Gal-3BP Levels in Hospitalized Patients Correlate With COVID-19

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Short Report

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Abstract

The ongoing Covid-19 pandemic disease is still lacking effective treatments and relying on a predictive diagnosis for early individuation of patients which will progress to a severe disease can be crucial. To this aim, the search and the identification of new molecular targets of inflammation and disease progression to be used as predictive biomarker of disease severity is important.

In this work Gal-3BP was explored as a potential biomarker for COVID-19 severity. We found highly increased circulating levels of Gal-3BP in COVID-19 patients compared to healthy controls. Furthermore, the serum levels of Gal-3BP were higher in those “non severe” patients which progressed to a “severe” disease and correlated with levels of IL-6, a known marker of disease progression in COVID-19 patients. These results suggest that Gal-3BP could be a predictor of Covid-19 severity in early infected patients contributing to extend the panel of the other already known biomarkers associated to Covid-19 severity and progression.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the strain of coronavirus that causes the novel coronavirus disease 2019 (COVID-19), which is the respiratory illness responsible for the global COVID-19 pandemic. By the second trimester of 2021, nearly 175 million verified infections and over 3.7 million deaths have been reported globally so far.

The spectrum of COVID-19 disease can range from asymptomatic infection to severe pneumonia with acute respiratory distress syndrome (ARDS). Early identification of patients who will progress to critical illness could facilitate clinical monitoring and early immunomodulatory intervention.

Although the pathogenesis of COVID-19 severity remains unclear, the up-regulation and secretion of IL-6 and other proinflammatory cytokines causing alveolar damage and extrapulmonary injury is the most credited hypothesis. Indeed, data have been presented to show that compared with patients with mild or moderate disease, here referred as “non severe”, those with “severe” disease have remarkably elevated IL-6 levels, although a high individual variability has been observed [1].

Gal-3BP (Uniprot ID – Q08380), also known as 90K, Mac-2 BP or LGALS3BP is a secreted glycoprotein belonging to the macrophage scavenger receptor cysteine-rich domain superfamily, which is expressed on several cell types, mostly in cavity lining respiratory and gastrointestinal epithelial cells [2]. The protein was originally identified, and mainly studied, in the context of neoplastic transformation and cancer progression [3]. Moreover, Gal-3BP expression is increased in viral infections and is induced by diverse molecules that either mimic or are characteristic of an ongoing inflammatory process [4]. Importantly, in asymptomatic HIV-infected patients, Gal-3BP serum level is an important biomarker of progression to AIDS [5].

In the present study, plasma Gal-3BP was explored as a potential biomarker for COVID-19 severity.

Methods

All Covid 19-positive patients (n = 84) were inpatients at the Hospital Senhora da Oliveira Guimarães (Portugal) from April 2020 to January 2021; they had infection with SARS-CoV-2 diagnosed and re-confirmed during hospitalization by RT-qPCR. Patients were admitted at the hospital about 2 days after symptoms onset and PCR test (average 2.1 ± 2.5 days). In addition, 28 age- and gender-matched healthy individuals were taken as controls. Patients were categorized according to the worst clinical presentation achieved during hospitalization. Patients were classified as having either “severe” or “non severe” disease. Patient data were anonymized for analysis and the study was approved by the ethical committee of hospitals Senhora da Oliveira Hospital (25/2020). An explanation of the project was provided to those individuals and participants signed an informed consent form. The informed consent was prepared according to the Declaration of Helsinki principles, the Oviedo Convention and the General Data Protection Regulation–Regulation (EU) 2016/679.

Gal-3BP and IL-6 plasma levels were assessed by Enzyme Linked Immunosorbent assay (ELISA).

Results

The baseline clinical characteristics of COVID-19 patients are shown in Table 1. The “severe” group included patients who required advanced respiratory support, including mechanical ventilation (invasive or noninvasive) or high-flow nasal cannula, within 12 hours of admission, and/or were admitted to intensive care unit and/or died during hospitalization. In patients classified as “severe”, high flow and noninvasive mechanical ventilation were adopted in 38%. In addition, among patients classified as “severe”, went ICU and 56 % died.

Table 1
Clinical characteristics of patients according to severity of the disease.

Parameter	Non severe	Severe
Total number	45	39
Men	47%	62%
Average age *	68.7 ± 16.2	76.8 ± 12.3
Lack of autonomy	11%	21%
Hypertension	69%	72%
Diabetes	31%	46%
Immunosuppression	13%	13%
Neoplasia	9%	10%
Autoimmune Disorder	2%	0%
HIV	0%	0%
Transplant	0%	3%
Other pulmonary disease	11%	23%
Smoking habits	4%	13%
Obesity	27%	13%
*p < 0.05		

A very large increase of Gal-3BP plasma levels was observed in hospitalized COVID-19 patients as compared with healthy controls. Using a statistically optimal cut-off value of 2.75 µg/ml, a ROC curve of Gal-3BP levels was drawn with an AUC of 0.97 (with 95% confidence interval from 0.99 to 0.95) (Fig. 1).

Furthermore, Gal-3BP plasma levels were higher in patients with “severe” than in those with “non severe” disease (19.2 ± 9.15 µg/ml, versus 15.29 ± 10.28 µg/ml; P < 0.05) (Fig. 2). Similarly, plasma levels of IL-6 were higher in “severe” patients (37.0 ± 50.44 pg/ml) than in “non severe” patients (12.55 ± 10.81 pg/ml) (p < 0.01) and correlated with those of Gal-3BP (p < 0.05).

Using a statistically optimal cut-off value of 10.5 µg/ml, a ROC curve of “non severe” vs “severe” patients was drawn with an AUC of 0.68 (with 95% confidence interval from 0.65 to 0.71) (Fig. 3). This cut off correctly classified 34 out of 39 as “severe” (PPV = 57% and Specificity = 42%) and 19 out of 45 as “non severe” patients (NPV = 79% and Sensitivity = 87%), with an Accuracy of 63%. Although the differences between Gal-3BP serum levels in “non severe” and “severe” patients are relatively low, it is worth noting that NPV and Sensitivity are relatively high.

Conclusions

The present study shows that Gal-3BP is associated with COVID-19. We found highly increased circulating levels of Gal-3BP in COVID-19 patients compared to healthy controls. Furthermore, the serum levels of Gal-3BP were higher in those “non severe” patients which progressed to a “severe” disease and correlated with levels of IL-6, a known marker of disease progression in COVID-19 patients [1]. Recently, using a platform for ultra-high-throughput serum and plasma proteomics, Gal-3BP was found to be upregulated in serum samples collected from a cohort of early hospitalized COVID-19 cases [6]. More importantly, the protein was identified as an interaction partners of SARS-CoV-2 spike glycoprotein and its overexpression inhibited spike-pseudoparticle uptake and spike-induced cell-cell fusion in vitro [7].

Currently, no effective treatment is available for COVID-19 patients. In light of the current pandemic and the emergence of drug needs, differentiation of “severe” from “non severe” clinical course of patients is important. High levels of diverse inflammatory markers, including C-reactive protein, procalcitonin, IL-6 and IL-10, have been associated with the severity of Covid-19 [8]. Here we observed that Gal-3BP could be another predictor of disease severity in early infected patients. In this context, it is worth noting that Gal-3BP induced expression and secretion of IL-6 has been documented in multiple different cell types [9]. Therefore, a model can be proposed thereby Sars-CoV-2 induces Gal-3BP in infected cells, which, in turn stimulates production and secretion of IL-6, a major contributor of inflammation and cytokine storm.

Limitations of this study include its single-centered retrospective nature and small sample size, and future efforts focused on the prospective analyses will strengthen our understanding of the prognostic utility of Gal-3BP. Additionally, the relatively low predicting value of Gal-3BP for progression of patients from “non severe” to “severe” disease could be due to the long interval between the initial positive RT-PCR result from oropharyngeal swabs and the evaluation of the biomarker. Results of our ongoing studies show increased levels of Gal-3BP as early as 12 hours from acute Sars-CoV-2 infection of sensitive cells. Therefore, many events could had occurred during the period thereafter.

Declarations

Ethics approval and consent to participate

Patient data were anonymized for analysis and the study was approved by the ethical committee of hospitals Senhora da Oliveira Hospital (25/2020). An explanation of the project was provided to those individuals and participants signed an informed consent form. The informed consent was prepared according to the Declaration of Helsinki principles, the Oviedo Convention and the General Data Protection Regulation–Regulation (EU) 2016/679.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

(I) GA, SI and ISERC Team designed and supervised the project; (II) VG performed the experiments; (III) GA, SI, VG and ISERC Team analyzed and interpreted data; (IV) ISERC Team collected specimen and clinical data; (V) Manuscript writing: all authors; (VI) All authors read and approved the final manuscript.

Availability of data and material

The dataset supporting the conclusions of this article is included within the article as additional file.

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Figures

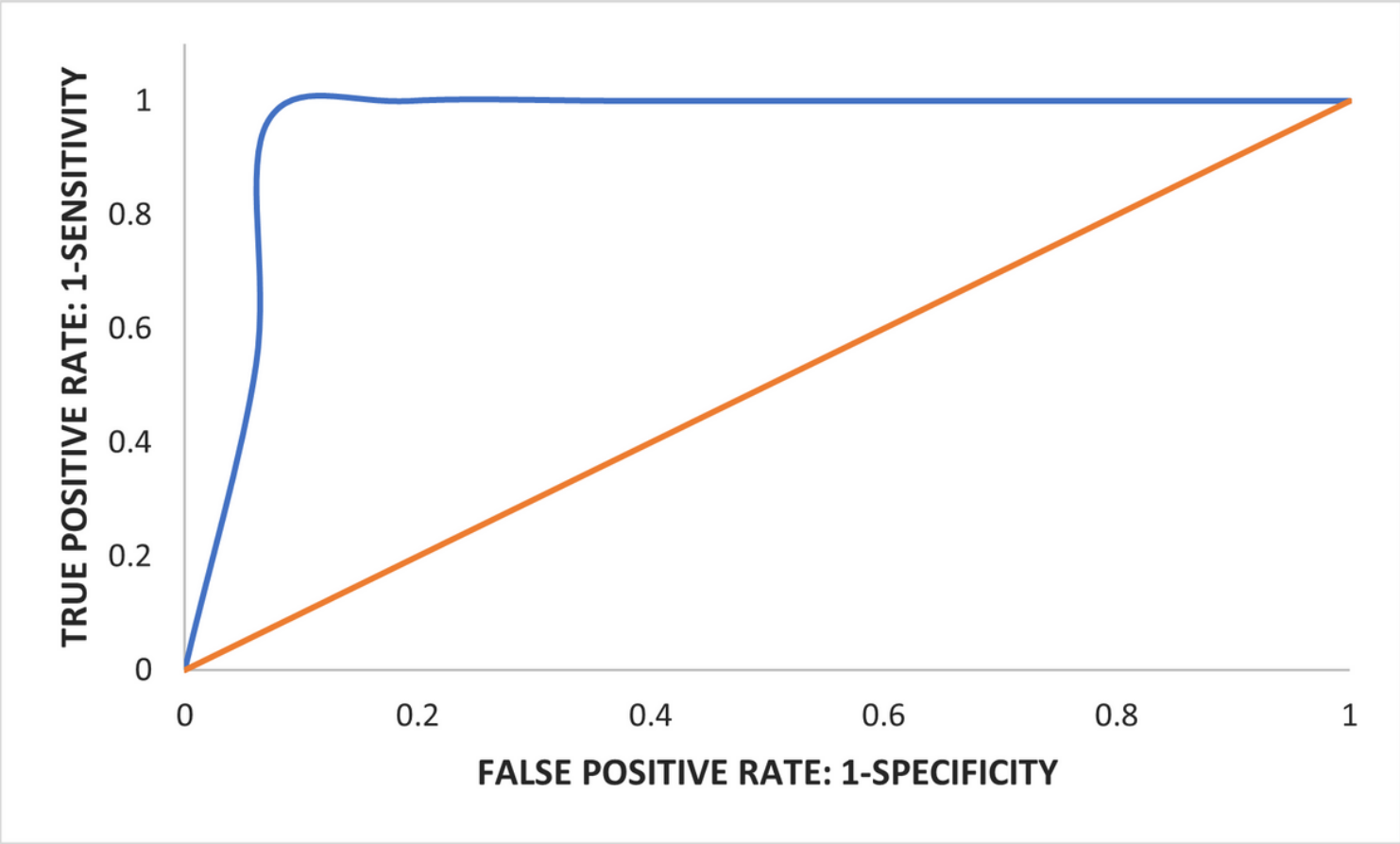

Figure 1

ROC curve of serum Gal-3BP levels in hospitalized COVID-19 patients compared with healthy controls (AUC = 0.97), using a cut-off value of 2.75 µg/ml.

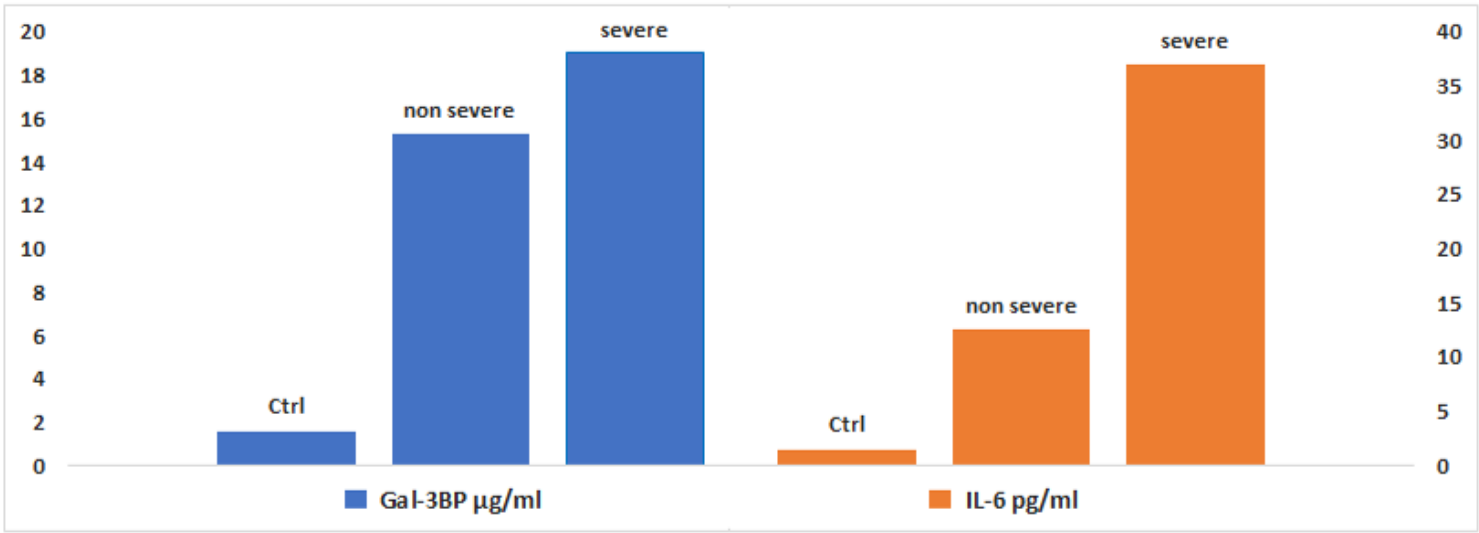

Figure 2

Mean plasma levels of Gal-3BP and IL-6 in healthy controls (Ctrl) and hospitalized “non severe” and “severe” COVID-19 patients. Samples were collected on average 3.9 days (+ 6.0 days) after SARS-CoV-2

detection by PCR test. Using ANOVA test, statistically significant differences were seen between “non severe” and “severe” patients for Gal-3BP ($p < 0.05$) as well as for IL-6 ($p < 0.01$). The correlation between Gal-3BP and IL-6 increases was significant ($p < 0.05$).

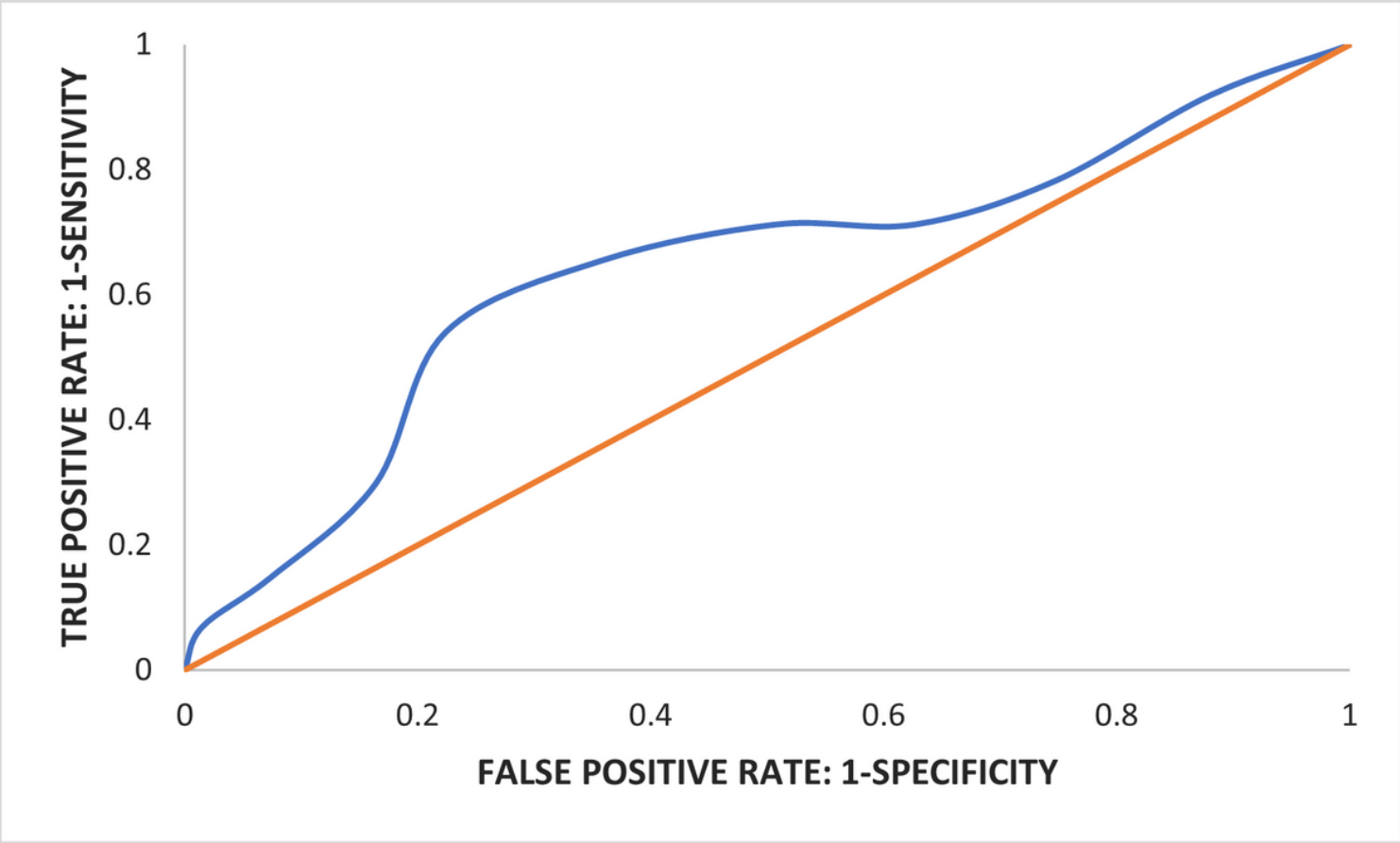

Figure 3

ROC curve of serum Gal-3BP levels in COVID-19 hospitalized patients classified as “severe” compared with patients classified as “non severe” (AUC = 0.68), using a cut-off value of 10.5 µg/ml.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Dataset.xlsx