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RESEARCH ARTICLE

Association between IL-10 systemic low level and highest pain score in patients during symptomatic SARS-CoV-2 infection

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Abstract

Background: Despite the wide variety of Covid-19 symptoms, pain and the related mechanisms underlying unsettled nociceptive status are still under-prioritized. Understanding the complex network of Covid-19-related pain may result in new lines of study. It is unknown whether patient's immunological background influences pain in the acute phase of Covid-19, including musculoskeletal pain. Thus, we evaluated the blood levels of selected molecules that are upregulated in SARS-CoV-2 infection and analyzed a possible correlation with pain during Covid-19.

Methods: A cohort of 20 hospitalized patients with confirmed diagnoses for Covid-19 were evaluated in the context of pain. Visual analogic scale (VAS) was applied to quantitate pain level. Blood tests were used to determine the systemic levels of cytokines (IL-10 and IL-1β), substance P, and leptin. The data were correlated when appropriate to determine the association between pain-related markers and assessed pain intensity.

Results: Our findings show that systemic levels of IL-10 have strong negative correlation with pain intensity on Covid-19 patients. Additionally, we also show that leptin systemic levels were increased in Covid-19 patients with pain, however, with moderate positive correlation between these events. IL-1β and SP levels did not differ between Covid-19 patients with or without pain. Men reported less pain compared to women. No differences were found between genders in the levels of the molecules evaluated in patients with pain.

Conclusion: IL-10 has been described over the years as an anti-inflammatory and analgesic cytokine. The present data support that low IL-10 levels might contribute to Covid-19-associated pain.

KEYWORDS
Covid-19, IL-10, nociception, pain, SARS-CoV-2

INTRODUCTION

Coronavirus disease 2019 (Covid-19) emerged and quickly spread all over the world as a new disease. The implication of Covid-19 infection urges to explore better the symptoms and outcomes during admission/treatment that influence the quality of life of patients. Among the intriguing features, reports in the literature describe Covid-19 patients suffer with intense pain that is persistent even after the disease has been cured. Despite several

studies in the comprehension of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the molecular mechanisms related to some clinical aspects, such as the pain evoked by the infection in the acute phase, remain elusive.

Severe acute respiratory syndrome coronavirus 2 infection is associated with myalgia, referred pain, and widespread hyperalgesia.[1] Since the beginning of the pandemic, painful manifestations in different forms, but specially myalgia, were described in the acute phase of the disease.[2] In a recent retrospective study, myalgia was reported by 24.1% individuals, and was the second most common symptom of hospitalized Covid-19 patients.[3] Myalgia is defined as diffuse muscular pain and tenderness. Pain-encoding nociceptive information from muscle tissue is conveyed via somatic nociceptor sensory neurons. Among other causes, myalgia typically may result from injuries related to muscle overload, trauma, or inflammation,[4] knowingly, this last one is exacerbated in Covid-19.[5] Alongside with other persistent or long-term complications, as respiratory, neuropsychiatric, and other sequelae, altered nociception underpins the new characterized post-acute Covid-19 syndrome.[6] Even after the resolution of the infection (qPCR negative diagnosis), reports of chronic pain symptoms highlight the importance of this issue.[7] This prolonged clinical condition hampers the quality of life of patients, leaving them more vulnerable to lifestyles related to sedentarism, unproductivity, dependence on medical care, and drug abusive consumption.[1,8] A previous study demonstrated that arthralgia, chest pain, myalgia, and headache are common painful symptoms reported during post-acute Covid-19 syndrome.[6] Regarding the relationship between Covid-19 and persistent musculoskeletal pain on post-Covid-19 some data is already found. Analysis of patients' symptoms upon admission to hospitals revealed an association between post-Covid-19 painful sequelae symptoms (present in 38% of individuals) in patients with myalgia during the infection period.[9] These data highlight a probable casual relation between infection course myalgia and chronic myalgia as one of the disease outcomes. Furthermore, these data indicate that understating pain in the acute phase of Covid-19 is important.

Covid-19 is characterized by an intense inflammatory response against SARS-CoV-2 infection, and it is presumed as being the main reason for severe cases and death in patients.[10] This hyperinflammatory state increases the production of inflammation-related products, including cytokines, neuropeptides, and metabolic hormones. This condition creates a favorable environment to increased nociceptive activity. Cytokines are well-known molecules related to inflammation and pain[11] and their levels are elevated in Covid-19 patients.[12] For instance, during acute phase of Covid-19, blood interleukin (IL)-1β starts to increase 1–5 days after the infection, with its levels reaching higher concentrations in severely ill patients when compared to moderately ill patients.[13] The anti-inflammatory cytokine IL-10 increases substantially in the first week after onset of symptoms in Covid-19 and its increased levels are related to diseases severity.[14] Evidence supports that inhibiting IL-1β and treatment with IL-10 reduce pain in clinical and/or pre-clinical studies.[15,16] In addition to cytokines, other classes of molecules are also elevated in Covid-19 and are potentially involved in pain during this infection. For instance, leptin is related to inflammation and pain, and the investigation about its role in muscle pain has been gaining attention.[17,18] Increased production of leptin is already observed in the hospital admission of Covid-19 patients, and its higher levels persist approximately until the end of the second week.[19] The neuropeptide substance P (SP) secreted by nociceptors in response to noxious stimulus sensitizes sensory neurons and increases immune response and cytokine production, thus boosting inflammatory response.[20] SP is involved in neurogenic inflammation.[20] Speculations were raised regarding the role of SP in Covid-19 pain mechanisms,[21] however, so far there are no studies evaluating its production in different time periods of the disease course.

Despite those important clinical observations, there is limited evidence regarding the association of patients' immune-inflammatory status and pain symptoms. We hypothesize that pro- and anti-inflammatory mediators, neuropeptides, or even hormones that have a role in pain in other diseases might contribute to pain symptoms in acute phase of Covid-19. Therefore, in the present study, we investigate the correlation between blood levels of the cytokines IL-1β and IL-10, the neuropeptide SP, and hormone leptin and pain quantitation using VAS in hospitalized Covid-19 patients up to 4 days of admission or of a positive RT-qPCR test.

Key Points

- Pain is a relatively common symptom of SARS-CoV-2 infection.
- Myalgia was the prevalent type of pain reported by Covid-19 patients.
- Leptin systemic levels are increased in Covid-19 patients, showing moderate positive correlation with pain.
- IL-10 systemic levels are reduced in Covid-19 patients with pain, showing strong negative correlation with pain intensity.
- The present data demonstrate an inverse correlation between IL-10 levels and pain symptoms during SARS-CoV-2 infection.

MATERIALS AND METHODS

Participants

This study recruited 20 hospitalized patients with a confirmed diagnosis of Covid-19. The patients were positive for Covid-19, as determined by real-time quantitative polymerase chain reaction (RT-qPCR).[22] All the patients evaluated in the present project were within the first 4 days of hospitalization after positive qPCR test. At the time of pain scoring and blood sampling, patients were allocated in the infirmary of University Hospital (HU) of State University of Londrina (UEL), Londrina, Paraná, Brazil. Table 1 summarizes patients' demographic data, associated comorbidities, laboratory parameters, and disease outcome. Table 2 presents the pharmacological interventions applied during the hospitalization period.

Ethical considerations

All procedures were approved by the Scientific Research Project Evaluation Committee of HU/UEL (under process number 1213/21) and by the National Ethics Committee, Brazil (CONEP, Certificate of Presentation for Ethical Appreciation [CAAE]): clinical registration number 45602721.0.0000.5231. All recruited patients signed a written informed consent.

Study design, pain assessment, and blood samples

In this study, we investigate the relationship of pain status with potential biological markers in the acute phase of Covid-19. Biological markers tested were IL-1β, IL-10, SP, and leptin. Subjects were grouped as pain-free and pain groups, according to pain outcome. The investigation of the systemic profile of potential biological markers of hospitalized, not intensive care unit (ICU) patients in the context of pain was considered the primary outcome while the association between the detected levels of these markers and pain reports constituted the secondary outcome. Pain scores were obtained using unidimensional-scale visual analogic scale (VAS), in millimeters (mm). The format used in the present study was a straight horizontal line of fixed length (100 mm). The ends are defined as the extreme limits of the pain measured, orientated from the left (pain as bad as it could possibly be) to the right (no pain). VAS ratings score was considered: no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm).[23] The use of VAS as an instrument to measure pain provides a high degree of resolution, being considered a sensitive single-item measure for clinical pain research.[24] A slide rule device was used to support pain scoring process. The patients were oriented to mark on the line the point that they feel represents their perception of their current state. Patients were considered pain free when the VAS score ranged from 0 to 4 mm. VAS was scored just once by each patient. The VAS scale was applied up to the 4th day of outpatient admission or the positive RT-qPCR test. Importantly, pain was scored by patients only when they showed no signs of mental confusion. The University Hospital's delirium assessment protocol

TABLE 1 Covid-19 patients' characteristics

General information	Values	%
Demographic data		
Number of patients	20	
Average age (years)	53.2 ± 3.5	
Average length of stay (days)	9.1 ± 1.1	
Sex	13(m)/7(w)	65/35
Associated comorbidities		
Arterial hypertension	9	45
Type 2 diabetes mellitus	6	30
Obesity	6	30
History of smoking	3	15
Type 1 diabetes mellitus	2	10
Chronic kidney disease	2	10
Autoimmune disease (RA)	2	10
Dyslipidemia	2	10
Heart disease (DHF)	1	5
Hepatic steatosis	1	5
Anemia	1	5
Cancer (thyroid adenoma)	1	5
Diverticular disease	1	5
Hypothyroidism	1	5
Without comorbidity	3	15
Mean of laboratory parameters		
CRP (mg/L)	99.38 ± 18.84	
Hemoglobin (g/dl)[a]	13.00 ± 0.4	
Neutrophils (µl)[b]	8840.15 ± 689.78	
Lymphocytes (µl)[c]	1079.15 ± 297.35	
Platelets (mil/µl)[d]	292.05 ± 25.71	
Outcomes		
Hospital discharges	16	80
Deaths	1	5
Transfer to ICU	4	20

Abbreviations: CRP, C-reactive protein (normal value <3.00 mg/L); DHP, decompensated heart failure; ICU, intensive care unit; m, men; RA, rheumatoid arthritis; w, women.

[a]Normal values 12.0–15.5 for w and 13.5–17.5 for m.

[b]Normal value 2000–7500 µl.

[c]Normal value 1000–2900 µl.

[d]Normal value 150–400 µl.

TABLE 2 Covid-19 patients-associated treatments during hospitalization

Pharmacological class	Number of patients	%
Steroids	20	100
Anti-coagulants	20	100
Non-opioid analgesic (dipyrone)	19	95
Insulin	19	95
Anti-helminthics	18	90
Anti-emetics	18	90
Proton pump inhibitors	18	90
Avermectins	15	75
Opioid analgesics	13	65
Anti-hypertensives	10	50
Diuretics	7	35
Antibiotics	5	25
β-adrenergic agonists	5	25
Benzodiazepines	5	25
SSRIs	3	15
Statins	2	10
Anti-fungal	2	10
Gastroprokinetic agents	2	10
Synthetic hormones[a]	2	10
Anti-psychotic	1	5

Abbreviation: SSRIs, selective serotonin reuptake inhibitors.
[a]Thyroxine.

considers, through clinical assessment by health professionals, the level of cognitive capacity associated with orientation in time and space and whether there are signs of hallucination. Consciousness evaluation was performed through Glasgow scale, applied by nurses in different shifts. All patients were classified as mild (score between 13 and 15; Table S1) on the day of the evaluations (blood collection and VAS score). Although it was not an initial aim of this study, we also performed additional analysis regarding pain and biological markers to investigate possible differences between genders. Pain measurement was performed during the period of hospitalization. Peripheral blood samples (4 ml) were obtained from all study participants and collected into tubes with ethylenediamine tetraacetic acid (EDTA). Blood samples were collected immediately after the evaluation of pain score to reliably equip the time of pain reporting with the blood analysis. This approach aimed to prevent large variations that could occur from one day to another in both analyses if the assessments (pain and blood) had been conducted on different days. Blood sample collection was conducted only once, which occurred always in the acute phase of the disease, up to the 4th of outpatient admission, after the RT-qPCR positive test in the hospital. Plasma was then separated, and subsequently stored at −80°C until processing.

TABLE 3 Evaluation of pain in Covid-19 patients

Patient	Pain
P1	No
P2	No
P3	Yes
P4	No
P5	Yes
P6	Yes
P7	No
P8	Yes
P9	Yes
P10	Yes
P11	No
P12[a]	No
P13	Yes
P14	No
P15	Yes
P16[a,b]	No
P17	No
P18	Yes
P19	No
P20	No
Average (%)	55(pf)/45(p)
Patients with pain per gender (%)	38.4(m)/57.1(w)
VAS score, median (IQR), mm (painful patients)	90 (75–95)
VAS score, median (IQR), mm (per gender, painful patients)	80 (65–90, m) 95 (82.5–100, w) $p = 0.1429$ (ns)

Abbreviations: m, men; ns, not significant; p, pain; pf, pain-free; w, women.
[a]Pain (myalgia) reported only in the initial phase of the disease, but not during hospitalization.
[b]Death.

Quantification of molecular targets

Human IL-1β (cat. #KHC0011; Invitrogen), IL-10 (IL-10; cat. #88-7106-88; Invitrogen), leptin (cat. #KAC2281; Invitrogen), and SP (SP; cat. #AE57630HU; Abebio) levels were evaluated by enzyme-linked immunosorbent assay (ELISA) following the manufacturers' instructions. The results were expressed as picograms (pg) of the target molecule per milliliter (ml) of plasma.

Statistical analysis

Quantitative variables are presented as median and interquartile range (IQR). Statistical significance between groups was determined by non-parametric two-tailed Mann–Whitney *U*-test. Pearson's correlation coefficient test was performed to establish the measure

of statistical dependence between variables. The change in the magnitude between the variables in the same or in the opposite directions for the interpretation of coefficient correlation was as follows: 0.00–0.10, negligible; 0.10–0.39, weak; 0.40–0.69, moderate; 0.70–0.89, strong; and 0.9–1.00, very strong.[25] $p < 0.05$ was considered statistically significant. Statistical analyses and graph plots were conducted using GraphPad Prism 7 software.

RESULTS

In this study we demonstrated that 55% of diagnosed Covid-19 patients were pain-free, while 45% presented painful conditions (Table 3). The most prevalent reported type of pain was myalgia (musculoskeletal pain) (88% of patients). It is important to mention that two patients who reported no pain at the time of data collection informed that they had myalgia only in the first days of the disease, and were not then grouped as painful (indicated by the asterisks in Table 3). These data highlight that pain may be detected in different time periods during the disease development. Among men, 38.4% reported pain, whereas among women, 57,1% reported pain. Regarding VAS score per gender in pain patients, median with IQR for men was 80 (65–90), and for women was 95 (82.5–100) ($p = 0.1429$) (Table 3).

Our next step was the evaluation of plasmatic levels of biological markers (Figure 1). IL-1β and SP levels did not differ between pain-free and pain groups (Figure 1A,B, respectively). On the other hand, leptin levels were significantly higher in pain Covid-19 patients compared to those without pain (Figure 1C; $p = 0.0310$). We also observed that IL-10 levels were reduced in Covid-19 patients with pain compared to pain-free Covid-19 patients (Figure 1D; $p = 0.0251$). Interestingly, we demonstrate that IL-10 levels have a strong negative correlation with pain intensity (Figure 2B; Pearson $r = -0.8609$; $p = 0.0029$), whereas there was moderate positive correlation between leptin levels and pain reported by Covid-19 patients (Figure 2A; $p = 0.1509$). Further extending on this topic, we evaluated whether there were differences in the molecules assessed between men and women (Figure 3). No differences were observed between genders for IL-1β and SP, when considered individually the pain-free, pain, and total (pain-free plus pain) patients (Figure 3A,B). However, pain-free men have lower levels of leptin when compared to pain-free women ($p = 0.0121$) and pain men ($p = 0.0109$) (Figure 3C). In statistical analysis covering pain-free and pain (total) individuals, leptin was also lower in men ($p = 0.0456$), however, showing weak and negligible correlations for men ($r = 0.1961$, $p = 0.6734$) and women ($r = 0.08768$, $p = 0.7758$), respectively. Finally, we did not observe differences between genders in IL-10 levels, although there

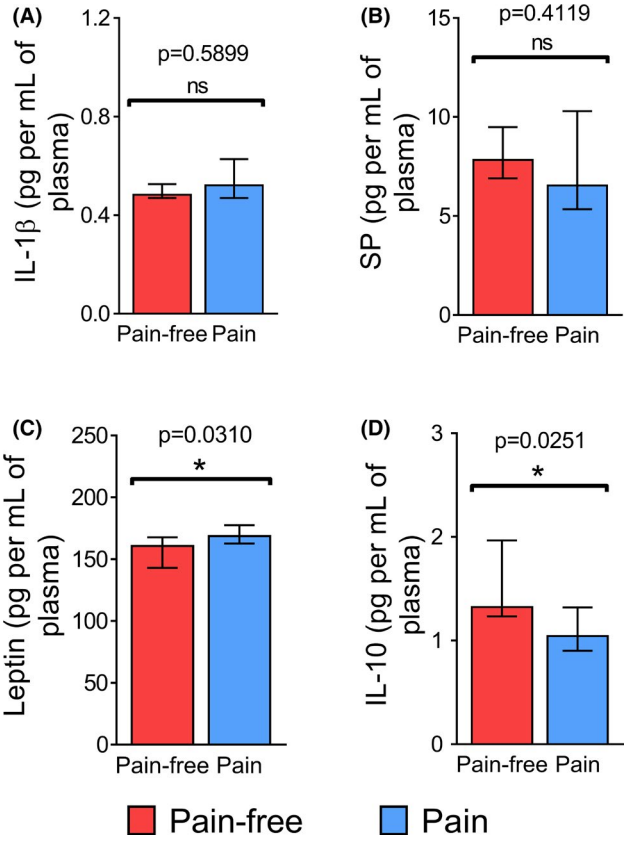

FIGURE 1 Plasmatic levels of molecules evaluated in pain-free and pain Covid-19 patients. IL-1β (A), SP (B), leptin (C), and IL-10 (D) plasmatic levels were determined in samples by ELISA. Data are presented as the median and IQR of eleven patients in the pain-free group and nine patients in the pain group. Two-tailed Mann–Whitney *U*-test. *$p < 0.05$ was considered as statistically significant. Results are presented as picogram of target molecules per ml of plasma

is a trend to be lower in women, as well as in individuals with pain (Figure 3D). Importantly, in pain-free men and women, IL-10 levels tend to be higher than in their pain-free counterparts (Figure 3D; $p = 0.1709$ and $p = 0.1143$, respectively).

DISCUSSION

The present study aimed to determine the relationship between pain and potential biological markers know to be upregulated in Covid-19 in the acute phase of the infection. Covid-19 literature uses myalgia (musculoskeletal pain) to describe one of the main pain symptoms of the acute phase of disease.[9,26] The increased inflammatory state that accompanies the disease may promote the sensitization of nociceptors innervating the musculoskeletal system, inducing myalgia. Our data indicate that myalgia was the main type of pain reported by patients in which IL-10 levels strongly negatively correlated with pain intensity during the SARS-CoV-2 infection. Although with moderate positive correlation with

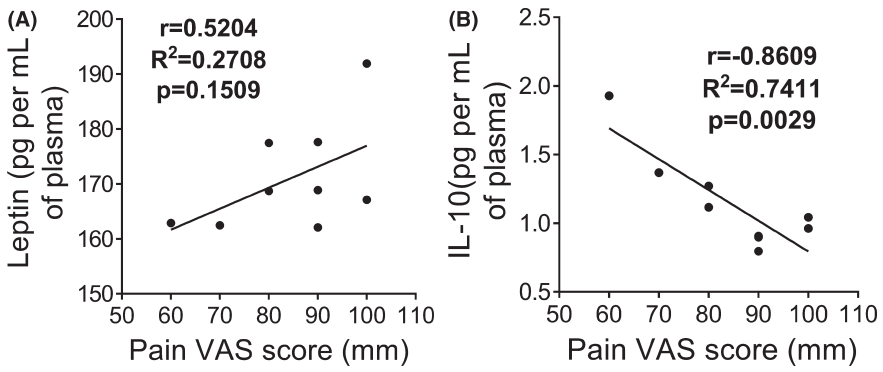

FIGURE 2 Correlations of leptin (A) and IL-10 (B) levels with pain Visual analogic scale score reported by Covid-19 patients during hospitalization. Pearson's correlation coefficient analysis of nine patients for IL-10 levels and leptin levels and respective pain scores in pain group were conducted. $p < 0.05$ was considered as statistically significant

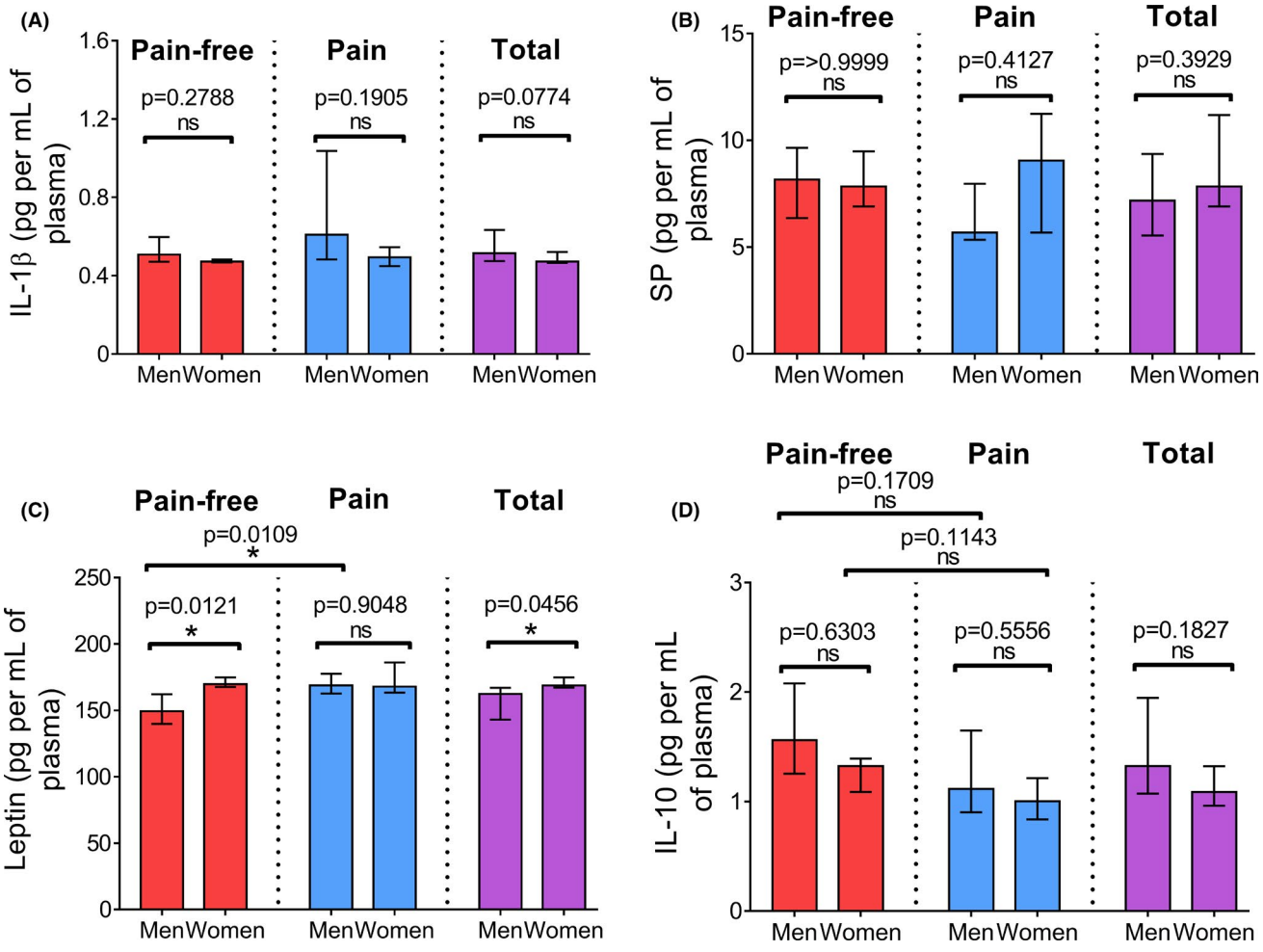

FIGURE 3 Levels of molecules evaluated by gender. IL-1β (A), SP (B), leptin (C), and IL-10 (D) plasmatic levels were determined for pain-free (red), pain (blue), and total (purple; pain-free plus pain) patients. Data are presented as the median and IQR of 11 patients in the pain-free group (8 men and 3 women), 9 patients in the pain group (5 men and 4 women), and 20 patients in total group (13 men and 7 women). Two-tailed Mann–Whitney *U*-test. *$p < 0.05$ was considered as statistically significant. Results are presented as picogram of target molecules per ml of plasma

pain, leptin levels were also increased during the infection in patients reporting pain. Surprisingly, the levels of IL-1β and SP were not increased in patients reporting pain compared to those who did not report pain. Men patients reported and scored less pain compared to women patients. Additionally, no differences were found between genders in the levels of the molecules evaluated in patients with pain.

Previous studies demonstrated that systemic levels of IL-10, IL-1β, and leptin are elevated in SARS-Cov-2-infected patients compared to non-infected controls.[10,27,28] Concerning the clinical evidence of SP in Covid-19, in-depth studies are until now scarce, thus making an interpretation about its role during the infection more complex. Leptin, IL-1β, and SP are described to be important molecules in pain processing, while IL-10 is potentially reduced in patients with chronic widespread pain.[16,29] Interestingly, in hospitalized Covid-19 patients who needed ICU assistance, the plasma levels of IL-10 were statistical reduced compared to community-acquired pneumonia ICU patients, which was not observed in the case of IL-1β levels.[10] This evidence suggests that these molecules may have distinct roles during SARS-CoV-2 infection in comparison to other pneumonia-related pathogens. In the present study, no differences in IL-1β and SP plasmatic levels were detected between Covid-19 pain-free and pain patients, indicating alternative pronociceptive mediators may be involved in this clinical aspect in the acute phase of the disease. Indeed, we found increased plasmatic levels of leptin in Covid-19 patients with pain. Although, in this group, moderate positive correlation was observed between these two variables, this result may represent an initial step highlighting this interface for the subsequent studies addressing the causal relationship between Covid-19 and pain regarding leptin. On the other hand, IL-10 is characteristically a robust anti-inflammatory and anti-nociceptive mediator in several inflammatory diseases contexts.[16] We observed in the present study that IL-10 levels are reduced in Covid-19 patients with pain, and that IL-10 levels presented an inverse correlation with pain in these patients. Thus, corroborating our findings and indicating that higher levels of systemic IL-10 may exert a protective role by limiting painful symptoms including myalgia during the acute phase of SARS-CoV-2 infection. It is important to highlight that there may be variations in the peak of each molecule, which can be assessed by performing serial blood sample collections to examine the levels of molecules.

We observed that the reports of pain in women were more frequent than in men. Nonetheless, considering gender, no differences were observed that could indicate a more relevant role of the molecules evaluated for men or for women with pain. An interesting fact was that pain-free men presented lower levels of leptin than men with pain, suggesting men with leptin resistance and/or obesity, for example, are more likely to have myalgia during Covid-19. A previous study showed that the serum levels of IL-10 in men are statistically higher than their levels in women during Covid-19,[27] which may explain, at least in part, what was evidenced in the present study. These data are in accordance with our results, that, although not significant, showed a tendency of higher levels of IL-10 in men patients than in women patients, in both pain-free and individuals with pain. Therefore, unlike leptin, which appears to be important for pain occurrence in men, IL-10 seems to play a role regardless of gender. Although we also separated the data to test whether there would be gender-specific effects and correlations, we could not find such differences. It is important to mention that our study was not designed to seek gender differences and the *n* was low to address such differences. This point remains to be determined. Of note, other factors including those linked to the immune background of the host cannot be neglected in dictating a favorable or adverse outcome regarding IL-10 effects and its causal relationship with pain symptoms in SARS-CoV-2 infection.

It is noteworthy to mention that 65% of patients in the present study received opioid analgesics (especially codeine) during hospitalization period. In most of the cases, codeine was administrated to suppress cough reflex, which consists of a lower dose than necessary to obtain analgesia (10 mg). According to the codeine sulfate package insert, adult dosage for the relief of mild to moderately severe pain ranges from 15 to 60 mg (https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022402s006lbl). Despite that, ≈77% composed the group of patients without pain, raising the possibility that codeine treatment could be a confounding factor, inhibiting the report of pain by a larger number of patients. Opioids such as morphine can reduce the production of cytokines by macrophages, including IL-10 and IL-12 levels.[30] In general, most of the studies demonstrated morphine suppresses the production of cytokines by macrophages and T cells.[31] Codeine does not produce a detectable influence on immune responses.[32] As mentioned above, the dose of codeine used (10 mg) was lower than its analgesic dose (ranging from 15 to 60 mg), thus, it is also reasonable that codeine dose was not as analgesic as it could be. This would normally occur in a disease of lesser intensity, but as there is an intense inflammatory response in Covid-19, the standard dose may not have been sufficient to reduce the production of the biological markers evaluated, and consequently, interfere with the pain perception.

Furthermore, 100% and 95% of patients evaluated in the present study received corticosteroids (eg, dexamethasone or prednisone) and analgesic drugs, respectively. Corticosteroid's interventions were described to reduce mortality in hospitalized patients with Covid-19 receiving oxygen.[33,34] They act by inhibiting nuclear factor κB (NFκB), a major immunoregulatory transcription factor in response to pro-inflammatory stimuli (including IL-1β as its downstream effector).[35] As Covid-19 induces a hyperinflammatory state,[36] inhibiting crucial inflammatory pathways reduces disease severity. Steroids upregulate IL-10 production in monocytes of healthy individuals,[37] explaining better rates

of survival in severe Covid-19 patients. Additionally, in bronchoalveolar lavage (BAL) fluid of moderate asthma patients, corticoids partially inhibited IL-10.[38] Thus, indicating its effect on IL-10 may vary according to the immune phenotype characteristic of each disease. Glucocorticoids acute treatment also increases leptin levels in overweight women,[37] but did not affect plasma leptin levels in healthy non-diabetic male,[39] indicating gender and metabolic parameters may interfere with its effects. Finally, dexamethasone does not inhibit SP in experimental skin neurogenic inflammation,[40] but inhibits SP expression in human cells during chronic tendinopathy,[41] making it hard to establish a position about its role in SP levels. Dipyrone was the only non-opioid analgesic drug used in patients during the hospitalization period. It is thought that dipyrone acts by both central and peripheral mechanisms to produce its analgesic effects. Peripherally, dipyrone may block hypersensitization by directly acting in neurons and modulating potassium channels and cannabinoid receptors.[42] In a model of neuropathic pain, dipyrone inhibited IL-1β at the mRNA but not protein levels.[43] However, the inflammatory response in Covid-19 is intense. When there is supramaximal response, not all treatments reduce it. This condition may evolve into upregulated mechanisms that are no longer inhibitable with standard doses. Therefore, a regular dose of a non-opioid analgesics (such as dipyrone), non-steroidal anti-inflammatory drugs (NSAID), or steroidal anti-inflammatory drugs can fail to fully inhibit the response. We understand that, since almost all patients in this study received both, corticosteroids and dipyrone, the clinical conditions for the obtained results were the same for all, not interfering with the results between the groups. Of course, pain levels without the concomitant use of these drugs may be different considering its mechanisms of action and biological repercussions. However, not giving to the patient the necessary treatment is unethical.

In this sense, notwithstanding the treatments being confounding factors, perhaps their relevance in the present study is smaller, considering the relationship of the dose used and the dose required to have an analgesic effect, given the intensity of the inflammatory response of the disease. Moreover, in light of SARS-CoV-2 infection influence on the integrity of skeletal muscle tissue [considering higher lactate dehydrogenase (LDH) levels detected in Covid-19 patients],[3,10] and whether the mechanism related to skeletal muscle damage also contributes to musculoskeletal pain in Covid-19, further investigations are warranted.

This study shed light on new alternatives to foster research and therapeutic actions regarding pain control in Covid-19 patients. Limitations of the present study include relatively small sample size, sampling limited to hospitalized patients (considering the response in non-hospitalized patients may be different), specific evaluations for the acute phase of disease, and single time point of VAS scoring and blood collection, since there is the possibility that multiple molecules at varied time points contribute to pain. The lack of follow-up after hospital discharge to check whether patients who had pain during the infection also had chronic pain after Covid-19 or if they needed a greater use of analgesics was also a limitation of the present study. Finally, other standardized and validated tools for pain measurements were not used in the study, eg, Douleur neuropathique 4 questions (DN4), neuropathic pain questionnaire (NPQ) and its short form (NPQ short form), painDetect, etc., since this type of pain may also be observed in Covid-19 patients.[44]

GENERAL CONCLUSION

In summary, these data demonstrate a hitherto unknown negative strong correlation between IL-10 blood levels and pain according to VAS score in hospitalized Covid-19 patients in the acute phase of disease. Increased systemic levels of leptin were detected in Covid-19 patients reporting pain, but only a moderate correlation between leptin levels and pain VAS score was detected. We envisage that a larger study than the present one could be helpful to analyze other variables such as gender; compare acute and chronic phases of disease; compare multiple time points since molecules can present varied peaks of production; then varied biological markers could contribute to pain or correlate with pain during the course and phases of disease; and expand the list of potential biological markers and methods to quantitate pain for deeper understanding of the correlations between biological markers and pain modalities and intensities. Future studies within this research field will elucidate potential molecules that can play a role in the connection between these two clinical conditions.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

AUTHOR CONTRIBUTIONS

AJCB, CRF, AVAL, JGSC, PDR, THZ, TSS, WAVJ, and SMB conducted data acquisition, analysis, and interpretation; wrote; and critically revised the manuscript. WAVJ and SMB conceptualized the study, critically revised the manuscript, and developed the final draft of the manuscript. All authors contributed to the content, critical review, and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.
Table S1

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