Title

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Authors

Yan, Peter D
Markovic, Daniela
Hixson, Roxana Y
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Original article

Eosinophil Recovery in Hospitalized COVID-19 Patients is Associated with Lower Rates of ICU Admission and In-Hospital Mortality: An Observational Cohort Analysis

Peter D. Yan[1], Daniela Markovic[2], Roxana Y. Hixson[3], Carolyn M. Shover[2,4], Russell G. Buhr[2,5], Ramin Salehi-Rad[2,5], Blake LeMaster[6], Donald P. Tashkin[2], Jennifer A. Fulcher[5,7], Igor Z. Barjaktarevic[2,*]

[1]David Geffen School of Medicine, University of California, Los Angeles, CA, United States

[2]Department of Medicine Statistics Core, David Geffen School of Medicine, University of California, Los Angeles, CA, United States

[3]Division of Pulmonary, Critical Care and Sleep Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA, United States

[4]Offsite Care, Santa Rosa, CA, United States

[5]Department of Medicine, Greater Los Angeles Veterans Affairs Health Care System, Los Angeles, CA, United States

[6]Division of Pulmonary and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN, United States

[7]Division of Infectious Diseases, David Geffen School of Medicine, University of California, Los Angeles, CA, United States

[*]**Corresponding author:** Igor Barjaktarevic, 10833 Le Conte Avenue, 43118 CHS, Westwood, 90095, CA, Email: ibarjaktarevic@mednet.ucla.edu

Abstract

Background: Admission eosinopenia (<100 cells/μL) is associated with poor clinical outcomes in hospitalized COVID-19 patients. However, the effects of eosinophil recovery (defined as reaching ≥50 eosinophils/μL) during hospitalization on COVID-19 outcomes have been inconsistent.

Methods: The study included 1,831 patients admitted to UCLA hospitals between February 2020 and February 2021 with PCR-confirmed COVID-19. Using competing risk regression and modeling eosinophil recovery as a time-dependent covariate, we evaluated the longitudinal relationship between eosinophil recovery and in-hospital outcomes including ICU admission, need for mechanical ventilation, and in-hospital mortality. All analyses were adjusted for covariates including age, BMI, tobacco smoke exposure, comorbidities known to be risk factors for COVID-19 mortality, and treatments including dexamethasone and remdesivir.

Results: Eosinophil recovery was evaluated in patients with <50 eosinophils/μL on admission (n=1282). These patients cumulatively amassed 11,633 hospital patient-days; 3,985 of those days qualified as eosinophil recovery events, which were represented by 781 patients achieving at least one instance of eosinophil recovery during hospitalization. Despite no significant difference in the rate of mechanical ventilation, eosinophil recoverers had significantly lower rates of in-hospital mortality (aHR: 0.44 [0.29, 0.65], P=0.001) and ICU admission (aHR: 0.25 [0.11, 0.61], P=0.002).

Conclusion: Trending eosinophil counts during hospitalization is simple and can be performed in resource-limited healthcare settings to track the inflammatory status of a patient. Lack of eosinophil recovery events can identify those at risk for future progression to severe COVID.

Keywords: *ARDS, critical illness, COVID-19, eosinophil, inflammation, survival*

Abbreviations: COVID-19 or COVID, (Coronavirus disease 2019); CRP, (C-reactive protein); ICD, (International Classficiation of Diseases); ICU, (intensive care unit); IL-6, (interleukin 6); LDH, (lactate dehydrogenase); NIH, (National Institutes of Health); LOS, (length-of-stay); NLR, (neutrophil-lymphocyte ratio); PAP, (positive airway pressure); PCR, (polymerase chain reaction); SARS-CoV-2, (severe acute respiratory syndrome coronavirus 2); UCLA, (University of California Los Angeles)

1. Introduction

COVID-19 (Coronavirus Disease 2019) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has upended the global community, infecting over 500 million people and causing over 6 million deaths worldwide at the time of writing[1]. Although much is now known about the pathogenesis of this infection, patterns of clinical presentation of diseased individuals range from minimal or no symptoms to severe clinical deterioration and death[2].

Great strides have been made to identify prognostic factors capable of predicting disease severity in patients with COVID-19. Older male adults with conditions including diabetes, end-stage renal disease, coronary artery disease, chronic respiratory diseases, and cirrhosis are at greater risk of developing severe COVID disease[3-7]. Other prognostic biomarkers associated with poor clinical outcomes include elevated admission inflammatory markers like C-reactive protein (CRP), D-dimer, and lactate dehydrogenase (LDH), along with lymphopenia and neutrophilia[8-11]. While multiple risk factors for severe COVID-19 have been identified, the search continues for additional biomarkers and better prognostic tools to better identify patients likely to become critically ill with COVID-19.

Peripheral blood eosinophil counts have garnered the interest of researchers as a prognostic biomarker for COVID-19 since eosinopenia (≤50 cells/μL) appears to be a unique

feature of COVID-19[12]. A study in Italy estimated approximately 75% of patients presenting with SARS-CoV-2 infection had undetectable levels of eosinophils (absolute eosinopenia) on hospital admission, while similar findings were observed in China during the outset of the pandemic[13,14].These persistent findings inspired some researchers to use admission eosinophil counts as a relatively specific diagnostic marker for SARS-CoV-2 infection when PCR kits were in short supply[15]. While no unifying rationale explains these observations, multiple studies have demonstrated an inverse relationship between admission blood eosinophil counts and COVID-19 severity[16,17].

Less is known about tracking changes in peripheral blood eosinophil counts throughout disease progression as a predictor for clinical outcomes in COVID-19 patients. Isolated reports in Spain have suggested eosinophil recovery during hospitalization to be associated with improved survival[18]. Although little is known about the relationship between eosinophils and COVID-19 progression, laboratory studies have postulated that eosinophils may play a role in viral mitigation through direct, cell-mediated manners or antigen presentation[19-23]. Therefore, it stands to reason that changes in blood eosinophil count throughout hospitalization may reflect disease evolution and can serve as an easily attainable biomarker capable of predicting and tracking disease severity in hospitalized COVID-19 patients.

Our aim was to evaluate the longitudinal relationship between eosinophil recovery (defined as reaching ≥50 cells/μL) and time-dependent in-hospital outcomes including intensive care unit (ICU) admission, mechanical ventilation, and in-hospital mortality. We hypothesized that eosinophil recovery in COVID-19 patients presenting with eosinopenia (<50 cells/μL) would be independently associated with positive clinical outcomes such as overall survival, less need for invasive ventilation, and decreased ICU admission for patients with COVID-19.

2. Materials and Methods:

2.1. Patients and Study Design

We performed a retrospective cohort analysis of 1,831 patients admitted to both UCLA Ronald Reagan and UCLA Santa Monica Medical Centers for COVID-19 infection between February 2020 and February 2021. This study was approved by the University of California, Los Angeles institutional review board (IRB 20-200473). Eligible patients were age ≥18 years with a positive COVID-19 PCR result upon hospital admission. Patients without peripheral blood eosinophil count data were excluded from the original cohort (n=180), leaving 1,651 patients in our final cohort for analysis.

2.2. Measurements

Prior comorbidities, patient demographics, tobacco exposure history, and lab values were available for analysis along with full, detailed hospital course (Table 1). Comorbidities were determined through documented International Classification of Diseases (ICD)-10 diagnosis per chart review (*Supplemental Table 2*). Routine laboratory results (i.e. metabolic panels, complete blood counts, etc.) were considered "admission" values if they were obtained during initial patient intake. Non-routine laboratory values (i.e. IL-6, procalcitonin, C-reactive protein, D-dimer, and other inflammatory markers) were considered admission values if they were collected during intake or within 48 hours of admission, whichever came first. We categorized the 1,651 patients with available blood eosinophil data into three groups based on their admission eosinophil counts: absolute eosinopenia (0 cells/μL), marked eosinopenia (<50 cells/μL but >0 cells/ μL), and non-eosinopenia (≥50 cells/μL). Cutoffs for each group were chosen to maximize equal distribution of patients into each group; absolute eosinopenia was an exception given its predominance in our cohort (n=910; 55.1% of cohort). Eosinophil recovery, defined as reaching

an eosinophil count ≥50 cells/μL, was assessed daily for each patient during hospitalization. We used 50 cells/μL as our cutoff for eosinophil recovery because it reflected our previous choice for delineating patients with marked eosinopenia from "non-eosinopenic" patients. Each patient was also assigned a score based on the NIH COVID-19 Severity Scale (*Supplemental Table 1*), an eight-category ordinal scale used by the Adaptive COVID-19 Treatment Trial to classify disease severity from 1 (not hospitalized, no limitations) to 8 (in-hospital death) at time of outcome[24].

Main outcomes included ICU admission, need for mechanical ventilation, and in-hospital mortality. Other metrics for COVID severity included length of hospitalization, NIH Severity Scores (*Supplemental Table 1*), and need for COVID-specific treatments such as remdesivir and dexamethasone. Mechanical ventilation was defined by invasive ventilation requiring endotracheal intubation, whereas non-invasive mechanical ventilation was defined by the use of high-flow nasal-cannula systems or positive airway pressure (PAP) ventilation with bi-level or continuous PAP. Criteria for ICU admission was multifaceted and included, but not limited to, need for ventilation, severe myocardia depression, neurological monitoring, and acute renal support.

2.3. Statistical Analysis

Summary statistics stratified by baseline eosinophil status (non-eosinopenia, marked eosinopenia, and absolute eosinopenia) were tabulated for key variables of interest with categorical variables analyzed by Chi-Squared test and continuous variables by Kruskal-Wallis or Mood's median tests. We utilized Fine and Gray competing risk regression to evaluate the relationship between baseline eosinophil status and time-dependent in-hospital outcomes (ICU admission, intubation, and in-hospital mortality) with adjustments for potential confounders, including age, sex, tobacco exposure, history of diabetes with end-organ damage, chronic

pulmonary conditions, coronary artery disease, liver disease, chronic kidney disease, and COVID-specific treatments remdesivir and dexamethasone (hereafter referred to as "covariates"). Competing risk models for ICU admission and mechanical ventilation were performed to account for competing events, including in-hospital death and hospital discharge. Meanwhile, competing risk analysis for in-hospital death was adjusted for hospital discharge. We also evaluated the relationship between eosinophil recovery and in-hospital outcomes among patients with low eosinophils at baseline (defined by <50 cells/μL) using a cause-specific competing risk model that modeled eosinophil recovery as a time-varying covariate. Non-eosinopenic patients were excluded from this analysis to highlight the effect of eosinophil recovery in patients with marked or absolute eosinopenia.

A non-parametric linear regression model with bootstrapping was used to evaluate the relationship between baseline eosinophil status and mean NIH Severity Score. Logistic models were used to evaluate the associations between baseline eosinophil status and in-hospital treatments, including dexamethasone, remdesivir, systemic antibiotics, and oxygen therapy. To control for potential confounding and moderating effects associated with prior immunosuppression, a sub-analysis was performed to compare outcomes in immunosuppressed transplant recipients (n=181) stratified by eosinophil status. This comparison was adjusted using propensity-score matching due to small sample size. Additional sub-analysis for eosinophil recovery was also performed on hospitalized patients treated with any systemic corticosteroid (n=1011) and adjusted accordingly with competing risk regression given sufficient sample size. Analyses were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

All models were fit using complete case analysis. We used a threshold of 15% missingness on key variables to trigger multiple imputation, which was not met for any of our analyses. Patients

who were transferred to another hospital were treated as censored observations in competing risk models, accounting for 252 patients (15.3% of cohort).

3. Results

3.1. Baseline characteristics of the study population

Of the 1,651 hospitalized COVID-19 patients in our cohort with eosinophil data, 910 presented with absolute eosinopenia (55.1% of overall cohort), 372 (22.5%) with marked eosinopenia, and 369 (22.4%) with non-eosinopenia. Admission eosinophil counts ranged from 0 to 1650 cells/μL with a median of 45 cells/μL and IQR (inter-quartile range) of 68 cells/μL (lower quartile: 0 cells/μL; upper quartile: 68 cells/μL). While a statistically significant difference in age (63.5±17.2 vs 62.2±20.4 and 58.6±20.3 years, P=0.001) and BMI (29.4±7.5 vs 28.3±7.2 and 28.3±7.5 kg/m2, P=0.001) was observed among patients with absolute eosinopenia when compared to the other eosinophil groups, the difference was likely not clinically significant. Current tobacco use was more prevalent among patients with absolute eosinopenia (7.9% vs 3.5% and 3.3%, P<0.001), while previous history of tobacco use was similar in all three groups (P=0.242). Patients in all three groups also had similar rates of comorbidities apart from congestive heart failure, which was found to be less prevalent among patients with absolute eosinopenia (P=0.01).

Baseline inflammatory state was also evaluated. In Table 1, non-specific markers for inflammation, including C-reactive protein, ferritin, IL-6, and procalcitonin, were increased in patients with absolute eosinopenia. Neutrophil-lymphocyte ratio (NLR) was also significantly higher in the absolute eosinopenia group (P<0.001). However, this was driven predominantly by lymphocyte depletion rather than neutrophil expansion.

3.2. Association between admission eosinophil status and clinical outcomes

Table 2, describes associations of eosinophil statuses with clinical outcomes. Rates of ICU admission, mechanical ventilation, and in-hospital mortality were higher in patients admitted with absolute eosinopenia before adjusting for any covariates. Other metrics for COVID severity, including length of hospitalization and need for COVID-specific treatments, were also increased in patients with absolute eosinopenia. After adjusting for pre-specified covariates and competing risks in Table 3, absolute eosinopenia remained strongly associated with ICU admission (aHR: 1.36 [1.05, 1.76], P=0.020), mechanical ventilation (aHR: 1.60 [1.04, 2.47], P=0.032), and in-hospital mortality (aHR: 2.53 [1.49, 4.29], P=0.001). Although patients with marked eosinopenia on admission had similar trends in these outcomes, they did not reach statistical significance when compared with outcomes in those without eosinopenia. As a result, any count-dependent relationship between admission eosinophil counts and clinical outcomes in our analysis was tenuous once appropriate adjustments were made. Similarly, effect size decreased when comparing NIH Severity Scores between absolute eosinopenia and non-eosinopenia groups.

A sub-analysis performed on transplant recipients receiving immunosuppressive treatments (n=181) revealed that absolute eosinopenia also had greater hazards for in-hospital mortality (aHR: 1.78 [0.58, 5.48], P=0.316), ICU admission (aHR: 1.62 [0.95, 2.74], P=0.075), and mechanical ventilation (aHR: 1.79 [0.66, 4.84], P=0.249) than transplant recipients without absolute eosinopenia. Although these differences did not reach statistical significance, the effect size was appreciable.

3.3. Eosinophil recovery as a predictor for specific outcomes

Finally in Table 4, we pooled patients with admission eosinophil counts <50 cells/μL (n=1,282) and found that they cumulatively amassed 11,633 hospital days; 3985 of those days

qualified as eosinophil recovery events (reaching ≥50 cells/μL) which were represented by 781 patients achieving at least one instance of eosinophil recovery during their hospital stay. These eosinophil recoverers were less likely to be admitted to the ICU (aHR: 0.25 [0.11, 0.61], P=0.002) or succumb to COVID during hospitalization (aHR: 0.44 [0.29, 0.65], P=0.001). Interestingly, eosinophil recovery did not result in any appreciable differences in mechanical ventilation.

In patients treated with systemic steroids (dexamethasone, methylprednisolone, prednisone, prednisolone, or hydrocortisone) during hospitalization (n=1,011), eosinophil recovery remained an independent prognostic factor for improved clinical outcomes including lower hazards for ICU admission (aHR: 0.17 [0.05, 0.57], P=0.004) and in-hospital mortality (aHR: 0.40 [0.25, 0.66], P=0.001) (Table 4).

4. Discussion

In this retrospective analysis of adults hospitalized with COVID-19 at a large academic institution between Feb 2020 and Feb 2021, recovery from admission eosinopenia was associated with improved overall survival and decreased need for ICU admission independent of age, major comorbidities, and in-hospital corticosteroid use. In addition, absolute eosinopenia upon admission was found to be an independent risk factor for ICU admission, mechanical ventilation, and in-hospital death. Our findings suggest that eosinophils are associated with risk reduction in developing severe COVID-19.

To our knowledge, this is the first study to evaluate the significance of peripheral blood eosinophil count recovery in SARS-CoV-2 infected hospitalized patients using a model that encapsulates dynamic eosinophil changes throughout hospitalization. Previous retrospective analysis performed on COVID patients admitted across 147 Spanish hospitals also assessed the association of eosinophil recovery with live discharge[18]. The latter study, however, assessed

eosinophil recovery seven days after hospital admission. A limitation of measuring eosinophil recovery within a fixed timeframe is data skewing that may result from placing more weight on the initial days of hospitalization, when in fact many patients may only begin to decompensate at a later point during their hospital stay[25]. Indeed, stratification of our own cohort based on NIH Severity Scores showed duration of hospitalization to be directly correlated with COVID disease severity (*Supplemental Fig. 1*). Using fixed time intervals to assess eosinophil recovery as a predictor for positive outcomes also fails to consider medication administration that can alter peripheral blood immune cell counts. As an example, corticosteroids cause fluctuations in leukocyte count by inhibiting neutrophil margination and extravasation[26,27]. We attempted to correct for this potential confounder by examining eosinophil recovery in a subset of patients treated with systemic corticosteroids during hospitalization. Hazard ratios for in-hospital mortality, ICU admission, and mechanical ventilation in this sub-analysis were unchanged from our results in our initial analysis (Table 4), suggesting that the relationship between eosinophil recovery and improved clinical outcomes was irrespective of corticosteroid treatment.

Mechanisms underlying the association between eosinophil recovery and improved clinical outcomes remain largely unknown, but early studies have found that eosinophils may play a role in viral clearance. Handzel et al. demonstrated for the first time that eosinophils derived from peripheral blood promoted clearance of human rhinovirus type 16 (RV16) by presenting viral antigens to RV16-specific T cells, causing T cell proliferation and IFN-γ secretion[20]. Eosinophil expression of nitric oxide and eosinophil-derived neurotoxin (EDN) were also shown to mitigate respiratory syncytial and parainfluenza viruses in mice[19,28]. Although these studies were performed in a laboratory setting, Pineros et al. demonstrated that human subjects with asthma who were treated with the anti-eosinophil drug mepolizumab (an anti–IL-5

humanized mAb) and subsequently challenged with rhinovirus demonstrated significant increases in viral titers in the upper airway compared to those treated with placebo[29]. Taken together along with our own findings, these data seem to substantiate the antiviral potential of eosinophils. Even so, the clinical significance of eosinophils in antiviral responses in COVID-19 remains a topic of debate.

In fact, others have argued that eosinopenia may instead be a product of underlying biological processes that promote severe COVID disease. For example, inflammation in severe COVID-19 may cause eosinopenia by promoting the migration of eosinophils into peripheral tissue[30]. One study examining lung tissue necropsies from patients with COVID pneumonia revealed moderate eosinophil counts and their activation products within the lung parenchyma, implying a pathogenic role for eosinophils in the setting of COVID-19[31]. However, studies beyond case reports are lacking in evaluating the pro-inflammatory effects of eosinophils in COVID-associated acute respiratory distress syndrome (ARDS). An oft-cited meta-analysis by Al Duhailib et al. correlating eosinophil activity with worsened outcomes in ARDS lacks specific studies on patients infected with SARS-CoV-2[32]. While COVID-associated ARDS bears some similarities to all-cause ARDS, many differences including lower systemic levels of IL-6, longer dependence on mechanical ventilation, and increased dead space ventilation exist[33,34]. Therefore, COVID-associated ARDS remains poorly characterized and more studies are needed to characterize the role of eosinophils in this setting.

Although prospective studies are needed, our initial findings support the use of eosinophil recovery as a favorable prognostic factor for hospitalized COVID-19 patients. With eosinophil count evaluation being a simple, inexpensive, and routinely available lab test, trending eosinophils can help clinicians identify COVID patients at risk for deterioration. This is

particularly helpful in resource-limited settings, where identification of at-risk patients is crucial for proper allocation of beds, mechanical ventilators, and healthcare staff[35].

Our study has limitations. Assessing outcomes other than mortality, ICU admission, and mechanical ventilation was beyond the scope of this retrospective analysis. Finer details on the time between symptom onset and admission eosinophil counts was also unreliable given that data collection started only upon hospital admission, and reported symptoms were not universally documented on initial patient encounter. The nature of retrospective analysis lends itself to inherent biases and unmeasured confounding that could influence our overall findings. Prior comorbidities were also based on ICD-10 coding with its inherent flaws of underreporting diagnoses[36]. But given the scope and length of our study, missing data from underreporting was likely random and unable to cause any significant confounding. Medication history prior to hospitalization was also often unknown, limiting our ability to directly evaluate drug-induced eosinopenia on clinical outcomes. Our attempt to control for this potential confounder by assessing eosinophil recovery in immunosuppressed transplant recipients was also likely sub-optimal given the relatively small sample size of transplant recipients. Even so, eosinophil recovery among steroid-treated patients during hospitalization continued to demonstrate protective effects against SARS-CoV-2 infection. However, corticosteroid data lacked dosage and schedule of steroid administration for each patient, thus limiting our ability to evaluate the relationship between corticosteroid use and eosinophil counts. Additionally, the relationship of blood eosinophil recovery with the recovery of other cell lines and overall relevance of the role of Th-2 immune pathway in the clinical recovery from COVID-19 is unclear. Trending specific immunologic and inflammatory markers such as IL1, IL12, IL6, IL5, IL17 or TNF-alpha could offer a better insight into the mechanisms of cellular immunity recovery in COVID-19,

nevertheless, none of these specific biomarkers has been routinely monitored and such data is missing here. Lastly, our pre-selected cutoffs for eosinophil recovery and our differing eosinophil groups were based off observation at our institution rather than clinical guidelines. We did not utilize any cutoffs from prior studies because there did not seem to be a universally accepted cutoff for eosinopenia in the context of SARS-CoV-2 infection.

Nevertheless, several strengths should be emphasized. This report is based on data from a large and well-characterized database from a large quaternary academic center from the early phase of the COVID-19 pandemic. Medical and supportive management were mandated to follow current CDC guidelines, and our medical centers did not have any major shortages or system limitations that could impact outcomes. In addition to comparing outcomes of patients with or without eosinophil recovery, we controlled for in-hospital corticosteroid use and correlated positive outcomes with eosinophil recovery beyond the first few days of hospitalization. This study, therefore, expands upon previous studies and further supports using eosinophil recovery as a readily available marker for improving disease that can be measured at all stages of hospitalization.

In conclusion, eosinophil recovery is associated with improved survival and decreased ICU admission rate in the setting of COVID-19. Peripheral blood eosinophil count, therefore, is a clinically relevant biomarker whose dynamic behavior may predict COVID-19 disease course and assist with disease risk stratification. Further studies are needed to determine whether eosinophils themselves should be potential targets of interest for future therapeutic approaches in COVID-19 as it is unclear whether eosinophils play an active role in viral mitigation or whether they are byproducts of underlying biological mechanisms that have yet to be elucidated.

Author contributions

DM, IZB, JAF, and PDY had full access to the data and take responsibility for the integrity of the data and accuracy of the analysis. PDY, DM, RGB, DPT, and IZB were involved in the design of the analysis. PDY, DM, RGB, and IZB contributed to the drafting of the manuscript; and DPT, RHY, and RSR were involved in the editing of the manuscript. All authors approved the manuscript for submission.

Role of the sponsors

Industry sponsors had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Other contributions

The authors thank the UCLA participants and participating physicians, investigators, and staff for making this research possible.

Ethics Approvals

This study was reviewed and approved by the UCLA Institutional Review Board (20-200473).

Disclosures

Drs. Buhr and Tashkin report personal consulting fees from Theravance Biopharma/Viatris, unrelated to this work. Drs. Buhr, Fulcher, and Salehi-rad are employed by the Veterans Health Administration. The views and positions in this manuscript do not necessarily represent those of the United States Government.

Dr. Barjaktarevic reports funding from NHLBI, PCORI and COPD foundation, Theravance, Viatris, Amgen, Aerogen and has received consulting fees from Astra Zeneca, Theravance, Viatris, Aerogen, GE Healthcare, Verona Pharma, Grifols and GSK, all unrelated to this project.

Drs. Hixson, LeMaster, and Shover, Ms. Markovic, and Mr. Yan report nothing to disclose.

Table 1. Baseline characteristics of study cohort by admission eosinophil status

	Non-	**Eosinopen**	**Absolute**	

	eosinopenia	ia	Eosinopenia	
	(n=369)	**(n=372)**	**(n=910)**	***P-value***
Demographics				
[1]Age, mean (SD)	58.6 (20.3)	62.2 (20.4)	63.5 (17.2)	0.001
[2]Male, %	57.5	53.2	56.3	0.476
[1]BMI, mean (SD)	28.2 (7.5)	28.3 (7.2)	29.4 (7.5)	0.001
[2]Active smoker, %	7.9	3.5	3.3	<0.001
[2]Former smoker, %	23.8	29.3	26.4	0.242
[2]*Comorbidities*				
Diabetes, %	37.7	35.5	40.3	0.246
Coronary artery disease, %	15.2	14.0	12.1	0.296
Heart failure, %	22.2	24.7	17.7	0.010
Chronic kidney disease, %	27.6	31.2	27.0	0.317
Chronic pulmonary condition, %	23.8	27.7	28.4	0.253
Liver disease, %	6.0	7.3	4.8	0.222
Cerebrovascular disease, %	17.6	18.5	17.1	0.835
Peripheral vascular disease, %	18.2	21.8	17.5	0.193
Immunosuppressed, %	8.1	11.8	11.7	0.142
[3]*Admission inflammatory Markers*				
Neutrophil-lymphocyte ratio, median (IQR)	3.31 (3.68)	4.60 (5.47)	6.55 (7.66)	<0.001
Neutrophils (x10^9cells/L), median (IQR)	4.77 (4.58)	4.22 (4.19)	4.66 (4.58)	0.063
Lymphocytes (x10^9cells/L), median (IQR)	1.38 (1.04)	0.93 (0.75)	0.74 (0.52)	<0.001
*Ferritin (ng/mL), median (IQR)	359 (717)	508 (831)	696 (993)	<0.001
*C-reactive protein (mg/L), median (IQR)	2.8 (7.7)	5.6 (9.1)	8.4 (9.2)	<0.001
*IL-6 (pg/mL), median (IQR)	4.1 (21.0)	5.0 (14.1)	7.1 (14.6)	0.035
*Procalcitonin (ng/mL), median (IQR)	0.11 (0.40)	0.11 (0.36)	0.17 (0.43)	<0.001
*D-dimer (ng/mL), median (IQR)	1,130 (1,891)	1,062 (1,660)	920 (1,290)	0.053
*Lactate (mg/dL), median (IQR)	13 (10)	13 (8)	13 (9)	0.821

[1]Kruskal-Wallis Nonparametric Test
[2]Chi-squared Test of Independence
[3]Mood's Median Test
*Inflammatory markers obtained within 48 hours of admission
SD = Standard Deviation; IQR = Interquartile Range

Table 2. Unadjusted outcomes and treatments stratified by baseline eosinophil status

	Non-eosinopenia	Eosinopenia	Absolute Eosinopenia	
	(n=369)	**(n=372)**	**(n=910)**	***P***
Outcomes				
[1]NIH Severity Score, mean (SD)	5.58 (1.18)	5.85 (1.09)	6.26 (0.90)	<0.001
[4]ICU admission, %	26.9	22.0	37.0	<0.001
[4]Mechanical ventilation, %	15.72	11.83	19.34	0.004
[4]In-hospital mortality, %	5.46	9.21	13.58	<0.001
[3]Hospital duration (days), median (IQR)	6 (9)	6 (9)	8 (8)	<0.001
[2]*Treatments*				
Remdesivir, %	23.6	47.0	77.7	<0.001
Dexamethasone, %	19.5	36.3	71.5	<0.001
Systemic steroids, %	30.4	49.2	78.7	<0.001
Antibacterials, %	63.7	71.0	69.0	0.080

[1]Kruskal-Wallis Nonparametric Test
[2]Chi-squared Test of Independence
[3]Mood's Median Test
[4]Gray's Test
SD = Standard Deviation; IQR = Interquartile Range

Table 3. Outcomes by eosinophil status after adjusting for covariates and competing events

		In-Hospital Death		ICU Admission		Mech. Ventilation	
	n	**aHR (95% CI)**	***P***	**aHR (95% CI)**	***P***	**aHR (95% CI)**	***P***
[1,a]Non-eosinopenia	369	1	—	1	—	1	—
[1,a]Eosinopenia	372	1.36 (0.78, 2.39)	0.282	0.78 (0.58, 1.03)	0.079	0.75 (0.47, 1.21)	0.244
[1,a]Abs. Eosinopenia	910	2.53 (1.49, 4.29)	0.001	1.36 (1.05, 1.76)	0.020	1.60 (1.04, 2.47)	0.032
		NIH Severity Score					
	n	**Mean (SE)**		***P***			
[2,a]Non-eosinopenia	369	5.80 (0.054)		—			
[2,a]Eosinopenia	372	5.92 (0.052)		0.11			
[2,a]Abs.	91	6.14 (0.037)		<0.001			

Eosinopenia	0					

[1]Fine and Gray Competing Risk Regression Model
[2]Non-parametric Linear Regression with Bootstrapping
[a]Adjusted for Age, Sex, BMI, Smoking Status, Comorbidities, Medications
aHR = Adjusted Hazard Ratio; CI = Confidence Interval; SE = Standard Error

Table 4. Outcomes by eosinophil recovery status after adjusting for covariates and competing events

		In-Hospital Death		**ICU Admission**		**Mechanical Ventilation**	
	n	**aHR (95% CI)**	**P**	**aHR (95% CI)**	**P**	**aHR (95% CI)**	**P**
[1,a,b]All-comers							
Eosinophil recoverer	781	0.44 (0.29, 0.65)	<0.001	0.25 (0.11, 0.61)	0.002	0.70 (0.42, 1.16)	0.165
Non-recoverer	501	1	—	1	—	1	—
[1,a,b]Corticosteroid-treated							
Eosinophil recoverer	716	0.40 (0.25, 0.66)	<0.001	0.17 (0.05, 0.57)	0.004	0.75 (0.41, 1.39)	0.363
Non-recoverer	295	1	—	1	—	1	—
		Days					
	n	**Median (IQR)**	**P**				
[2]Hospital duration							
Eosinophil recoverer	781	8 (12)	0.232				
Non-recoverer	501	6 (11)					

[1]Competing Risk Regression Model
[2]Mood's Median Test
[a]Adjusted for Age, Sex, BMI, Smoking History, Comorbidities, Treatments
[b]Eosinophil Recovery Modeled as a Time-dependent Covariate
aHR = Adjusted Hazard Ratio; CI = Confidence Interval

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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References

1. Coronavirus Disease (COVID-19) Situation Reports. Accessed January 4, 2022. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

2. Esakandari H, Nabi-Afjadi M, Fakkari-Afjadi J, Farahmandian N, Miresmaeili SM, Bahreini E. A comprehensive review of COVID-19 characteristics. *Biol Proced Online*. 2020;22:19. doi:10.1186/s12575-020-00128-2

3. Singh AK, Singh R. Hyperglycemia without diabetes and new-onset diabetes are both associated with poorer outcomes in COVID-19. *Diabetes Res Clin Pract*. 2020;167:108382. doi:10.1016/j.diabres.2020.108382

4. Rastad H, Ejtahed HS, Shafiee G, Safari A, Shahrestanaki E, Khodaparast Z, et al. The risk factors associated with COVID-19-Related death among patients with end-stage renal disease. *BMC Nephrol*. 2021;22:33. doi:10.1186/s12882-020-02221-w

5. Loffi M, Piccolo R, Regazzoni V, Di Tano G, Moschini L, Robba, D, et al. Coronary artery disease in patients hospitalised with Coronavirus disease 2019 (COVID-19) infection. *Open Heart*. 2020;7(2):e001428. doi:10.1136/openhrt-2020-001428

6. Guan W jie, Liang W hua, Shi Y, Gan L, Wang H, He J, et al. Chronic Respiratory Diseases and the Outcomes of COVID-19: A Nationwide Retrospective Cohort Study of 39,420 Cases. *J Allergy Clin Immunol Pract*. 2021;9(7):2645-2655.e14. doi:10.1016/j.jaip.2021.02.041

7. Iavarone M, D'Ambrosio R, Soria A, Triolo M, Pugliese N, Poggio P, et al. High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol*. 2020;73(5):1063-1071. doi:10.1016/j.jhep.2020.06.001

8. Mosquera-Sulbaran JA, Pedreañez A, Carrero Y, Callejas D. C-reactive protein as an effector molecule in Covid-19 pathogenesis. *Rev Med Virol*. Published online February 17, 2021:e2221. doi:10.1002/rmv.2221

9. Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H, et al. Diagnostic Value of D-Dimer in COVID-19: A Meta-Analysis and Meta-Regression. *Clin Appl Thromb Hemost*. 2021;27:10760296211010976. doi:10.1177/10760296211010976

10. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, et al. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. *Am J Emerg Med*. 2020;38(9):1722-1726. doi:10.1016/j.ajem.2020.05.073

11. Penttilä PA, Van Gassen S, Panovska D, Vanderbeke L, Van Herck Y, Quinterlier K, et al. High dimensional profiling identifies specific immune types along the recovery trajectories

of critically ill COVID19 patients. *Cell Mol Life Sci*. Published online March 13, 2021:1-16. doi:10.1007/s00018-021-03808-8

12. Cazzaniga M, Fumagalli LAM, D'angelo L, Cerino M, Bonfanti G, Fumugalli R, et al. Eosinopenia is a reliable marker of severe disease and unfavourable outcome in patients with COVID-19 pneumonia. *Int J Clin Pract*. Published online February 5, 2021. doi:10.1111/ijcp.14047

13. Outh R, Boutin C, Gueudet P, Suzuki M, Saada M, Aumaître H. Eosinopenia <100/µL as a marker of active COVID-19: An observational prospective study. *Journal of Microbiology, Immunology and Infection*. 2021;54(1):61-68. doi:10.1016/j.jmii.2020.12.005x

14. Zhang J jin, Dong X, Cao Y yuan, Yuan Ya dong, Yang Yi bin, Yan You qin, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*. 2020;75(7):1730-1741. doi:10.1111/all.14238

15. Xie G, Ding F, Han L, Yin D, Lu H, Zhang M. The role of peripheral blood eosinophil counts in COVID-19 patients. *Allergy*. Published online June 20, 2020:10.1111/all.14465. doi:10.1111/all.14465

16. Nair AP, Soliman A, Masalamani MAA. Clinical Outcome of Eosinophilia in Patients with COVID-19: A Controlled Study : Eosinophilia and COVID -19. *Acta Biomed*. 2020;91(4):e2020165-e2020165. doi:10.23750/abm.v91i4.10564

17. Le Borgne P, Abensur Vuillaume L, Alamé K, Lefebvre F, Chabrier S, Berard L, et al. Do Blood Eosinophils Predict in-Hospital Mortality or Severity of Disease in SARS-CoV-2 Infection? A Retrospective Multicenter Study. *Microorganisms*. 2021;9(2):334. doi:10.3390/microorganisms9020334

18. Mateos González M, Sierra Gonzalo E, Casado Lopez I, Arnalich Fernandez F, Beato Perez J, Monge Monge D, et al. The Prognostic Value of Eosinophil Recovery in COVID-19: A Multicentre, Retrospective Cohort Study on Patients Hospitalised in Spanish Hospitals. *J Clin Med*. 2021;10(2):305. doi:10.3390/jcm10020305

19. Drake MG, Bivins-Smith ER, Proskocil BJ, Nie Z, Scott GD, Lee JJ, et al. Human and Mouse Eosinophils Have Antiviral Activity against Parainfluenza Virus. *Am J Respir Cell Mol Biol*. 2016;55(3):387-394. doi:10.1165/rcmb.2015-0405OC

20. Handzel ZT, Busse WW, Sedgwick JB, Vrtis R, Lee WM, Kelly EA, et al. Eosinophils bind rhinovirus and activate virus-specific T cells. *J Immunol*. 1998;160(3):1279-1284.

21. Yang D, Rosenberg HF, Chen Q, Dyer KD, Kurosaka K, Oppenheim JJ. Eosinophil-derived neurotoxin (EDN), an antimicrobial protein with chemotactic activities for dendritic cells. *Blood*. 2003;102(9):3396-3403. doi:10.1182/blood-2003-01-0151

22. Rosenberg HF. Eosinophil-Derived Neurotoxin (EDN/RNase 2) and the Mouse Eosinophil-Associated RNases (mEars): Expanding Roles in Promoting Host Defense. *International Journal of Molecular Sciences*. 2015;16(7):15442-15455. doi:10.3390/ijms160715442

23. Pozo VD, de Andrés B, Martín E, Cardaba B, Fernandez J, Gallardo S, et al. Eosinophil as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. *European Journal of Immunology*. 1992;22(7):1919-1925. doi:10.1002/eji.1830220736

24. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the Treatment of Covid-19 — Final Report. *N Engl J Med*. 2020;383(19):1813-1826. doi:10.1056/NEJMoa2007764

25. Chiam T, Subedi K, Chen D, Best E, Bianco F, Dobler G, et al. Hospital length of stay among COVID-19-positive patients. *J Clin Transl Res*. 2021;7(3):377-385.

26. Ronchetti S, Ricci E, Migliorati G, Gentili M, Riccardi C. How Glucocorticoids Affect the Neutrophil Life. *Int J Mol Sci*. 2018;19(12):4090. doi:10.3390/ijms19124090

27. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol*. 2011;335(1):2-13. doi:10.1016/j.mce.2010.04.005

28. Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF. Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J Infect Dis*. 1998;177(6):1458-1464. doi:10.1086/515322

29. Sabogal Piñeros YS, Bal SM, van de Pol MA, Dierdorp BS, Dekker T, Dijkhuis A, et al. Anti-IL-5 in Mild Asthma Alters Rhinovirus-induced Macrophage, B-Cell, and Neutrophil Responses (MATERIAL). A Placebo-controlled, Double-Blind Study. *Am J Respir Crit Care Med*. 2019;199(4):508-517. doi:10.1164/rccm.201803-0461OC

30. Wen T, Rothenberg ME. The Regulatory Function of Eosinophils. *Microbiol Spectr*. 2016;4(5):10.1128/microbiolspec.MCHD-0020-2015. doi:10.1128/microbiolspec.MCHD-0020-2015

31. MCCRAE C, CANN J, ZHAO W. EOSINOPHILS AND IL-5 RECEPTOR IN COVID-19 POSTMORTEM LUNG TISSUE. *Chest*. 2021;160(4):A568-A569. doi:10.1016/j.chest.2021.07.548

32. Al Duhailib Z, Farooqi M, Piticaru J, Alhazzani W, Nair P. The role of eosinophils in sepsis and acute respiratory distress syndrome: a scoping review. *Can J Anaesth*. 2021;68(5):715-726. doi:10.1007/s12630-021-01920-8

33. Bain W, Yang H, Shah FA, Suber T, Drohan C, Al-Yousif N, et al. COVID-19 versus Non–COVID-19 Acute Respiratory Distress Syndrome: Comparison of Demographics, Physiologic Parameters, Inflammatory Biomarkers, and Clinical Outcomes. *Ann Am Thorac Soc*. 18(7):1202-1210. doi:10.1513/AnnalsATS.202008-1026OC

34. Bertelli M, Fusina F, Prezioso C, Cavallo E, Nencini N, Crisci S, et al. COVID-19 ARDS Is Characterized by Increased Dead Space Ventilation Compared With Non-COVID ARDS. *Respir Care*. 2021;66(9):1406-1415. doi:10.4187/respcare.08786

35. Siow WT, Liew MF, Shrestha BR, Muchtar F, See KC. Managing COVID-19 in resource-limited settings: critical care considerations. *Crit Care*. 2020;24:167. doi:10.1186/s13054-020-02890-x

36. Gothe H, Rajsic S, Vukicevic D, Schoenfelder T, Jahn B, Geiger-Gritsch S, et al. Algorithms to identify COPD in health systems with and without access to ICD coding: a systematic review. *BMC Health Serv Res*. 2019;19:737. doi:10.1186/s12913-019-4574-3