Presepsin as a predictive indicator of severity in Coronavirus disease-2019 (COVID-19)

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Abstract

Coronavirus disease 2019 (COVID-19) currently represents a major health emergency worldwide. Early recognition of severe forms of this virus is essential to align effective management and treatment strategies. Presepsin (PSP), the soluble cluster of differentiation (CD14) subtype; is a useful biomarker not only for early diagnosis of sepsis but also could be used as a predictive for the severity and mortality in septic patients, as well as in pneumonia. This study aimed to investigate the potential utility of PSP as a predictive indicator of disease severity in COVID-19 patients. A total of 42 COVID-19 patients were enrolled in this study and stratified into moderate and severe groups, in addition to 15 healthy patients as controls. The PSP levels were measured using Enzyme Linked Immuno-Sorbent Assay (ELISA) within 24 h (1 day) as well as on the 5th day of admission to the Geriatrics hospital, Ain Shams University, Cairo, Egypt, in addition to other relevant laboratory tests performed during the study period from July to October, 2020. Results showed that the PSP levels were significantly higher in COVID-19 patients compared to the controls (p< 0.001), and were also noticeably elevated in severe group than in moderate group on the 1st day (p= 0.008) and the 5th day (p= 0.003) of hospital admission. Significant correlation between PSP level and hospital stay (r = 0.332, p= 0.032) was detected; however, no significant correlation was recorded with the different laboratory parameters. For severity prediction, PSP revealed significant values for the 1st day and the 5th day (AUC 0.737; p= 0.003 and AUC 0.810; p< 0.001), respectively. Data obtained in this study suggested the potential utility of PSP as a predictive indicator of severity in COVID-19 patients, thus allowing for earlier identification of high-risk patients and those who will be hospitalized for longer periods.

Keywords: COVID-19, Enzyme Linked Immuno-Sorbent Assay (ELISA), Presepsin, Moderate and severe COVID-19
1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by the new severe acute respiratory distress syndrome coronavirus (SARS-CoV-2); represents the major current health emergency worldwide since late December, 2019. A recent study of Liu et al., (2020a) reported that COVID-19 was first identified and caused an outbreak of respiratory illness cases in Wuhan City, China, and was later declared as a global pandemic by the World Health Organization (WHO) on 11th of March, 2020. The clinical spectrum of COVID-19 ranges from an asymptomatic state or mild respiratory symptoms to a severe or critical disease (Kwok et al., 2021). Viral sepsis has been hypothesized as being crucial for the disease mechanism of severe COVID-19 (Li et al., 2020). Many severe or critically ill COVID-19 patients met the diagnostic criteria for sepsis and septic shock (Guan et al., 2019). In addition, Huang et al., (2020); Liu et al., (2021) highlighted that pro-inflammatory cytokines and chemokines were significantly elevated in severe COVID-19 patients; suggesting the role of cytokine storm in the immunopathology of COVID-19, and its association with severity of this disease (Li et al., 2020).

Presepsin (PSP), a small soluble peptide generated from the monocyte/macrophage specific CD14 receptor complex; is known to function as a regulatory factor that can modulates the immune responses by interacting with the B and T cells (Chenevier-Gobeaux et al., 2015). Several previous studies have reported that PSP is not only useful for the diagnosis of sepsis but also could be predictive for the severity and mortality of sepsis (Endo et al., 2012; Masson et al., 2014), as well as in those patients suffering from pneumonia (Huang et al., 2020). Recently, Zaninotto et al., (2020) have also revealed that elevated PSP level could be a biomarker in the prognostic assessment of COVID-19 patients. Early identification of COVID-19 progression and patients that are at high-risk of severe complications is of great importance; to align an effective management and treatment strategies at an early stage (Feng et al., 2020). Thus, the objective of this study was to investigate the potential utility of PSP as a predictive indicator of disease severity in COVID-19 patients; in an attempt to provide additional early information about the prognostic and clinical implications.

2. Patients and methods

2.1. Study population

This descriptive study was conducted on 42 patients of COVID-19 confirmed through detecting SARS-CoV-2 RNA in the nasopharyngeal swab specimens, in addition to 15 healthy controls (group matched). Patients were enrolled from the Geriatric’s hospital Ain Shams University during the period from July to October, 2020. Informed consents were obtained from all the participants. Patients with immunosuppressive conditions or suffering from autoimmune diseases were excluded from this study. Patients were stratified into two groups including 22 moderate and 20 severe COVID-19 patients, according to Ain Shams University Hospitals Consensus Statement on Management of Adult COVID-19 patients.

2.2. Collection of blood samples

Blood samples for PSP measurements were taken from each patient within 24 h of admission (1st day) as well as on the 5th day. From the 22 moderate COVID-19 patients; 16 samples only were collected on the 5th day (6 patients were discharged), while from the 20 severe patients; 15 samples were collected (3 were discharged and 2 died), as demonstrated in Fig. (1). Relevant clinical data and laboratory results including; white blood cell (WBC), platelet and lymphocyte counts, C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH) and D-dimer were recorded.
2.3. Measurement of serum PSP levels

From each subject of the studied populations, about 1 ml of aseptically collected peripheral venous blood samples was transferred into serum separation tube; left to clot for 30 min. before centrifugation at 2000-3000 rpm for 15 min. Serum was separated and then stored at -80°C for quantitative measurement of PSP. The serum PSP level was measured through the sandwich Enzyme Linked Immuno-Sorbent (ELISA) serological assay (Shirakawa et al., 2011); using Human presepsin ELISA Kit (Bioassay Technology Laboratory, Cat. No: E3754Hu, China), according to the kit manufacturer’s instructions. A standard curve was generated with each assay by plotting the average optical density for each standard on the Y-axis of the curve; measured using Digital and analog systems (das) plate reader, Roma, Italy, versus the corresponding standard values on the X-axis, obtained by serially diluting the standard stock solution (1600 pg/ml) to produce; 100 pg/ml, 200 pg/ml, 400 pg/ml and 800 pg/ml. The concentration of PSP in the tested samples was determined by interpolation from the standard curve and expressed in pg/ml.

2.4. Statistical analysis

All statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) version 26.0. Quantitative variables were presented as medians or mean ± standard deviation (SD), whereas the qualitative variables were described as numbers and percentages. Results were analyzed using unpaired student's t-test; Mann-Whitney U test, Chi square test and correlation coefficient (r) test. The receiver operator characteristic (ROC) curve analysis was used to determine the cut off values; sensitivity and specificity for PSP as an indicator of the viral severity. For all the used analyses, a probability (p) value of less than 0.05 was considered significant.

3. Results

3.1. Demographic and clinical data

The demographic and clinical data of the 42 enrolled patients are presented in Table (1), while the laboratory findings on hospital admission are shown in Table (2). Results of some relevant routine laboratory tests including; the complete blood count (CBC), C reactive proteins (CRP) and D-Dimer revealed lymphocytopenia (lymphocytes < (1×10³/µl) in 47.4 % (18/38) of patients, thrombocytopenia (platelets <150×10³/µl) in 10 % (4/40), increased CRP (> 6 mg/l in 86.7 % (26/30)), and increased D-dimer (> 0.5 µg FEU (forty-foot equivalent unit)/ml) in 65 % (17/26) of patients.
Farag et al., 2021

**Table 1:** Demographic and clinical data of the 42 enrolled COVID-19 patients

<table>
<thead>
<tr>
<th>Demographic and clinical data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Males, n (%); Females, n (%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Co-morbidities within cases</strong></td>
</tr>
<tr>
<td>Diabetes mellitus; Hypertension</td>
</tr>
<tr>
<td>Chronic heart diseases; Chest diseases; Renal disorders</td>
</tr>
<tr>
<td><strong>Clinical outcomes</strong></td>
</tr>
<tr>
<td>Improved n (%); Died n (%)</td>
</tr>
<tr>
<td>Discharged upon request n (%)</td>
</tr>
<tr>
<td>Unknown outcome (transferred into another hospital)</td>
</tr>
</tbody>
</table>

**Table 2:** Laboratory data of the enrolled COVID-19 patients on hospital admission

<table>
<thead>
<tr>
<th>Laboratory parameters unit (Reference interval)</th>
<th>No. of available results</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White cell count, 10^3/µl (4-11)</td>
<td>42</td>
<td>10.65 (6.5 - 14.6)</td>
</tr>
<tr>
<td>Lymphocyte count, 10^3/µl (1-3)</td>
<td>38</td>
<td>1.2 (0.75 - 1.54)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>40</td>
<td>244 (200.5-302)</td>
</tr>
<tr>
<td>CRP, mg/l (0-6)</td>
<td>30</td>
<td>86.05 (16.73 - 194.6)</td>
</tr>
<tr>
<td>Ferritin, ng/ ml (10-291)</td>
<td>23</td>
<td>550 (276.7 - 1064)</td>
</tr>
<tr>
<td>LDH, U/l (140-270)</td>
<td>34</td>
<td>359.5 (279 - 507)</td>
</tr>
<tr>
<td>D-dimer, µg FEU/ ml (0-0.5)</td>
<td>26</td>
<td>0.9 (0.35 - 3.57)</td>
</tr>
<tr>
<td>Creatinine, mg/ dl (0.6-1.2)</td>
<td>41</td>
<td>1.3 (0.9 – 2.1)</td>
</tr>
<tr>
<td>ALT, IU/l (7-52)</td>
<td>40</td>
<td>25 (11 - 44.5)</td>
</tr>
<tr>
<td>AST, IU/l (13-39)</td>
<td>40</td>
<td>27.5 (17 - 45.5)</td>
</tr>
</tbody>
</table>

Where; CRP: C reactive proteins; LDH: Lactate dehydrogenase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase
In regards to the moderate and severe COVID-19 groups, the male: female ratio was 13:9 in moderate group, and 13:7 in severe group. Age was significantly higher in severe group (mean 67.3±13.44) compared to moderate group (52.64±17.56), \( p=0.004 \). A significant difference was also observed in regards to hypertension (severe; 16/19 (84.2\%) patients vs moderate; 9/22 (40.9\%) patients, \( p=0.05 \)), and chronic heart disease (severe; 8/19 patients (42.1\%) vs moderate; 2/22 (9\%) patients, \( p=0.026 \)). While there were no significant differences regarding the other comorbidities.

### 3.2. Laboratory data

Most of the laboratory parameters of patients on hospital admission including; white cell, lymphocyte and platelet counts, creatinine, ALT and ferritin; demonstrated no notable differences between the moderate and severe COVID-19 groups. However, the CRP, LDH and D-dimer were higher in the severe group compared to the moderate group. Moreover, the D-dimer showed a statistically significant difference (median (IQR); 3.09 (0.83 - 7.95) vs 0.47 (0.32 - 0.93), \( p=0.048 \)), as demonstrated in Table (3).

#### Table 3: Laboratory data of moderate and severe COVID-19 groups on hospital admission

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>COVID-19 groups (n. 42)</th>
<th>( U ) value</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate (n. 22)</td>
<td>Severe (n. 20)</td>
<td></td>
</tr>
<tr>
<td>White cell count, ( \times10^9/\mu l ) (4-11)</td>
<td>10.65 (6 - 13.9)</td>
<td>11.2 (8.1 - 15.4)</td>
<td>161</td>
</tr>
<tr>
<td>Lymphocyte count, ( \times10^3/\mu l ) (1-3)</td>
<td>0.9 (0.59 - 1.4)</td>
<td>1.39 (0.93 - 1.78)</td>
<td>99.5</td>
</tr>
<tr>
<td>Platelet count</td>
<td>236 (194.5 - 297.5)</td>
<td>275 (192 – 311)</td>
<td>177</td>
</tr>
<tr>
<td>CRP, mg/ l (0-6)</td>
<td>67.8 (16.73 -105.3)</td>
<td>140.65 (17.5 - 229.8)</td>
<td>93.5</td>
</tr>
<tr>
<td>Ferritin, ng/ ml (10-291)</td>
<td>570 (479.4 - 1256)</td>
<td>541 (256.85 - 714.05)</td>
<td>54.0</td>
</tr>
<tr>
<td>LDH, U/ l (140-270)</td>
<td>336.5 (229 - 506)</td>
<td>381.5 (324 - 513)</td>
<td>109.0</td>
</tr>
<tr>
<td>D-dimer, ( \mu g FEU/ ml ) (0-0.5)</td>
<td>0.47 (0.32 - 0.93)</td>
<td>3.09 (0.83 - 7.95)</td>
<td>45.5</td>
</tr>
<tr>
<td>Creatinine, mg/ dl (0.6-1.2)</td>
<td>1.25 (0.9 - 2.1)</td>
<td>1.3 (0.8 - 2.4)</td>
<td>207.5</td>
</tr>
<tr>
<td>ALT, IU/ l (7-52)</td>
<td>25 (8 - 37)</td>
<td>25 (17 - 58)</td>
<td>160.5</td>
</tr>
<tr>
<td>AST, IU/ l (13-39)</td>
<td>21.5 (15 - 42)</td>
<td>29 (21 - 51)</td>
<td>138.0</td>
</tr>
</tbody>
</table>

Where; Data were presented as Median (IQR); *Significant \( p \) value; \( U \) value: Mann-Whitney U Test; CRP: C reactive proteins; LDH: Lactate dehydrogenase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.
Considering the clinical outcomes; a significant difference ($p=0.001$) between both groups of patients was demonstrated. Of the 22 moderate group patients, 20 (90.91%) were improved and one patient (4.55%) died. However, in the severe group (20 patients), while 2 patients were transferred into another hospital; 7/18 (38.89%) were improved and 10/18 (55.56%) died. One patient from each group was discharged upon request.

3.3. Estimation of serum PSP levels

The PSP levels (pg/ ml) demonstrated a statistically significant difference between COVID-19 patients (median, IQR= 465, 280-1300) and healthy controls (median, IQR=120, 50-160) ($p<0.001$) (Fig. 2). On the other hand, on comparing both patient groups, the PSP was significantly elevated in the severe group than in the moderate group on the 1st day (median, IQR = 950, 350-1500; vs 390, 190-560, $p=0.008$), and the 5th day (median, IQR = 450: 200-550; vs 200, 150 -270. $p=0.003$) (Fig. 3).

Fig. 2: PSP levels (pg/ ml) in patients and controls
Of all 42 enrolled COVID-19 patients; 31 second sample was collected on the 5th day (Fig. 1). On comparing the PSP levels on the 1st and 5th days; the PSP remained the same in 2(6 %) patients, increased in 2(6 %) patients and decreased in 27(88 %) patients. With regard to the clinical outcomes in each set; the 2 patients who remained with the same PSP levels were improved, while those with increased PSP; one patient was improved and the other died. For those patients with decreased PSP values; 17(63 %) were improved, 8(29.6 %) died and 2(7.4 %) were discharged on their request, as shown in Fig. (4).

Studying the association between the PSP levels and the period of hospital stay has evidenced a statistically significant correlation ($r = 0.332, p 0.032$), where patients with relatively higher PSP levels stayed more at the hospital (Fig. 5). However, no statistically significant correlation was revealed among the different laboratory parameters.

Using the receiver operator characteristic (ROC) curve analysis for the PSP level as a predictor of severity in COVID-19 patients; the PSP revealed significant values for the 1st and 5th days.

**Fig. 3:** PSP levels (pg/ml) on 1st day (A) and the 5th day (B) of hospital admission, in moderate and severe COVID-19 patient groups
Fig. 4: Clinical outcome among patients with same; increased and decreased PSP levels on the 5th day

Fig. 5: Correlation between the PSP levels and the period of hospital stay
For the 1st day; area under the curve (AUC) 0.737, 
\(p=0.003\) and at a PSP cut off value of >1300 pg/ ml, 
the specificity; sensitivity, PPV and NPV were; 100 
\%, 50 \%, 100 \% and 68.7 \%, respectively. On the 
other hand, on the 5th day; AUC 0.810, \(p<0.001\), 
and at a cut off value of > 330 pg/ ml, the 
specificity; sensitivity, PPV and NPV were 100 \%, 
60 \%, 100 \% and 72.7 \%; respectively, as presented 
in Table (4) and Fig. (6).

### Table 4: ROC curve analysis of PSP as a predictor of severity in COVID-19 patients

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95 % CI</th>
<th>(p) value</th>
<th>Cut off pg/ ml</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>LR +</th>
<th>LR -</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>0.737</td>
<td>0.579 - 0.861</td>
<td>0.003</td>
<td>&gt;1300</td>
<td>50</td>
<td>100</td>
<td>--</td>
<td>0.50</td>
<td>100</td>
<td>68.7</td>
</tr>
<tr>
<td>5th day</td>
<td>0.810</td>
<td>0.630 - 0.928</td>
<td>&lt;0.001</td>
<td>&gt;330</td>
<td>60</td>
<td>100</td>
<td>--</td>
<td>0.40</td>
<td>100</td>
<td>72.7</td>
</tr>
</tbody>
</table>

Where; AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; (+): LR positive 
likelihood ratio; (−): LR negative likelihood ratio

**Fig. 6:** ROC curve of PSP as a predictor of severity in COVID-19 patients on 1st day (A) and 5th day (B) of admission to the 
hospital
4. Discussion

The dramatic increases in numbers of COVID-19 patients worldwide and treatment in Intensive care units (ICUs) have become great challenges of major concern (Liu et al., 2020b). Thus, early recognition of severe forms of COVID-19 is essential to establish realistic treatment goals and clinical decision-making.

According to Li et al., (2020), lots of theories have suggested the role of cytokines and inflammatory mediators that could contribute to severity of COVID-19. Presepsin (PSP); is a small soluble peptide generated from the monocyte/macrophage-specific CD14 receptor complex (Chenevier-Gobeaux et al., 2015). This peptide has been indicated in many clinical studies as a useful biomarker not only for early diagnosis of sepsis, but also for risk stratification and prognosis prediction in patients with sepsis, in addition to patients suffering from pneumonia, as revealed by Carpio et al., (2015).

In order to investigate the potential utility of PSP as a predictive indicator of COVID-19 severity; the current study assessed the PSP level and several relevant laboratory parameters in patients with both moderate and severe COVID-19 on the 1st and 5th day of hospital admission. The demographic and clinical data revealed that age was significantly higher in severe than in moderate COVID-19 patients, in accordance with the previous study conducted by Song et al., (2020). Moreover, a significant difference was recorded as well regarding hypertension and chronic heart disease. On the other hand, Gao et al., (2020) reported a significant difference between moderate and severe cases of COVID-19 regarding diabetes; however, there was no difference considering the other comorbidities.

Among the enrolled COVID-19 patients; laboratory data on admission demonstrated lymphocytopenia; thrombocytopenia, increased CRP and D-dimer in 47.4 %, 10 %, 86.6 % and 65 % of patients, respectively. Similarly, Zaninotto et al., (2020) observed lymphocytopenia; thrombocytopenia, increased CRP and D-Dimer in 83 %, 18 %, 85 % and 31 % of COVID-19 patients, respectively.

Laboratory parameters revealed no notable differences between moderate and severe COVID-19 groups regarding WBC; platelet counts, creatinine, ALT and ferritin. However, CRP, LDH and D-dimer were elevated in the severe group compared to the moderate group, but the D-dimer showed a significant difference. These results were consistent with the recent study of Gao et al., (2020); who reported no significant difference among COVID-19 cases regarding WBC count; creatinine, ALT and AST, while the D-dimer increased significantly in the severe cases. However, these COVID-19 cases demonstrated a significant difference with respect to CRP. In the previous study of Song et al., (2020) a significant difference among the cases was demonstrated regarding WBC count and ferritin in addition to D-dimer; however, they showed no difference regarding CRP; LDH, ALT, AST and creatinine. This discrepancy in laboratory results might be attributed to the difference of sample size in each study, and unavailability of some laboratory data in the current work.

Considering the PSP serum levels; a high statistically significant difference was detected between the enrolled COVID-19 patients and the controls. In accordance, Nagiub et al., (2019) also demonstrated a significant difference in the PSP levels between the pneumonic patients and the healthy controls. On comparing the moderate and severe COVID-19 groups; PSP was significantly higher in the severe cases on the 1st and 5th day of hospital admission. In accordance with the current results; Zaninotto et al., (2020) reported a significantly higher PSP values in the severely infected COVID-19 patients, compared to the...
moderately infected ones. In a case series study conducted by Fukada et al., (2020); they demonstrated a higher PSP level in the severe than the mild COVID-19 patients. In addition, they also suggested that the PSP levels may correlate with the lung damage caused by COVID-19 pneumonia, and thus may be useful as a prognostic biomarker for severe cases of COVID-19; although the detailed mechanism of PSP elevation in patients of COVID-19 pneumonia is not known. While assessing the PSP levels on the 5th day, most of the patients (88%) exhibited lower PSP values compared to that on the 1st day; however, 63% of the patients were improved. In accordance, the case study of Fukada et al., (2020) reported that PSP levels decreased along with improvement in 2 out of 3 severe COVID-19 cases. These preceding data collectively revealed that higher PSP levels were associated with worse clinical presentation in COVID-19 patients, in accordance with the previous studies that proposed a relation between PSP levels and disease severity in COVID-19 patients, in accordance with the previous studies that proposed a relation between PSP levels and disease severity in pneumonia (Martínez et al., 2013; Nagiub et al., 2019), and in sepsis (Ulla et al., 2013; Klouche et al., 2016).

In the current study, no correlation was revealed between PSP levels and the different laboratory parameters including: CRP, D-dimer, ferritin, LDH, creatine, ALT and AST. Correspondingly, Zaninotto et al., (2020) reported that PSP levels were poorly correlated with CRP and LDH, while Fukada et al., (2020) showed no correlation between PSP and CRP in COVID-19 patients. On the other hand, the current study demonstrated a significant correlation between the PSP levels and the hospital stay. This comes in agreement with Zaninotto et al., (2020); who also noted that COVID-19 patients with higher PSP values stayed in ICU for significantly a longer period, and were also at high risk of poor outcomes than those exhibiting lower values. Moreover, their data demonstrated the role of PSP level in allowing us to identify COVID-19 patients that will be hospitalized for a longer period.

The data obtained in the current and the other recent studies conducted by Zaninotto et al., (2020); Fukada et al., (2020) showed that PSP has potential as a good predictor of severity in COVID-19 patients, as already described in several different diseases reported by Behnes et al., (2014); Nagiub et al., (2019); Zhao et al., (2020). Moreover, the PSP level allows early identification of COVID-19 patients suffering from a more severe infection, and will be hospitalized for a longer period. Song et al., (2020) reported that elevation of the PSP level resulting from the host-pathogen interaction occurs in the initial phase of pathogen recognition, and remains elevated for several days on the basis of the disease severity. This underlies the additional value of the PSP biomarker in the prognostic assessment of septic patients (Liu et al., 2013; Ulla et al., 2013; Carpio et al., 2015; Song et al., 2020).

Although the present study is one of the initial follow up research works that detect PSP levels in COVID-19 patients; however, there are some limitations, namely small sample size; unavailability of some data, as well as inaccessibility to mild cases that had been isolated at home. If these cases were considered; they would have allowed for a more reliable evaluation of using the PSP level as a predictor of severity in COVID-19 patients.

Conclusion

The data obtained in this study suggest the potential utility of PSP as a predictive indicator of severity in COVID-19 patients; thus allowing earlier identification of high-risk patients and those who will be hospitalized for a longer period. Accordingly, current results can provide better treatment strategies and clinical decision-making for COVID-19 patients at an early stage. The present work might serve as a preliminary report for further future investigations on a larger scale and with a longitudinal design; to confirm and validate the clinical indications of PSP level in COVID-19 patients.
Conflicts of interest

There is no conflict of interests among authors of this study.

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Ethical approval

This study was approved by the Ethical and Moral Committee of the Faculty of Medicine, Ain Shams University, Cairo, Egypt (No: FMASU M S 417/ 2020).

5. References


