Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection 82 (2021) e40–e42

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Circulating levels of GDF-15 and calprotectin for prediction of in-hospital mortality in COVID-19 patients: A case series

Dear Editor,

We read the recent articles by Li and colleagues and Lin and colleagues about the role of two inflammatory biomarkers, serum amyloid A[1] and ferritin[2], in the evaluation of severity in coronavirus disease 2019 (COVID-19) patients. We fully agree with authors that early identification of patients with a higher risk for severity remains crucial to define an optimal strategy for the management of COVID-19 patients.

In severe cases, the interaction of SARS-CoV-2 with the immune system contributes to a dysfunctional immune response, which triggers a cytokine storm that mediates widespread inflammation and multi-organ damage, major cause of disease severity and death in infected patients[3,4]. Higher levels of inflammatory biomarkers in blood, such as C-reactive protein (CRP), ferritin and D-dimer, have been reported as predictors of a poor outcome in COVID-19 patients[5]. The role of biomarkers associated to inflammation other than those above mentioned, such as calprotectin or growth differentiation factor 15 (GDF-15), is less known.

The association of inflammation and calprotectin and GDF-15 has been previously reported[6-8]. During inflammatory response, neutrophils and monocytes quickly arrive to the site of inflammation. The heterodimeric protein S100A8/A9, named as calprotectin, is an alarmin mainly derived by both cell types which play a critical role in inflammatory response, exerting its functions by binding to two patterns recognition receptors: Toll-like receptor and Receptor of Advanced Glycation Endproducts and activating pro-inflammatory signaling pathways leading to further recruitment and activation of immune cells[6]. Infection-induced inflammation is one of the main triggers that leads to calprotectin liberation[8]. In a recent study, calprotectin levels were significantly higher in COVID-19 patients who required mechanical ventilation, similarly to CRP[9]. GDF-15 is a member of the Tumor Growth Factor-beta (TGF-β) family, primarily expressed under conditions of inflammation and oxidative stress. Recently, an experimental study showed a novel function of GDF-15 in the promotion of lung human rhinovirus and virus-associated inflammation, contributing to the severity of respiratory viral infection[10].

In the present observational study, we aimed to explore a potential role of calprotectin and GDF-15 for prediction of in-hospital mortality in a cohort of COVID-19 patients admitted to Santa Lucia University Hospital (Cartagena, Spain).

From March 14th to April 12th, 66 consecutive patients were admitted to our hospital by confirmed SARS-CoV-2 infection. COVID-19 was confirmed either by a positive result of real–time reverse transcriptase-polymerase chain reaction (RT-PCR) testing of a nasopharyngeal swab specimen or by positive result of serological testing and a clinically compatible presentation. Patients were followed until discharge or death. In all patients, a blood sample was collected in the Emergency Department (ED) for measurement of inflammatory biomarkers, including CRP, ferritin, calprotectin, GDF-15 and D-dimer. Serum calprotectin levels were measured by a particle enhanced turbidimetric immunoassay (PETIA) (GentianAS, Norway) in a Cobas c502 instrument (Roche Diagnostics, Mannheim, Germany) and serum GDF-15 levels by electrochemiluminescent immunoassay, in a Cobas e602 analyzer (Roche Diagnostics, Mannheim, Germany), according to manufacturersfecommendations.

To analyze the data, firstly we compared the crude values of biomarkers in both patient groups (those who died during admission versus those discharged alive) by means of a Mann-Whitney test. Then, we built a binary logistic regression analysis being in-hospital mortality the dependent variable and SOFA score as unique covariate. This model showed a discrimination capacity equal to 0.964. We obtained the estimated individual probability of the occurrence of in-hospital mortality for each patient and then we weighted each value of the panel of circulating biomarkers with that probability obtained from the logistic regression model (inverse probability weighting [IPW]). We further compared values between groups by applying the IPW method. The discrimination ability for those biomarkers that showed a significant IPW-adjusted p-value was evaluated by Receiver Operating Characteristic (ROC) curve analysis (Hanley&Mc Neil method). We finally used a binary non-adjusted logistic regression model for the prediction of in-hospital mortality entering each biomarker dichotomized according to the optimal threshold maximizing the Youden index. The p-values < 0.05 were considered statistically significant. Software package SPSS version 20 (SPSS Inc., Chicago, USA) was used for statistical analyses.

In our cohort, the mortality rate was 12.1% (8/66). Median time from symptoms onset to ED admission was 7 (5–10) days. Table 1 shows the differences in demographics and comorbidities between survivors and non-survivors.

Table 2 shows the circulating biomarker levels in both groups. Notably, all inflammatory biomarkers evaluated in the present study (serum calprotectin, GDF-15, CRP, ferritin and plasma D-

https://doi.org/10.1016/j.jinf.2020.08.010

Table 1
Baseline characteristics of COVID-19 patients on admission.

	Total $n=66$	Survivors $n=58$	Non-survivors $n=8$	p-value
Age, years	61 (16)	60 (16)	74 (9)	0.013
Gender, male	43 (65.2)	35 (57.7%)	8 (67.7%)	0.027
Comorbidities				
Hypertension	31 (47.0)	23 (39.7)	8 (100)	0.001
Diabetes mellitus	18 (27.3)	13 (22.4)	5 (62.5)	0.017
Non-asthma respiratory disease	5 (7.6)	4 (6.9)	1 (12.5)	0.574
Cardiovascular disease	15 (22.7)	10 (17.2)	5 (62.5)	0.004
Chronic kidney disease	8 (12.1)	1 (1.7)	7 (100)	<0.001
Immunosupression	6 (9.1)	6 (10.3)	0 (0)	0.340
SOFA score	1 (1–2)	1 (1–2)	8 (6–9)	<0.001

Note: Data are expressed as frequency (percentage) or mean (standard deviation).

Table 2
Inflammatory biomarkers in patients who died during admission versus those who survived. Crude and inverse probability weighted estimation.

	Non-survivors ($n=8$)	Survivors ($n=58$)	Crude p-value	IPW-adjusted p-value[1]	ROC AUC (CI95%; p-value)	Youden index derived cutoff[1]	Unadjusted OR ratio (CI 95%; p-value)[2]
Ferritin (ng/mL)	769 (501–1301)	360 (223–1256)	0.080	<0.001	0.692 (0.566–0.800) $p=0.080$	–	–
CRP (mg/dL)	19.3 (10.4–30.5)	6.0 (2.8–10.8)	0.008	<0.001	0.791 (0.673–0.881) $p=0,003$	≥ 10	15.56 (1.78–136) $p=0,002$
D-dimer (μg/L FEU)	3465 (995–4432)	570 (404–848)	0.001	0.031	0.869 (0.763–0.939) $p<0.001$	≥ 936	38.11 (4.17–348) $p<0.001$
Calprotectin (mg/L)	7.1 (4.5–10.3)	3.1 (1.9–4.4)	0.005	<0.001	0.801 (0.691–0.894) $P=0.001$	≥ 3.9	13.30 (1.53–116) $p=0.004$
GDF-15 (ng/L)	9448 (6462–17,707)	2590 (1886–4811)	<0.001	<0.001	0.892 (0.792–0.955) $P<0.001$	≥ 7789	40.50 (6.09–270) $p<0.001$

AUC: Area under the curve, CI: Confidence interval, CRP: C-reactive protein, OR: Odd ratio, ROC: Receiver operating characteristic.
Biomarker levels are expressed as median (interquartile range).
[1] Weighted by inverse probability of dying during admission (obtained from a propensity score model using SOFA scale as covariate, C statistic of the model 0.964, 95% CI: 0.919–1).
[2] For the calculation the Youden index derived cutoffs for each of the biomarkers were used.

Dimer) showed significant differences between patients who died and those who survived after adjustment by the SOFA score by means of the IPW method. Calprotectin levels correlated positively with ferritin ($r=0.359$; $p=0.003$), CRP ($r=0.686$; $p<0.001$), GDF-15 ($r=0.441$; $p<0.001$) and D-dimer ($r=0.330$; $p=0.007$), whilst GDF-15 levels correlated positively with ferritin ($r=0.334$; $p=0.006$), CRP ($r=0.527$; $p<0.001$) and D-dimer ($r=0.260$; $p=0.035$). Calprotectin and GDF-15 showed a good discrimination capacity (see Table 2), as assessed by the analysis of the AUC of ROC curve for in-hospital mortality, similar to both D-dimer and CRP but numerically higher than ferritin (Table 2). Table 2 shows the unadjusted odds ratio for each of the studied biomarkers stratified by a cut off obtained by means of the Youden Index for the prediction of in-hospital mortality.

We have shown that circulating levels of two emerging inflammatory biomarkers, calprotectin and GDF-15, are significantly higher in COVID-19 patients who died, suggesting a potential role in the evaluation of prognosis in these patients. Our study is the first, to our knowledge, exploring a potential prognostic value of both in COVID-19 patients.

This early report has some limitations, namely the small sample size. Hence, we did not perform multivariable analysis, due to the small number of included patients and outcomes. However, the IPW method allowed us to adjust for the SOFA score, that was shown to be a powerful predictor of mortality. The aim was not to generate a predictive model, but rather to explore the potential role of these novel biomarkers.

Our findings suggest that calprotectin and GDF-15 might have a potential role in the assessment of prognosis in COVID-19 patients.

Declaration of Competing Interest

The authors declare no competing interests.

References

1. Li H, Xiang X, Ren H, Xu L, Zhao L, Chen X, et al. Serum amyloid a is a biomarker of severe coronavirus disease and poor prognosis. *J Infect* 2020;**80**:646–55.
2. Lin Z, Long F, Yang Y, Chen X, Xu L, Yang M. Serum ferritin as an independent risk factor for severity in COVID-19 patients [published online ahead of print. Jun 24. *J Infect* 2020 2020;S0163-4453(20)30434-5.
3. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol* 2020;**20**:355–62.
4. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* 2020;**20**:363–74.
5. Bohn MK, Lippi G, Horvath A, Sethi S, Koch D, Ferrari M, et al. Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence. *Clin Chem Lab Med* 2020;**58**:1037–52.
6. Pruenster M, Vogl T, Roth J, Sperandio M. S100A8/A9: from basic science to clinical application. *Pharmacol Ther* 2016;**167**:120–31.
7. Wischhusen J, Melero I, Fridman WH. Growth differentiation factor-15 (GDF-15): from biomarker to novel targetable immune checkpoint. *Front Immunol* 2020;**11**:951.
8. Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in inflammation. *Front Immunol* 2018;**9**:1298.
9. Shi H, Zuo Y, Yalavarthi S, Gockman K, Zuo M, Madison JA, et al. Neutrophil calprotectin identifies severe pulmonary disease in COVID-19. Preprint. medRxiv. 2020;2020.05.06.20093070. Published 2020 May 10. doi:10.1101/2020.05.06.20093070.

10. Wu Q, Jiang D, Schaefer NR, Harmacek L, OĆonnor BP, Eling TE, et al. Overproduction of growth differentiation factor 15 promotes human rhinovirus infection and virus-induced inflammation in the lung. *Am J Physiol Lung Cell Mol Physiol* 2018;**314**:L514–27.

Luis García de Guadiana Romualdo*
Medicine Laboratory Department, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

María Dolores Rodríguez Mulero
Intensive Care Unit, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Marta Hernández Olivo
Pneumology Department, Hospital Universitario Santa Lucía, Cartagena, Spain C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Carlos Rodríguez Rojas, Verónica Ramos Arenas, Mercedes González Morales
Medicine Laboratory Department, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Ana Blazquez Abellán
Microbiology and Parasitology Department, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Pablo Conesa-Zamora
Medicine Laboratory Department, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Josefina García-García
Infectious Diseases Unit, Internal Medicine Department, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Andrés Conesa Hernández
Emergency Department ,Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Daniel Morell-García
Medicine Laboratory Department, Hospital Universitario Son Espases, Palma de Mallorca, Spain. Carretera de Valldemossa 79, Palma 07120, Spain

María Dolores Albaladejo-Otón
Medicine Laboratory Department, Hospital Universitario Santa Lucía, Cartagena, Spain. C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

Luciano Consuegra-Sánchez
Cardiology Department, Hospital Universitario Santa Lucía, Cartagena, Spain C/ Mezquita, s/n, Paraje Los Arcos, Cartagena, Santa Lucía 30202, Spain

*Corresponding author.
E-mail address: guadianarom@yahoo.es