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CLINICAL STUDY

Serum tryptase levels in patients with post-acute COVID-19 syndrome

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ABSTRACT

OBJECTIVES: to investigate the difference in serum tryptase levels between post-acute COVID-19 syndrome (PACS) patients and controls.

BACKGROUND: PACS has been defined as symptoms that persist for more than 3 months after the onset of COVID-19. The pathogenesis is still unknown, but mast cell activation has been proposed as one of the mechanisms, and increased serum tryptase levels have been demonstrated in PACS patients.

METHODS: A total number of 133 patients were included: 50 with PACS, 37 asymptomatic COVID-19 convalescents, and 46 controls with a negative history of COVID-19. Serum tryptase levels were determined in all participants.

RESULTS: There was no significant difference in serum levels of tryptase among the groups.

CONCLUSION: the role of mast cell activation in PACS remains unclear and further research is needed to fill the gaps in understanding the pathogenesis of this complex and heterogeneous disorder *(Tab. 2, Ref. 17)*.

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KEY WORDS: post Acute COVID-19 syndrome, tryptase, mast cells, lactate dehydrogenase, ferritin.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which spread rapidly in 2020, causing a global pandemic (1). A significant proportion of patients experienced severe disease and death, but in the majority of cases, COVID-19 presents with mild flu-like illness or is asymptomatic (2, 3). Many reports emerged of patients who struggled with prolonged symptoms after the acute phase of infection, adding to the medical and socioeconomic burden of the pandemic. Post-acute COVID-19 syndrome (PACS) or long COVID-19 has been defined as symptoms that persist for more than 3 months after the onset of COVID-19 (4, 5). As over 70% of COVID-19 survivors experienced symptoms resolution by 12 weeks, a period of 3 months was set as necessary for the diagnosis (6).

The clinical presentation of PACS is highly variable and the pathogenesis remains poorly understood. It has been proposed that PACS may be driven by long-term damage to lung, brain, or heart tissues and pathological inflammation due to viral persistence, immune dysregulation, or autoimmunity (5). It has been shown that SARS-CoV-2 can persist in the body for months. Although the highest burden of the virus was in the respiratory tract, persistent SARS-CoV-2 ribonucleic acid (RNA) has also been demonstrated in other tissues, such as the myocardium, lymph nodes, and central nervous system (7).

The role of mast cells (MCs) in the pathogenesis of PACS has been proposed. MCs are heterogenous innate leukocytes that produce many inflammatory mediators, and their role is substantial not only in the context of allergies but also in infections, immune regulation, and tissue fibrosis (7–9). Upon activation, MCs release preformed granules containing inflammatory mediators, vasoactive autacoids, and catalytically active MC-specific proteases, including tryptase, chymase, and carboxypeptidase 3 (9). Viruses have the potential to stimulate MCs both directly and indirectly, through viral inflammatory products, and trigger MC degranulation, protease release, and cytokine production. Indeed, MC activation has been shown in the lungs during acute infections with certain viruses, such as influenza H5N1 or enterovirus EV71, and MC responses can influence long-term homeostasis in the respiratory tract (8).

There is emerging evidence indicating MC activation in sera and lung tissue in patients with acute COVID-19, which has been linked to a hyperinflammatory state (10–12). Moreover, increased levels of MC-derived proteases, including active tryptase, have been demonstrated in the serum of PACS patients, compared to asymptomatic controls, which is highly suggestive of systemic MC activation (14).

It has been hypothesized that COVID-19 could exaggerate existing undiagnosed MC activation syndrome, or activate normal mast cells due to the persistence of viral particles (15).

In this study, we aimed to compare serum tryptase levels between patients with PACS, patients who recovered from

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COVID-19 without PACS, and patients with a negative history of COVID-19. Additionally, we sought to evaluate the potential differences in the lactate-dehydrogenase (LDH) levels as a marker of cell damage, and ferritin (16), as an indicator of oxidative stress and systemic inflammation, between the groups. We also aimed to determine the differences in pulmonary function between the groups, as measured by spirometry and diffusion capacity of carbon monoxide.

Tab. 1. Baseline characteristics, pulmonary function tests, and laboratory test results across the groups

	PACS	ACC	Control group	p
Subjects	50	37	46	
Baseline characteristics				
Male	19 (38)	13 (35.1)	10 (21.7)	
Female	31 (62)	24 (64.9)	36 (78.4)	
Age	55.8±15.09[c]	57.54±17.2	63.72±15.17[a]	**0.041**
Pulmonary function tests				
FVC %	100.86±21.73	103.52±22.08	102.51±23.57	0.863
FVC L	3.48±1.14	3.61±1.27	3.06±1.09	0.085
FEV1 %	96.65±24.93[c]	85.88±31.15	79.11±33.31[a]	**0.018**
FEV1 L	2.74±1.06[c]	2,54±1.35	1.95±1.1[a]	**0.004**
FEV1/FVC	0.78±0.13[c,b]	0.66±0.19[a]	0.6±0.18[a]	**0.001**
DLCO	83.84±26.9	84,44±32	71.17±33.54	0.090
KCO %	93 (77–107)[c]	92.5 (77–100.5)	84.5 (51–97)[a]	**0.029**
VA %	96 (82–104)	93,5 (78.5–109.5)	98 (82–103)	0.983
Laboratory test results				
Leukocytes x10^9/L	7.54±2.58	7.02±2.7	7.48±1.77	0.567
Neutrophils %	64.52±9.03	60.89±7.99	63.57±13.39	0.278
Lymphocytes %	26.25±8.34	28.39±8.16	25.03±10.18	0.246
Eosinophils %	3±2.58	2.98±1.8	2.68±1.7	0.716
LDH U/L	191.55±54.87	189.22±43.2	188.07±58.78	0.949
Ferritin ug/L	104 (51–155)	79 (30–152)	133 (71–259)	0.100
Tryptase ug/L	4.8±3.2	4.7±2.58	4.4±2.66	0.777

Data are presented as n, n(%), mean±sd, or median (interquartile range). PACS: post-acute COVID-19 syndrome; ACC: asymptomatic COVID-19 convalescents; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; DLCO: diffusing capacity of the lungs for carbon monoxide; KCO: carbon monoxide transfer coefficient; VA: alveolar volume; LDH: lactate-dehydrogenase. [a]: statistically significant difference versus PACS; [b]: statistically significant difference versus ACC; [c]: statistically significant difference versus the control group.

Materials and methods

In this cross-sectional study, we included patients with PACS, who were referred to our center from 26th May 2022 to 15th November 2022. PACS was defined as the presence of symptoms (Tab. 2) that persisted 12 weeks or more after acute COVID-19 (the first day of acute COVID-19 was defined as the day of the onset of symptoms). We also included a group of asymptomatic COVID-19 convalescents (ACC) consisting of patients who recovered from COVID-19 without developing PACS and a control group of patients with a negative history of COVID-19 and negative serology to SARS-CoV-2. The patients in the second and third groups were recruited among outpatients who were referred to our center for reasons other than PACS.

Exclusion criteria for all groups were the history of conditions that could result in elevation of serum tryptase levels (recent anaphylaxis, chronic kidney disease, systemic mastocytosis, myelodysplastic syndrome, myeloproliferative neoplasm, acute myeloid leukemia, chronic myeloid leukemia, and chronic eosinophilic leukemia), as well as unwillingness to participate in the study.

The study was approved by the Institutional Review Board of the Special Hospital for Pulmonary Diseases with approval number 02-374/2022-5. All procedures were following the 1975 Helsinki Declaration (17). Written informed consent was obtained from all participants before enrolment.

Demographic data and clinical history including comorbidities were collected from each participant. Also, in PACS and ACC groups data regarding the clinical presentation and the course of acute COVID-19 were obtained. All participants with PACS were asked to fill in a questionnaire indicating the presence of the following PACS symptoms: malaise, diffuse aching, chest discomfort, muscle cramps, shortness of breath, breathlessness on exertion, difficulties of concentration, excessive sweating, headache and difficulties of sleeping.

All participants underwent pulmonary function tests (PFTs) that involved spirometry and diffusing capacity of the lungs for carbon monoxide (DLCO). Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), the ratio of FEV1/FVC, DLCO, the carbon monoxide transfer coefficient (KCO), alveolar volume determined by the volume of gas containing CO measured by the dilution of inert tracer gas in the inspired volume (VA) were recorded. PFTs were performed and measured following the American Thoracic Society (ATS) and European Respiratory Society (ERS) technical standards on interpretive strategies for routine lung function tests.

Blood samples were taken from each participant, for determination of serum levels of tryptase, ferritin, LDH, creatinine, urea,

Tab. 2. Prevalence of PACS symptoms.

Symptom	Prevalence
Malaise	42 (84)
Diffuse aching	14 (28)
Chest discomfort	27 (54)
Muscle cramps	18 (36)
Shortness of breath	25 (50)
Breathlessness on exertion	40 (80)
Difficulties of concentration	30 (60)
Difficulties of sleeping	18 (36)
Headache	12 (24)
Excessive sweating	23 (46)

Data are presented as n (%).

and complete blood count (CBC). Serum tryptase levels were determined by ImmunoCAP (Phadia 200). In subjects without a history of COVID-19 serologic tests for SARS-CoV-2 (nucleocapsid and spike protein antigens) were also performed by qualitative ELISA test to confirm each participant's group assignment.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) when normally distributed or as median with interquartile range (IQR) when not normally distributed. Groups were compared by using the ANOVA test with post hoc comparison using Tukey's method or with the Kruskal Wallis test followed by the Mann-Whitney test, as appropriate. The chi-square test was used to compare categorical data. Statistical analysis was performed using GraphPad Prism version 6 for Windows (GraphPad Software Inc., La Jolla, CA). All tests were two-sided and $P<0.05$ was considered statistically significant.

Results

A total number of 133 participants were included: 50 PACS patients, 37 ACC, and 46 controls. Demographic data, laboratory findings, and pulmonary function test parameters are shown in Table 1.

In the PACS group, the most prevalent symptoms were malaise (84%) and breathlessness on exertion (80%), followed by difficulties of concentration (60%) and chest discomfort (54%) (Tab. 2).

There was no significant difference in serum tryptase levels between the PACS, ACC, and controls (Tab. 1). Also, serum levels of LDH, ferritin, and other laboratory parameters were similar among the groups.

Regarding pre-existing pulmonary conditions, in the PACS group, 3/50 (6%) patients had chronic obstructive pulmonary disease (COPD), and 11/50 (22%) had asthma. In the ACC group, 11/37 (29.7%) patients had COPD and 9/37 (24.3%) had asthma, and in the control group, 19/46 (41.3%) patients had COPD, and 13/46 (28.3%) had asthma. As for PFTs, patients with PACS had significantly higher FEV1/FVC compared with ACC and controls ($p<0.001$), as well as significantly higher FEV1 compared with controls ($p=0.018$).

Discussion

Evidence for MC activation in acute COVID-19 is emerging. Autopsy findings revealed a higher number of tryptase-secreting MCs in COVID-19 patients, compared with the H1N1 patients and control group, suggesting the role of these cells in acute lung injury and diffuse alveolar damage (11).

Also, the role of MC activation in PACS has been proposed. This peculiar syndrome encompasses a myriad of symptoms, and pathogenesis remains largely unknown. Weinstock et al showed that symptoms compatible with MC activation were increased in patients with PACS, suggesting that activation of aberrant MCs induced by SARS-CoV-2 infection may partly underlie the pathophysiology (13). Furthermore, Wechsler et al found significantly elevated serum levels of MC proteases (active tryptase and carboxypeptidase A3) in patients with PACS compared with healthy controls, which was highly suggestive of MC activation. Also, the study revealed that the levels of tryptase, but not the levels of carboxypeptidase, were significantly elevated in PACS compared with patients who recovered from COVID-19 without developing PACS (14), implicating the potential usefulness of serum tryptase as a biomarker for PACS in COVID-19 convalescents.

However, in our study, we found no significant difference in the serum levels of tryptase in PACS patients compared with APC and control groups. There were also no significant differences in LDH and ferritin levels among the groups.

In comparison to the study by Wechsler et al (14), our study included a larger sample of patients, and a different set of inclusion criteria was used, reflecting inconsistency in PACS definitions (12 weeks post-acute COVID-19 vs 4 weeks), which might have affected the results. Further studies are needed with a serial determination of serum tryptase during the post-acute COVID-19 period.

Some limitations to our study should be noted. Firstly, in the ACC and control groups, we included outpatients referred to the pulmonologist at our center for a reason other than PACS, many of which were treated for COPD or asthma. A higher proportion of patients in the control groups had pre-existing obstructive lung disease, compared with the group with PACS, and this is the most probable explanation for the significant difference in PFT parameters FEV1 and FEV1/FVC ratio between the groups. However, we would not expect that the presence of obstructive lung disease would affect the serum tryptase levels. The patients with conditions that may result in elevated tryptase levels were not included in the study. Additionally, it should be noted that the PACS group had a significantly lower mean age than the control group, which could also partly explain the lower measured values of FEV1.

Regarding the significant burden of PACS and the lack of effective treatment, further research is needed to fill the gaps in understanding the complex pathogenesis of this heterogeneous disorder.

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