Long-term symptom severity and clinical biomarkers in post-COVID-19/chronic fatigue syndrome: results from a prospective observational cohort

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Summary

Background Post-COVID-19 syndrome (PCS) is characterised by a wide range of symptoms, primarily fatigue and exertion intolerance. While disease courses in the early months post-infection have been well-described, the long-term health consequences for patients with PCS with disabling fatigue remain unclear.

Methods In this prospective observational cohort study, we evaluated symptom severity and various biomarkers, including hand grip strength (HGS), cardiovascular function, and laboratory parameters, in 106 patients with PCS with moderate to severe fatigue and exertion intolerance at three time points after infection (3–8, 9–16, and 17–20 months). The study was conducted at the Charité's Fatigue Centre and the Charité's outpatient clinic for neuroimmunology at Berlin, Germany from July 16, 2020, to February 18, 2022. A subset of patients (PCS-ME/CFS) met the diagnostic criteria for myalgic encephalomyelitis/chronic fatigue syndrome according to the Canadian Consensus Criteria (CCC). The aim was to determine differences in the disease course between the two patient groups (i.e., PCS vs PCS-ME/CFS) and identify correlating biomarkers.

Findings Patients with PCS-ME/CFS reported persistently high severity of most symptoms up to 20 months after infection, while patients with PCS showed overall health improvement. Although fatigue and post-exertional malaise (PEM), hallmarks of post-infectious fatigue syndromes, were still evident in both groups, they remained more pronounced in PCS-ME/CFS. Inflammatory biomarkers decreased in both groups, but not antinuclear antibodies. Lower HGS at onset correlated with symptom persistence, particularly in patients with PCS-ME/CFS.

Interpretation Our findings suggest that PCS can persist beyond 20 months post-infection and encompass the full scope of post-infectious ME/CFS as defined by the CCC. Sub-classifying patients with PCS based on the CCC can assist in the management and monitoring of patients with PCS-ME/CFS due to their persistently higher symptom severity.

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Research in context

Evidence before this study

We conducted a comprehensive search on PubMed from the emergence of SARS-CoV-2 until April 31, 2023, with no restrictions to language or article type, using the terms "post COVID" OR "long COVID" AND "fatigue", yielding 1383 articles. Previous studies have highlighted the presence of (long-lasting) symptoms such as fatigue, headache, cognitive and emotional impairment, orthostatic intolerance, physical disability, and social dysfunction following SARS-CoV-2 infection, significantly impacting patients' quality of life. However, those studies mostly relied on cross-sectional analyses, solely focused on observation periods up to 12 months or lacked in-depth analyses only using self-assessment questionnaires. Furthermore, clear sub-classifications for post-COVID-19 syndrome (PCS) are often missing, which poses challenges in identifying appropriate subgroups to accurately predict disease progression and narrow down diagnostics and potential treatments.

Added value of this study

We assessed symptom severity using physician-supervised questionnaires and collected clinical, functional, and laboratory parameters in 106 patients at three time points over a duration of 20 months. To our knowledge, this study is the first to specifically evaluate long-term disease courses of patients with PCS experiencing significant and persistent fatigue and exertion intolerance, including extensive clinical parameters in addition to (self-reported) symptom assessments. We classified this PCS cohort based on the Canadian Consensus Criteria (CCC) for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and found that PCS progressed to encompass PCS-ME/CFS in 55 out of 106 patients. Fatigue and post-exertional malaise (PEM) remained more pronounced in PCS-ME/CFS, and while most inflammatory biomarkers decreased, antinuclear antibodies (ANA) remained elevated. Postural orthostatic tachycardia syndrome (POTS) exclusively persisted in patients with PCS-ME/CFS, indicating the need for further investigations.

Implications of all the available evidence

Our findings suggested that sub-classifying patients with PCS based on the CCC can assist in the management and monitoring of patients with PCS-ME/CFS due to their persistently higher symptom severity. We suggest that hand grip measurements could serve as a simple and accessible method for estimating prognosis of patients with PCS-ME/CFS and warrant further evaluation. Our study thus provides directions for clinical practice for the large number of patients with PCS world-wide.

Introduction

Post-COVID-19 syndrome (PCS) is worldwide recognised as sequela of coronavirus disease 19 (COVID-19) caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2).[1] There is a worrying number of patients with various persistent symptoms following mild or moderate COVID-19 mainly presenting as fatigue, exertion intolerance, headache, myalgia, neurological and cognitive deficits as well as orthostatic disturbances, which can severely impact the patients' quality of life.[2–7] Reports estimate a proportion of 2%–10% of all COVID-19 patients to be still impaired one year after infection.[2,3,8]

More than two years into the SARS-CoV-2 pandemic, the WHO led the way to standardise the definition of PCS as part of the WHO International Classification of Diseases (ICD-10)[4]: The post-COVID condition occurs within three months after a probable SARS-CoV-2 Infection, lasts for at least two months with an impact on everyday functioning and cannot be explained by alternative diagnoses. Acknowledging the post-COVID condition was a crucial first step towards recognizing and improving the health care situation of the patients affected. While the short- and medium-term clinical presentations of PCS between 3 and 9 months after SARS-CoV-2 infection have been concisely described,[3,5,9] little is known to date about potential long-term health consequences that may prevail beyond 12 months.

We previously reported on the first results of our ongoing prospective observational cohort study initiated in August 2020 in order to characterise patients with persisting debilitating fatigue and exertion intolerance following COVID-19.[9] Our first analyses revealed that a subset of patients with PCS developed the full scope of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) 6 months after initial infection.[9] ME/CFS is a complex multisystemic disease with an estimated pre-pandemic worldwide prevalence of 0.2–0.8%. Approximately 3 million people were diagnosed with ME/CFS in Europe by 2020 alone.[10,11] It is characterised by pronounced fatigue and post exertional malaise (PEM), cognitive impairment, orthostatic intolerance, pain and sleep disturbance while lacking evidence of macroscopic organ damage. The key symptom of ME/CFS is an

intolerance to mental and physical exertion, which triggers PEM.[12] Infections with various pathogens can cause ME/CFS such as Epstein–Barr virus (EBV), enteroviruses, human herpes virus (HHV)-6, dengue viruses, intracellular bacteria and SARS-CoV-1.[13] The pathomechanism is still only partially elucidated: Infection-triggered autoimmunity, viral mimicry, latent virus reactivation, and autonomous dysfunction including a dysregulation in β2-adrenergic vasoconstriction are concepts currently debated.[14] Specifically, for PCS-ME/CFS, studies have provided first evidence that autoantibodies to G protein–coupled receptors and endothelial dysfunction may play a role.[15] Of interest, reactivation of EBV during COVID-19 frequently occurs and is considered a risk factor for developing PCS.[13,16–19] Here we present the follow-up data up to 20 months after SARS-CoV-2 infection and describe biomarkers correlating with the disease course. We hypothesised that the subgroup of patients with PCS-ME/CFS develops a chronic condition with distinct clinical and paraclinical features.

Methods

Study design and cohort characteristics

This work's data was collected as part of the PA-COVID study of the Charité—Universitätsmedizin Berlin and approved by the ethics committee of the Charité in accordance with the 1964 Declaration of Helsinki and its later amendments (ethics approval number EA2/006/20). The current manuscript analyses follow-up data from a prospective observational cohort study of patients with severe fatigue and exertion intolerance post COVID-19 diagnosis.[9] A formal sample size calculation or power analysis was not feasible due to the exploratory nature of the study. Patients were recruited via the Charité's Fatigue outpatient clinic's website. From a total of 250 patients who were first seen at the Charité's Fatigue Centre and the Charité's outpatient clinic for neuroimmunology at Berlin, Germany, between July 16, 2020 and February 18, 2022, 171 fulfilled the inclusion criteria of (Supplementary Fig. S1a): (1) confirmed previous diagnosis of mild to moderate COVID-19 according to WHO criteria I or II, (2) persistent moderate to severe fatigue according to Chalder Fatigue Score (CFQ) and exertion intolerance with PEM, (3) absence of COVID-19-related organ dysfunction (as examined in case of indicative symptoms) and 4) absence of preexisting fatigue or relevant cardiac, respiratory, neurological, or psychiatric comorbidities according to the European Network on ME/CFS (EUROMENE) guidelines.[10] COVID-19 diagnosis was confirmed by SARS-CoV-2 polymerase chain reaction (PCR) or anti-SARS-CoV-2 IgG serology (only prior to vaccination). To exclude COVID-19-related organ dysfunction, patients with severe headache or cognitive impairment were evaluated by a neurologist, patients with respiratory problems underwent comprehensive pulmonological examination including chest computer tomography and pulmonary function tests with diffusing capacity and patients with chest pain, postural tachycardia, palpitations, or elevated NT-pro BNP had a cardiological examination and were assessed by electrocardiogram (ECG), 24 h ECG and echocardiography. Baseline assessment was conducted 3–8 months post COVID-19 manifestation. Follow-up visits were scheduled 9–16 months (follow-up 1) and 17–20 months (follow-up 2) post COVID-19 diagnosis (see Supplementary Fig. S1b). All visits included questionnaires as well as functional and laboratory tests (see Supplementary Fig. S1b). An additional questionnaire-based assessment was conducted 9 months post COVID-19 to standardise diagnoses of patients with baseline visits at months 3 and 4 post infection as ME/CFS can only be diagnosed after symptom persistence of more than 6 months. These assessments were not used for analysis. A total of 65 patients were excluded from the final analyses. Out of these, 19 participants were dropouts (PCS n = 12; PCS-ME/CFS n = 7), while the remaining 46 were excluded due to a missing second data point at the time of analysis, which prevented longitudinal evaluation (PCS n = 20; PCS-ME/CFS n = 26). This resulted in 106 patients with complete datasets from at least two different assessment timepoints (baseline and one follow-up). For cohort sample sizes for each assessment, group and time point, please refer to the Supplement (Supplementary Table S1). All participants provided their informed consent to participate.

Diagnosis and symptom assessment

PCS was diagnosed based on fatigue, exertion intolerance with PEM and, optionally, additional key symptoms following COVID-19 persisting for at least 3 months and impairing daily live according to the WHO "clinical case definition of post-COVID-19 condition".[4] The diagnosis of PCS-ME/CFS was based on the Canadian Consensus Criteria (CCC) and PEM, which lasted until the next day. The minimum PEM duration required for diagnosis was set at 14 h as this cut-off value has been shown to be reliable in distinguishing ME/CFS fatigue from fatigue associated with other diseases.[20] In contrast, patients with PCS did not fulfill all CCC and most presented with PEM less than 14 h.

Post-COVID-19 cardinal symptoms and their severity were assessed (on a scale from 1 to 10, no symptoms to extreme symptoms) using the quantitative CCC (qCCC) from 2003.[21] Symptom complexes were summed up as follows: Fatigue, impaired performance, need for rest and post exertional malaise were summarised as qCCC fatigue score. Painful lymph nodes, sore throat and flu-like symptoms were summed up as qCCC immune score. Concentration impairment, memory/wordfinding problems and mental fatigue were summarised as qCCC mental score. The CFQ is a broadly recognised

measuring tool for the diagnosis of ME/CFS fatigue and contains 11 items on an ordinal scale of 0–3 with a minimal total score of 0 (no fatigue) to a maximum total score of 33 (strong fatigue).[22,23] The two subscales are rated as follows: mental fatigue (CFQA) contains 4 items with a range of 0–12 and physical fatigue (CFQB) 7 items with a range of 0–21. Frequency, severity and duration of PEM symptoms were assessed according to Cotler et al. (range 0–46, no PEM to frequent, severe or long-lasting PEM).[20] Impairment in daily life due to chronic fatigue was rated based on the Bell disability scale with increasing impairment from 100 (no symptoms present) to 50 (moderate symptoms at rest and moderate to severe symptoms with exercise or activity; unable to perform strenuous duties, but able to perform light duty or desk work 4–5 h a day) and 0 points (unable to get out of bed independently).[24] Physical and social dysfunction, bodily pain, emotional well-being, general health perception and health change were evaluated by the Short Form-36 (SF-36), which is used as a generic measurement tool to assess health perception, disease progression and experienced impairment through illness[25] and ranges from 0 (greatest possible health limitation) to 100 (no relevant health limitation) points. The subitems orthostatic intolerance and gastrointestinal function of the Composite Autonomic Symptom Score 31 (COMPASS 31) were used to detect symptoms of autonomic dysfunction often reported by patients with PCS with a minimum total score of 0 (no symptoms) and a maximum total score of 100 (strong autonomic dysfunction).[26] Furthermore, we used the Patient Health Questionnaire-9 (PHQ-9), a questionnaire to screen for depressive symptoms[27]: Due to the overlap of several PCS symptoms with depressive disorders, the PHQ-9 score was used descriptively only.

Functional tests and biomarker assessment

Postural tachycardia syndrome (POTS), orthostatic hypotension (OH) and diminished hand grip strength (HGS) were used as clinical markers to comprehensively characterise the broad variety of symptoms and their severity seen in patients with PCS. POTS and OH describe different phenotypes of autonomic dysfunction and are often seen in patients with ME/CFS.[28–30] For evaluation, blood pressure and heart rate were measured in seated position, immediately after standing up and after two, five and 10 min (standing). Orthostatic intolerance was defined as increase of more than 30 bpm or above 120 bpm within 10 min after standing up.[30,31] OH was defined as decrease of systolic pressure of more than 20 mmHg or diastolic pressure of more than 10 mmHg at any measurement.[30] HGS, which is a meaningful marker for evaluation of muscle exertion and fatigability in fatigue patients, was assessed using an electronic dynamometer.[32] In detail, patients gripped the measuring device 10 times with maximum force with their leading hand. This procedure was repeated after 60 min. Maximum (fmax1, fmax2) and mean (fmean1, fmean 2) force of each session were determined in kg. Previous studies by Jäkel et al.[32] identified reference values in healthy females (fmean1 = 25.6 kg; fmean2 = 25.9 kg; fmax1 = 28.2 kg; fmax2 = 28.7 kg) and determined cut-off values for the diagnosis of ME/CFS in patients 20–39 years and 40–59 years of age respectively. Cut-offs: 20–39 years fmean1 < 19.74 kg, fmean 2 <19.95 kg, fmax1 < 23.55 kg, fmax2 < 24.40 kg; 40–59 years fmean1 < 15.73 kg, fmean2 < 16.75 kg, fmax1 < 25.05 kg, fmax2 < 19.95 kg.[32] Furthermore, we calculated the fatigue ratio (fmax/fmean) as correlate of decreased force after repeated measurements (cut-offs: 20–39 years > 1.161 session 1, >1.189 session 2; 40–59 years > 1.117 session 1, >1.157 session 2) and the recovery ratio (fmean2/fmean1) indicating lower force during the second measurement (cut-offs: 20–39 years < 0.914; 40–59 years < 0.9003), thus impaired muscle recovery.[32] We decided to investigate multiple laboratory parameters, which have previously been associated with postinfectious fatigue syndromes including ferritin (reference range 13–150 µg/l), interleukin 8 (IL-8) in erythrocytes (reference <150 pg/ml), mannose-binding lectin MBL (reference >50 ng/ml), antinuclear antibodies (ANAs) (reference negative 1:0 dilution) and serum phosphate (PO4) (reference range 0.87–1.45 mmol/l).[33] They were determined at the Charité diagnostics laboratory (Labor Berlin GmbH, Berlin, Germany).

Statistical analysis

Study data were collected and managed using REDCap, an electronic data collection system, and last accessed on June 22, 2023. Non-parametric rank-based ANOVA tests for factorial longitudinal data were used for data analysis. These tests served as the effect measure underlying the well-established Wilcoxon-Mann-Whitney test for longitudinal data. We tested for group effects, time effects, and an interaction effect between the group and time effect. A group effect corresponds to a significant difference in the distribution of data between the PCS and the PCS-ME/CFS cohort, a time effect to a significant change in the data distribution over time, and an interaction effect to a temporal trend difference between the PCS and the PCS-ME/CFS cohort. The effects measured should be interpretated as follows: When the effect is ½, it indicates that the values in one group are approximately equal to those in the combined sample. If the effect of group 1 is smaller than the effect of group 2, it suggests that the data in group 1 tend to be smaller than those in group 2. Therefore, by sorting the effects from smallest to largest, we could determine which groups had the smallest and largest data values. Empirically, the effects are computed using joint ranks of the data and base classical rank tests. Note that the reported effect sizes are relative (i.e., the probability of observing larger values for a given group at a given

time). Again, due to the exploratory nature of this study, and the multitude of parameters evaluated, the effects of the main analysis were not adjusted for multiple comparisons. In subsequent post-hoc analyses, Wilcoxon-Mann-Whitney tests were used to analyse group differences per time point and time differences per group. The corresponding p-values were adjusted according to the Holm-Bonferroni correction (see Table 1). Correlation analyses were performed using Kendall's Tau correlation. p-values <0.05 were considered statistically significant. All data analyses were performed in Prism version 9 and R version 4.2.1 with the packages tidyverse version 1.3 and nparLD version 2.2.[34]

Role of the funding source

The funders did not have any role in design and conduct of the study, analysis and interpretation of the data, preparation, review, or approval of the manuscript and decision to submit the manuscript for publication. All authors had full access to the data set of the study and had final responsibility for the decision to submit for publication.

Results

Cohort and symptom characteristics

We examined a total of 106 patients suffering from persistent moderate to severe fatigue and exertion intolerance 6 months post COVID-19 at up to two follow-up time points (9–16 months, and 17–20 months) after SARS-CoV-2 infection (Supplementary Fig. S1). 55 patients fulfilled the CCC for ME/CFS and are referred to as PCS-ME/CFS; the remaining 51 patients are referred to as PCS. For demographic characteristics see Table 2. Symptoms frequently reported by both post-COVID-19 and patients with ME/CFS are shown in

Canadian Consensus Criteria quantified (CCCq)	Baseline				p-value between groups[a]	Follow-up 1				p-value between groups[a]	Follow-up 2				p-value between groups[a]	PCS time effect[a]	PCS-CFS/ME time effect[a]
	PCS		PCS-ME/CFS			PCS		PCS-ME/CFS			PCS		PCS-ME/CFS				
	Median	%	Median	%		Median	%	Median	%		Median	%	Median	%			
Fatigue	7	**100**	8	**100**	0.18	6	**98**	8	**100**	**<0.01**	5	**100**	8	**100**	**<0.01**	**<0.01**	0.58
PEM	7	**88**	8	**100**	**<0.01**	6	**98**	8	**100**	**<0.01**	5	**96**	8	**98**	**<0.01**	**<0.01**	**0.04**
Need for rest	8	**96**	8	**100**	0.13	7	**87**	8	**100**	**<0.01**	6	**100**	8	**100**	**<0.01**	**<0.01**	0.51
Impaired performance	8	**98**	8	**100**	0.20	6	**89**	8	**100**	**<0.01**	6	**96**	8	**96**	0.07	**<0.01**	0.18
Stress intolerance	8	**94**	8	**98**	**<0.01**	7	**97**	8	**100**	**<0.01**	6	**100**	8	**98**	**< 0.01**	**0.04**	0.97
Muscle pain	5	**78**	6	**85**	0.10	4	**98**	6	**83**	0.10	3	**79**	6	**90**	**0.01**	0.60	0.91
Headache	5	**82**	6	**93**	0.16	4	**83**	5	**96**	0.16	4	**77.7**	5	**98**	**0.01**	**<0.01**	0.19
Joint pain	4	**69**	5	**64**	0.29	2	**63**	5	**77**	0.29	3	**59**	5	**65**	0.29	0.75	0.75
Memory/word finding problems	5	**88**	5	**98**	0.72	5	**91**	5	**91**	0.91	3	**83**	6	**88**	0.15	**<0.01**	0.46
Concentration impairement	6	**94**	7	**100**	0.60	6	**93**	7	**98**	0.60	5	**86**	7	**98**	**<0.01**	**<0.01**	0.62
Mental Fatigue	7	**96**	7	**100**	0.92	6	**95**	7	**100**	0.16	5	**100**	8	**97**	**<0.01**	**<0.01**	0.86
Visual disturbance	3	**66**	3	**67**	1	2.5	**71**	3	**64**	1	2	**69**	2	**66**	0.90	0.81	0.81
Mood change	5	**88.2**	5	**89**	0.79	5	**89**	5	**85**	0.79	4	**79**	5	**88**	0.36	**<0.01**	0.73
Reading concentration	5	**90**	6	**100**	0.19	5	**91**	6	**96**	0.19	3	**86**	6	**90**	**0.01**	**<0.01**	0.81
Palpitations	3	**65**	3	**89**	0.08	2.5	**74**	5	**85**	0.07	3	**76**	5	**80**	**0.04**	0.49	0.49
Standing up dizziness	4	**75**	5	**76**	1	3	**71**	3	**74**	1	2	**65**	3	**69**	0.42	**0.05**	0.40
Walking dizziness	2	**63**	4	**76**	0.36	2	**65**	3	**74**	0.62	2	**59**	4	**66**	0.08	0.29	0.29
Sleep disturbance	7	**86**	7	**91**	1	6	**82**	6	**89**	1	5	**76**	7	**90**	**<0.01**	**<0.01**	0.68
Temperature hypersensitivity	4	**57**	5	**84**	0.33	4	**66**	5	**82**	0.33	3	**65**	6	**90**	**0.01**	0.42	0.16
Light hypersensitivity	2	**63**	4	**65**	0.19	2	**56**	3	**83**	0.06	2	**65**	5	**66**	**0.02**	0.52	0.52
Noise hypersensitivity	5	**73**	6	**91**	0.27	6	**76**	6	**94**	0.41	4	**96**	7	**90**	0.06	0.43	0.55
Breathing difficulty	5	**72.5**	6	**80**	0.34	3	**65**	5	**76**	0.25	4	**82**	5	**78**	0.32	0.06	0.15
Irritable bowel	4	**55**	5	**74**	0.51	3	**64**	4	**80**	0.51	2	**51.8**	4	**66**	**0.01**	**0.02**	0.66
Fever	1	**20**	1	**16**	0.76	1	**17**	1	**25**	0.66	1	**10**	1	**23**	0.47	0.50	0.50
Painful lymph nodes	1	**30**	1	**27**	1	1	**20**	1	**28**	1	1	**14**	1	**42**	**0.04**	0.18	0.18
Sore throat	2	**55**	2	**62**	0.50	1	**39**	3	**62**	0.13	1	**31**	3	**68**	**<0.01**	**0.01**	0.29
Flu-like symptoms	4	**74.5**	6	**83**	**0.04**	2	**52**	5	**71**	**<0.01**	1.5	**50**	5	**85**	**<0.01**	**<0.01**	0.30
Symptom severity	7	**88.2**	8	**91**	**0.02**	6	**97**	7	**100**	**0.01**	5	**100**	7	**100**	**<0.01**	**<0.01**	0.29

Group differences per time point and time differences per group are reported as p-values and significant results (<0.05) are marked in bold. [a]p-values adjusted using the Bonferroni-Holm correction.

Table 1: **Frequency (in %) and severity (median scores) of symptoms as quantified by the quantitative Canadian Consensus Criteria (qCCC) for all three time periods.**

Table 1 assessing symptom prevalence, severity, and evolution over time for both cohorts in detail. During the study, a total of 19 participants dropped out. Among these dropouts, 12 (63%) were initially diagnosed with PCS, while 7 were diagnosed with PCS-ME/CFS. 70% of the dropouts reported an improvement in symptom severity and therefore chose not to continue participating in the study. The remaining dropouts cited reasons unrelated to their disease activity. Only two participants, both diagnosed with PCS-ME/CFS, were unable to continue due to severe disease symptoms. For a comparison of demographic and symptom characteristics between the study and the drop-out cohort, please refer to the Supplement (Supplementary Table S2).

Fatigue and PEM remain key symptoms in PCS-ME/CFS after 18 months

Fatigue and PEM are among the key symptoms of PCS and indispensable for the diagnoses of ME/CFS. Patients diagnosed with PCS-ME/CFS were significantly more affected by fatigue than patients with PCS over the entire study period. At baseline, patients with PCS presented on average a lower CFQ than patients with PCS-ME/CFS with 33% of PCS and 55% of PCS-ME/CFS reporting severe (≥ 28 points) fatigue (Fig. 1A and Supplementary Table S3). While CFQ scores remained on a similarly high level in the PCS-ME/CFS cohort over time, they significantly decreased in patients with PCS. At follow-up 2, only 3% of PCS but 46% of patients with PCS-ME/CFS still reported CFQ scores ≥ 28 points. The course of symptom severity was similar for the CFQ sub-score of physical fatigue (Fig. 1B), the SF-36 fatigue score (Fig. 1C) and the qCCC fatigue score (Supplementary Fig. S2a). Mental fatigue slightly improved also in PCS-ME/CFS over time (Fig. 1D).

PEM duration, severity and frequency were all significantly higher in patients with PCS-ME/CFS compared to patients with PCS at baseline and remained at a higher level up to follow-up 2 (Fig. 2A–C). While neither of the two cohorts experienced a reduction of PEM duration over time, PEM frequency and severity improved. Interestingly, in the PCS-ME/CFS cohort, PEM duration decreased below 10 h in 7 individuals (17%) at follow-up 2 (thus no longer fulfilling CCC; Fig. 2A). Five of these seven individuals showed an equally strong improvement in PEM severity and frequency.

Continuously reduced functional disability in both cohorts

At baseline, both patients with PCS and PCS-ME/CFS presented a low median Bell disability scale of 40 with 4 of 55 patients with PCS-ME/CFS and 1 of 51 patients with PCS reporting an inability to leave the house (Bell scale 20/100; Fig. 3A). 53 of 55 PCS-ME/CFS and 43 of 51 PCS reported that they are unable to work full- or part-time (Bell scale <70/100). At follow-up 2, the Bell scale remained at 40 in PCS-ME/CFS but increased to 60 in PCS with only 12% of PCS-ME/CFS (5/41) but 43% of PCS (12/28) reporting a Bell scale ≥70. Patient reports on performance capacity as assessed with the qCCC confirmed this course (Supplementary Fig. S2b). Again, the seven patients with PCS-ME/CFS who reported less PEM duration over time also considerably improved on the Bell scale (Fig. 3A).

Various sub-scores of the SF-36 were lower in patients with PCS-ME/CFS compared to PCS at baseline (Supplementary Table S1) and group differences increased over time, mainly due to higher scores in PCS at both follow-ups (Fig. 3A–D). Specifically, perception of physical functioning (SF-36) was more reduced in patients with PCS-ME/CFS compared to patients with PCS from the beginning with an only minor improvement at follow-up 2 in PCS-ME/CFS (median 45–50/100) and moderate in PCS (median 55–70/100) (Fig. 3C). Despite group differences in fatigue (Fig. 1A–C), PEM (Fig. 2A–C) and (perception of) functional disability (Fig. 3A, C), overall health perception as measured by the SF-36 was equally reduced to levels below 50% in both cohorts at baseline (Fig. 3D). Taken together, while PCS reported improved health perception up to follow-up 2, patients with PCS-ME/CFS stagnated at their initial level.

Emotional well-being improves in patients with PCS only

Perception of emotional well-being as measured by the SF-36 was equally reduced by 50% in both cohorts at baseline (Fig. 4A). Further, no differences in PHQ-9, which is used as a screening tool for affective disorders, was found at baseline (Fig. 4B). Seven patients (6 PCS-

	PCS (n = 51)		PCS-ME/CFS (n = 55)		Total (n = 106)		p-values between groups
	Median	Range	Median	Range	Median	Range	
Sex (m/f)	15/36		6/49		21/85		**0.01**
Age (yrs)	43	19–66	43	20–62	40	19–66	0.93
BMI (kg/m^2)	25	18–40	24	15–35	24	15–40	0.97

Median and range are reported for both cohorts separately as well as all participants together. Group differences are reported as p-values and significant results (<0.05) are marked in bold. Sex is indicated based on participants' self-reports.

Table 2: **Demographic participant characteristics.**

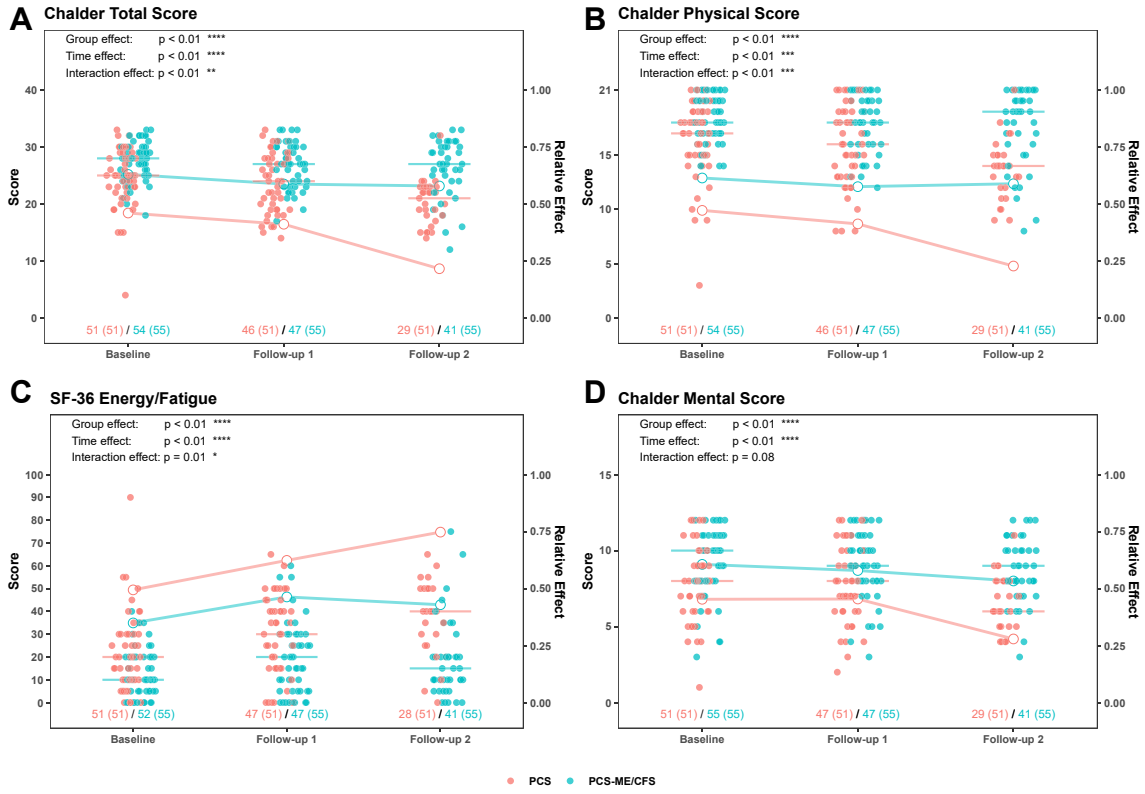

Fig. 1:* Fatigue**. **A**, Chalder Fatigue Total Score, ranging from 0 (no fatigue) to 33 (severe fatigue); **B**, Chalder Fatigue Physical Score (range 0–21); **C**, SF-36 Energy/Fatigue, ranging from 0 (most impaired) to 100 (no impairment); **D**, Chalder Fatigue Mental Score (range 0–12). Dots represent absolute score values (red for PCS, blue for PCS-ME/CFS) as quantified on the left Y axis. Bars depict group medians. Lines (red for PCS, blue for PCS-ME/CFS) depict main relative time, group, and interaction effects as quantified on the right Y axis. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = *.

ME/CFS; 1 PCS) reached more than 20 points on the PHQ-9 score at baseline, which suggests a PCS-associated affective burden. However, the PHQ-9 includes symptoms of fatigue, cognition and sleep and thus has a low specificity for depression in PCS and ME/CFS. Impairment according to the PHQ-9 improved in patients with PCS from baseline to follow-up 2 but remained largely unchanged in the PCS-ME/CFS cohort (Fig. 4A and B). Together, these scores indicate a considerable emotional burden due to illness, which over time improved in patients with PCS while patients with PCS-ME/CFS remained severely impaired.

PCS-ME/CFS remain more severely affected by pain than PCS

According to the SF-36 pain sub-score, patients with PCS-ME/CFS were more affected by pain than patients with PCS at all time points and while the PCS cohort improved from baseline to follow-up 2 there was just a minor improvement among patients with PCS-ME/CFS (Fig. 5A). In detail, muscle pain (qCCC) was reported by 85% of patients with PCS-ME/CFS and 78% of patients with PCS at baseline while joint pain (qCCC) was reported by 64% of patients with PCS-ME/CFS and 69% of patients with PCS (Table 1, Supplementary Fig. S2c and d). None of these symptoms improved significantly over time in either of the two cohorts: Both patient groups reported joint and muscle pain with a severity of more than 5/10 up to follow-up 2. Headaches were mentioned by 82% PCS and 93% PCS-ME/CFS at baseline with decreasing severity over time in both cohorts (Supplementary Fig. S2e).

Prevalence of neurological symptoms remains at a high level in both cohorts

At baseline, the prevalence of cognitive symptoms such as concentration (Supplementary Fig. S2f), memory/wordfinding difficulties (Table 1), mental fatigue (Supplementary Fig. S2g), and difficulties while reading (Table 1) was comparable in both cohorts. Assessing symptom evolution over time, overall cognitive impairment as summarised in the qCCC cognitive score (Supplementary Fig. S2h) ameliorated solely in patients

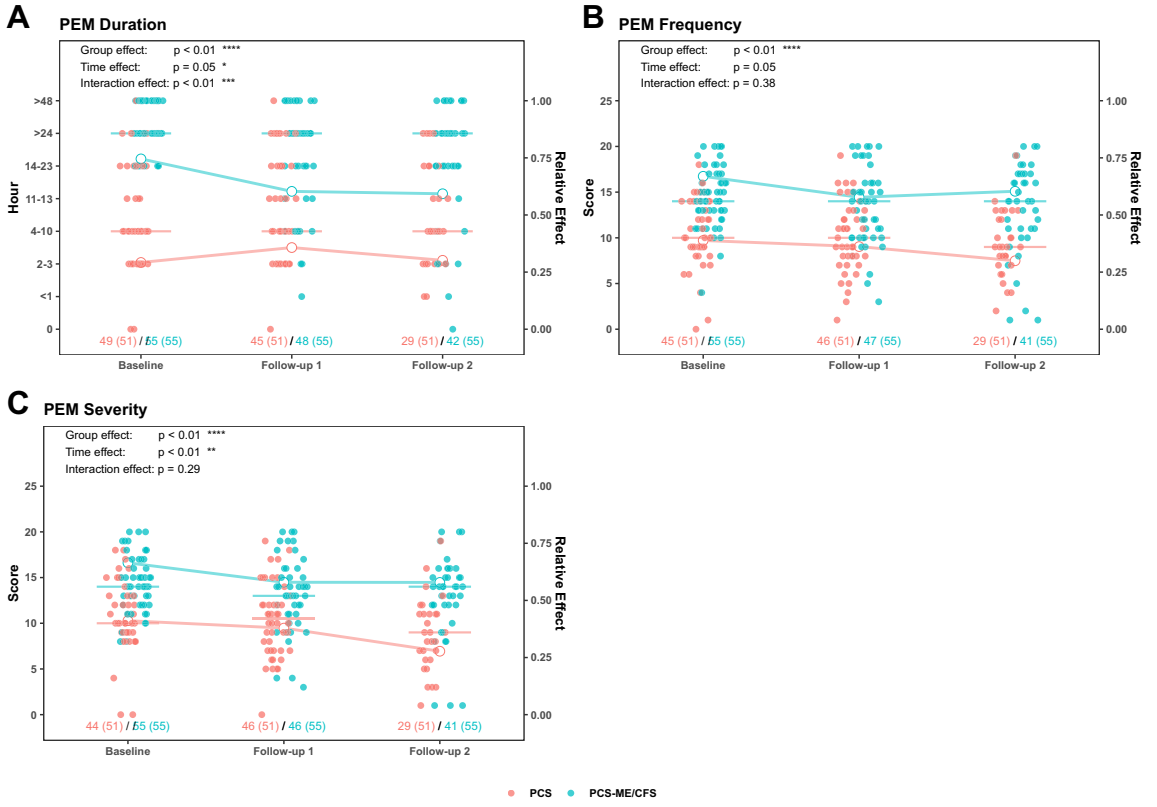

Fig. 2:* Post-exertional malaise**. **A**, PEM duration (in hours). Please note that 7 PCS-ME/CFS patients reported <14h of PEM at baseline, which was earlier than 6 months after symptom onset, but fulfilled CCC for ME/CFS at an additional visit 9 months post infection to ensure standardized diagnosis (see 'Study design and cohort characteristics' in Methods) **B,** frequency (stated as occurring: never, rarely, half of the time, most of the time, always), and **C,** severity (stated as: not at all, mild, moderate, severe, very severe) of exhaustion experienced post exertion. Dots represent absolute score values (red for PCS, blue for PCS-ME/CFS) as quantified on the left Y axis. Bars depict group medians. Lines (red for PCS, blue for PCS-ME/CFS) depict main relative time, group and interaction effects as quantified on the right Y axis. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = *.

with PCS. Hypersensitivities to noise, light and temperature, which are characteristic symptoms in ME/CFS were more frequent in patients with PC-ME/CFS (Table 1) than patients with PCS and all of them remained more pronounced in PCS-ME/CFS at second follow-up. High median symptom severity scores at follow-up 2 indicated a continuous burden from neurological impairment despite overall improvement (Table 1).

Clinical signs of ongoing inflammation persist in patients with PCS-ME/CFS

Patients suffering from postinfectious fatigue syndromes often state persisting flu-like symptoms, painful lymph nodes and a sore throat, which can be signs of ongoing inflammation. These symptoms were here summed up as qCCC immune score (Supplementary Fig. S2i), which was equally elevated in both cohorts at baseline. However, symptoms only decreased in patients with PCS and persisted in patients with PCS-ME/CFS (Supplementary Fig. S2i).

Regression of autonomic dysfunctions in both patients with PCS and PCS-ME/CFS

Autonomic dysfunction is a common feature of postinfectious fatigue syndromes. At baseline, patients with PCS and PCS-ME/CFS showed comparable signs of autonomic dysfunction reflected by an overall COMPASS 31 indicative of moderate to severe complaints. Despite an improvement of this overall score over time in both cohorts, patients with PCS-ME/CFS were more affected at second follow-up than patients with PCS (Fig. 5B and Supplementary Table S2). Gastrointestinal complains only improved in PCS with an almost unchanged level of impairment in PCS-ME/CFS at follow-ups (Fig. 5C and Supplementary Fig. S2j). On a similar note, patients with PCS-ME/CFS continued to suffer from more severe sleep disturbances compared to PCS (qCCC; Supplementary Fig. S2k).

To further characterise autonomic dysfunction in our patients and investigate potential clinical implications, we measured the adaption of blood pressure and pulse

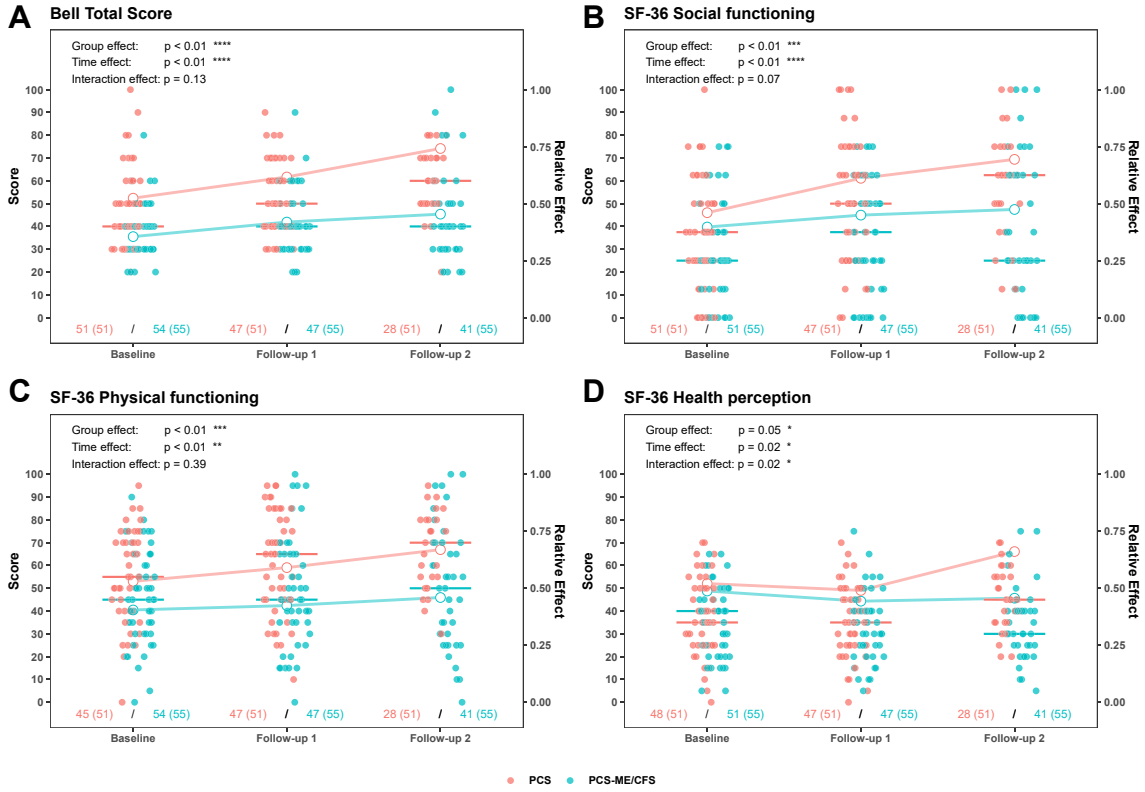

Fig. 3: **General health and functional impairment**. **A**, Bell scale assessing disability due to chronic fatigue from 100 (no symptoms present) to 50 (moderate symptoms at rest and moderate to severe symptoms with exercise or activity) to 0 points (unable to get out of bed independently); **B**, SF-36 social functioning; **C,** SF-36 physical functioning; **D,** SF-36 health perception, 0 points (greatest possible health limitation)—100 points (no health limitation). Dots represent absolute score values (red for PCS, blue for PCS-ME/CFS) as quantified on the left Y axis. Bars depict group medians. Lines (red for PCS, blue for PCS-ME/CFS) depict main relative time, group, and interaction effects as quantified on the right Y axis. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = ****.

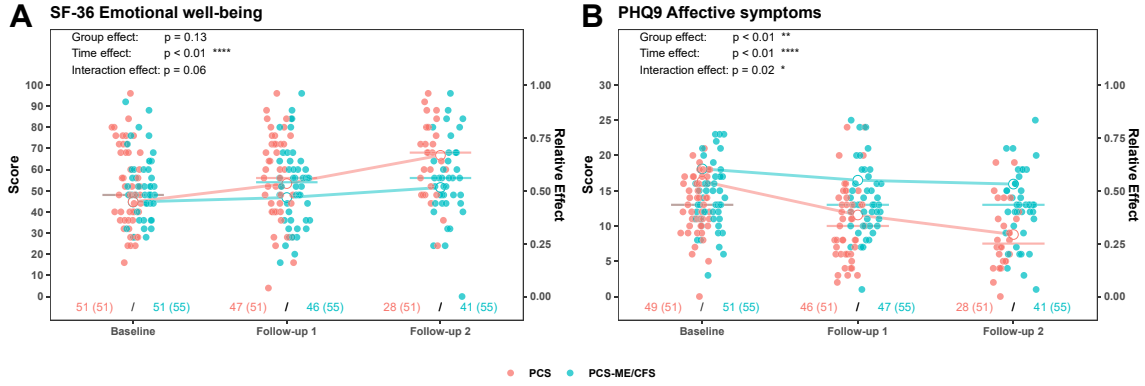

Fig. 4: **Emotional impairment**. **A**, SF-36 emotional well-being, 0 points (greatest possible health limitation)—100 points (no health limitation); **B,** Patient Health Questionnaire 9 (PHQ-9), minimal depressive symptoms (1–4), mild depressive symptoms (5–9), moderate depressive symptoms (10–14), moderately severe depressive symptoms (15–19), or severe depressive symptoms (20–27). Dots represent absolute score values (red for PCS, blue for PCS-ME/CFS) as quantified on the left Y axis. Bars depict group medians. Lines (red for PCS, blue for PCS-ME/CFS) depict main relative time, group, and interaction effects as quantified on the right Y axis. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = ****.

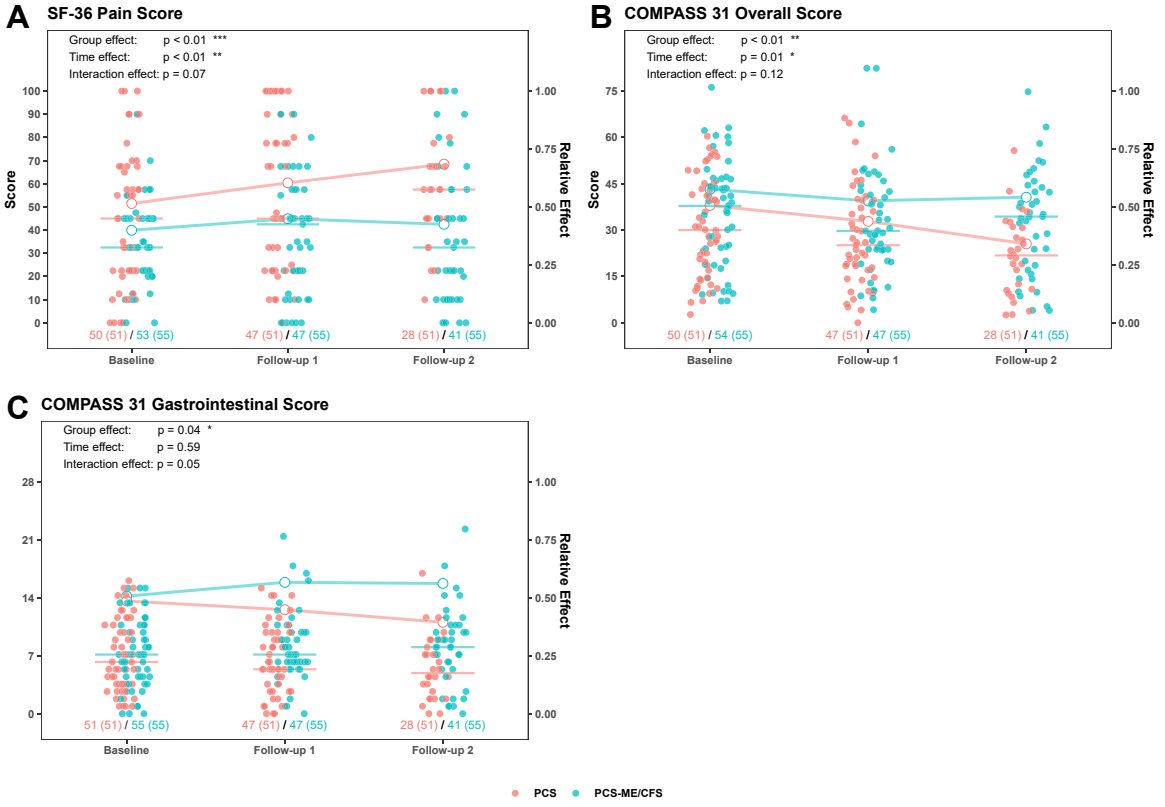

Fig. 5: **Pain and autonomic dysfunction**. **A**, SF-36 pain score, 0 points (greatest possible health limitation)–100 points (no health limitation); **B**, COMPASS 31 overall score, 0 (no symptoms)–100 (severe autonomic dysfunction); **C,** COMPASS 31 gastrointestinal score (range 0–25). Dots represent absolute score values (red for PCS, blue for PCS-ME/CFS) as quantified on the left Y axis. Bars depict group medians. Lines (red for PCS, blue for PCS-ME/CFS) depict main relative time, group, and interaction effects as quantified on the right Y axis. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = ****.

to postural change. At baseline, 7% (5/42) of PCS and 11% (5/44) of PCS-ME/CFS showed signs of POTS and 4% (2/42) of PCS and 6% (3/44) of PCS-ME/CFS showed signs of OH. Up to follow-up 2, none of the patients with PCS continued to show signs of POTS or OH (but two patients showed newly emerged symptoms of OH or POTS at follow-up 1). In contrary, 6% (3/44) of PCS-ME/CFS presented POTS symptoms also at follow-up 2 suggesting a persistent autonomic dysfunction in this cohort (one patient showed new signs of OH).

Persistently diminished HGS but improvement of muscle fatiguability over time

HGS, a reliable parameter to quantify frailty and mortality, was evaluated (for female patients only to avoid gender bias and due to a low number of male patients) in two consecutive sessions at baseline (n = 35 PCS, n = 49 PCS-ME/CFS) and follow-up 1 (n = 25 PCS, n = 37 PCS-ME/CFS; Fig. 6) and results were compared to age-dependent reference values as reported by Jäkel et al.[29] We found no significant differences in mean (fmean) and maximum force (fmax) between PCS and PCS-ME/CFS and no changes in HGS over time in either cohort. A remarkable number of patients with PCS (63%) and PCS-ME/CFS (67%) showed measurements below their respective cut-offs, i.e., for mean force in the second session (fmean2) at baseline and follow-up 1 (PCS 40%; PCS/ME/FCS 67%; Fig. 6A and B).

The fatigue ratio (fmax/fmean) was determined for each session as a correlate for muscle fatigability with higher values indicating a stronger decline in force (Fig. 6E and F).[29] Patients with PCS-ME/CFS showed higher fatigue ratios at baseline (session 2) and at follow-up (session 1) than patients with PCS. The fatigue ratio decreased over time in both cohorts. No changes over time (or group differences) were found for the recovery ratio, which serves as marker for muscle strength recovery (Fig. 6G).[29]

Reduction of inflammation markers over time in both cohorts

We investigated a range of biomarkers linked to postinfectious fatigue in a subset of patients (PCS n = 35; PCS-ME/CFS n = 31). Previous studies have

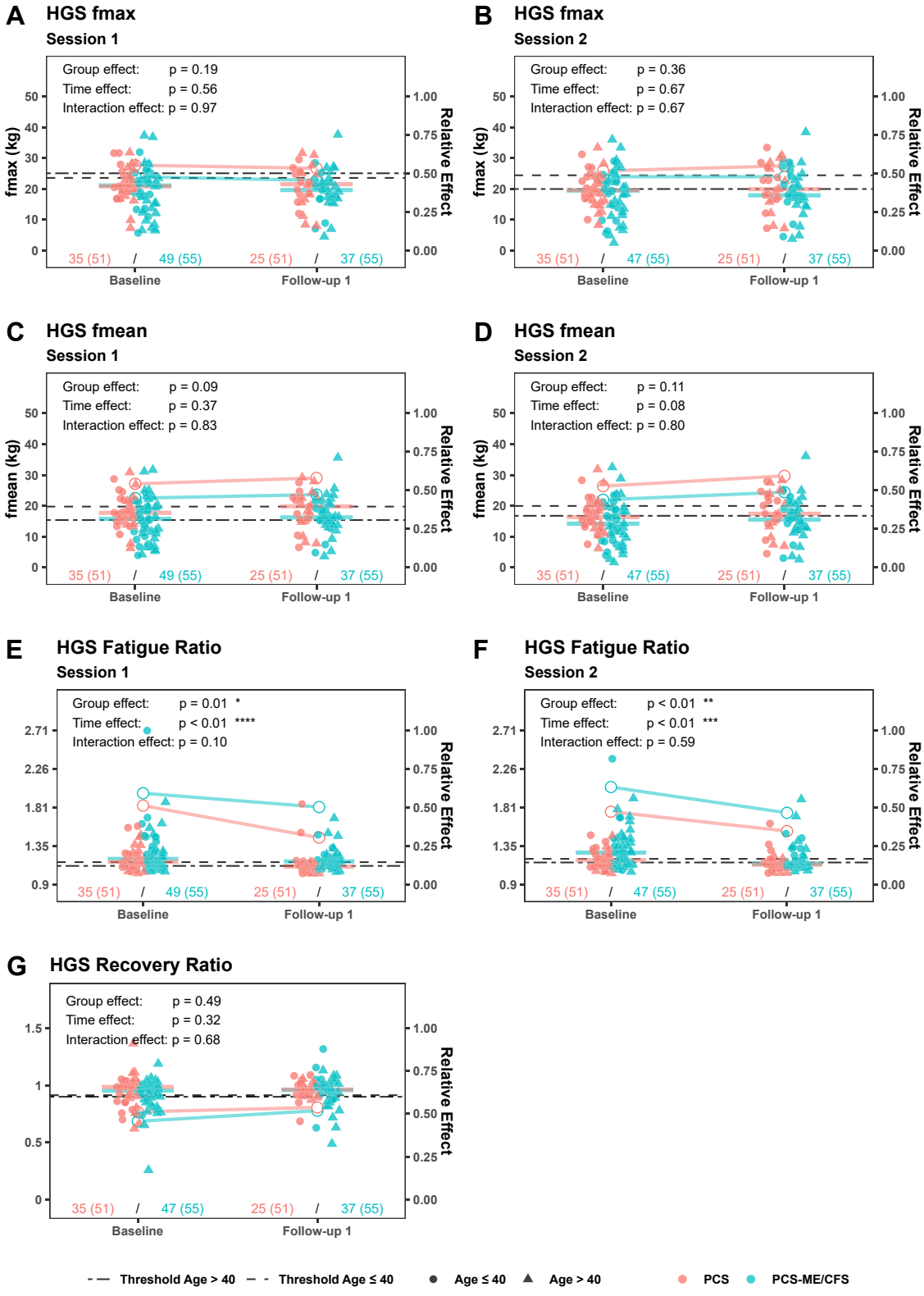

Fig. 6: **Hand grip strength**. **A-D**, mean (**A and B**) and maximum (**C and D**) force in kg of all 10 measurements per session for session 1 (**A and C**) and 2 (**B and D**) for baseline and follow-up 1; **E and F,** fatigue ratio (fmax/fmean) per session for session 1 (**E**) and 2 (**F**) and for baseline and

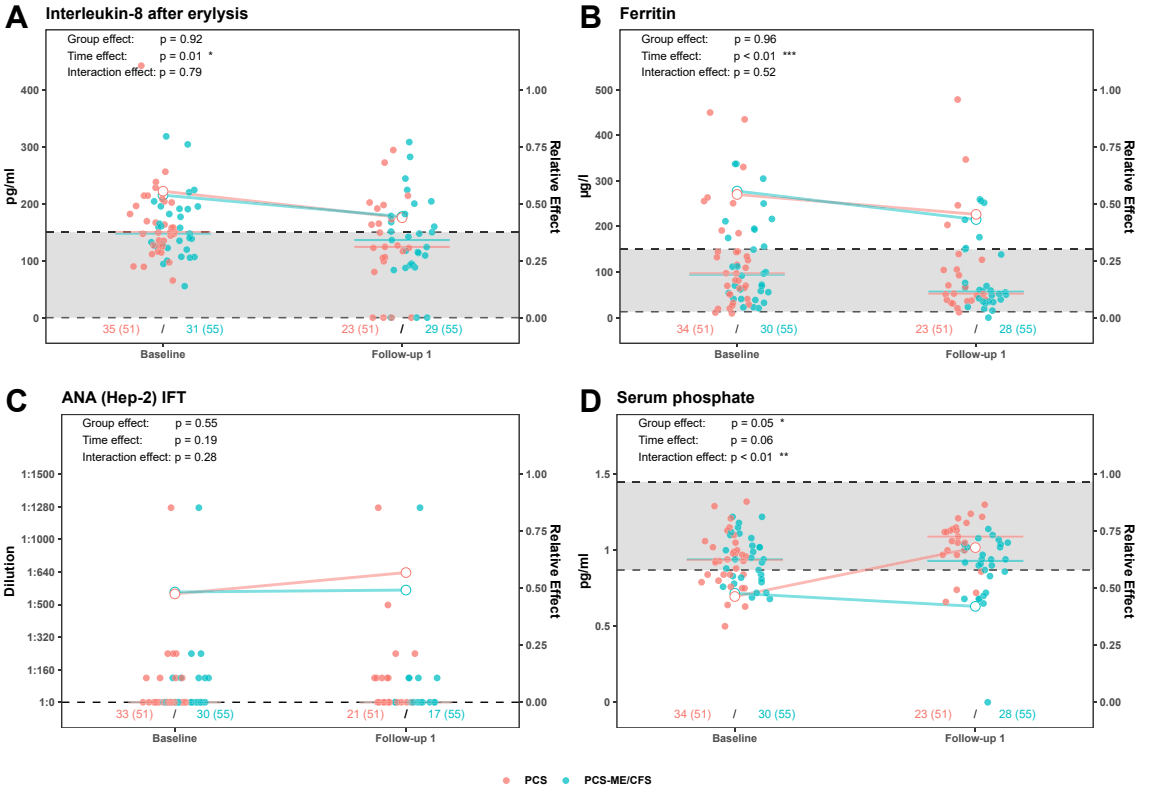

Fig. 7: **Inflammatory biomarkers associated with postinfectious fatigue**. **A**, interleukin 8 (IL-8) after erylysis, reference <150 pg/ml, **B**, ferritin, reference range 13–150 µg/l, **C,** antinuclear antibodies (ANA), negative reference 1:0 dilution, **D**, serum phosphate (PO4), reference range 0.87–1.45 mmol/l, for baseline and follow-up 1 respectively. Dots represent absolute score values (red for PCS, blue for PCS-ME/CFS) as quantified on the left Y axis. Bars depict group medians. Lines (red for PCS, blue for PCS-ME/CFS) depict main relative time, group, and interaction effects as quantified on the right Y axis. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = ****.

found that altered levels of the proinflammatory and neutrophil-recruiting IL-8, ANA and ferritin were associated with the post-COVID condition.[35–37] Among these patients, IL-8, which was determined after erythrocyte lysis to reflect its level of production for a duration of up to 3 months, was equally elevated in almost half of both patient cohorts at baseline.[38] Over time, IL-8 levels decreased significantly in both groups (Fig. 7A). Consistently, ferritin, another inflammatory marker, was equally increased in almost a third of all patients at baseline, but again decreased in both cohorts until follow-up 2 (Fig. 7B). ANAs were detected in 25% (8/32) of patients with PCS and 26% (8/30) patients with PCS-ME/CFS at baseline without significant changes over time (Fig. 7C). Furthermore, 35% of all patients were deficient of PO4 at baseline, which is associated with increased mortality in COVID-19.[39] Up to follow-up 1, this deficiency receded in most patients with PCS while persisting in PCS-ME/CFS (Fig. 7E).

Initial hand grip strength is associated with symptom burden at follow-up in PCS-ME/CFS

As disease courses and outcomes vary widely between individual patients, we aimed at identifying an objective marker, which could be used to estimate disease prognosis. We found HGS to be diminished in patients with PCS-ME/CFS and to be associated with disease severity at baseline.[32] In line with this, we observed HGS baseline measurements at baseline to strongly correlate with symptom burden in patients with PCS-ME/CFS at follow-up 1: Low HGS mean and maximum force at baseline correlated with increased fatigue (CFQ), PEM, functional disability, pain, sleep disturbance and emotional impairment at follow-up 1. Consistently, a high HGS fatigue ratio indicating faster fatiguability at

follow-up 1; **G,** recovery ratio (fmean2/fmean1) for baseline and follow-up 1. Triangle data points depict patients <40 years. Age-dependent cut-offs are depicted according to Jäkel et al.[29] Red dots represent PCS cohort, blue dots PCS-ME/CFS cohort. Bars depict group medians with a 95% confidence interval. $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = ****.

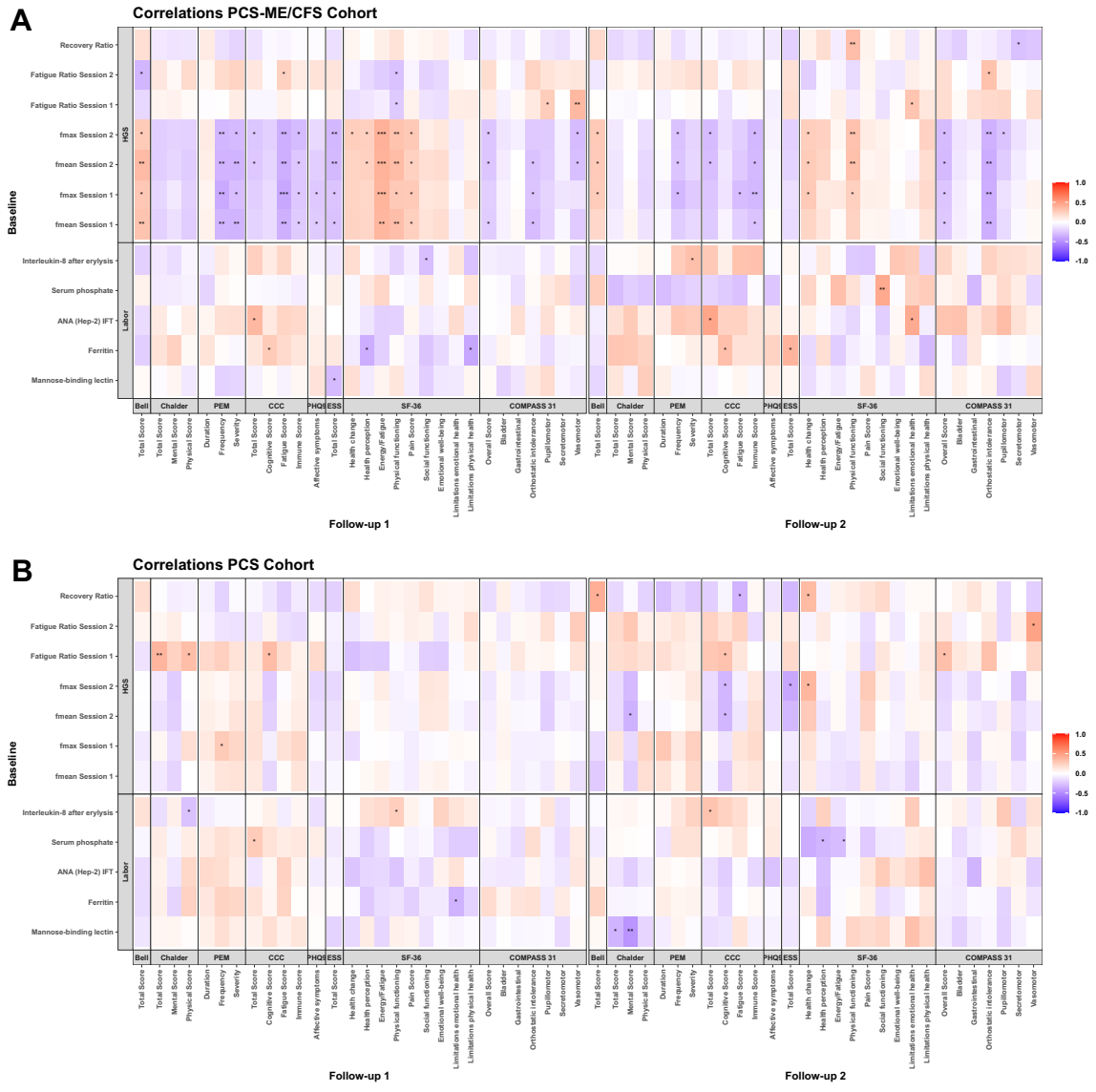

Fig. 8: **Correlations of initial symptom severity, HGS and biomarkers with longitudinal symptom severity in a, PCS-ME/CFS patients and b, PCS patients**. Markers at baseline are shown on the x axis, parameters at follow-up 1 and 2 on the y axis. Blue colouring indicates negative correlations, red colouring indicates positive correlations, see colour legend below the plot; $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, $p \leq 0.0001$ = ****.

baseline correlated with increased fatigue (CFQ) and functional disability at follow-up 1, and low baseline HGS recovery ratios with increased fatigue (CFQ) and disability at follow-up 2 (Fig. 8A). In patients with PCS, we found fewer and weaker correlations of HGS parameters at baseline with symptom outcomes at follow-up (Fig. 8B).

Next, we evaluated potential associations between biomarkers at baseline and persisting disease burden. In the PCS-ME/CFS cohort, high IL-8 levels at baseline were associated with decreased social functioning at follow-up 1 and high PEM severity at follow-up 2. High ferritin levels correlated with increased cognitive impairment at both follow-ups as well as with poor health perception and more limitation due to physical health at follow-up 1. Further, elevated ANA levels correlated with high symptom severity at follow-up 1 and 2 and low serum PO4 levels were linked to reduced social functioning and increased gastrointestinal complaints at follow-up 2. Surprisingly, in patients with PCS, high IL-8 levels at baseline correlated with high total symptom scores (qCCC) at follow-up 2 but

improved physical functioning and less physical fatigue (CFQ) at follow-up 1. Likewise, reduced serum PO4 was associated with low total symptom scores (qCCC) at follow-up 1 while at follow-up 2 (in line with PCS-ME/CFS), the link was to decreased health perception and health change. High MBL levels were associated with less fatigue (CFQ) at follow-up 2.

Discussion

We here provide a comprehensive longitudinal characterization of the post-COVID-19 condition in patients with pronounced fatigue and exertion intolerance over a period of 20 months following COVID-19. All patients suffered from PCS with a subgroup fulfilling the CCC for ME/CFS.

While both patients with PCS and PCS-ME/CFS continued to report post-COVID-19 symptoms throughout the observation period,[27,40] clinical improvement was observed to variable degrees and mostly restricted to the non-ME/CFS sub-cohort. This is in line with Tran et al.[8] who monitored symptom evolution in patients with persisting symptoms after acute COVID-19 based on an online survey over a 12-month period. The proportion of patients with persisting symptoms in their cohort was about 85%. After an initial decrease, symptoms plateaued 6–8 months after onset.[8] Consistently, Seeβle et al. reported that neurocognitive deficits following COVID-19 can persist beyond 12 months and lead to a marked reduction of quality of life.[41]

We here showed that patients fulfilling criteria for ME/CFS continued to be more affected than patients with PCS by a wide range of symptoms including fatigue, physical disability, impaired social functioning, and emotional well-being. Importantly, exertion intolerance and PEM as the hallmark of post-infectious fatigue syndromes remained more pronounced in PCS-ME/CFS up to 20 months after initial infection. However, the extent of PEM did not improve in either cohort. Considering this persistence of PEM in most patients with PCS, our study provides evidence that despite early diagnosis, prognosis is poor for most patients. Due to the lack of effective causal therapies, non-pharmacological interventions are important. This includes symptom management by determining individual activity limits and balancing rest and activity (i.e., pacing). It is, however, important that pacing is approached with caution to yield beneficial effects and overseen by trained personnel to avoid over-exertion and rebound effects of symptom worsening. All patients in this study were seen in specialist outpatient clinics and received recommendations for symptomatic treatment and self-management strategies. However, symptomatic therapy in ME/CFS requires prompt clinical follow-ups, which have not been available for many patients due to a lack of knowledge among most primary care physicians.[42] We are currently evaluating in a clinical trial if comprehensive care and close monitoring can improve physical functions and well-being in patients with ME/CFS.

CCC, PEM, CFQ and Bell scale were found to discriminate best between patients with PCS and PCS-ME/CFS 16–20 months post infection. POTS was only found in patients with PCS-ME/CFS, at a prevalence of 6% consistent with other reports. Given these characteristic features and the distinct disease course of the PCS-ME/CFS sub-group, classification of patients with the post-COVID condition based on the CCC is useful for further diagnostics and treatment. In line with other studies, patients fulfilling the CCC were more impaired and more symptomatic.[43] 16 of the 51 patients with PCS not fulfilling the CCC would have fulfilled the IOM (Institute of Medicine) criteria for ME/CFS as these criteria do not predefine the length of PEM and require only fatigue, sleep disturbance and cognitive or orthostatic symptoms as mandatory symptoms.

The majority of patients reported newly emerged affective symptoms and poor emotional well-being after COVID-19 diagnosis, which were thus directly related to their post-COVID-19 condition. These symptoms improved only in patients with PCS along with their overall clinical condition and therefore must rather be considered a consequence of the burdening disease impacting PCS-ME/CFS patients' quality of life than any primary condition. Consequently, psychological support should be integrated into PCS management.

We found baseline HGS to be linked to persisting disease severity, particularly in the PCS-ME/CFS cohort. Patients with PCS-ME/CFS with initially reduced HGS were more likely to experience high disease burden up to 20 months after infection. Specifically, higher hand grip force correlated with less fatigue, exertion intolerance, physical functioning and disability, the characteristic hallmarks of chronic postinfectious fatigue syndromes. In patients with PCS, links of HGS to these symptom measures were not found or were much less pronounced than in PCS-ME/CFS. We thus assume that HGS is a more accurate prognostic parameter for patients with PCS-ME/CFS patients. Consequently, HGS measurements could serve as an easy to perform method to estimate prognosis of PCS-ME/CFS patients. However, these correlations should be considered observational as we could not control for potential confounding values or multiplicity due to the limited number of participants. Therefore, these results need to be validated in further studies.

We observed a distinct improvement of symptoms (fatigue, PEM, and disability) in 7 out of patients with 55 PCS-ME/CFS. These patients initially presented with severe symptoms and fulfilled the CCC for ME/CFS. At follow-up 2, their Bell scale improved above a value of 60 points. 5 of them also demonstrated improved hand grip strength over time. We were, however, unable to identify any defining clinical or

demographical characteristics that could explain the improvement in this sub-group.

Our evaluation of biomarkers associated with post-infectious fatigue supports the presumption of ongoing inflammation in post COVID-19. We observed elevated ANA titers in 25% of patients, which is above the prevalence in the general population[44,45] and which correlated positively with symptom severity up to 20 months post COVID-19. This is consistent with other studies that found elevated ANA titers at 12 months post-COVID, which also correlated with persisting symptoms and inflammation[35] Together, this indicates that ANA could be a relevant marker for autoimmunity in PCS. The evidence of autoimmunity and proinflammatory cytokines in post-COVID suggests potential therapeutic approaches focused on immune modulation and anti-inflammatory therapies, such as immunoadsorption. In order to explore these possibilities and to gain a better understanding of the disease's pathophysiology, randomised controlled clinical trials have been initiated, which are accompanied by comprehensive biomarker analysis.[46] Hypophosphatemia was found in one third of patients at baseline and interestingly persisted only in the PCS-ME/CFS cohort. The etiology of hypophosphatemia is complex and potential causes are mitochondrial dysfunction with depletion of adenosine triphosphate (ATP), insulin resistance, and respiratory alkalosis.[39] Complications of hypophosphatemia include impaired cellular ATP metabolism and increased affinity of hemoglobin to oxygen in red blood cells, which may exacerbate fatigue as well as neurologic, cardiovascular and muscle dysfunction. Thus, the effect of phosphate supplementation should be further evaluated in ME/CFS. In acute COVID, results on supplementation approaches are still inconsistent.[47] With increasing data and knowledge about the pathogenesis of post-COVID, the need to constantly re-evaluate the effectiveness of identified biomarkers increases.

The higher number of women in both study cohorts aligns with the higher prevalence of the post-COVID syndrome in women. This unequal gender distribution further supports the hypothesis of an autoimmune component, which is also more commonly observed in women. However, it is important to note that the limited sample size of male patients (15/51 in PCS and 6/55 in PCS-ME/CFS) prevented statistical comparisons between women and men and thus constrains the discussion on gender aspects.

Another limitation of this study is the drop-out of 19 participants during the study period. Among these dropouts, 63% were initially diagnosed with PCS, while the remaining were diagnosed with PCS-ME/CFS. Another 46 participants provided incomplete data sets. Notably, in a subsequent drop-out investigation, 70% of the dropouts reported symptom improvement as the reason for not continuing the study, which aligns with the higher clinical improvement observed in patients with PCS in our study cohort and highlights the favorable disease course of patients with PCS who do not meet the CCC. While caution should be exercised when interpreting statistical comparisons between the study cohort (n = 106) and dropouts (n = 19) due to the unequal sample sizes, it is noteworthy that the dropouts, primarily diagnosed with PCS, displayed milder symptoms at baseline compared to the study cohort. Despite these limitations, a great strength of this study is the comprehensive disease evaluation including an extensive and interdisciplinary set-up of questionnaires and on-site clinical examinations and functional and laboratory tests.

Taken together, the post-COVID-19 condition can develop into a chronic syndrome with long-lasting symptoms and impairment. The chance of relevant improvement during the time period of 20 months investigated is particularly low in patients fulfilling the CCC, despite symptomatic therapy. Against the backdrop of over 763 million documented SARS-CoV-2 infections worldwide (status April 2023), these results suggest that the post-COVID-19 syndrome continues to present a heavy burden for those affected as well as on our healthcare systems. Further studies on the pathomechanism, therapy approaches and how to establish comprehensive and interdisciplinary care networks are urgently needed.

Contributors

C.S., C.K., and F.P. developed the concept of the study. J.B.-S. and L.M.-A. gave important input into study concept and objectives. F.L. and L.M.-A. were responsible for data curation and analyses of data. C.K., U.H., C.S., J.B.-S., L.M.-A., F.P., R.R., E.S., J.B., K.W. and N.S. were involved in clinical investigation. F.L. was involved in data transfer, data management and collection of further information for revision. H.F. was also involved in data transfer. C.K., J.B.-S. and C.S. verified the data. L.M. and F.L. performed statistical analyses. F.L. and L.M. were involved in data visualization. F.L. and L.M.-A wrote the original draft of the paper. C.S. and J.B.-S. reviewed and edited the paper. F.K supervised statistical analyses. A.T provided resources. All authors had access to all the data in the study, revised and approved the paper and accepted responsibility to submit for publication. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. J.B.-S. is the guarantor.

Data sharing statement

Deidentified data is available upon reasonable request to the corresponding author with scientific rationale and sound methodology beginning 9 months and ending 5 years following Article publication. Request for data sharing will be handled according to data access and sharing policy of Charité–Universitätsmedizin Berlin.

Furthermore, the manuscript's guarantor affirms that the paper is honest, accurate, and transparent; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained. Dissemination to participants and related patient and public communities is encouraged by open access publication and citing the study on our site https://cfs.charite.de/. We are engaging with print and internet press, television, radio, news, and documentary program makers. All items of the STROBE checklist are covered in this manuscript.

Declaration of interests
The authors declare no competing interests.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102146.

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