n; range)	2	1-8	3	1-23	2	1-28	1	1-8
Time from 1 st dose to onset (days; SD) ^c	33	31	71	88	73	110	21	15
Median (days; range)	23	8-60	39	5-322	28	7-420	14	8-60
Symptoms								
Fatigability / diurnal fluctuation (n; %)	7	30%	0	0%	0	0%	1	7%
Oculomotor ^d	21	91%	1	5%	9	31%	10	71%
Bulbar (any) ^e	11	48%	4	20%	10	34%	6	43%
Head drop	3	13%	1	5%	6	21%	4	29%
Respiratory/dyspnea	13	57%	3	15%	12	41%	9	64%
Proximal weakness	7	30%	2	10%	14	48%	6	43%
Distal weakness	1	4%	4	20%	0	0%	0	0%
Unspecified weakness	5	22%	5	25%	8	28%	3	21%
Muscle pain	0	0%	1	5%	20	69%	4	29%
Sensory loss	0	0%	13	65%	0	0%	0	0%
Cardiac involvement ^f	3	13%	0	0%	11	38%	5	36%
Laboratory work-up								
AChR-Ab positive (n; % ^g)	10	50%	0	0%	2	17%	9	64%
CPK elevated	6	26%	0	0%	26	90%	14	100%
Mean CPK (U/L; range)	2427	(1156-6566)	NA	-	7328	(72-43130)	7939	(478-14229)
EMG (n; %)	6	26%	6	30%	12	41%	8	57%

SNF	4	17%	0	0%	1	3%	2	14%
RNS	8	35%	0	0%	8	28%	6	43%
ENG	5	22%	17	85%	5	17%	5	36%
Histological evaluation	1	4%	3	15%	19	66%	6	43%
Treatment and outcome								
IV corticosteroids (n; %)	7	30%	7	35%	16	55%	10	71%
PO corticosteroids	14	61%	11	55%	9	31%	3	21%
IVIG	8	35%	11	55%	7	24%	8	57%
Plasmapheresis	8	35%	2	10%	6	21%	7	50%
Pyridostigmine	11	48%	0	0%	1	3%	9	64%
Other immunomodulatory treatment	3	13%	2	10%	4	14%	2	14%
Improvement	19	83%	19	95%	20	69%	8	57%
Death at last follow-up	11	48%	0	0%	12	41%	7	50%

Table 3: Comparison of demographics, anti-PD-1 treatment, symptoms, diagnostic workup, treatment and outcome for the four categories of NMDs. AChR-ab,

Acetylcholine receptor antibody; CPK, creatine phosphokinase; EMG, electromyography;

ENG, electroneuronography; IVIG, intravenous immunoglobulin; RNS, repetitive nerve

stimulation; SNF, single nerve fiber electromyography. ^a This group including 11 with

myasthenia gravis/myopathy, 2 with myopathy/neuropathy and 1 with myasthenia

gravis/myopathy/neuropathy. ^b Data available from $n=21$ (myasthenia gravis), $n=17$

(neuropathy), $n=26$ (myopathy) and $n=14$ (combination). ^c Data available from $n=15$

(myasthenia gravis), $n=12$ (neuropathy), $n=22$ (myopathy) and $n=13$ (combination). ^d

Including ptosis, ophthalmoplegia, diplopia. ^e Including dysphagia and dysarthria. ^f Any

form of cardiac symptom or laboratory finding. ^g Positives as percentage of number tested.

Table 4										
	Myasthenia gravis		Neuropathy		Myopathy		Combination		Total	
<i>1. Diagnosis likely</i>	13	57%	16	80%	26	90%	12	86%	67	78%
<i>2. Diagnosis likely, but signs of co-diagnosis</i>	5	22%	0	0%	3	10%	0	0%	8	9%
<i>3. Diagnosis possible, but not corroborated</i>	3	13%	3	15%	0	0%	1	7%	7	8%
<i>4. Diagnosis possible, but not corroborated, signs of other NMD</i>	2	9%	1	5%	0	0%	1	7%	4	5%
<i>Total</i>	23	100%	20	100%	29	100%	14	100%	86	100%

Table 4: Evaluation of case reports in respect to diagnostic criteria of NMDs. NMD, neuromuscular disorder.