Received: 12 April 2024 | Revised: 19 August 2024 | Accepted: 23 August 2024

DOI: 10.1111/sji.13407

Scandinavian Journal of Immunology
An International Monthly Journal
WILEY

ORIGINAL ARTICLE

No signs of mast cell involvement in long-COVID: A case–control study

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Funding information
EkstraStiftelsen Helse og Rehabilitering (Stiftelsen Dam), Grant/Award Number: SDAM_HEL45920; Norwegian Brain Council

Abstract

Long-COVID caused by SARS-CoV-2 infection has significant and increasing effects on human health worldwide. Although a unifying molecular or biological explanation is lacking, several pathophysiological mechanisms have been proposed. Involvement of mast cells—evolutionary old "multipurpose" innate immune cells—was reported recently in studies of acute infection and post-acute-COVID-19 syndrome. Mast cell activity has been suggested in long-COVID. In this case–control study, we compared data from 24 individuals with long-COVID (according to the NICE criteria) and 24 age- and sex-matched healthy individuals with a history of SARS-CoV-2 infection without developing sequelae. Serum levels of the proteases beta-tryptase (TPSB2) and carboxypeptidase (CPA3), which are mast cell specific, were measured using immunoassays. The values were compared between the two groups and correlated to measures of physical exertional intolerance. TPSB2 and CPA3 levels were median (range) 26.9 (2.0–1000) and 5.8 (1.5–14.0) ng/mL, respectively, in the long-COVID group. The corresponding values in the control group were 10.9 (2.0–1000) ($p = 0.93$) and 5.3 (3.5–12.9) ng/mL ($p = 0.82$). No significant correlations between TPSB2 or CPA3 levels and scores on the ten physical subscales of SF-36, 3.1–3.10 were revealed. We found no significant differences in the levels of mast cell activation markers TPSB2 and CPA3 between the long-COVID and control groups and no correlations with proxy markers of exercise intolerance. Mast cell activation does not appear to be part of long-term pathogenesis of long-COVID, at least in the majority of patients.

KEYWORDS

exertional intolerance, long-COVID, mast cell-derived proteases, mast cells

1 | INTRODUCTION

Long-COVID continues to be a major concern for people who do not recover after the initial SARS-CoV-2 infection. This entails significant health burden at the level of the individual, in addition to imposing substantial personal and societal costs. Prevalence is presently estimated at approximately 10%, and the condition is generally regarded as a multi-organ disorder that manifests with a plethora of signs and symptoms. Cognitive dysfunction, fatigue, dizziness, shortness of breath, irregular heart rate, exertional intolerance, and joint and muscle pain are among the most frequently reported phenomena.[1,2]

The National Institute for Health Care Excellence (NICE) criteria define long-COVID as "signs and symptoms that develop during or after an infection consistent with COVID-19, continue for more than 12 weeks and are not explained by an alternative diagnosis." At least 65 million individuals, most between 36 and 50 years old, are living with impairments and ailments resulting from long-COVID.[2,3] Although the pathogenesis is poorly understood, several mechanisms appear to be involved, including autoimmune activation, sustained antigen stimulation through a persistent CoV-2 reservoir, reactivation of EBV, intestinal dysbiosis, or endothelial dysfunction triggering coagulopathies.[4]

Mast cells are evolutionary old, tissue-resident innate immune cells that are mostly present in the skin, lungs, and intestines. They are activated not only by allergens but also by a broad range of structures produced by pathogens (pathogen-associated molecular patterns [PAMPs]) as well as endogenous biomolecules that can be released under conditions of cellular stress (danger-associated molecular patterns [DAMPs]). When activated, mast cells release several highly bioactive molecules, such as histamine, proteases, proinflammatory cytokines, and chemokines.[5]

Mastocytosis and mast cell activation syndrome are disease entities characterized by strong and persistent mast cell activation. Clinically, patients experience urticaria, abdominal pain, bronchospasm, joint and muscle pain, and exertional intolerance, as well as malaise, fatigue, and cognitive disturbances. These clinical characteristics are also frequently experienced by patients with long-COVID.[6] Mast cells express pattern recognition receptors (PRRs) that recognize viral DNA and have been hypothesized to be involved in long-COVID pathogenesis.[1] One study of post-acute COVID-19 syndrome reported elevated blood levels of "active" tryptase and carboxypeptidase (CPA3), which are biomolecules highly associated with mast cell activation.[7] In addition, a recent authoritative review listed mast cell activation as one of eight hypothetical mechanisms underlying long-COVID pathogenesis.[1]

To explore whether persistent activation of mast cells is a pathogenetic mechanism, we measured the blood levels of beta-tryptase (TPSB2) and CPA3 in 24 individuals with long-COVID and compared them with measures in 24 sex- and age-matched controls who had recovered from COVID-19 without developing residual complications or conditions. Moreover, we aimed to explore the peculiar observation that pain, malaise, and mental exhaustion occur in many patients after muscular exertion. We thus investigated whether serum levels of TPSB2 and CPA3 in the long-COVID group were associated with measures of physical activity intolerance based on the knowledge that mast cells can be activated via mechanical stress/strain.[8]

2 | METHODS

2.1 | Study design and recruitment of participants

This was a single-center, age- and sex-matched, case–control study. The inclusion criteria were age between 16 and 80 years and confirmation of previous SARS-CoV-2 infection through PCR, immunoassay self-testing, or healthcare-provided testing. The exclusion criteria were as follows: (1) presence of autoimmune or chronic inflammatory diseases, cancer, anaemia (haemoglobin levels <100 g/L), or other conditions associated with fatigue (such as untreated hypothyroidism); (2) inability to consent or adhere to the study protocol; and (3) individuals considered not eligible at the discretion of the study physician.

The participants were recruited from the Stavanger community area through regional general practitioners who had been informed about the study via direct contact, email, and local meetings with district medical officers. The candidates selected by these doctors received a letter with information about the study and the opportunity to contact the study center. Individuals presumed to have long-COVID syndrome were selected according to the NICE criteria,[9] while the controls were recruited from nonfamilial friends of the patients with long-COVID or from friends and neighbours of the hospital staff. Vaccination data were insufficient to be analysed.

2.2 | Patient consent and data handling

Eligible participants were informed about the study and provided written informed consent before inclusion. Of the 30 individuals referred with long-COVID and considered for inclusion, six were excluded due to lack of age- and sex-matched controls and one due to anaemia,

resulting in a final study sample of 24. All participants were examined at the Clinical Research Unit at Stavanger University Hospital. Demographic and clinical data were recorded, and blood samples were collected from the participants in the long-COVID and healthy groups. All participants completed selected health questionnaires under supervision before blood sampling.

2.3 | Proxy measures for exercise intolerance

As we had no validated measure for exercise intolerance, we explored potential associations between serum levels of TPSB2 or CPA3 and items on the Physical Functioning Scale of the 36-Item Short-Form Health Survey (SF-36). SF-36 is a widely used instrument for measuring various dimensions of health status.[10] The physical functioning scale consists of 10 items (3.1–3.10) that are measures of different levels of physical activity, from "vigorous activity" (SF3.1) to "bathing and dressing" (SF3.10).

2.4 | Laboratory methods

Routine haematological and biochemical analyses were performed in the hospital laboratory. For the analyses of TPSB2 and CPA3 levels, venous blood samples were allowed to clot for 30 min at room temperature before centrifugation (2500 *g*, 10 min, 4°C). The sera were aliquoted and immediately stored at −80°C until analysis. CPA3 and TPSB2 levels were measured using commercially available ELISA kits from Abbexa LTD (Cambridge, UK) and Invitrogen (Life Technologies Europe BV, Stockholm, Sweden), respectively. Freshly thawed serum samples were analysed in duplicate according to the manufacturer's instructions. The lower limits of detection for CPA3 and TPSB2 in serum were 0.39 and 2 ng/mL, respectively, and the upper limits of detection for CPA3 and TPSB2 in serum were 25 and 1000 ng/mL, respectively.

2.5 | Statistical analyses

Normal distribution of the data was tested using the Shapiro–Wilk test. Demographic and clinical data were reported as means and 95% CI if normally distributed, otherwise as medians and ranges. Paired differences for normally distributed data were analysed using the paired Student's *t*-test. Paired differences for non-normally distributed data were analysed using the Wilcoxon signed rank test. Differences in categorical variables between the groups were analysed using the chi-square test. Correlations were analysed using the Spearman's rho for non-normally distributed and categorical data. A *p*-value <0.05 was regarded to indicate statistical significance. Statistical analyses were performed using the SPSS Statistics 26 statistical software (IBM®). Bonferroni correction for multiple analyses was performed on the correlation results for TPSB2 and CPA3.

3 | RESULTS

A total of 48 participants were consecutively included in the study between March 2022 and December 2023. The mean (95% CI) age was 46 (43–49) years, and 75% were women. The long-COVID and control groups comprised 24 participants each, who were matched for sex and age (±5 years), and were equally aged, 46 (42–51) years. The demographic data, level of completed education, and clinical and routine laboratory data are presented in Table 1.

3.1 | Protease profiling

For all participants, the median (range) TPSB2 level was 14.1 (2.0–1000) ng/mL. In the long COVID vs. control group, TPSB2 levels were 26.9 (2.0–1000) vs. 10.9 (2.0–1000) ng/mL, respectively ($p = 0.93$, Figure 1). CPA3 levels were 5.5 (1.5–14.0) ng/mL for all participants, and 5.3 (3.5–12.9) vs. 5.8 (1.5–14.0) ng/mL in the long-COVID vs. control group, respectively ($p = 0.82$, Figure 1). We found no correlation between time since COVID-19 diagnosis (in weeks) and CPA3 or TPSB2 levels in patients or controls (data not shown).

3.2 | Exercise intolerance (proxy) and levels of mast cell markers

In a preliminary correlation analysis, high TPSB2 levels correlated significantly with low SF 3.1 scores (vigorous activity; $p = 0.03$). High CPA3 levels correlated significantly with low SF3.10 scores (walking > 100 m; $p = 0.04$). However, applying the Bonferroni-adjusted *p*-value for statistical significance (0.05/number of analyses, $n = 10$), defined as 0.005, no significance remained between TPSB2 or CPA3 concentration and the scores on the different physical functioning subscales of SF-36 (Table 2).

4 | DISCUSSION

The main finding of this study was that there was no sign of mast cell activation in individuals with long-COVID

TABLE 1 Characteristics of patients with long COVID ($n=24$) and controls ($n=24$).

	All ($n=48$)	Long COVID ($n=24$)	Controls ($n=24$)	p-value (LC vs. CS)
Age, years (mean, 95% CI)	46.2 (42.9–49.4)	46.2 (41.6–50.8)	46.2 (41.6–50.8)	1.0
Sex, female, *n* (%)	36 (75)	18 (75)	18 (75)	1.0
Education (completed) *n* (%)				
Secondary school	1 (2)	1 (4)	0 (0)	0.09 (all categories)
High school	5 (10)	4 (17)	1 (4)	
College	23 (46)	7 (29)	16 (63)	
University	21 (42)	13 (50)	8 (33)	
Time since COVID-19 diagnosis, weeks (median, range)	65 (3–188)	60 (22–152)	69 (3–188)	0.33
Haemoglobin levels, g/dL (median, range)	13.6 (12.0–17.0)	13.8 (12.2–16.6)	13.4 (12.0–16.9)	0.36
Leucocyte level, 10^9/L (median, range)	5.5 (3.2–11.6)	5.6 (3.2–10.7)	5.4 (3.8–11.6)	0.67
Thrombocyte level, 10^9/L (mean, 95% CI)	281 02 (261–300)	286 (252–319)	276 (252–299)	0.67
CRP levels, mg/L (median, range)	0 (0–16.0)	0.5 (0–16.0)	0 (0–11.0)	0.32
ESR (mm) (median, range)	5 (1–34)	5 (2–34)	5 (1–11)	0.27
TSH levels, mIU/L (mean, 95% CI)	1.1 (0.9–1.3)	1.2 (0.9–1.5)	1.1 (0.9–1.2)	0.35
Free-T4 levels, pmol/L (mean, 95% CI)	13.0 (12.6–13.4)	13.5 (12.8–14.2)	12.5 (12.0–13.0)	0.004

Abbreviations: CRP, C-reactive protein; CS: controls; ESR, erythrocyte sedimentation rate; LC, patients with long-COVID; TSH, thyroid-stimulating hormone.

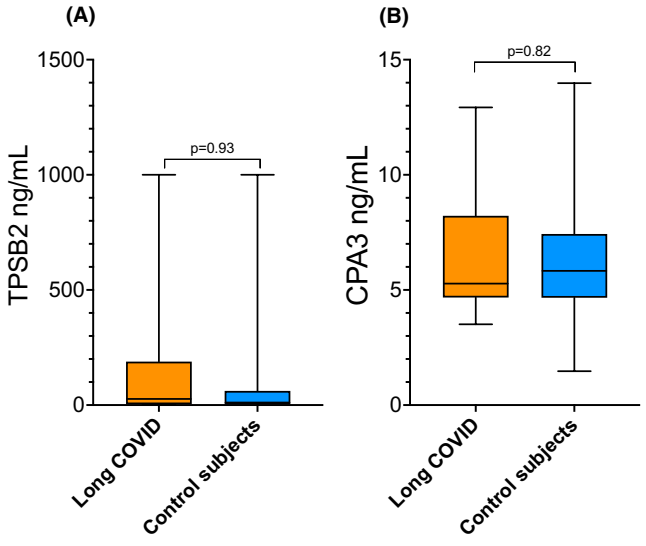

FIGURE 1 Serum concentrations of beta-tryptase (TPSB2; A) and carboxypeptidase A3 (CPA3; B) in 24 patients with long COVID and 24 age- and sex-matched controls. Plots with median, 25th, and 75th percentiles (box), and ranges (whiskers) are shown. CPA3, carboxypeptidase; TPSB2, beta-tryptase.

compared with those who completely recovered from CoV-2 infection and did not present with long-standing sequelae. This conclusion is based on the measurement of serum levels of the proteases TPSB2 and CPA3, which are specifically released from mast cells during activation and/or degranulation and closely reflect the activation status of these cells.[5] Further, in this case–control study, we did not observe any signs of systemic inflammatory activity in the long-COVID group based on laboratory markers such as C-reactive protein (CRP) content, erythrocyte sedimentation rate (ESR), or leukocyte or platelet numbers (Table 1).

It could be argumented that TPSB2 and CPA3 can be retained in tissues upon mast cell activation. Also, activation through TLRs could lead to mast cell activation without degranulation, and release of proinflammatory cytokines, only. However, in that instance we would have expected that IL-1, TNF-α, and IL-6 had lead to systemic inflammatory activity. We did not observe that.

These findings are in contrast with observations in a small group of patients with post-acute COVID-19 syndrome ($n=13$),[7] as well as with measures of mast cell activation in acute CoV-2 infection,[11,12] in which significantly elevated levels of mast cell proteases were evident. Importantly, the median observation time since diagnosis for the patients with long-COVID in our study was much longer (60 weeks) than that reported in the study of post-acute COVID-19 syndrome (62 days). This finding implies that mast cell activation is a transient phenomenon that may be involved as long as viral persistence or other immune activation is present but does not seem to contribute to the pathophysiology of long-COVID along a longer timeframe.

Exertional intolerance and post-exertional malaise are characterized by limited physical capacity that leads to worsening pain, fatigue, cognitive symptoms, and malaise during or after physical exertion. These phenomena are not specific to long-COVID but also occur in conditions

TABLE 2 Correlation between carboxypeptidase A3 (CPA3) and beta-tryptase (TPSB2) levels with SF-36 items 3.1–3.10 (proxy for exercise intolerance) in 24 patients with long COVID.

	CPA3 levels		TPSB2 levels	
SF-36 item	**Spearman's rho**	***p*-value***	**Spearman's rho**	***p*-value***
SF3.1	0.19	0.36	−0.44	0.03
SF3.2	−0.24	0.25	−0.19	0.37
SF3.3	−0.27	0.20	−0.02	0.91
SF3.4	−0.17	0.43	0.03	0.89
SF3.5	−0.25	0.24	0.04	0.87
SF3.6	−0.31	0.14	−0.07	0.75
SF3.7	0.05	0.81	0.21	0.33
SF3.8	−0.42	0.04	0.14	0.53
SF3.9	−0.26	0.22	−0.10	0.66
SF3.10	−0.29	0.17	0.20	0.35

Abbreviation: SF-36, 36-item Short Form Health Survey.

*Bonferroni correction for multiple testing requires $p < 0.005$ for statistical significance; 0.05: 10 (number of analyses) = 0.005.

without inflammation, such as chronic pain syndromes (fibromyalgia), and in patients suffering from fatigue due to chronic inflammatory or malignant diseases.[13,14] In a recent study, patients with long-COVID who were exercised on ergometer cycles displayed exercise-induced myopathy and infiltration of amyloid-containing deposits in the skeletal muscle. In addition, local and systemic metabolic disturbances have been reported, but no pathogenetic mechanisms have been identified.[15]

We hypothesized that exertional intolerance and post-exertional malaise could be caused by an increased release of bioactive molecules due to mechanical/physical stress on activated mast cells or on mast cells with a lower threshold for activation or degranulation. The individual 10 elements in the SF-36 Physical Functioning Scale do not allow absolute and objective measures of physical activity in each participant. An indication of physical capacity and tolerance was nonetheless obtained through identification of the most intensive activities, including "vigorous activities such as running, lifting heavy objects, or participating in strenuous sports" and "climbing several flights of stairs," or "walking more than a mile." As these activities are representative of demanding physical exercise, we consider these items as relevant surrogate markers for exertional intolerance in an explorative setting.

Although we found no consistent signs of exertional intolerance among patients with long-COVID, two significant associations appeared in the preliminary uncorrected analysis, between high levels of TPSB2 and CPA3 and low tolerance for vigorous and moderate exercise, respectively. The final corrected analysis did not confirm these findings. Whether exercise intolerance can be influenced to some degree by mast cell activation in a subgroup of patients with long-COVID should be further pursued in larger studies.

These observations strongly indicate that long-COVID—at least in the majority of our patients—is not driven by any overt inflammatory processes, for example due to viral persistence or autoimmune induction. More likely, biological mechanisms that do not involve direct immune activation are operative, with major influence on the subjective phenomena that many patients report, such as fatigue, exercise intolerance, depression, and cognitive disturbances, often referred to as "brain fog" by the patients. Myalgic encephalomyelitis, also called chronic fatigue syndrome (ME/CFS) is a much debated and disputed condition in which fatigue, brain fog, and exercise intolerance are dominant features, among many other symptoms. This condition is often reported to occur in long-COVID.

In addition to the dominating fatigue, most of the numerous subjective phenomena in long-COVID fit into the "sickness behavior response," a genetically controlled set of uncontrollable and automatic responses that occur during the course of infection, sterile inflammation, and injury.[16,17] Sickness behaviour is highly conserved in evolution and is thought to enhance an animal's survival by reducing exposure to pathogens and predators. Fatigue constitutes a substantial component of this behaviour. Several studies have explored the pathways involved in sickness behaviour and demonstrated the fundamental role of IL-1β signalling and other biomolecules that act on cerebral neurons to initiate the response.[18,19] However, fatigue also occurs in conditions without obvious systemic inflammation; therefore, inducers other than proinflammatory factors are operative.

A reasonable assumption is that long-COVID represents post-viral fatigue, a well-known phenomenon observed as a sequela of EBV, CMV, and other viral infections.[20–22] While most people recover completely after

such viral infections, some continue to experience fatigue for years. A relevant hypothesis for post-viral fatigue is that "fatigue-genes" are not turned off in genetically predisposed individuals after the virus is eliminated and that evolutionary, deeply conserved neuronal pathways in the brain continue to generate the sickness response.[23] In this case, no inflammatory drive would be expected.

Among the strengths of the study, we mention the age- and sex-matched case–control design, physical examination of the participants, and patient selection according to the NICE criteria. An initial COVID-19 infection in all individuals documented by a positive COVID-19 test and the long follow-up time from diagnosis (median, 60 weeks) are additional strengths. This study has some limitations. First, the limited numbers of patients may have influenced the results. Second, validated measures of physical activity or limitations in physical capacity were lacking for our cohort. It would have been advantageous to perform controlled exercise testing to explore the involvement of mast cells in exercise intolerance and post-exertional malaise.

AUTHOR CONTRIBUTIONS

OBL, TG, GSB, and RO contributed to the conceptualization of the study. OBL recruited the study individuals and conducted the clinical investigations. GJ performed the laboratory analyses of TPSB2 and CPA3. OBL, TG, and RO performed formal analysis and interpreted the data. RO applied for financial support. OBL, GJ, TG, EAMJ, GSB, FB, and RO wrote and reviewed the manuscript prior to submission.

ACKNOWLEDGEMENTS

The project is funded by Foundation Dam (Stiftelsen Dam) (grant number #SDAM_HEL45920) and The Norwegian Brain Council. We are grateful to Anne Gro Larsen, Maombi Mirindi Mongane, Karina Fuglestein Bru, and Stina Kvalheim at the Clinical Research Unit at Stavanger University Hospital for their excellent assistance.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL

The study was approved by the regional ethics committee (REC West, Norway 489,162) and conducted in compliance with the principles expressed in the Declaration of Helsinki.

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How to cite this article: Lenning OB, Jonsson G, Grimstad T, et al. No signs of mast cell involvement in long-COVID: A case–control study. *Scand J Immunol*. 2024;100:e13407. doi:10.1111/sji.13407