Serological biomarkers of COVID-19 severity at hospital admission are not related to long-term post-COVID pain symptoms in hospitalized COVID-19 survivors

Fernández-de-Las-Peñas, César; Ryan-Murua, Pablo; de-la-Llave-Rincón, Ana I; Gómez-Mayordomo, Víctor; Arendt-Nielsen, Lars; Torres-Macho, Juan

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Corresponding Author:	César Fernández de las Peñas, PT, PhD Universidad Rey Juan Carlos Alcorcón, Madrid SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidad Rey Juan Carlos
Corresponding Author's Secondary Institution:	
First Author:	César Fernández de las Peñas, PT, PhD
First Author Secondary Information:	
Order of Authors:	César Fernández de las Peñas, PT, PhD
	Pablo Ryan-Murua, MD
	Ana I De-la-Llave-Rincón, PT, PhD
	Victor Gómez-Mayordomo, MD
	Lars Arendt-Nielsen, PhD
	Juan Torres-Macho, MD, PhD
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Abstract

This study investigated the association between serological biomarkers at hospital admission with the development of long-term post-COVID pain symptoms in previously hospitalized COVID-19 survivors. A cohort study including patients hospitalised due to COVID-19 in one urban hospital of Madrid (Spain) during the first wave of the outbreak was conducted. Hospitalisation data, clinical data and eleven serological biomarkers were collected at hospital admission. Participants were scheduled for an individual telephone interview after hospital discharge for collecting data about post-COVID pain symptoms. A total of 412 (mean age: 62, SD: 15 years; 46.1% women) were assessed twice, a mean of 6.8 and 13.2 months after discharge. The prevalence of post-COVID pain symptoms was 42.7% (n=176) and 36.2% (n=149) at 6.8 and 13.2 months after hospital discharge. Patients reporting post-COVID pain exhibited a greater number of COVID-19 associated symptoms at hospital admission, more medical comorbidities, higher lymphocyte count, and lower glucose and creatine kinase (CK) levels (all, $P<0.01$) than those not reporting post-COVID pain. The multivariate analysis revealed that lower CK and glucose levels were significantly associated, but just explaining 6.9% of the variance of suffering post-COVID pain. In conclusion, the association between serological biomarkers associated with COVID-19 severity at hospital admission and the development of post-COVID pain is small. Other factors, e.g., higher number of COVID-19 onset symptoms (higher symptom load) could be more relevant for the development of post-COVID pain. As inflammatory biomarkers were not directly analyzed, they may have stronger predictive strengths for the development of post-COVID pain symptoms.

Key words: COVID-19, pain, post-COVID, biomarkers.

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César Fernández-de-las-Peñas[1,2] Dr. Med, PhD; Pablo Ryan-Murua[3] MD; Ana I de-la-Llave-Rincón[1] PT, PhD; Víctor Gómez-Mayordomo[4] MD; Lars Arendt-Nielsen[2,5] Dr. Med, PhD; Juan Torres-Macho[3,6] MD

[1] Department of Physical Therapy, Occupational Therapy, Physical Medicine and Rehabilitation, Universidad Rey Juan Carlos (URJC), Madrid. Spain.

[2] Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark

[3] Department of Internal Medicine, Hospital Universitario Infanta Leonor-Virgen de la Torre, Madrid Spain

[4] Department of Neurology, Hospital Clínico San Carlos. Madrid, Spain

[5] Department of Medical Gastroenterology, Mech-Sense, Aalborg University Hospital, Aalborg, Denmark

[6] Department of Medicine, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain.

Corresponding / reprint requests author:

César Fernández-de-las-Peñas
Facultad de Ciencias de la Salud
Universidad Rey Juan Carlos
Avenida de Atenas s/n
28922 Alcorcón, Madrid, SPAIN
Email: cesar.fernandez@urjc.es

Telephone number: + 34 91 488 88 84

Fax number: +34 91 488 89 57

Introduction

Symptoms associated with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) affect different systems [25]. Different biomarkers had been investigated at the acute phase to identify individuals at a risk for developing a worse hospital course during the infection. Hematological (lymphocyte count, neutrophil count), inflammatory (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], procalcitonin [PCT]), immunological (interleukin IL-6) and biochemical (D-dimer, troponin, creatine kinase [CK]) markers have been investigated. A meta-analysis (56 studies, n=8,719 patients) found that patients with severe COVID-19 exhibited higher levels of some inflammatory biomarkers such as white blood cell count, CRP, ESR, PCT, or IL-6 than those with mild COVID-19 [13].

Most studies have investigated biomarker relevance during the acute phase of the infection; however, whether biomarkers of a worse infection prognosis were correlated with development of post-COVID symptoms is less known. Different meta-analyses reported that 60% of COVID-19 survivors develop post-COVID symptoms six months following the infection [4,15]. The association between post-COVID symptoms and serological biomarkers associated with COVID-19 severity is controversial. Mandal et al. reported that 30.1% and 9.5% of COVID-19 survivors showed elevated D-dimer and CRP two months after hospital discharge but no association with any post-COVID symptom was found [16]. Townsend et al. did not find an association between post-COVID fatigue and laboratory biomarkers of inflammation and cell turnover at hospital admission [23].

One relevant post-COVID symptom, which is not specifically reported in previous COVID-19 literature is chronic pain [4,15]. Just one published meta-analysis has focused on post-COVID pain symptoms and reported a prevalence of 10.9% and 7.7% for myalgia and arthralgia as post-COVID pain symptoms the first six months after the infection [10].

Interestingly, studies specifically focusing on post-COVID pain symptoms have reported prevalence rates of 60% the first three months after infection [2,14,22]. It seems that post-COVID pain maybe underreported in general cohort post-COVID studies [10].

Several of the proinflammatory signaling molecules elevated in COVID-19 patients due to the cytokine storm could impact skeletal muscle. Preliminary evidence suggests an association between laboratory biomarkers and the presence of pain symptoms at hospital admission and at the post-COVID phase. Batur et al. found an increase in CK levels and lymphocyte count in patients presenting myalgia as a symptom at hospital admission [3]. Bakılan et al. showed lower lymphocyte count and higher D-dimer levels in individuals developing post-COVID pain symptom [2]. However, both studies included small sample sizes with short-term follow-ups [2,3]. Monitoring serological biomarkers of COVID-19 severity at the acute phase could help for identifying patients at a higher risk of developing post-COVID pain, and, hence, indicate the need for timely interventions. We present a cohort study of hospitalized COVID-19 survivors assessed at 6- and 12-months after discharge for the presence of post-COVID pain. Our aim was to investigate the association between serological biomarkers of COVID-19 severity at hospital admission with post-COVID pain symptoms in previously hospitalized COVID-19 survivors. We hypothesized that biomarkers related to COVID-19 severity could serve as antecedent biomarkers (risk of developing a condition) for post-COVID pain symptoms.

Methods

Participants

This cohort study included patients hospitalized because of SARS-CoV-2 infection during the first wave of the pandemic (from March 20 to June 30, 2020) from an urban hospital in Madrid (Spain). All participants have been diagnosed with real-time reverse transcription-polymerase chain reaction (PCR) assay of nasopharyngeal/oral swab sample and the presence of clinical and radiological findings at hospital admission. The study was approved by the Ethics Committee of the Hospital Universitario Infanta Leonor (HUIL/092-20). Participants were informed of the study and all provided their respective informed consent.

Hospitalization Data

Clinical and hospitalization data including age, gender, height, weight, COVID-19 associated onset symptoms at hospital admission, pre-existing comorbidities, intensive care unit [ICU] admission were systematically collected at hospital admission. Further, serological values of hemoglobin, lymphocyte count, neutrophil count, platelet count, glucose, CRP, CK, lactate dehydrogenase (LDH), D-dimer, alanine transaminase (ALT) and aspartate transaminase (AST) were also systematically collected. The number of days in hospital was collected from medical records.

Post-COVID Pain Symptoms Assessment

Participants who agreed to participate in the study were scheduled for a telephone semi-structured interview by trained healthcare researchers. Patients were asked to report the three most bothersome post-COVID symptoms. A specific questionnaire focusing on pain symptoms was developed. Participants were asked for the presence of pain symptoms appearing after hospital discharge and whether the reported pain symptom persisted at the time of the study. We focused on the presence of post-COVID pain symptoms, e.g., neck pain, shoulder pain, widespread pain, differentiating from headache (i.e., migraine-like

pain). We did not include headache due to the need for a proper diagnosis according to agreed classifications.

We defined post-COVID pain as: 1) pain symptoms compatible with a diagnosis of chronic primary musculoskeletal pain, as defined by the International Association for the Study of Pain (IASP) [19]; 2) symptoms experienced for at least three consecutive months after hospital discharge, and 3) absence of any underlying medical condition which could best explain pain, e.g., arthritis. Participants were asked to differentiate the symptoms beginning after SARS-CoV-2 infection from their previous pain condition.

Statistical Analysis

The STATA 16.1 program (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LP. USA) was used for the analysis. Data are presented as means (standard deviation, SD) or percentages as appropriate. McNemar's chi-squared test and paired Student t-tests were conducted to compare proportions and means between patients with and without post-COVID pain symptoms at 6- and 12-months follow-ups. Missing values were imputed by using median imputation. A multiple lineal hierarchical regression analysis including all variables (age, gender, height, weight, COVID-19 onset symptoms at hospital admission, pre-existing comorbidities, ICU admission, serological biomarkers, and days in hospital) was conducted to determine which of these variables contributed significantly to the presence of post-COVID pain symptoms. The significance criterion of the critical F value for entry into the regression equation was set at $P<0.05$. Changes in adjusted R^2 were reported after each step of the regression model to determine the association of the additional variables.

Results

From a total of 450 hospitalized patients invited to participate, ten refused to participate, eight could not be contacted after three attempts, and twenty had deceased after hospital discharge. Finally, 412 patients (mean age: 62, SD: 15 years; 46.1% women) were included in the study.

Participants were assessed at a mean of 6.8 (range 6 to 8) and 13.2 (range 12 to 14) months after hospital discharge. At the time of the evaluation, 176 (42.7%) patients reported post-COVID pain symptoms six months after hospital discharge, whereas 149 (36.2%) reported post-COVID pain symptoms twelve months after.

Table 1 compares clinical and hospitalization data between individuals developing and not developing post-COVID pain at 6-months. A similar distribution at 12-months was observed (data not shown). Patients developing post-COVID pain 6-months after hospital discharge exhibited a greater number of symptoms at hospital admission, particularly a higher prevalence of myalgia and headache ($P<0.01$) and a greater number of comorbidities than those not exhibiting post-COVID pain at 6-months (**Table 1**). Additionally, a greater proportion ($P=0.005$) of patients developing post-COVID pain symptoms (n=91, 51.7%) reported previous pain symptoms. From these patients suffering from previous pain symptoms, 62 (35.2%) reported that post-COVID pain was different from previous symptomatology (new-onset post-COVID pain), whereas the remaining 29 (16.4%) experienced an increase of their previous symptoms (exacerbated post-COVID related-pain). The remaining 84 patients (48.3%) reported new-onset post-COVID related-pain, since they did not suffer from previous symptoms before the infection. Accordingly, the prevalence of new-onset post-COVID pain symptoms was up to 83.6%. Further, no significant differences in the prevalence of the most bothersome post-COVID symptoms, being these fatigue, dyspnea and brain fog, were seen between those experiencing or not experiencing post-COVID pain (**Table 2**).

Patients with post-COVID pain exhibited higher lymphocyte count and lower glucose and CK levels on hospital admission (all, P<0.01) than those not developing post-COVID pain symptoms at both 6- and 12-months (**Table 3**). The stepwise regression analysis revealed that lower levels of CK (step 1: r^2 adj: 0.05; B: -0.337; 95%CI -0.566, -0.109; P=0.004) and glucose (step 2: r^2 adj: 0.069; B: -0.003; 95%CI -0.005, -0.001; P=0.047) were significantly associated, but just explained 6.9% of the variance, of suffering from long-term post-COVID pain.

Discussion

This study found that post-COVID pain symptoms were present in almost 40% of COVID-19 survivors the first year after hospital discharge. In addition, patients reporting post-COVID pain exhibited higher lymphocyte count, and lower levels of glucose and CK at hospital admission than those not reporting post-COVID pain symptoms, although this association was small.

Our prevalence data are slightly lower than those previously reported by small cohort studies providing prevalence rates of post-COVID pain up to 60% at one [2,14] and three [22] months after the infection, but much higher than the prevalence rates (10% to 15%) reported in a recent meta-analysis including general cohort studies [10]. Data may vary significantly depending on how focused the study is on specifically pain or general post-COVID symptoms.

Potential pathophysiologic mechanisms proposed for explaining post-COVID pain symptomatology include a systemic immune response with prolonged inflammation, viral toxicity, hypercoagulability, and microvascular injury [1]. Supporting some of these hypotheses, lower lymphocyte count (lower immune response) and higher D-dimer levels (coagulopathy) have been found in individuals reporting post-COVID pain three months

after SARS-CoV-2 infection [2]. Our results are contrary to the data reported by Bakılan et al [2] since higher lymphocyte count (lymphocytosis) suggesting an "exaggerated" immune response, was observed in individuals developing long-term post-COVID pain.

Additionally, since the presence of angiotensin-converting enzyme-2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) receptors is higher in the muscle tissue than in other tissues [8], another mechanism for developing post-COVID pain could be the presence of skeletal muscle injury. Skeletal muscle injury is associated with elevated CK levels (i.e., hyperckemia). In fact, hyperckemia has been associated with respiratory failure and fatal outcomes in COVID-19 patients [7]. The current study found lower CK levels at hospital admission in patients developing long-term post-COVID pain, suggesting that skeletal muscle injury seems to be not associated with post-COVID pain symptoms. In fact, it should be recognized that differences in CK values between pain developing and no-pain developing individuals were extremely low (i.e., a few tenths of mg/mL).

Similarly, patients reporting post-COVID pain also showed lower glucose levels. Since increased blood glucose is associated with severe COVID-19 [6], our results would suggest that individuals with less severe COVID-19 would develop post-COVID pain. Again, between-group differences in glucose levels were also low, hence, their clinical impact on the development of post-COVID pain symptoms seems to be small.

Other biomarkers included in our study were not associated with the presence of post-COVID pain. For instance, higher levels of CRP [17], higher D-dimer concentration [9], and lower platelet count [18], have been associated with more severe COVID-19. No differences in these biomarkers were seen depending on the development or not of long-term post-COVID pain symptoms.

The biomarker levels observed in our study suggest a greater immune response (higher lymphocyte count) against the SARS-CoV-2 infection and a lower COVID-19 severity (lower glucose and CK levels) in individuals developing long-term post-COVID pain symptoms [20]; however, associations were small, after adjusting for all the variables during the multivariate regression analyses. It is possible that the fact that our sample was relatively young (<65 years old), with a low number of medical comorbidities and low death rate explain the lack of association between serological biomarkers and long-term post-COVID pain symptoms.

Other potential risk factors associated with post-COVID symptoms in general such as female gender, higher number of onset symptoms at hospital admission (higher symptom load) or longer hospital stay [12] could also influence the development of post-COVID pain. In fact, a greater number of acute onset symptoms at hospital admission, i.e., higher symptom load, was seen in patients developing post-COVID pain 6- and 12-months after hospital discharge. Interestingly, myalgia and headache were the symptoms at hospital admission with a greater prevalence in patients with post-COVID pain. In line with our results, previous studies reported that the presence of pain symptoms at the acute phase is a marker associated with good prognosis for hospitalization [21], but also is associated with post-COVID pain symptoms [11]. Based on current evidence, post-COVID pain has a multifactorial genesis where factors related to the pathogen (SARS-CoV-2 associated-factors) intersect with the host response (immune and biological responses), as well as with hospitalization (treatment-associated factors) and emotional (COVID-19 outbreak surrounding elements) factors [5].

The current study did not include headache due to its specific diagnostic criteria, which could limit the generality of the results. In fact, Trigo et al observed that patients experiencing headache as an onset symptom at hospital admission exhibited higher levels

of IL-10, but not other pro-inflammatory biomarkers, suggesting a more intense immune response in these patients [24]. We do not currently know the biomarker profile of those individuals developing post-COVID headache.

Current data should be considered according to limitations of the study design. First, data can be only applicable to previously hospitalized COVID-19 patients. Further, the number of individuals requiring ICU admission was small. Similarly, hospitalization treatments, e.g., amount of sedation, medication intake received for the acute infection, or presence of neuromuscular symptoms associated with ICU admission) were not collected. Second, post-COVID symptoms were collected by telephone, a procedure with a potential bias in population-based survey studies. Nevertheless, telephone interview is a common method used in cohort studies investigating post-COVID pain [10]. Third, although we collected data on post-COVID pain symptoms at two different follow-up periods, it would be difficult exclusively to attribute to SARS-CoV-2 infection to the development of post-COVID pain symptoms. Fourth, we focused on pain symptoms potentially considered of musculoskeletal origin; however, due to the use of telephone interviews, characterization of the pain symptoms is not available and we are not able to properly classify the observed post-COVID pain as musculoskeletal or neuropathic in origin. In fact, factors that could potentially influence the development of post-COVID pain, such as depression or anxiety, were not evaluated in this study. Studies characterizing and classifying the nature of post-COVID pain symptoms are clearly needed. Finally, as specific inflammatory biomarkers, e.g., cytokines, were not assessed in the current study, they may exhibit strong predictive strengths for the development of post-COVID pain. Similarly, we did not collect data about the intensity or severity of post-COVID pain symptoms; therefore, we were not able to determine the proportion of patients showing disabling symptomatology.

Conclusions

This study found a weak association between serological biomarkers associated with COVID-19 severity at hospital admission and the development of long-term post-COVID pain symptoms in previously hospitalized patients. Other factors such as higher number of acute onset symptoms at hospital admission (higher symptom load) could be more relevant for the development of post-COVID pain symptoms.

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Declaration of interests

No conflict of interest is declared by any of the authors

References

1. Afrin LB, Weinstock LB, Molderings GJ. COVID-19 hyperinflammation and post-Covid-19 illness may be rooted in mast cell activation syndrome. Int J Infect Dis 2020; 100: 327-332.
2. Bakılan F, Gökmen İG, Ortanca B, Uçan A, Eker Güvenç Ş, Şahin Mutlu F, Gökmen HM, Ekim A. Musculoskeletal symptoms and related factors in postacute COVID-19 patients. Int J Clin Pract. 2021: e14734.
3. Batur EB, Korez MK, Gezer IA, Levendoglu F, Ural O. Musculoskeletal symptoms and relationship with laboratory findings in patients with COVID-19. Int J Clin Pract 2021; 75: e14135.
4. Cares-Marambio K, Montenegro-Jiménez Y, Torres-Castro R, Vera-Uribe R, Torralba Y, Alsina-Restoy X, et al. Prevalence of potential respiratory symptoms in survivors of hospital admission after coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. Chron Respir Dis. 2021; 18: 14799731211002240.
5. Cascella M, Del Gaudio A, Vittori A, Bimonte S, Del Prete P, Forte CA, Cuomo A, De Blasio E. COVID-Pain: Acute and late-onset painful clinical manifestations in COVID-19: Molecular mechanisms and research perspectives. J Pain Res 2021; 14: 2403-2412.
6. Chen J, Wu C, Wang X, Yu J, Sun Z. The impact of COVID-19 on blood glucose: A systematic review and meta-analysis. Front Endocrinol 2020; 11: 574541.
7. De Rosa A, Verrengia EP, Merlo I, Rea F, Siciliano G, Corrao G, Prelle A. Muscle manifestations and CK levels in COVID infection: results of a large cohort of patients inside a Pandemic COVID-19 Area Acta Myol. 2021; 40: 1-7.

8. Disser NP, De Micheli AJ, Schonk MM, Konnaris MA, Piacentini AN, Edon DL, Toresdahl BG, Rodeo SA, Casey EK, Mendias CL. Musculoskeletal consequences of COVID-19. J Bone Joint Surg Am 2020; 102: 1197-1204.
9. Du WN, Zhang Y, Yu Y, Zhang RM. D-dimer levels is associated with severe COVID-19 infections: A meta-analysis. Int J Clin Pract 2021; 75: e14031
10. Fernández-de-las-Peñas C, Navarro-Santana M, Plaza-Manzano G, Palacios-Ceña, Arendt-Nielsen L. Time course prevalence of post-COVID pain symptoms of musculoskeletal origin in patients who had survived to SARS-CoV-2 infection: A systematic review and meta-analysis. Pain 2021 doi: 10.1097/j.pain.0000000000002496
11. Fernández-de-las-Peñas C, Rodríguez-Jiménez J, Fuensalida-Novo S, Palacios-Ceña M, Gómez-Mayordomo V, Florencio LL, Hernández-Barrera V, Arendt-Nielsen L. Myalgia as a symptom at hospital admission by severe acute respiratory syndrome coronavirus 2 infection is associated with persistent musculoskeletal pain as long-term post-COVID sequelae: a case-control study. Pain. 2021 doi: 10.1097/j.pain.0000000000002306
12. Iqbal FM, Lam K, Sounderajah V, Clarke JM, Ashrafian H, Darzi A. Characteristics and predictors of acute and chronic post-COVID syndrome: A systematic review and meta-analysis. EClinicalMedicine 2021; 36: 100899.
13. Ji P, Zhu J, Zhong Z, Li H, Pang J, Li B, Zhang J. Association of elevated inflammatory markers and severe COVID-19: A meta-analysis. Medicine 2020; 99: e23315
14. Karaarslan F, Demircioğlu GF, Kardeş S. Postdischarge rheumatic and musculoskeletal symptoms following hospitalization for COVID-19: prospective follow-up by phone interviews. Rheumatol Int 2021; 41: 1263-1271

15. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, Villapol S. More than 50 Long-term effects of COVID-19: a systematic review and meta-analysis. Sci Rep 2021; 11: 16144.
16. Mandal S, Barnett J, Brill SE, Brown JS, Denneny EK, Hare SS, Heightman M, Hillman TE, Jacob J, Jarvis HC, Lipman MCI, Naidu SB, Nair A, Porter JC, Tomlinson GS, Hurst JR; ARC Study Group. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax. 2021; 76: 396-398.
17. Melo AKG, Milby KM, Caparroz ALMA, Pinto ACPN, Santos RRP, Rocha AP, Ferreira GA, Souza VA, Valadares LDA, Vieira RMRA, Pileggi GS, Trevisani VFM. Biomarkers of cytokine storm as red flags for severe and fatal COVID-19 cases: A living systematic review and meta-analysis. PLoS One. 2021; 16: e0253894.
18. Mitra S, Ling RR, Yang IX, Poon WH, Tan CS, Monagle P, MacLaren G, Ramanathan K. Severe COVID-19 and coagulopathy: A systematic review and meta-analysis. Ann Acad Med Singap 2021; 50: 325-335.
19. Perrot S, Cohen M, Barke A, Korwisi B, Rief W, Treede RD; IASP Taskforce for the Classification of Chronic Pain. The IASP classification of chronic pain for ICD-11: chronic secondary musculoskeletal pain. Pain 2019; 160: 77-82.
20. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci 2020; 57: 389-399
21. Rubio-Rivas M, Corbella X, Mora-Luján JM, Loureiro-Amigo J, López Sampalo A, Yera Bergua C et al. Predicting clinical outcome with phenotypic clusters in COVID-19 pneumonia: an analysis of 12,066 hospitalized patients from the spanish registry SEMI-COVID-19. J Clin Med 2020; 9: 3488.

22. Soares FHC, Kubota GT, Fernandes AM, Hojo B, Couras C, Costa BV, Lapa JDDS, Braga LM, Almeida MM, Cunha PHMD, Pereira VHH, Morais ADS, Teixeira MJ, Ciampi de Andrade D; "Pain in the Pandemic Initiative Collaborators". Prevalence and characteristics of new-onset pain in COVID-19 survivours, a controlled study. Eur J Pain 2021; 25: 1342-1354

23. Townsend L, Dyer AH, Jones K, Dunne J, Mooney A, Gaffney F, O'Connor L, Leavy D, O'Brien K, Dowds J, Sugrue JA, Hopkins D, Martin-Loeches I, Ni Cheallaigh C, Nadarajan P, McLaughlin AM, Bourke NM, Bergin C, O'Farrelly C, Bannan C, Conlon N. Persistent fatigue following SARS-CoV-2 infection is common and independent of severity of initial infection. PLoS One 2020; 15: e0240784.

24. Trigo J, García-Azorín D, Sierra-Mencía Á, Tamayo-Velasco Á, Martínez-Paz P, Tamayo E, Guerrero AL, Gonzalo-Benito H. Cytokine and interleukin profile in patients with headache and COVID-19: A pilot, CASE-control, study on 104 patients. J Headache Pain. 2021; 22: 51.

25. Zhu J, Ji P, Pang J, Zhong Z, Li H, He C, Zhang J, Zhao C. Clinical characteristics of 3062 COVID-19 patients: a meta-analysis. J Med Virol 2020; 92: 1902-14

Serological Biomarkers of COVID-19 Severity at Hospital Admission are not Related to Long-Term Post-COVID Pain Symptoms in Hospitalized COVID-19 Survivors

Summary

Long-term post-COVID pain seems to be not associated with serological biomarkers of COVID-19 severity at hospital admission in previously hospitalized COVID-19 survivors. Other factors such as higher symptom load at the acute phase of the infection could be more relevant for the development of post-COVID pain symptoms.

Table

Table 1: Demographic, clinical and hospitalisation data of COVID-19 patients according to the presence or absence of post-COVID pain at 6 months follow-up

	Post-COVID Pain (n=176)	**No Post-COVID Pain (n=236)**
Age, mean (SD), years	62.5 (14.0)	62.0 (16.5)
Gender, male/female (%)	78 (44.3%) / 98 (55.7%)	95 (40.2%) / 141 (59.8%)
Weight, mean (SD), kg.	75.1 (18.4)	75.6 (15.9)
Height, mean (SD), cm.	164.0 (12.0)	165 (10.0)
Number of medical comorbidities*	1.0 (0.85)	0.7 (0.80)
Medical co-morbidities		
Hypertension	52 (29.5%)	61 (25.8%)
Cardiovascular Diseases	26 (14.8%)	30 (12.7%)
Diabetes	18 (10.3%)	22 (9.3%)
Asthma	15 (8.5%)	23 (9.7%)
Obesity	10 (5.7%)	14 (5.9%)
Chronic Obstructive Pulmonary Disease	10 (5.7%)	15 (6.4%)
Migraine	5 (2.9%)	7 (2.9%)
Other (Cancer, Kidney Disease)	30 (17.0%)	37 (15.7%)
Previous Pain Symptomatology, n (%)*	91 (51.7%)	86 (33.4%)
Number of COVID-19 symptoms at hospital admission, mean (SD)*	2.3 (0.8)	2.0 (0.7)
Symptoms at hospital admission, n (%)		
Fever	132 (75.0%)	186 (78.8%)
Dyspnoea	65 (36.9%)	89 (37.7%)
Myalgias*	59 (33.5%)	54 (23.3%)
Cough	36 (20.4%)	55 (23.3%)
Headache*	46 (26.1%)	38 (16.1%)
Diarrhoea	22 (12.5%)	31 (13.1%)
Anosmia	15 (8.5%)	20 (8.4%)
Ageusia	11 (6.3%)	15 (6.3%)
Throat Pain	5 (2.8%)	8 (3.4%)
Vomiting	5 (2.8%)	7 (3.0%)
Dizziness	8 (4.5%)	11 (4.7%)
Stay at the hospital, mean (SD), days	7.5 (4.5)	7.0 (4.5)
Intensive Care Unit (ICU) admission		
Yes/No, n (%)	10 (5.7%) / 166 (94.3%)	9 (4.0%) / 227 (96.0%)
Stay at ICU, mean (SD), days	4.3 (2.7)	4.5 (4.4)

n: number; SD: Standard Deviation; * Statistically significant differences between groups ($P<0.01$)

Table 2: Location of post-COVID Pain Symptoms and other post-COVID symptoms according to the presence or absence of post-COVID pain at 6 months follow-up

	Post-COVID Pain (n=176)	**No Post-COVID Pain (n=236)**
Location of post-COVID Pain		
Cervical Spine	15/176 (8.5%)	
Thorax-Chest	35/176 (19.9%)	
Lumbar Spine	14/176 (7.9%)	
Widespread Pain	40/176 (22.7%)	
Upper Extremity	12/176 (6.8%)	
Shoulder Area	15/176 (8.5%)	
Wrist-Elbow	10/176 (5.7%)	
Lower Extremity	20/176 (11.5%)	
Hip Region	5/176 (2.8%)	
Knee	10/176 (5.7%)	
Other post-COVID Symptoms		
Fatigue	125 (71.0%)	163 (69.1%)
Dyspnea	29 (16.5%)	42 (17.8%)
Brain Fog	26 (14.8%)	33 (14%)

Table 3: Laboratory biomarkers of COVID-19 patients according to the presence or absence of post-COVID pain at 6- and 12-months follow-up

6 months follow-up period		
	Post-COVID Pain (n=176)	**No Post-COVID Pain (n=236)**
Haemoglobin (g/dL)	13.9 (1.5)	14.0 (1.6)
Lymphocyte ($x10^9$/L)*	1.15 (0.5)	1.05 (0.4)
Neutrophils ($x10^9$/L)	5.15 (2.6)	5.25 (2.8)
Platelets ($x10^9$/L)	281.7 (80.9)	290 (83.8)
Glucose (mg/mL)*	112.0 (31.0)	124.0 (37.5)
Creatine (mg/L)*	97.5 (36.4)	108.0 (44.5)
Alanine transaminase (ALT, U/L)	49.0 (39.4)	48.5 (37.6)
Aspartate transaminase (AST, U/L)	47.0 (34.1)	48.6 (30.6)
Lactate dehydrogenase (LDH, U/L)	271.8 (97.7)	286.7 (91.6)
C-reactive protein (mg/L)	78.9 (80.7)	84.7 (88.3)
L-dimer (ng/mL)	935.2 (848.9)	992.1 (993)
12 months follow-up period		
	Post-COVID Pain (n=149)	**No Post-COVID Pain (n=263)**
Haemoglobin (g/dL)	13.9 (1.6)	14.0 (1.5)
Lymphocyte ($x10^9$/L)*	1.2 (0.45)	1.02 (0.4)
Neutrophils ($x10^9$/L)	4.95 (2.5)	5.35 (2.8)
Platelets ($x10^9$/L)	327.0 (95.0)	265.0 (74.5)
Glucose (mg/mL)*	114.0 (26.0)	122.0 (40.0)
Creatine (mg/L)*	92.1 (24.4)	110.0 (47.5)
Alanine transaminase (ALT, U/L)	51.0 (49.5)	47.5 (30.0)
Aspartate transaminase (AST, U/L)	50.2 (41.3)	46.6 (25.5)
Lactate dehydrogenase (LDH, U/L)	272.4 (85.8)	285.0 (98.5)
C-reactive protein (mg/L)	75.0 (79.5)	86.2 (87.8)
L-dimer (ng/mL)	818.5 (737.7)	1056.0 (1020.1)

n: number; SD: Standard Deviation; * Statistically significant differences between groups ($P<0.01$)

STROBE Statement—checklist of items that should be included in reports of observational studies
YOU MUST NOTE THE PAGE NUMBER WHERE EACH ITEM IS REPORTED INSIDE BRACKETS [ ] FOR EACH ITEM #. IF NOT APPLICABLE WRITE N/A

	Item #	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract [Title Page, page 1]
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found [Abstract]
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported [1,2]
Objectives	3	State specific objectives, including any pre-specified hypotheses [2]
Methods		
Study design	4	Present key elements of study design early in the paper [3]
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection [3]
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up [3] *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed [3] *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable [3-4]
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group [3-4]
Bias	9	Describe any efforts to address potential sources of bias [N/A]
Study size	10	Explain how the study size was arrived at [N/A]
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why [3-4]
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding [4]
		(*b*) Describe any methods used to examine subgroups and interactions [4]
		(*c*) Explain how missing data were addressed [N/A]
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed [4] *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses [4]

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed [5]
		(b) Give reasons for non-participation at each stage [5]
		(c) Consider use of a flow diagram [N/A]
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders []
		(b) Indicate number of participants with missing data for each variable of interest [5]
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) [5]
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time [5]
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included [5]
		(*b*) Report category boundaries when continuous variables were categorized [5]
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period [N/A]
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses [5]
Discussion		
Key results	18	Summarise key results with reference to study objectives [5-7]
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias [8]
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence [5-7]
Generalisability	21	Discuss the generalisability (external validity) of the study results [5-7]
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based [9]

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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