SARS-CoV-2-RNA viremia is associated to hypercytokinemia and critical illness in COVID-19

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GR, RH, JB, PE, RC, JA, JGB, NM, NBL, LJV, BFC, MAM recruited the patients and/or collected the clinical data. MDG, AO, RO, LMR and JME performed the assays for the detection of SARS-CoV-2 IgG and viremia. CD and NJ profiled the immunological mediators. SR and MMF developed the statistical analysis and drafted the figures. AAK, ATO, AM and LF performed the literature search. All the authors critically reviewed the manuscript.

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**Scientific Knowledge on the Subject:** there is a limited number of works evaluating the presence of SARS-CoV-2 RNA in the serum or plasma of patients with COVID-19, involving few patients, most of them with non-severe disease. In consequence, the impact that the presence of SARS-CoV-2 RNA viremia has on disease biology and severity is largely unknown.

**What This Study Adds to the Field:** to our knowledge, this is the first study comparing the prevalence of SARS-CoV-2-RNA viremia in outpatients (n=50), patients admitted to the ward (n=100), and critically ill patients (n=100) with COVID-19. Multivariate analysis demonstrates that the presence of SARS-CoV-2-RNA viremia in those COVID-19 patients needing hospitalization translates into an 8-fold increase in the risk of presenting critical illness. Presence of viremia in COVID-19 patients is associated with high levels of ferritin and LDH, hypercytokinemia (increased levels of CXCL10, CCL2, IL-10, IL-1ra, G-CSF & IL-15), lymphopenia and low monocyte and platelet counts. These data indicate that SARS-CoV-2-RNA viremia could be a driver of immunological dysregulation and severe disease in COVID-19

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org
Abstract

Rationale: whether systemic dissemination of SARS-CoV-2 has any impact on COVID-19 severity and also on the immunological alterations observed in this disease is largely unknown.

Objectives: We determined the association of plasma SARS-CoV-2 RNA with clinical severity, laboratory findings and immunological parameters in a cohort of 250 patients with confirmed COVID-19 infection.

Methods: Three groups of patients were studied: 50 outpatients, 100 hospitalised ward patients, and 100 critically ill. The association between plasma SARS-CoV-2 RNA and severity was evaluated using multivariate ordinal logistic regression analysis and Generalized Linear Model (GLM) analysis with a binomial distribution. The association between plasma SARS-CoV-2 RNA and laboratory parameters was evaluated using multivariate GLM with a gamma distribution.

Measurements and Main Results: The prevalence of SARS-CoV-2-RNA viremia increased in parallel with severity of infection (22% in outpatients, 36% in those hospitalised in wards, and 82% in those at the ICU). In hospitalised patients, the presence of SARS-CoV-2-RNA viremia was independently associated to critical illness: (adjusted OR= 8.30 [CI95%=4.21 - 16.34], p < 0.001). SARS-CoV-2-RNA viremia was an independent predictor of higher levels of ferritin, LDH and cytokines (involving CXCL10, CCL-2, IL-15, IL-10, IL-1ra and GCS-F), and lower of lymphocytes, monocytes and platelets counts.

Conclusions: SARS-CoV-2-RNA viremia is a robust marker of critical illness in COVID-19. Our findings support that hypercytokinemia in COVID-19 is a reactive event in response to the dissemination of viral material at the systemic level.
Abstract word count: 234

Key words: Coronavirus, plasma, ICU, cytokines.
Introduction

With well over 17 million cases and 715,013 deaths globally, Coronavirus disease 2019 (COVID-19) has become the top economic and health priority worldwide. As the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic continues to emerge in low income countries and resource-poor settings, tools for the early identification and management of severe COVID-19 cases are of paramount importance. It is unclear how many affected individuals need hospitalization. Amongst hospitalized patients, around 10–20% are admitted to the intensive care unit (ICU), 3–10% require intubation and 2–5% die.

SARS-CoV-2 RNA detection in nasopharyngeal swabs is the most common diagnostic test for SARS-CoV-2 infection; however viral RNA can be found in sputum, lung samples, peripheral blood, serum, stool samples, and to a limited extent urine. While the lungs are most often affected, severe COVID-19 can also induce inflammatory cell infiltration, haemorrhage, degeneration or necrosis in other organs (spleen, lymph nodes, kidney, liver, central nervous system). Whether systemic spreading of the virus or viral components has any role in the pathogenesis of the “sepsis-like” failure in different organs or in immunological dysregulation observed in severe COVID-19 is currently unknown.

In this study, by using an integrative approach, we evaluated first the association between the presence of SARS-CoV-2 RNA in peripheral blood plasma from COVID-19 patients and severity. We next studied the impact of SARS-CoV-2 RNA viremia on a number of biological parameters denoting tissue damage and immunological dysregulation in this disease.
Methods

Study design: 250 adult patients with a positive nasopharyngeal swab PCR test for SARS-CoV-2 administered at participating hospitals were recruited during the first pandemic wave in Spain from March 16th to the 15th of April 2020. The patients recruited were of three different categories. The first corresponded to patients examined at an emergency room and discharged within the first 24 hours (outpatients group, n=50). The second group were patients hospitalized to pneumology, infectious diseases or internal medicine wards. This group neither required critical care not died during hospitalization (wards group, n=100). The third group corresponded to patients admitted to the ICU (n=100). Patient’s recruited by participating hospital are detailed in Table E1 in the online data supplement. The study was registered at Clinicaltrials.gov (NCT04457505).

Blood samples: Plasma from blood collected in EDTA tubes samples was obtained from the three groups of patients at a median collection day of 7, 8 and 10 respectively, and also from 20 blood donors (10 men and 10 women).

Laboratory works: Immunological mediators were profiled in plasma using the Ella-SimplePlex™ (San Jose, California, U.S.A) immunoassay. RNA was extracted from 150 µl of plasma using an automated system, eMAG® from bioMérieux® (Marcy l'Etoile, France). Detection of SARS-CoV-2 RNA was performed in five µl of the eluted solution using the CLART® COVID-19 platform from Genomica® (Madrid, Spain). IgG specific for the Nucleocapsid Protein of SARS–CoV-2 was detected in 150 µl of plasma using the Abbott Architect SARS-CoV-2 IgG Assay (Illinois, U.S.A).

Ethical aspects: The study was approved by the Committee for Ethical Research of the coordinating institution, “Comite de Etica de la Investigacion con Medicamentos del Area de Salud de Salamanca”, code PI 2020 03 452. Informed consent was obtained orally when
clinically possible. In the remaining cases, the informed consent waiver was authorized by the Ethics committee.

**Statistical analysis:** Statistical analysis was performed by using Statistical Package for the Social Science (SPSS) 20.0 (SPSS INC, Chicago, IL, U.S.A), Stata 15.0 (StataCorp, Texas, U.S.A) and Minitab 19.2 software. For the demographic and clinical characteristics of the patients, the differences between groups were assessed using the Chi-square test for categorical variables. Differences for continuous variables were assessed by using the Kruskal-Wallis test with post hoc tests adjusting for multiple comparisons. Statistical association analysis was performed using different regression models to assess the relationship between viremia and outcome variables. An ordinal logistic regression (OLR) model was used considering the outcome variable as ordinal (outpatients, wards, and ICU), providing an odds ratio (OR). Generalized Linear Models (GLM) with binomial distribution were used when the outcome variable was dichotomous (outpatients vs. wards, and wards vs. ICU), also providing an odds ratio (OR). GLM with a gamma distribution (log-link) was used when the outcome variable was continuous, providing arithmetic mean ratios (AMR). In all cases, the analysis was performed first without adjustment and was later adjusted for the most relevant covariates in our study. P-values were corrected using the false discovery rate (FDR) with the Benjamini and Hochberg (q-values) procedure.

**Results**

**Clinical characteristics of the patients** (Table 1): Patients diagnosed with SARS-CoV-2 infection based on a positive nasopharyngeal test and requiring hospitalization (either general ward or ICU) were older (median 64 years of age for ward and 66 years for ICU) than those patients discharged to their home from the ER (median age 48 years of age).
There were no significant differences regarding age between ward and ICU hospitalised patients. Critically ill patients (ICU admitted) were more frequently male than those in the other groups. Comorbidities of obesity, hypertension, dyslipidemia and type II diabetes were more commonly found in patients requiring hospitalization, with no significant differences found in the comorbidities profile between critically ill and non-critically ill hospitalized patients. ICU patients showed significantly lower levels of O₂ saturation at the time of admission to the ICU compared to other patients admitted to the ER or the ward. 100% of ICU patients presented with pulmonary infiltrates of whom 93 % also had bilateral pneumonia, these findings were significantly higher than the incidence of pulmonary infiltrates and bilateral pneumonia found in the other two groups. Glucose levels were higher in the group of critically ill patients, who also showed higher values of INR, D-dimers, LDH, GPT, ferritin, C-reactive protein and lower haematocrit. ICU patients showed pronounced lymphopenia and lower monocyte counts; however, neutrophil counts were increased. ICU patients more frequently received experimental treatments during their hospitalization period, including hydroxichloroquine, corticoids, remdesivir, tocilizumab, lopinavir/ritonavir or beta-interferon. ICU patients stayed longer in the hospital, with 48 % fatalities reported in this group. The number of missing values for the variables registered in this study are reported in Table E2 in the online data supplement.

Prevalence of SARS-CoV-2-RNA viremia and specific SARS-Cov-2 IgG. To assess the possible systemic nature of SAS-CoV-2 infections in hospitalized patients we determined the presence of SARS-CoV-2 RNA in plasma. As depicted in table 1 and figure 1, the frequency of SARS-CoV-2-RNA viremia was higher in the critically ill patients (82%) compared to ward patients (36%) and outpatients (22%) (p<0.001). No statistical differences were found in plasma viral RNA between the outpatients and the patients in the
ward ($p = 0.081$). Critically ill patients also had a higher frequency of specific SARS-CoV-2 IgG responses than the other groups (70% in ICU compared to 52% and 49% in the outpatients and ward groups, $p < 0.05$, table 1 and figure 1). No significant differences were found between the group of outpatients and those admitted to the ward.

**SARS-CoV-2-RNA viremia and disease severity.** When the association between viremia and clinical status was evaluated, multivariate ordinal logistic regression analysis revealed that SARS-CoV-2-RNA viremia was a predictor of severity across the three categories considered in our study [OR = 8.24, $p < 0.001$, (CI 95% = 4.71; 14.41)] (see Table E3 in the online data supplement). When we compared outpatients with admitted ward patients, multivariate GLM analysis showed that viremia was not significantly associated with either group (Table 2). In contrast, when the group of ward patients was compared to ICU patients, multivariate GLM analysis showed that viremia was strongly associated with patient severity requiring critical care [OR = 8.3, $p < 0.001$, (CI 95% = 4.21-16.34)] (Table 2). In the patients admitted to the ICU, no significant difference in the presence of SARS-CoV-2-RNA was found between survivors and non survivors: 42 out of 48 of non survivors had viremia (87.5%), while 40 out of 52 survivors (76.9%) had viremia, $p = 0.169$.

**Impact of SARS-CoV-2-RNA viremia on laboratory and immunological parameters:**

Multivariate GLM analysis showed that SARS-CoV-2-RNA viremia was an independent predictor of higher levels of ferritin, LDH and higher levels of chemokines (CXCL10, CCL-2), cytokines (IL-15, IL-10, IL-1ra) and GCS-F (Figure 2 and Table E4 in the online data supplement). In contrast, viremia predicted lower lymphocytes, monocytes and platelets counts and lower concentration of IL-4 in plasma (Figure 2 and Table E4 in the online data supplement). Patients requiring hospitalization at the ward showed significantly increased levels of IL-10, CXCL0, IL-1ra, IL-6 and TNF-α compared to
outpatients. Critically ill patients showed significantly higher levels of GM-CSF, CXCL10, CCL2, IL-10, IL-6 and IL-15 compared to ward patients and outpatients (figure 3 and Table E5 in the online data supplement).

**Discussion**

Here we report that SARS-CoV-2 viral RNA is detected in the plasma of the vast majority of those COVID-19 patients admitted to the ICU (82%). In hospitalized COVID-19 patients, presence of SARS-CoV-2-RNA viremia translates into an 8-fold increase in the risk of presenting critical illness, independently of age, sex and major comorbidities. Importantly, these findings suggest that detection of viral RNA in plasma may serve as a simple test to identify those patients needing critical care.

Whether the finding of viral RNA is “true viremia” with the live virus found in the plasma and peripheral blood is unknown; however, the SARS-CoV-2 virus has been reported to be difficult to culture from blood. Alternatively, but not mutually exclusive, the presence of viral RNA in the blood may represent a substantial spill over event from virally infected tissue. Importantly, SARS-CoV-2-RNA viremia was associated with higher levels of plasma LDH (a marker of necrosis and cellular injury) and lower O2 saturation, which supports viral involvement in the genesis of tissue damage and respiratory failure in patients with severe COVID-19.

Interestingly, SARS-CoV-2-RNA viremia maybe a contributing factor in the development of hypercytokinemia in patients with severe COVID-19, since the presence of viral RNA in plasma predicted higher levels of CXCL10, CCL2, IL-15, IL-10, IL-1ra and G-CSF. Recognition of viral RNA by endosomal receptors such as TLR7 in human plasmacytoid dendritic cells and B cells, or TLR8 in myeloid cells, activate the intracellular signalling pathways enhancing cytokine production. In fact, it has recently been demonstrated that
SARS-CoV-2 genome has more single-stranded RNA fragments that could be recognized by TLR7/8 than the SARS-CoV-1 genome, which suggest the potential of SARS-CoV-2 to induce hyperactivation of innate immunity.  

From a cohort of SARS-CoV-1 patients in 2003, we previously demonstrated that severe SARS patients had increased levels of CXCL10 and CCL2 in serum during the early onset of symptoms. CXCL10 is a potent chemoattractant for activated Th1 lymphocytes and natural killer cells and is thought to play a role in the temporal development of innate and adaptive immunity. Signalling via the CXCL10 cognate receptor, CXCR3, mediates immunopathology during other highly pathogenic respiratory virus infections such as H5N1 influenza virus. In our current cohort, CXCL10 was the most accurate identifier of SARS-CoV-2-RNA viremia in plasma (area under the curve (AUC), [CI95%], \( p = 0.85 \) [0.80 – 0.89], <0.001), suggesting its potential role as a surrogate biomarker of viremia. CCL2 is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages. Interestingly, during SARS-CoV-1 infection, the presence of high levels of CXCL10 and CCL2 was coincident with the presence of lymphopenia, as occurs in patients with severe COVID-19. In SARS-CoV-1 severe disease, self-sustaining expression of proinflammatory chemokines has been suggested to represent a compensatory mechanism for an ineffective adaptive immune response to clear the virus. IL-10 is a major immunomodulatory cytokine inducing immunosuppression. Zhao Y et al reported IL-10 (along with IL-1ra, another immunomodulatory cytokine capable of suppressing the IL-1 signalling pathway) to be associated with the severity of COVID-19. In our study, both cytokines were elevated in the plasma of patients requiring hospitalization, with higher levels of IL-10 in those patients admitted to the ICU. Whether elevation of IL-10 and IL-1RA represents a mechanism of viral evasion or an attempt of
the immune system to control an exuberant inflammatory response remains to be elucidated.  

The association between SARS-CoV-2-RNA viremia and IL-15 is also especially intriguing, since IL-15 was the cytokine better differentiating clinical ward patients from ICU patients (AUC: 0.82 [0.76 – 0.88], <0.001). Previously, we identified IL-15 as a signature of critical illness in the 2009 influenza pandemics. IL-15 is a pleiotropic cytokine that induces T-cell proliferation and enhances natural killer (NK) cell cytotoxicity. It may also play an essential role in T cell or NK cell mediated tissue destruction. During respiratory distress, elevated systemic levels of IL-15 is observed in non survivors. Moreover, during Hantavirus infection, IL-15 drives massive NK cell response and high levels of IL-15 have been associated with severe disease and fatal outcome. Recently, it was reported that circulating NK cells were elevated in COVID-19 severe patients illustrating the potentially important role of IL-15 in rendering NK function during COVID-19 pathogenesis. IL-15 also induces the formation and release of neutrophil extracellular traps (NETs), which could mediate endothelial damage and thrombosis activation. Endothelial cells can produce IL-15, which promotes transendothelial migration of NK cells and T Cells. Future work will examine the therapeutic importance of modulating IL-15 function in COVID-19 disease.

The presence of hyperferritinaemia has been highlighted in COVID-19, since it is, along with hypercytokinemia and high LDH, a marker of macrophage activation syndrome (MAS). Another signature of MAS is the decrease in platelet concentrations in blood. Even though platelet counts did not show significant variation across the three severity groups (Table 1), those patients with SARS-CoV-2-RNA viremia showed significantly lower platelet counts than the other patients (198,000 vs 230,000 cells/mm3, p = 0.003). In
turn, critically ill non survivors showed lower platelet counts than survivors (179,000 vs 221,000 cells/mm3, $p = 0.001$).

Severe COVID-19 is characterised by the frequent finding of lymphopenia\textsuperscript{15}. Since in our study SARS-CoV-2-RNA viremia was associated with low lymphocyte and monocyte counts, it is intriguing to posit the question as to whether lymphopenia and the decrease in the monocyte counts are a result of direct cytopathic events or emigration due to localized chemokine and IL-15 expression, or a combination of these processes. Cytopenias in blood are also a shared feature between severe COVID-19 and MAS.

Our findings revealing the association between SARS-Cov-2 RNA viremia, hypercytokinemia, higher ferritin and LDH levels and lower lymphocyte, monocyte and platelets counts suggest that systemic dissemination of the virus or viral material could be the driver of the MAS-like syndrome observed in severe COVID-19.

In our work, most ICU patients with SARS-CoV-2-RNA viremia had already developed a specific IgG response against the virus (70.7 %), which would support the notion that continued viral replication is a persistent event in the course of the of antibody responses. Additional information on cytotoxic T cells and the role of NK cells in controlling COVID-19 disease will help link the cellular immune events with viral replication and control in disease progression.

A limitation of our study is that we did not evaluate viral load. In consequence we could not assess differences in the amount of viral RNA between severity groups, and also between survivors and non survivors. In addition, neither the potential infectivity of plasma nor the presence of live virus in plasma was assessed. Follow up studies should investigate the presence of viral RNA in specific blood cells as well.
In conclusion, the high prevalence of SARS-CoV-2-RNA viremia in critically ill patients suggests that these patients are unable to control SARS-CoV-2 replication in tissues or blood cells. Viremia is associated with hypercytokinemia and other signatures typically found in conditions characterised by immunological dysregulation such as MAS. These findings suggest a potential role of SARS-CoV-2-RNA viremia as a driver of severity in COVID-19. Our results have major potential clinical repercussions that deserve to be investigated. 1) The presence of SARS-CoV-2-RNA viremia could help to promptly identify those patients needing critical care with a simple one step test. 2) Early control of the virus with either antivirals, hyperimmune plasma or monoclonal antibodies could decrease the risk of further development of critical illness in this disease. 3) Early antiviral treatment could also prevent immunological derangement / dysregulation.

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References:

1 COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). https://coronavirus.jhu.edu/map.html.

2 Guan W-J, Ni Z-Y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020; published online Feb 28. DOI:10.1056/NEJMoa2002032.

3 Cevik M, Bamford CGG, Ho A. COVID-19 pandemic—a focused review for clinicians. Clinical Microbiology and Infection 2020; 26: 842–7.

4 Zhang W, Du R-H, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerging Microbes & Infections 2020; 9: 386–9.

5 Chen X, Zhao B, Qu Y, et al. Detectable Serum Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load (RNAemia) Is Closely Correlated With Drastically Elevated Interleukin 6 Level in Critically Ill Patients With Coronavirus Disease 2019. Clin Infect Dis DOI:10.1093/cid/ciaa449.

6 Li H, Liu L, Zhang D, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. Lancet 2020; 395: 1517–20.

7 Yang L, Liu S, Liu J, et al. COVID-19: immunopathogenesis and Immunotherapeutics. Signal Transduct Target Ther 2020; 5: 128.

8 Birra D, Benucci M, Landolfi L, et al. COVID 19: a clue from innate immunity. Immunol Res 2020; 68: 161–8.

9 Moreno-Eutimio MA, López-Macías C, Pastelin-Palacios R. Bioinformatic analysis and identification of single-stranded RNA sequences recognized by TLR7/8 in the SARS-CoV-2, SARS-CoV, and MERS-CoV genomes. Microbes Infect 2020; 22: 226–9.

10 Cameron MJ, Bermejo-Martin JF, Danesh A, Muller MP, Kelvin DJ. Human immunopathogenesis of severe acute respiratory syndrome (SARS). Virus Res 2008; 133: 13–9.

11 Cameron CM, Cameron MJ, Bermejo-Martin JF, et al. Gene expression analysis of host innate immune responses during Lethal H5N1 infection in ferrets. J Virol 2008; 82: 11308–17.

12 Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. J Interféron Cytokine Res 2009; 29: 313–26.

13 Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. J Virol 2007; 81: 8692–706.
14 Liu J, Li S, Liu J, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* 2020; 55: 102763.

15 Bermejo-Martin JF, Almansa R, Menendez R, Mendez R, Kelvin DJ, Torres A. Lymphopenic community acquired pneumonia as signature of severe COVID-19. *Journal of Infection* 2020.

16 Zhao Y, Qin L, Zhang P, et al. Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. *JCI Insight* 2020; 5. DOI:10.1172/jci.insight.139834.

17 Rojas JM, Avia M, Martín V, Sevilla N. IL-10: A Multifunctional Cytokine in Viral Infections. *J Immunol Res* 2017; 2017. DOI:10.1155/2017/6104054.

18 Couper KN, Blount DG, Riley EM. IL-10: The Master Regulator of Immunity to Infection. *The Journal of Immunology* 2008; 180: 5771–7.

19 Bermejo-Martin JF, Ortiz de Lejarazu R, Pumarola T, et al. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. *Crit Care* 2009; 13: R201.

20 Perera P-Y, Lichy JH, Waldmann TA, Perera LP. The role of interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use. *Microbes Infect* 2012; 14: 247–61.

21 Jabri B, Abadie V. IL-15 functions as a danger signal to regulate tissue-resident T cells and tissue destruction. *Nat Rev Immunol* 2015; 15: 771–83.

22 Agouridakis P, Kyriakou D, Alexandrakis MG, Perisinakis K, Karkavitsas N, Bouros D. Association between increased levels of IL-2 and IL-15 and outcome in patients with early acute respiratory distress syndrome. *Eur J Clin Invest* 2002; 32: 862–7.

23 Klingström J, Smed-Sörensen A, Maleki KT, et al. Innate and adaptive immune responses against human Puumala virus infection: immunopathogenesis and suggestions for novel treatment strategies for severe hantavirus-associated syndromes. *J Intern Med* 2019; 285: 510–23.

24 Garley M, Jabłońska E, Surażyński A, et al. Cytokine Network & NETs. *Folia Biol (Praha)* 2017; 63: 182–9.

25 Gardiner EE, Andrews RK. Neutrophil extracellular traps (NETs) and infection-related vascular dysfunction. *Blood Rev* 2012; 26: 255–9.

26 Oppenheimer-Marks N, Brezinschek RI, Mohamadzadeh M, Vita R, Lipsky PE. Interleukin 15 is produced by endothelial cells and increases the transendothelial migration of T cells In vitro and in the SCID mouse-human rheumatoid arthritis model In vivo. *J Clin Invest* 1998; 101: 1261–72.

27 Estess P, Nandi A, Mohamadzadeh M, Siegelman MH. Interleukin 15 induces endothelial hyaluronan expression in vitro and promotes activated T cell
extravasation through a CD44-dependent pathway in vivo. *J Exp Med* 1999; **190**: 9–19.

28McGonagle D, Sharif K, O’Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev* 2020; : 102537.

29Cron RQ, Davi S, Minoia F, Ravelli A. Clinical features and correct diagnosis of macrophage activation syndrome. *Expert Rev Clin Immunol* 2015; **11**: 1043–53.

30Fardet L, Galicier L, Lambotte O, *et al.* Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis & Rheumatology (Hoboken, NJ)* 2014; **66**: 2613–20.

**Conflicts of interests:** the authors declare no conflicts of interests regarding this submission.
| Outpatients (1) | Ward (2) | ICU (3) | $p$ value (1 vs 2) | $p$ value (1 vs 3) | $p$ value (2 vs 3) |
|----------------|----------|---------|-------------------|-------------------|-------------------|
## Characteristics

| Age [years, median (IQR)] | 48.50 [19] | 64 [20] | 66 [19] | < 0.001 | < 0.001 | n.s. |
|---------------------------|------------|--------|--------|----------|----------|-----|
| Male [% (n)]              | 46 (23)    | 50 (50)| 64 (64)| n.s.     | 0.035    | 0.046|
| Alcoholism                | 2 (1)      | 0 (0)  | 1 (1)  | n.s.     | n.s.     | n.s.|
| Smoking                   | 4 (2)      | 5 (5)  | 6 (6)  | n.s.     | n.s.     | n.s.|
| Drug abuse                | 2 (1)      | 0 (0)  | 0 (0)  | n.s.     | n.s.     | n.s.|
| Cardiac disease           | 4 (2)      | 13 (13)| 9 (9)  | n.s.     | n.s.     | n.s.|
| Chronic vascular disease  | 2 (1)      | 2 (2)  | 5 (5)  | n.s.     | n.s.     | n.s.|
| COPD                      | 2 (1)      | 2 (2)  | 3 (3)  | n.s.     | n.s.     | n.s.|
| Asthma                    | 8 (4)      | 6 (6)  | 2 (2)  | n.s.     | n.s.     | n.s.|
| Obesity                   | 4 (2)      | 26 (26)| 26 (26)| 0.001    | 0.001    | n.s.|
| Hypertension              | 24 (12)    | 44 (44)| 45 (45)| 0.017    | 0.012    | n.s.|
| Dyslipidemia              | 16 (8)     | 41 (41)| 34 (34)| 0.002    | 0.021    | n.s.|
| Chronic renal disease     | 2 (1)      | 3 (3)  | 3 (3)  | n.s.     | n.s.     | n.s.|
| Chronic hepatic disease   | 2 (1)      | 0 (0)  | 3 (3)  | n.s.     | n.s.     | n.s.|
| Neurological disease      | 0 (0)      | 6 (6)  | 3 (3)  | n.s.     | n.s.     | n.s.|
| HIV                       | 0 (0)      | 0 (0)  | 1 (1)  | n.s.     | n.s.     | n.s.|
| Autoimmune disease        | 2 (1)      | 1 (1)  | 1 (1)  | n.s.     | n.s.     | n.s.|
| Chronic inflammatory bowel disease | 0 (0) | 2 (2) | 1 (1) | n.s. | n.s. | n.s. |
| Type 1 diabetes           | 0 (0)      | 0 (0)  | 3 (3)  | n.s.     | n.s.     | n.s.|
| Type 2 diabetes           | 0 (0)      | 23 (23)| 22 (22)| < 0.001  | < 0.001  | n.s.|
| Cancer                    | 6 (3)      | 2 (2)  | 1 (1)  | n.s.     | n.s.     | n.s.|
| Invasive mechanical ventilation | 0 (0) | 0 (0) | 96 (96)| n.a.     | < 0.001  | < 0.001|
| Non-invasive mechanical ventilation | 0 (0) | 15.1 (14)| 34 (34)| 0.004    | < 0.001  | 0.002|
| Hydroxychloroquine        | 77.6 (38)  | 89 (89)| 99 (99)| n.s.     | < 0.001  | 0.003|
| Chloroquine               | 4.1 (2)    | 7 (7)  | 0 (0)  | n.s.     | 0.042    | 0.007|
| Corticoids                | 6.7 (3)    | 29.6 (29)| 85 (85)| 0.002    | < 0.001  | < 0.001|
| Azithromycin              | 15.9 (7)   | 84.8 (84)| 84 (84)| < 0.001  | < 0.001  | n.s.|
| Remdesivir                | 0 (0)      | 1 (1)  | 9 (9)  | n.s.     | 0.029    | 0.009|
| Tocilizumab               | 0 (0)      | 13 (13)| 33 (33)| 0.008    | < 0.01    | 0.001|
| Lopinavir/ritonavir       | 74 (37)    | 35.4 (35)| 96 (96)| < 0.001  | < 0.001  | < 0.001|
| Beta Interferon           | 0 (0)      | 0 (0)  | 55.6 (55)| n.a.     | < 0.001  | < 0.001|

### Treatment during hospitalization, [% (n)]

| Hospital stay [days, median (IQR)] | - | 9 (6) | 24 (19) | n.a. | n.a. | < 0.001 |
| Viral RNA in plasma [% (n)]        | 22 (11) | 36 (36) | 82 (82) | n.s. | < 0.001 | < 0.001 |
| SARS-CoV-2 IgG, [% (n)]            | 52 (26) | 49 (49) | 70 (70) | n.s. | 0.030 | 0.002 |
| Hospital mortality, [% (n)]        | 0 (0) | 0 (0) | 48 (48) | n.a. | < 0.001 | < 0.001 |

## Time course and outcome

| Temperature (ºC) [median (IQR)] | 36.50 (1.0) | 36.80 (1.4) | 37.00 (1.4) | - | - | - |
| Systolic Arterial Pressure (mmHg) [median (IQR)] | 120 (29) | 126 (25) | 120 (26) | n.s. | n.s. | 0.013 |
| Oxygen saturation (%) [median (IQR)] | 96 (3) | 94 (5) | 92 (6) | 0.002 | < 0.001 | 0.001 |
| Pulmonary infiltrate [% (n)] | 72 (36) | 93 (93) | 100 (100) | < 0.001 | < 0.001 | 0.007 |
| Bilateral pulmonary infiltrate [% (n)] | 26 (13) | 67 (67) | 93 (93) | < 0.001 | < 0.001 | < 0.001 |
| Glucose (mg/dl) [median (IQR)] | 99.5 (22) | 112 (31) | 160.50 (83) | 0.004 | < 0.001 | < 0.001 |
| Creatinine (mg/dl) [median (IQR)] | 0.84 (0.18) | 0.91 (0.33) | 0.88 (0.57) | - | - | - |
| Na (mEq/L) [median (IQR)] | 138 (4) | 138 (5) | 138.50 (7) | - | - | - |
| Measurements at diagnosis | K (mEq/L) [median (IQR)] | Platelets (cell x 10^3/µl) [median (IQR)] | INR [median (IQR)] | D Dimer (pg/ml) [median (IQR)] | LDH (UI/L) [median (IQR)] | GPT (UI/L) [median (IQR)] | Ferritin (pg/ml) [median (IQR)] | CRP (mg/dl) [median (IQR)] | Haematocrit (%) [median (IQR)] | WBC (cells/mm3) [median (IQR)] | Lymphocytes (cells/mm3) [median (IQR)] | Neutrophils (cells/mm3) [median (IQR)] | Monocytes (cells/mm3) [median (IQR)] | Eosinophils (cells/mm3) [median (IQR)] | Basophils (cells/mm3) [median (IQR)] |
|--------------------------|--------------------------|------------------------------------------|----------------|------------------------|--------------------------|--------------------------|-------------------------------|--------------------------|-----------------------------|---------------------------------|---------------------------------|----------------------------------|--------------------------------|-----------------------------------|---------------------------------|
|                          | 3.90 (0.50)              | 4.10 (0.68)                              | 3.95 (0.90)     |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 223 [97]                 | 207 [113]                                | 204 [126]       |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 1.04 (0.10)              | 1.11 (0.13)                              | 1.22 (0.22)     |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 795278 [828234]          | 1597362.50 [2024704]                    | 6182104.50 [52690922] | < 0.001               | < 0.001                  | < 0.001                  |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 214 (73)                 | 278 (138)                                | 496 (285)       |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 27 (43)                  | 29 (29)                                  | 44 (44,50)      |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 359507 [458748]          | 523805 [534757]                          | 923687 [1526492] | < 0.001               |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 1.40 (3.50)              | 40.90 (89.18)                            | 91 (182.10)     |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 43.15 (4.72)             | 42.50 (6.50)                             | 38.15 (6.48)    |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 6450 (2815)              | 7005 (4115)                              | 9145 (6613)     |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 1400 (805)               | 1000 (433)                               | 540 (445)       |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 4260 (2625)              | 5250 (3918)                              | 8300 (5880)     | 0.046                  |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 500 (300)                | 400 (300)                                | 300 (280)       |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 0 (100)                  | 0 (40)                                   | 0 (6)           |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |
|                          | 0 (0)                    | 0 (20)                                   | 5.25 (17)       |                        |                          |                          |                               |                          |                             |                                  |                                  |                                  |                                  |                                  |                                  |

Table 1: Clinical characteristics of the patients: continuous variables are represented as [median, (interquartile range, IQR)]; categorical variables are represented as [% (n)].

INR, International Normalized Ratio; n.s., not significant; n.a., not applicable. COPD (Chronic obstructive pulmonary disease), HIV (Human Immunodeficiency Virus), INR (International Normalized Ratio), LDH (Lactic Acid Dehydrogenase), GPT (glutamic-pyruvate transaminase); CRP (C-reactive protein), WBC (white blood cell).
Table 2: Multivariate generalized linear model with binomial distribution to assess the association between viremia and hospitalization at the wards in the comparison (outpatients vs wards) (left) and the association between viremia and hospitalization at the ICU in the comparison (wards vs ICU) (right).

|                | GLM (outpatients vs wards) | GLM (wards vs ICU) |
|----------------|----------------------------|--------------------|
|                | OR  | p    | CI 95%          | OR  | p    | CI 95%          |
|                |     |      | lower  | upper |     |      | lower  | upper |
| Viremia        | 2.12| 0.10 | 0.86   | 5.20  | 8.30| < 0.001 | 4.21   | 16.34 |
| Age            | 1.06| < 0.001 | 1.03  | 1.09  | 1.00| 0.97    | 0.98   | 1.02  |
| Sex            | 1.06| 0.88 | 0.48   | 2.32  | 1.86| 0.06    | 0.97   | 3.56  |
| Obesity        | 4.89| 0.05 | 1.02   | 23.53 | 1.11| 0.78    | 0.52   | 2.36  |
| Hypertension   | 1.19| 0.71 | 0.48   | 2.95  | 0.90| 0.77    | 0.44   | 1.83  |
| Cardiac disease| 1.69| 0.55 | 0.29   | 9.83  | 0.69| 0.51    | 0.23   | 2.07  |
| Asthma         | 1.02| 0.98 | 0.23   | 4.43  | 0.26| 0.15    | 0.04   | 1.62  |
| Dyslipidemia   | 1.63| 0.33 | 0.60   | 4.40  | 0.84| 0.66    | 0.40   | 1.79  |
Figure 1: Prevalence of SARS-CoV-2-RNA viremia and SARS-CoV-2 IgG antibodies in each severity group.

Figure 2: Forest plot showing the result from the multivariate generalized linear model with a gamma distribution (log-link) to assess the association between viremia and laboratory and immunological parameters. Arithmetic mean ratios (AMR) adjusted by major comorbidities (Age, Sex, Obesity, Hypertension, Type II Diabetes, Cardiovascular disease, Asthma, Dyslipidemia) and disease severity category (outpatients, ward and ICU) are showed in the plot.

Figure 3: Box plots showing the immunological mediators’ levels across severity groups.
SARS-CoV-2 RNA viremia (%)

SARS-CoV-2 IgG (%)

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