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Evidence from quantitative and single fiber EMG and muscle histopathology

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Myopathy as a cause of Long COVID fatigue: Evidence from quantitative and single fiber EMG and muscle histopathology

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HIGHLIGHTS

- Myopathic changes in qEMG and/or increased jitter in sfEMG were seen in 63% of 84 patients with Long COVID neuromuscular symptoms.
- Low quality of life score correlated with higher mean jitter values in sfEMG but not with qEMG measures.
- Electron microscopy showed damage of terminal nerves and motor endplate.

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ABSTRACT

Objective: To describe neurophysiological abnormalities cin Long COVID and correlate quantitative electromyography (qEMG) and single fiber EMG (sfEMG) results to clinical scores and histopathology.
Methods: 84 patients with non-improving musculoskeletal Long COVID symptoms were examined with qEMG and sfEMG. Muscle biopsies were taken in a subgroup.
Results: Mean motor unit potential (MUP) duration was decreased in $\geq$ 1 muscles in 52 % of the patients. Mean jitter was increased in 17 % of the patients in tibialis anterior and 25 % in extensor digitorum communis. Increased jitter was seen with or without myopathic qEMG. Low quality of life score correlated with higher jitter values but not with qEMG measures. In addition to our previously published mitochondrial changes, inflammation, and capillary injury, we show now in muscle biopsies damage of terminal nerves and motor endplate with abundant basal lamina material. At the endplate, axons were present but no vesicle containing terminals. The post-synaptic cleft in areas appeared atrophic with short clefts and coarse crests.
Conclusions: Myopathic changes are common in Long COVID. sfEMG abnormality is less common but may correlate with clinical scores. sfEMG changes may be due to motor endplate pathology.
Significance: These findings may indicate a muscle pathophysiology behind fatigue in Long COVID.

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1. Introduction

Long term symptoms following COVID-19 (Long COVID) now affects a large number of patients with estimates of up to 100 million people world-wide (Chen et al., 2022; Office for National Statistics, 2022; WHO, 2022; ZOE Health Study, 2022). Patients suffer significant disability (Davis et al., 2021) with a number of persistent symptoms for more than three months described in hospitalized (Huang et al., 2021; Mølhave et al., 2021) as well as in patients with mild initial COVID-19 (Augustin et al., 2021). Fatigue, myalgia, headache, problems with memory and concentration (“brain fog”), chest pain, palpitations, sensory disturbances, depression and anxiety, joint pain and insomnia are the most prevalent described symptoms (Groff et al., 2021; Lopez-Leon et al., 2021; Nasserie et al., 2021). A pathophysiological mechanism has not yet been established.

We previously found myopathic changes in quantitative electromyography (qEMG) in 11 of 20 patients referred for electrophysiological investigation with suspicion of polyneuropathy due to paresthesia (Agergaard et al., 2021) and suggested that myopathy might explain physical fatigue. Recently we published light and electron microscopy results of muscle biopsies from 16 Long COVID patients with fatigue, in which we found pathological findings in all patients including microvascular changes as well as affection of the myofibrils with mitochondrial changes, atrophy, inflammation and multiple basal membrane as a sign of regeneration (Hejbøl et al., 2022).

In order to further explore whether myopathy might be a common finding in Long COVID patients, we aimed to examine a larger cohort of Long COVID patients in a Post COVID Clinic with fatigue and musculoskeletal symptoms (Agergaard et al., 2022). We aimed also to examine a possible neuromuscular transmission failure which could be a cause of fatigue. We here present the qEMG and single fiber EMG (sfEMG) investigations of patients referred for electrophysiological evaluation from a Post COVID Clinic and describe their clinical and electrophysiological characteristics including presentation of histology in a small subgroup of these patients.

2. Methods

In Denmark, hospital based multidisciplinary regional clinics (Post COVID Clinics) receive patients referred from their general practitioners, with unexpected or complex and prolonged symptoms for more than 12 weeks after initial COVID-19. The Post COVID Clinic at Aarhus University Hospital receives patients from 2/3 of Central Denmark Region with a population of one million inhabitants. In the clinic, patients with severely affected and non-improving daily function, as well as physical/muscular exhaustion, myalgia or reduced muscle force, were referred for qEMG investigation. Patients who have been critically ill and who had known neurological disease were excluded. Referrals were on clinical indication. Patients included in this study had EMG performed between the time period December 2020 and March 2022.

In the Post COVID Clinic, medical history for all patients were registered by a physician, and patient reported questionnaire data were collected at the first clinical visit, including symptom severity (31 symptoms constituting a Post COVID Questionnaire (PCQ) score), Fatigue Assessment Scale (FAS), Post-COVID-19 Functional Status (PCFS), and Health-Related Quality of Life (EQ-5D-5L index). This data collection was first started in February 2021 (Agergaard et al, 2022).

2.1. Clinical examination

All patients underwent a detailed clinical evaluation and neurological examination including bilateral manual muscle strength testing using the Medical Research Council (MRC) Scale (0–5) of all major muscle groups (shoulder abduction, elbow and wrist extension and flexion, hip flexion, knee extension and flexion and ankle dorsal and plantar flexion).

2.2. Electrophysiological examination

All electrophysiological tests took approximately 2 hours for each patient.

A Keypoint.net EMG machine was used for all electrophysiological examinations. The skin temperature was maintained above 32 °C during all tests. The examinations were done by a clinical neurophysiologist (HT) with more than 20 yearś experience in performing qEMG and sfEMG. The clinical neurophysiologist was blinded to the questionnaire data.

Peroneal nerve sensory and motor nerve conducting studies (NCS) including the knee segment, sural sensory and tibial motor NCS were performed in all subjects to exclude entrapment neuropathies and polyneuropathy. Additionally, all patients were tested with repetitive nerve stimulation in median and accessory nerves recorded from abductor pollicis brevis and trapezius muscles, respectively. The stimulation rate was 3 Hz and 10 stimuli and repeated just after maximal voluntary contraction and up to 3 minutes with 1-minute intervals.

qEMG was performed using a 38 mm Ambu Neuroline disposable concentric needle electrode (diameter 0.45 mm (26G), recording area 0.07 mm^2) and standard filter settings of 20 Hz–10 kHz, gain (100 μV/division) and sweep speed (10 ms/division). In all patients, biceps brachii, vastus medialis and anterior tibial muscles were examined. Examination of other muscles were included to the diagnostic work-up when necessary. The presence of spontaneous activity was assessed at 10 separate sites. Quantitative motor unit potential (MUP) analysis (Stålberg et al., 2019) was done by sampling at least 20 MUPs during weak voluntary contraction. Mean duration, amplitude, and percentage of polyphasic potentials were evaluated. The mean MUP duration was calculated for the simple potentials. The results were compared with laboratory reference material.

sfEMG was performed during voluntary contraction using a 25 mm Ambu Neuroline disposable concentric needle electrode (diameter 0.30 mm (30G), recording area 0.02 mm^2) and standard filter setting of 1 kHz-10 kHz, gain (200 μV/division) and sweep speed (1 ms/division). After slight movements of the electrode, a pair of single fiber action potentials was recorded, with both potentials belonging to the same motor unit. The trigger was put to the higher amplitude spike, and then the temporal variation on the second spike was measured. If multispikes were recorded, the spike that produces the lowest summed jitter between spikes was chosen as the time reference. Acceptable potentials were based on the published criteria (Sanders et al., 2019). For each action potential pair measurement, 50–100 consecutive discharges were recorded for analysis and 20 different pairs were collected. Action potentials with amplitude < 100 μV, rise time > 300 ms, jitter value < 5 μs and inter-spike intervals > 4 s were excluded according to published guidelines (Stålberg et al., 2019). The jitter value was calculated by the mean consecutive differences (MCD) for each pair. The mean jitter was compared with the published reference values (Stålberg et al., 2016; Zambelis and Anagnostou, 2021), the test was interpreted as either normal or abnormal accordingly.

In all patients, anterior tibial muscle was examined. In March 2021, sfEMG of the extensor digitorum communis (EDC) muscle was added to the diagnostic work-up.

2.3. Muscle biopsy, light microscopy

Biopsies were taken from biceps brachii muscle and processed for paraffin embedding or frozen. Immunohistochemistry was performed on a Ventana Benchmark Ultra platform. Paraffin sections of 2 μm were used for double staining of fast (Sigma-Aldrich, My32, 1:8000) and slow (Novocastra, wb-MHCs, 1:50) fibers, while 5 μm cryosections were used for HLA-ABC (Dako, Agilent, W6/32, 1:100). In enzyme histochemistry for COX/Succinate dehydrogenase we used 7 μm cryosections.

2.4. Muscle biopsy, electron microscopy

Biopsy material for epoxy embedding was fixed in glutaraldehyde and postfixed for one hour in OsO_4 before embedding. The ultrathin sections, 60 nm, were contrasted with uranyl acetate and lead citrate.

2.5. Data analyses

Data was entered in a secured REDCap database and analyzed using Stata Intercooled version 17. Median, means and frequencies were reported. Mean MUP duration and mean jitter in tibialis anterior muscle were compared with symptom and health scores using correlation coefficient (r), p-values, and two-way linear prediction plots.

2.6. Ethical considerations

Patients were entered in the study following informed consent of using data from the patients' record. The study was approved by the Data Protection Authority in Central Denmark Region (reference 1–16-02–4-21).

3. Results

3.1. Patient demographics

From December 2020 to March 2022 a total of 659 patients were examined in the Post COVID clinic and 107 patients were received for EMG investigation. In all patients, qEMG and sfEMG were performed, and 99 patients accepted the use of their clinical data for this research study. From the 99 patients, 15 were excluded due to: other diseases with risk of or proven peripheral neuropathy (diabetes in three patients, neuropathy in five patients and hereditary neuropathy in one patient), no positive SARS-CoV-2 test (two patients), qEMG investigation less than 12 weeks after initial COVID-19 (one patient), age below 18 (one patient), or insufficient investigation due to patient compliance (two patients). Thus, 84 patients had qEMG performed and were included in analyses.

Median age of the 84 patients was 49 (Inter Quartile Range (IQR) 41–54) years, 64 (76 %) patients were female, and 11 of 84 (13 %) patients were hospitalized during the acute phase of COVID-19. Co-morbidity was reported in 18 of 84 (21 %). Patient demographics are shown in Table 1.

In 46 of the 84 patients (55 %), the strength was reduced (MRC = 4 or 4 +) in shoulder abduction (5 patients) or in hip flexion (9 patients) or both in shoulder abduction and hip flexion (32 patients). Of these, in 8 patients, the strength was decreased in elbow flexion/extension and knee flexion/extension too. None of the patients had decreased force in distal muscles.

Patient reported questionnaire information were available from 71 of patients included for EMG.

3.2. Electrophysiological examinations

Electrophysiological examinations were performed 9.8 (IQR 7.8–11.0) months after the acute phase of COVID-19.

None of the 84 patients had mononeuropathy or entrapment neuropathy in peroneal nerve or signs of polyneuropathy. None of the patients had decrement in repetitive nerve stimulation. In all patients, the decrease in amplitude was < 7 % in both median and accessory nerves.

None of the patients had fibrillation potentials or positive sharp waves in EMG.

In Fig. 1, the qEMG and sfEMG results are depicted as dot-plots.

The mean MUP duration was 9.79 ms ± 1.18 in biceps brachii, 10.63 ms ± 1.72 in vastus medialis and 11.29 ms ± 1.43 in tibialis anterior muscles. The mean jitter was 35.37 μs ± 11.79 in tibialis anterior and 27.97 μs ± 6.85 in extensor digitorum communis muscles.

In 53 patients (63 %), qEMG or sfEMG or both were abnormal in at least one muscle, while in the remaining 31 patients (37 %), the sfEMG was normal and qEMG did not show myopathic changes. None of our patients was examined in the acute phases or for any other acute peripheral nervous system disorder. Therefore, we interpreted the decreased MUP duration as myopathic changes compared to laboratory controls.

For the individual muscles, the mean MUP duration was decreased in 37 % of the patients in biceps brachii, 32 % in vastus medialis and 50 % in tibialis anterior muscles (Fig. 1A).

In six patients, the MUP duration was decreased in one muscle, in 16 patients in two muscles and in 21 patients in all three muscles examined. In total, there was decreased MUP duration in $\geq$ 1 muscles in 43 patients (52 %).

The most prominent finding was the increased incidence of polyphasic potentials in tibialis anterior muscle (76 %), while in biceps brachii (29 %) and vastus medialis (19 %), this was less pronounced (Fig. 1B).

There were two patients with reduced MUP amplitude in biceps brachii, whereas no patients showed decreased amplitude in vastus medialis or tibialis anterior muscles (data not shown).

Regarding the sfEMG, the mean jitter was increased in 14 of the 84 patients (17 %) in tibialis anterior and in 14 of the 57 patients (25 %) in EDC (Fig. 1C). In 19 patients, mean jitter was increased either in tibialis anterior or EDC muscle whereas in 10 of these, both muscles were abnormal.

Of the 19 patients (23 %) with increased mean jitter in tibialis anterior or EDC or both, the MUP duration was normal in 12 patients while qEMG was myopathic in seven patients in $\geq$ 1 muscles.

For the number of pairs with increased jitter, in tibialis anterior, 18 of the 84 patients (21 %) had $\geq$ 3 outliers with increased jitter for individual pairs. Of these, in 14 patients, the mean jitter was also increased. In EDC, 16 patients (28 %) had $\geq$ 3 pairs of outliers with increased jitter (Fig. 1C). Of these, in 14 patients the mean jitter was also increased.

In tibialis anterior, there were $\geq$ 2 pairs with blocking in seven patients, and in EDC, there were two patients with blocking in $\geq$ 2 pairs.

3.3. Correlation between clinical scores and electrophysiological examinations

There was no correlation between MUP duration and health scale or symptom scores for any of the muscles examined (Fig. 2-A-D).

Table 1
Demographic and clinical characteristics of patients referred for electromyography (EMG) examination due to severely affected and non-improving daily function, as well as physical/muscular exhaustion, myalgia or reduced force.

Total	N = 84
Time COVID-19 to EMG, months	9.8 (IQR 7.8–11.0)
Sex (male)	24 % (20/84)
Age, median (IQR)	49 (IQR 41–54)
Hospitalization in the acute phase of COVID-19	13 % (11/84)
Ethnic origin, other than Danish[1]	15 % (13/84)
Current smoker	9 % (7/81)
Alcohol, 7 units per week	8 % (6/76)
BMI above 25	68 % (55/81)
Familial predispositions	
Metabolic	14 % (12/83)
Allergy	16 % (13/83)
Autoimmunity	8 % (8/83)
Comorbidity[2]	21 % (18/84)
Diabetes	0 %
Asthma and COPD	11 % (9/83)
Hypertension	8 % (7/84)
Coronary dis.	<1 %
Cerebrovascular dis.	<1 %
Previous depression	9 % (7/81)
Medicine	
ACE or AT2 receptor inhibitor	6 % (5/79)
Statin[2]	10 % (8/82)
Steroids	0 % (0/80)
Biochemical analyses, abnormal[3]	
Creatine kinase	16 % (13/81)
Myoglobin	8 % (6/79)
Long COVID Symptoms	
Headache	86 % (70/81)
Concentration difficulties	92 % (77/84)
Paresthesia	64 % (54/84)
Dyspnoea during physical activity	90 % (75/83)
Chest pain	58 % (45/78)
Myalgia	88 % (74/84)
Physical fatigue	95 % (80/84)
Standardized health scale scores	
Post COVID questionnaire (IQR)	40 (27–52)
Post COVID Functional Scale 3–4	42 % (22/41)
Fatigue Assessment Scale $\geq$ 35	67 % (41/61)
EQ-5D-5L index, mean (SD)	0.69 (0.17)
Objective examination	
Reduced muscle force	55 % (46/84)

[1] Seven from middle East, four from other countries.
[2] Seven patients prescribed statins had normal EMG.
[3] Creatine kinase were above normal reference level in 9 patients without and 4 patients with abnormal EMG. Abnormal biochemistry values were slightly above reference normal value (data not shown). dis.: disease, IQR: Inter Quartile Range, SD: standard deviation.

Higher mean jitter values in sfEMG in tibialis anterior correlated with results of more severely affected health evaluated by EQ-5D-5L index and movement item in EQ-5D-5L (Fig. 2E-F) but the correlation was not significant for FAS and PCQ (Fig. 2G-H).

3.4. Laboratory tests

In cases where creatine kinase (CK) or myoglobin were abnormal, results were only slightly above the normal reference value (data not shown). Acetylcholine receptor antibodies were evaluated and not present in the 16 patients who had the most prominent sfEMG changes.

3.5. Histopathological findings

We performed muscle biopsies in eight patients. The patients were selected for biopsy based on a clinical indication. The results have recently been published (Hejbøl et al., 2022), and showed a large variety of changes. In the present study, we re-evaluated the previously published biopsies with focus on visualising motor end-plate cross sections.

Electrophysiological examinations showed three groups of patients, one with myopathic qEMG and normal sfEMG, one with abnormal sfEMG but not-myopathic qEMG and one showing both qEMG and sfEMG abnormalities. Of the eight patients with muscle biopsy, 5 patients had myopathic EMG but normal sfEMG, two patients had myopathic EMG and abnormal sfEMG and one patient had normal EMG but abnormal sfEMG. We selected three of the eight patients to represent each group of electrophysiological pathology (Fig. 3).

Patient 1 with normal EMG and abnormal sfEMG demonstrated very discrete changes including type 2 atrophy (Fig. 4A) small signs of muscle fiber membrane damage seen as small basal lamina duplications (Fig. 4B and C), and indications of capillary remodelling (D). No inflammatory infiltration was found and the HLA-ABC expression was insignificant (Fig. 4E) thus no indication of inflammation.

Patient 2 with myopathic qEMG and abnormal sfEMG showed type 2 fiber atrophy (Fig. 4F) but also the presence of increased number of internal nuclei indicated regenerative activity (Fig. 4G). COX negative fibers (Fig. 4H) suggested an involvement of mitochondria. Capillary involvement (Fig. 4I) was seen as basal lamina duplications and endothelial activation seen as prominent presentation of Weibel-Palade bodies and cytoplasmic projections. The presence of mature collagen in the endomysium indicated chronic changes (Fig. 4J), however there were only slight indications of inflammations seen as a weak HLA-ABC expression (Fig. 4K).

Patient 3 with myopathic qEMG and normal sfEMG showed on ultrastructural level signs of damage of terminal nerves and motor endplate. A prominent sign was increased amount of basal lamina both in relation to perineurial cells and at the motor end plate (Fig. 5). In the small nerves, loss of unmyelinated fibers was seen (Fig. 5B), but no changes in myelinated nerves were observed. At the end plate, axons were present (Fig. 5C, D, and E) but no vesicle containing terminals. The post synaptic cleft in areas appeared atrophic with short clefts and coarse clefts (Fig. 5D).

4. Discussion

This study showed myopathic changes in qEMG and/or increased jitter in sfEMG in 53 of the 84 patients (63 %) referred to electrophysiological testing for neuromuscular Long COVID symptoms. Myopathic qEMG was more common (52 %) than sfEMG (23 %), and seven of the 84 patients (8 %) showed both. There was no correlation between the clinical scores and qEMG measures while more pronounced sfEMG changes correlated with some of the more severe Long COVID symptom scores. Muscle biopsy in a subgroup showed as previously published (Hejbøl et al., 2022) a wide variety of histological findings including mitochondrial changes, inflammation, and capillary injury. The novelty in the present study is that we show now damage of terminal nerves and motor endplate.

Muscle damage is not surprising following severe acute COVID-19 with rhabdomyolysis, which is reported in patients hospitalized in the acute phase (Albaba et al., 2021). Only 13 % of patients in this study required hospital admission during their acute course of COVID-19, none required intensive care unit therapy or had signs of rhabdomyolysis. Thus, our findings are not related to severe acute COVID-19 disease.

Fatigue, muscle weakness, and muscle aches are among the most common symptoms in Long COVID patients (Buttery et al., 2021; Davis et al., 2021). Whitaker et al. estimate a population prevalence of Long COVID of 5.75 %, and tiredness, muscle aches and heavy arms/legs were three of 15 symptoms which they corre-

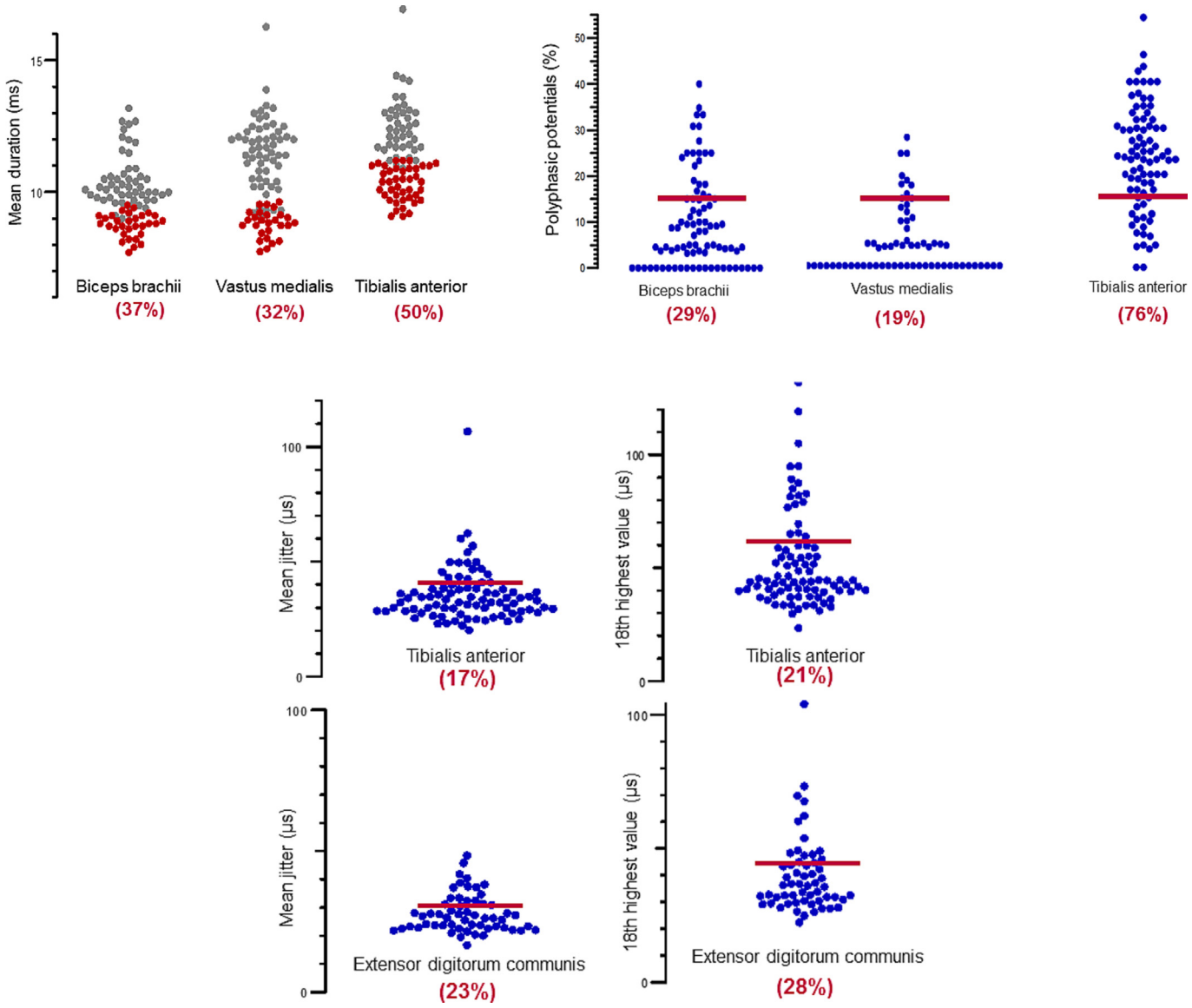
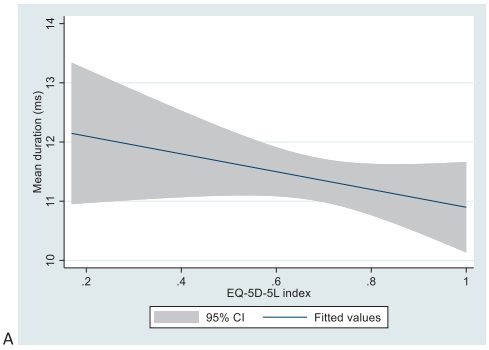


Fig. 1. Dot plots of quantitative electromyography (qEMG) and single fiber EMG (sfEMG) measurements in 84 Long COVID patients. Numbers in parenthesis show the number of patients with abnormal findings. A) Dot plots of motor unit potential duration. Red plots indicate the patients with decreased values compared with age-matched laboratory controls and gray plots indicate the patients with normal motor unit potential duration, B) Dot plots of percentage of polyphasic potentials. Red lines show 15 % laboratory limit for abnormally increased percentage of polyphasic potentials, and C) Dot plots of sfEMG results for mean jitter and number of outliers. Red lines show reference limits compared with published normal values for extensor digitorum communis (Stålberg et al., 2016) and tibialis anterior (Zambelis and Anagnostou, 2021). Upper limit for mean jitter is 30 µs for extensor digitorum communis and 41.6 µs for tibialis anterior while the upper limit for outliers is 43 µs for extensor digitorum communis and 59.7 µs for tibialis anterior.

lated with a self-reported positive SARS-CoV-2 PCR test (Whitaker et al., 2022). An increased prevalence of musculoskeletal symptoms following COVID-19 is also reported compared to control groups. In a health care register cohort of almost 5 million with 181,384 COVID-19 patients, excess burden of fatigue in 13.59 of 1000, and muscle weakness in 9.8 of 1000 people, were estimated 6 months after COVID-19 infection, compared with negative controls (Xie et al., 2021). Taquet et al. found an increased absolute risk of fatigue and myalgia in COVID-19 patients compared with influenza patients (Taquet et al., 2021a), and a significantly higher odds for at diagnose of myoneuronal junction and muscular disease (Taquet et al., 2021b). Later, Kaspersen et al found fatigue and muscle or joint pain more commonly to persist $\geq$ 30 days in SARS-CoV-2 seropositive than in SARS-CoV-2 seronegative health care workers (Kaspersen et al., 2021). In the light of evidence of neuromuscular problems following COVID-19 in this study and our previous studies (Agergaard et al., 2021; Hejbøl et al., 2022) and no established pathophysiological explanation for Long COVID symptoms, we consider our results to be important in the search for an explanation of Long COVID.

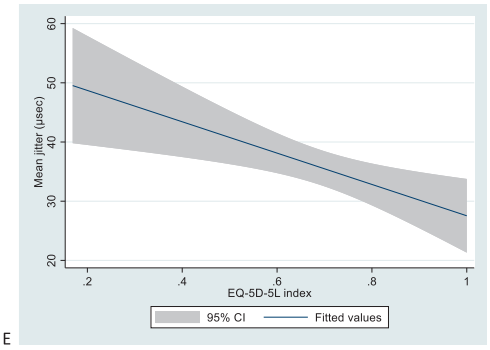
Prevalence of comorbidity, medicine intake, depression, smoking or drinking alcohol in the study group (Table 1) indicated the investigated patients had few health problems or health risks. The prevalence of these health risk factors were not more prevalent in the study group than health risk factors in the general Danish population (Syddansk Universitet, 2021). It is consistently found in several studies that female sex/gender predisposes for Long COVID symptoms (Pelà et al., 2022) and a 76 % of patients investigated by qEMG were female.

In a previous study, we found a wide variety of histological changes including mitochondrial changes, inflammation, and capillary injury in muscle biopsies that could cause fatigue in part due to reduced energy supply (Hejbøl et al., 2022), and we reported myopathic changes in EMG (Agergaard et al., 2021). We found a similar



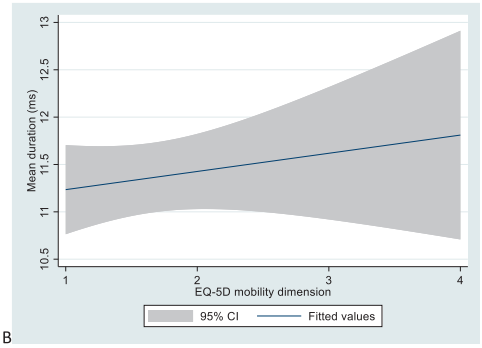
A

r= - 0.17, p=0.17



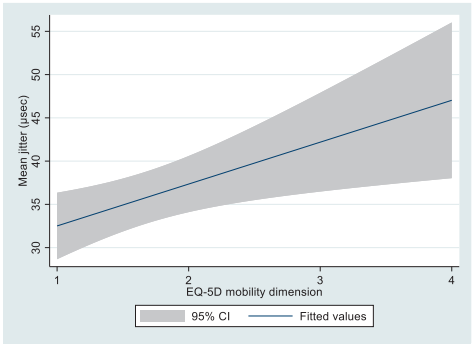
E

r= - 0.35, p=0.004



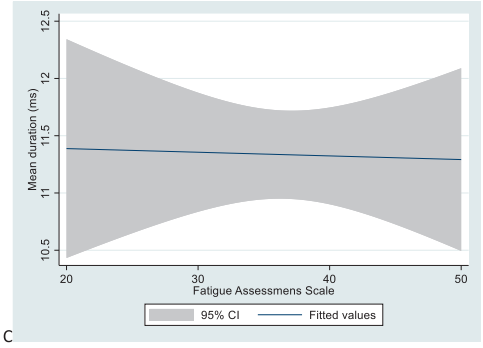
B

r=0.10, p=0.39



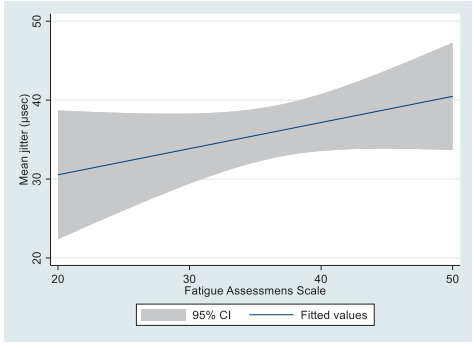
F

r=0.32, p=0.01



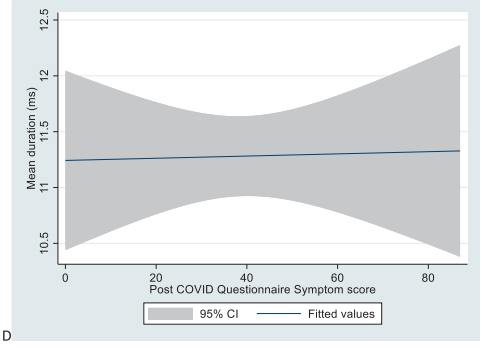
C

r= - 0.02, p=0.90



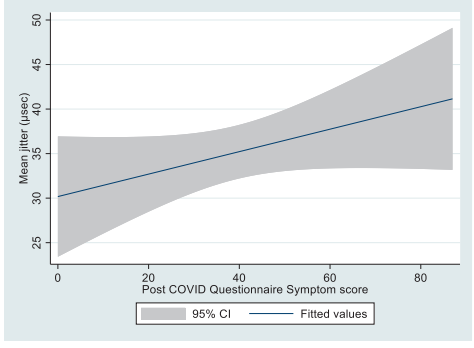
G

r=0.19, p=0.14



D

r=0.01, p=0.91



H

r=0.20, p=0.11

Fig. 2. Correlation plots. Mean motor unit potential (MUP) (A-D) and mean single fiber electromyography (sfEMG) (E-H) correlations with Health-Relates Quality of Life (EQ-5D-5L)-index, movement item of EQ-5D-5L, Fatigue Assessment Scale (FAS) score, and Post COVID Questionnaire symptom (PCQ) score. Two-way linear prediction plots with 95 % confidence intervals. Pearson correlation coefficient (r) and p-value for the correlation presented below each plot. Worse health condition is indicated by a lower EQ-5D-5L index, higher score in movement item of EQ-5D-5L index, higher score in FAS and higher PCQ.

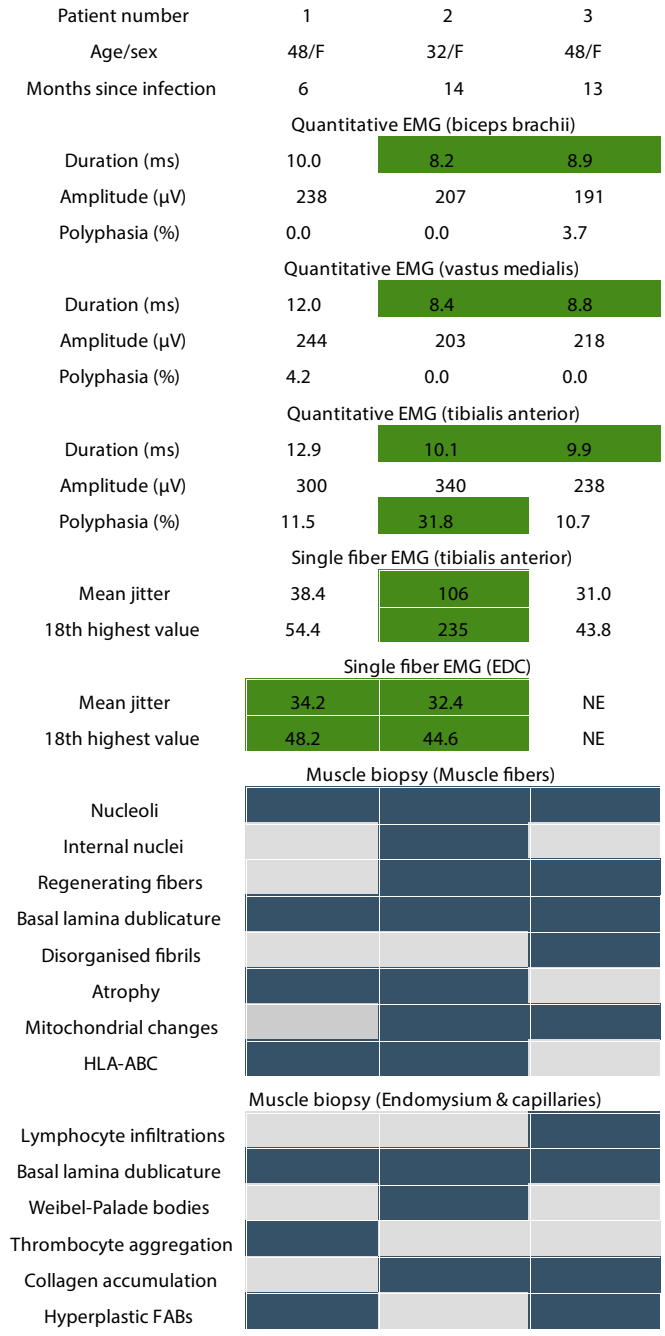


Fig. 3. Summary of electrophysiological and histopathological findings. The heat map shows findings from 3 patients with Long COVID symptoms. Electromyography (EMG) was performed, with quantitative motor unit potential analyses. Dark green cells indicate abnormal EMG results compared with age matched laboratory controls. Single fiber EMG (sfEMG) in tibialis anterior was performed in all patients. Dark green cells indicate abnormal increased jitter compared with published reference values (Zambelis and Anagnostou, 2021). sfEMG in extensor digitorum communis was performed in two patients and both patients showed increased mean jitter compared with published reference values (Stålberg et al., 2016). Muscle fibers and endomysium and capillaries show the histopathological changes based on light microscopy and electron microscopy. EDC = extensor digitorum communis; F = female; FAP = fibroadipogenic progenitor; HLA-ABC = human leukocyte antigen ABC; M = male; NE = Not examined.

pattern in the qEMG and sfEMG findings in the present study. Patients had myopathic qEMG or abnormal sfEMG or both. We present here the histological results in patients from each group. The myopathic changes in the patients with only abnormal sfEMG suggest that the primary pathology may be in the muscle fibers. In the present study, up to 76 % of the patients had increased polyphasia on qEMG, which may be explained by unequal muscle fiber diameter due to muscle fiber atrophy or changes of regeneration such as basal lamina duplications detected by histology. We also showed in our previous study uncommon amounts of basal lamina not only surrounding muscle fibers but also around nerves and capillaries.

In one patient with myopathic qEMG but normal sfEMG, we were lucky to visualise the motor endplate in electron microscopy with abundant basal lamina material and atrophic post synaptic cleft. These changes may suggest a similar mechanism in patients with normal as well as abnormal sfEMG. Most interestingly, the sfEMG measures correlated with the severity of clinical scores as MUP durations did not. From these results, one may speculate that abnormalities in motor endplate might be giving most of the symptoms, and the patients particularly with abnormal sfEMG may benefit from medications such as Pyridostigmine.

Previous studies of possible mechanisms behind Long COVID are not incompatible with myopathy. SARS-CoV-2 has been detected in skeletal muscle including diaphragm muscle in autopsies (Aschman et al., 2021; Shi et al., 2021), but an indirect inflammatory process seems also to be a likely cause of disturbance of cellular functions (Mehandru and Merad, 2022). In the acute phase of COVID-19 there is an increased risk for thromboembolism (Malas et al., 2020); neuroinflammation is reported (Kanberg et al., 2021; Pilotto et al., 2021); and entry mechanisms have been investigated and discussed (Kumar et al., 2022). A defect in the mitochondria leading to metabolic disturbances has been hypothesized (Hejbøl et al., 2022; Paul et al., 2021) and clinical trials has been established (ClinicalTrials.gov, 2022; Hansen et al., 2022). Reduced oxygen consumption after COVID-19 supports this mechanism (Singh et al., 2022). Viral effects on mitochondrial function was also previously described (Li et al., 2021), and remains to be further explored in SARS-CoV-2. Yet, few studies guide the establishment of paraclinical diagnostic criteria for the long-term post-COVID-19 sequelae, in which neurophysiological examination may play a role (Haykal and Menkes, 2022).

Among the most common Long COVID symptoms, memory disturbances and headache have often been reported together with fatigue (Nasserie et al., 2021). In our study, headache was seen in 86 % and memory disturbances in 92 % of our patients (Table 1). Neurophysiological methods may contribute shedding a light on the mechanisms of both central (Versace et al., 2021; Ortelli et al., 2022) and peripheral (Agergaard et al., 2021) components of Long COVID.

An obvious weakness of this study is the lack of systematic inclusion of consecutive patients and the lack of comparison with another patient cohort with physical fatigue not related to COVID-19. Another limitation of our study is that we did the muscle biopsy in one muscle while sfEMG was performed in another muscle, therefore, we cannot directly correlate the histopathological and electrophysiological findings. Additionally, we cannot draw any concrete conclusions from the small number of muscle biopsies available in this study cohort. In 55 % of the patients, we found decreased force in the proximal muscles. Since full patient effort being given on the strength examination could not be ensured, isokinetic and isometric muscle strength examination using a dynamometer would be a more objective measure. However, these clinical investigations showed that the majority of patients referred for investigation had myopathic qEMG. Biopsies suggest that muscle pathology may occur even in Long COVID patients with normal qEMG (Hejbøl et al., 2022), and patients not referred for qEMG might also present neuromuscular pathology if they were to be examined. Our findings may be generalizable for other Long COVID patients, and a considerable number of individuals may suffer from neuromuscular disease.

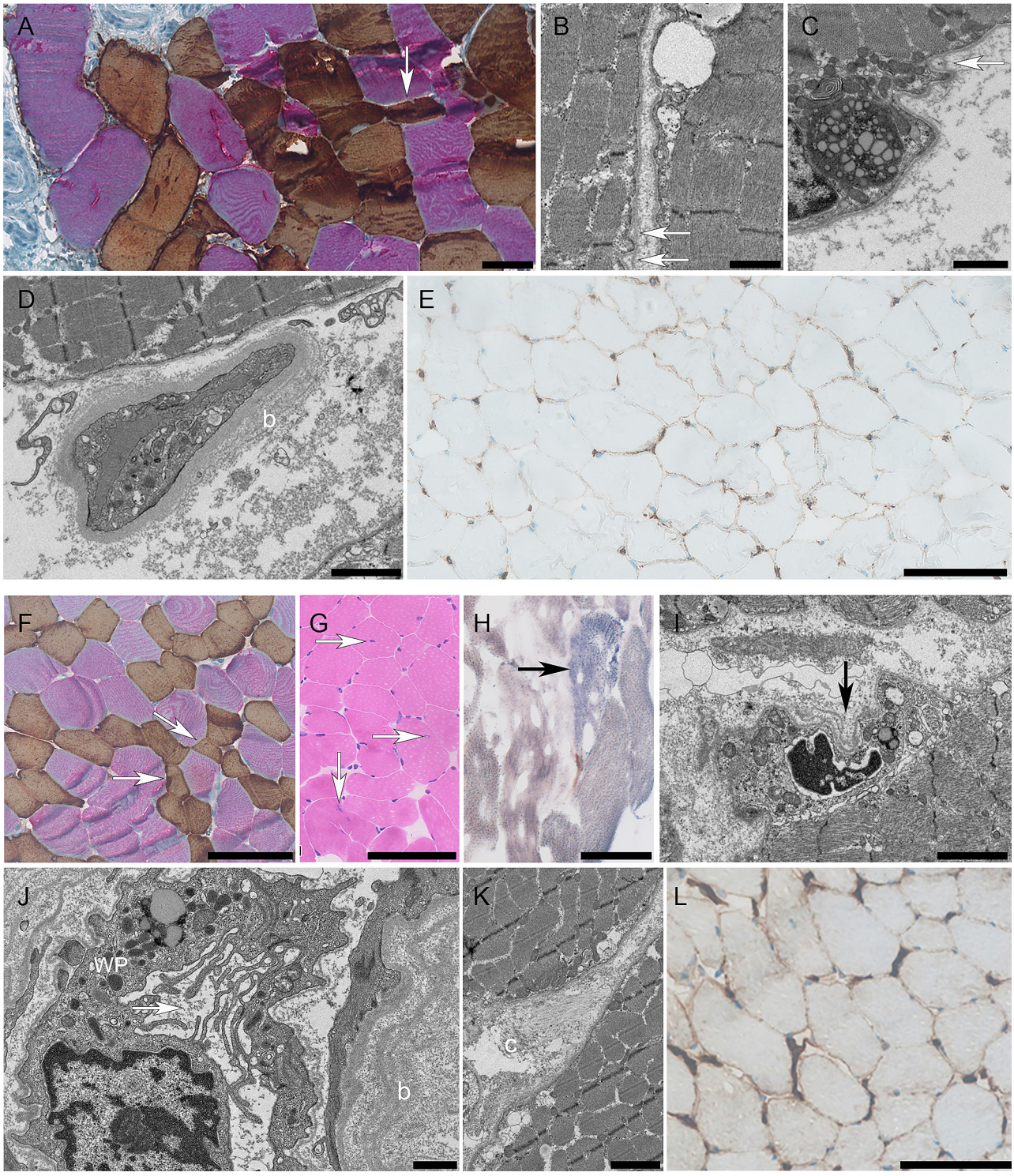


Fig. 4. 48-year old female, disease duration 6 months without signs of myopathy in quantitative electromyography (qEMG) but abnormal single fiber EMG (sfEMG) (only in extensor digitorum communis) (A-E) and a 32-year old female, disease duration 14 months with myopathic qEMG and abnormal sfEMG (F-L). A: IHC for slow (red) and fast (brown) MHC. Arrows point to atrophic fast (type 2) fibers. B and C: Electron microscopy demonstrating small basal lamina duplications (arrows) indicating membrane damage. D: Capillary in the endomysium with multiple basal lamina (b), indicating demodulation. E: IHC for HLA-ABC showing insignificant membrane reaction in the muscle fibers. F: IHC for slow (red) and fast (brown) MHC. Arrows point to atrophic fast (type 2) fibers. G: H&E showing internal nuclei (arrows). H: COX/ succinate dehydrogenase enzyme stain showing a COX negative succinate dehydrogenase positive (blue) fiber (arrow). I: Duplication of muscle fiber basal lamina (arrow). J: Capillary with multiple basal lamina (b), and endothelial cell with Weibel – Palade bodies (WP) and multiple, long cytoplasmic projections into the lumen (arrow). K: Aggregation of collagen (c) between muscle fibers. L: IHC for HLA-ABC showing a slight but continues reaction of the muscle fiber membranes. Scale bars. A, E, F, G, and L: 100 μm, B and C: 1 μm, D, I, J, and K: 2 μm, H: 50 μm.

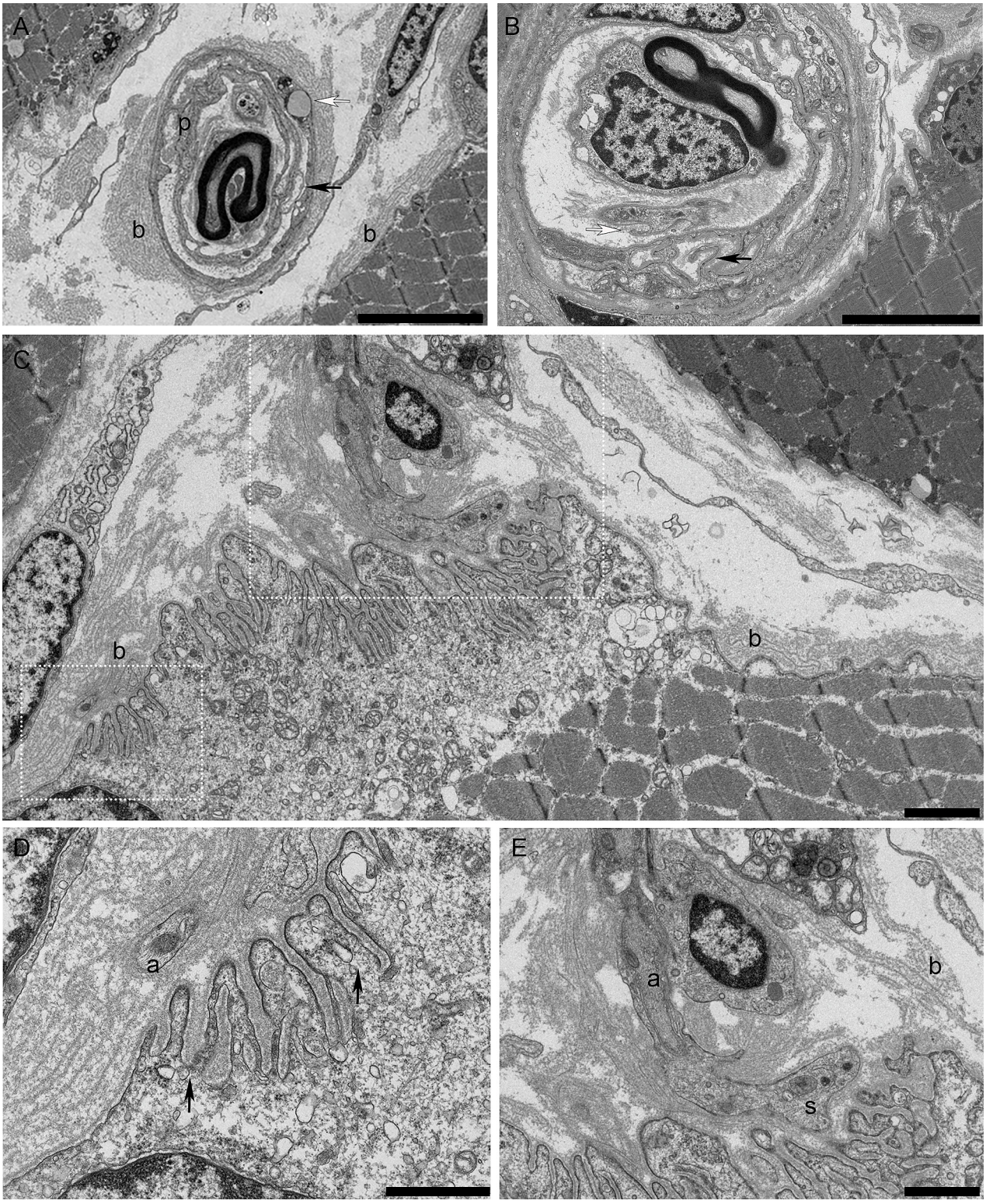


Fig. 5. Ultrastructure of Intramuscular nerves and motor endplate from 48-year old female with myopathic quantitative electromyography and normal single fiber EMG (disease duration 13 months). A: A nerve with perineurial cells (p) with multiple layers of basal lamina (b) on the external side and basal lamina material in the dilated space between perineurial cells (black arrow). The adjacent muscle fiber is also covered with multiple layers of basal lamina (b). The white arrow points to secondary lysosomes in a perineurial cell. B: A nerve with increased space between the perineurial cells, some of which have small cytoplasmic projections into the intercellular space (example at the black arrow). The endoneurium contains part of a non-myelinating Schwann cell that lacks the axons in the cleft (white arrow). C: A motor endplate with abundant basal lamina material (b). No terminal boutons are seen. The areas in the dot frames are magnified in D and E. D: An axon (a) is seen close to the post synaptic side that presents short clefts and broad crests (black arrow). E: An Axon (a) is seen close to the synaptic cleft basal lamina folds (b) and a part of a Schwann cell (s) is seen in the synaptic cleft. Scale bars. A and B: 5 μm, C: 2 μm, and D and E: 1 μm.

5. Conclusion

We showed myopathic changes in qEMG in majority of patients while abnormal sfEMG was less common. However, mean jitter was correlated with the clinical scores. Our results suggest that neuromuscular pathology may be common in Long COVID patients, which may be an important cornerstone in the search for treatment options.

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Appendix

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