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Prevalence of ARDS Phenotypes in Critically-Ill COVID-19 Patients: A Prospective Observational Cohort Study.

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Contributors
All authors conceived and designed the study. SC, DB, SC, CK, CK, OR, YC, CB, TSz collected the data. All authors contributed to data analysis and interpretation. PS drafted the manuscript and authors contributed to revision of the manuscript and approved the final version.

Abstract (282 words)

Rationale: In non-COVID-19 ARDS, two phenotypes, based on the severity of systemic inflammation, have been described. The hyperinflammatory phenotype is known to be associated with increased multi-organ failure and mortality. In this study, we aimed to identify these phenotypes in COVID-19 ARDS.

Methods: Patients with ARDS due to COVID-19 at two U.K. ICUs were recruited to the study. Demographic, clinical, and laboratory data were collected at baseline. Plasma samples were analysed for Interleukin-6 (IL-6) and soluble tumour-necrosis-factor receptor-1 (sTNFR-1) using a novel point-of-care assay. A parsimonious regression classifier model was used to calculate the probability for the hyperinflammatory phenotype in COVID-19 using IL-6, sTNFR-1 and sodium bicarbonate levels. Data from this cohort was compared to patients with ARDS recruited to a UK multicentre, randomised controlled trial of simvastatin (HARP-2).

Results: 39 patients were recruited to the study. Median PaO_2/FiO_2 was 18 kpa (IQR: 15 – 21) and APACHE II score was 12 (IQR: 10 – 14.5). 17/39 patients (44%) had died by day 28 of the study. Patients that died were older and had lower PaO_2/FiO_2. The median probability for the hyperinflammatory phenotype was 0.03 (IQR 0.01 – 0.2). Depending on the probability cut-off used to assign class, the prevalence of the hyperinflammatory phenotype was between 10-21% (4-8/39) which is lower than in HARP-2 (186/539, 35%). Mortality in the hyperinflammatory phenotype was 5/8 (63%) and 12/31 (39%) in the hypoinflammatory phenotype. Compared to

matched patients recruited to HARP-2, in COVID-19 levels of IL-6 were similar, whereas sTNFR-1 was significantly lower.

Summary: In this exploratory analysis of 39 patients, ARDS due to COVID-19 is not associated with higher systemic inflammation and is associated with a lower prevalence of the hyperinflammatory phenotype compared to historical ARDS data.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel virus leading to coronavirus disease-19 (COVID-19) that has resulted in a global pandemic and is associated with high mortality and morbidity.[1-3] SARS-CoV-2 pneumonia in its most severe form can lead to profound hypoxia and acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation.[1,3] Little is understood about the pathophysiology of COVID-19, though many have speculated that a central pathophysiological abnormality associated with severe COVID-19 is an exaggerated systemic inflammatory response or a "cytokine storm". [4-6] Objective, data-driven evidence to support this theory is currently lacking.[7]

Considerable evidence does, however, exist for the presence of subgroups of ARDS with exaggerated inflammation. In secondary analyses of five ARDS randomized controlled trials (RCTs), two phenotypes, termed hyperinflammatory and hypoinflammatory, have been consistently identified using latent class analysis (LCA).[8-11] The hyperinflammatory phenotype is associated with exaggerated inflammation evidenced by highly elevated levels of circulating pro-inflammatory cytokines and increased incidence of shock. Mortality rates in the phenotype with lower systemic inflammatory responses are approximately 20% and consistently 20% lower than in the hyperinflammatory phenotype. Further, in three of these RCTs, differential treatment responses to randomized interventions were observed in the two phenotypes.[8-10] These findings suggest that the hyperinflammatory phenotype may be useful for prognostic and predictive enrichment in ARDS.

LCA-derived phenotypes are usually identified using “large data” algorithms that are dependent on research biomarkers. Recently, parsimonious classifier models have been developed to identify ARDS phenotypes using a small number of variables.[12] We leveraged these models and novel point-of-care (POC) assays[13] in order to identify ARDS phenotypes in patients with COVID-19 in real-time. The main objective of the study was to describe the prevalence of ARDS phenotypes in COVID-19 associated ARDS. A second objective of the study was to compare the clinical and biological characteristics of COVID-19 patients with ARDS to a previously characterized ARDS patient population, patients enrolled in the HARP-2 clinical trial of simvastatin vs placebo.

Methods

Study Design and Population

This was a prospective observational study conducted at two centres. The study was a subset of an ongoing multi-centre study titled- clinical evaluation of a POC assay to identify phenotypes in the acute respiratory distress syndrome (PHIND study; ClinicalTrials.gov number: NCT04009330). All patients lacked capacity, and consent was gained using the appropriate emergency consent mechanisms in line with the ethical approval of the study by the Bromley Research Ethics Committee, U.K (reference number: 19/LO/0672). The study sites were the Royal Gwent Hospital (RGH), a district general hospital in Newport, Wales and University College Hospital (UCH), a university hospital serving an inner-city population in London. Both intensive care units (ICUs) were operating at surge capacity for the duration of the study (see supplement for details p1).

Patients were eligible for recruitment if they were positive for SARS-CoV-2 and met the Berlin definition of ARDS. Patients were excluded from the study if they were under the age of 18, onset of ARDS was > 48 hours, receiving extra-corporeal membrane oxygenation, or had a do not resuscitate order in place. Diagnosis of ARDS was established by the attending physicians caring for the patient. All patients were recruited to the study between March 17 and April 25, 2020.

Data Collection

Comprehensive data at baseline were collected, including demographics, chronic health conditions, vital signs, ventilatory and laboratory investigations. In addition to standard laboratory investigations, data were also available for acute makers of inflammation widely described in COVID-19. These included D-dimer, ferritin, C-reactive protein (CRP), procalcitonin, lactate dehydrogenase (LDH), fibrinogen and troponin. Biospecimens were also collected at baseline to quantify additional protein biomarker levels (see below). The study was censored at day 28 and vital status was adjudicated at this point.

Protein Biomarker Quantification and Phenotype Classification

Probabilities for belonging to the hyperinflammatory phenotype were generated using a novel rapid POC platform. In a pre-specified two-step process performed in real-time, plasma samples were first used to quantify IL-6 and sTNFR-1 concentrations. Second, these values were entered into a classifier model, which in turn generated the probabilities of phenotype assignment. Plasma levels of the two biomarkers were quantified at the time of study recruitment using a novel POC assay measured using the Evidence Multistat Analyser (Randox Laboratories, Country

Antrim, UK). As per the study protocol, a three-variable classifier model comprised of IL-6, serum bicarbonate, and sTNFR-1 was used to generate the probabilities.[12] Values for serum bicarbonate were measured in clinical laboratories. Clinical staff at both sites were blinded to the biomarker data and generated probabilities. The POC platform-generated probabilities have been validated against probabilities generated using ELISA-based biomarker quantification and the same classifier model.[13] These studies showed good correlation between the probabilities generated by the two methods, and both methods classified ARDS phenotypes accurately. Details of assay specific procedures and the validation of the probabilities can be found in the supplementary material (see p1).

As per the PHIND protocol, patients were classified into the hyperinflammatory phenotype using one of two pre-specified probability cut-offs: 1) ≥ 0.5; 2) the Youden index generated during model development (≥ 0.274). During previous model validation, classification based on a cut-off of ≥ 0.5 led to higher specificity, whereas the Youden index cut-off led to higher sensitivity.[12] Once classified, differences in measured variables and mortality at day 28 were compared between the phenotypes.

Previous findings from the secondary analysis using LCA of a phase 2b randomised trial of simvastatin for treatment of ARDS (the HARP-2 study)[14] were used as a historical reference standard to compare proportion of phenotypes, and clinical outcomes in the COVID-19 phenotypes. HARP-2 was specifically selected because data were available for IL-6 and sTNFR-1 quantified by the Multistat analyser in a selection of patients and would allow direct comparison

with the studied cohort. First, phenotype proportions, APACHE II scores, PaO_2/FiO_2, and clinical outcomes from the current study were compared with the entire HARP-2 cohort (n = 539). For HARP-2, phenotypes described are those derived using LCA. It was not possible to use the parsimonious model used in the COVID-19 cohort in HARP-2 due to bicarbonate not being measured. Next, biomarker levels, phenotype proportions, APACHE II scores, and clinical outcomes in the COVID-19 cohort were compared to an equivalent number of matched patients from HARP-2 that had IL-6 and sTNFR-1 levels measured using the Evidence Multistat Analyser (herein referred to as the 'HARP-2 matched' cohort; n = 39). This matched analysis permitted comparison of biomarker levels quantified using the same assay across two independent populations. Of the entire HARP-2 cohort, Multistat biomarker analysis was available in 98 patients. In an effort to compare aetiologically similar groups to COVID-19, only patients with pneumonia as the primary risk factor for ARDS were selected for matching from this subset. Matching of patients to the COVID-19 cohort was performed based on a logistic-regression derived score using age, gender and PaO_2/FiO_2, as predictor variables (see online supplement p2).

Statistical Analysis

Clinical data from the time of study enrollment were used for analysis. Given the small sample size in the analysed subgroups, data are only presented as median (interquartile range) for all continuous variables. Characteristics between groups were compared using Wilcoxon-rank test or Fisher's exact test depending on the nature of the variable. Spearman's rank correlation was

used to assess association between biomarkers. All analyses were performed on R Studio (version 1.1.453) using R (version 3.4.1).

Results

A total of 39 patients were recruited to the study. Of these, 32 patients were from RGH and seven were from UCH. All samples were collected within 2 hours of enrollment into the study and within 24 hours of diagnosis of ARDS / meeting study enrollment criteria. The median time from the onset of symptoms to study enrollment was 10 days (IQR 7 – 13). The population summary of demographics and baseline characteristics are presented in **Table 1**. Of note, 35/39 patients (90%) were receiving invasive mechanical ventilation and 4 patients were non-invasively ventilated at the time of recruitment to the study. All four patients receiving non-invasive ventilation were subsequently intubated in their stay in the ICU. 24/39 patients (62%) were on vasopressors at baseline (median dose 0.08 $mcg.kg^{-1}.min^{-1}$). The median APACHE II score was 12 (IQR 10 – 16). At day 28, 17/39 patients (44%) had died. Of the survivors, seven remained in the ICU on day 28 of the study and have subsequently been discharged alive. Mortality was 12/32 (38%) in the RGH cohort compared to 5/7 (71%) in the UCLH cohort. Differences in key baseline characteristics of the two sites are summarised in **Table S1** (supplement p3).

Differences in baseline characteristics between survivors and non-survivors are summarized in **Table 1**. Median age of survivors (54, IQR: 45 – 57) was significantly lower compared to non-survivors (60, IQR: 56 – 64, p = 0.0036). Of the respiratory variables, only the PaO_2/FiO_2 was significantly different, with lower levels in non-survivors (p = 0.0040). Of the biomarkers, IL-6 (p

= 0.0048), sTNFR-1 (p = 0.0197), D-Dimer (p=0.0187), and bilirubin (p=0.0235) were all significantly higher in non-survivors compared to survivors (**Table 1**). Significant correlations were noted between many of the measured biomarkers (**Figure 1**). D-dimer, ferritin, CRP, LDH and procalcitonin showed association with one another with correlation coefficients approaching 0.5. The highest correlations were observed between fibrinogen and CRP (r = 0.63) and sTNFR-1 and creatinine (r = 0.60).

Applying the parsimonious classifier model to the COVID-19 cohort resulted in a median probability for the hyperinflammatory classification of 0.03 (IQR 0.01 – 0.2), suggesting low prevalence of the phenotype in this population. Using a probability cut-off of $\geq$ 0.5 to assign phenotype, 4/39 patients (10%) were in the hyperinflammatory phenotype. With this cut-off, mortality at day 28 in the hyperinflammatory phenotype was 75% (3/4 patients) and 40% (14/35 patients) in the hypoinflammatory phenotype. Key differences in the baseline characteristics between the two phenotypes derived using this cut-off are summarised in **Table S2** (supplement p4). Using the Youden Index cut-off ($\geq$ 0.274) to assign class led to eight patients (21%) being classified as the hyperinflammatory phenotype. It is worth noting that without LCA-derived phenotypes, it is not possible to ascertain which of the two cut-offs is more accurate. Given that more patients were in the hyperinflammatory phenotype using the Youden Index cut-off, to enhance interpretability of comparative statistics, for the remainder of the manuscript only classification using this cut-off are presented.

Differences between key characteristics of the two phenotypes are summarised in **Table 2**. As with prior studies, APACHE II score was higher in hyperinflammatory phenotype compared to the hypoinflammatory phenotype (17 vs 12; p = 0.0223). Mortality was also higher in the hyperinflammatory phenotype, although this did not reach statistical significance (63% vs 39%; p = 0.26; **Table 3**).

Creatinine and LDH were significantly higher in the hyperinflammatory phenotype (**Figure 2A** and **2B**). Lymphocyte counts were lower in the hypoinflammatory phenotype; however, these differences did not reach statistical significance (**Figure 2C**). Values of D-dimer (hypoinflammatory 1601 ng/mL vs 1643 ng/mL hyperinflammatory; p = 0.91) and CRP (hypoinflammatory 206 mg/dL vs 255 mg/dL hyperinflammatory; p = 0.78) were similar between the phenotypes. Vital signs and respiratory variables were also similar between the two phenotypes (**Table 2**). In contrast to prior studies, vasopressor-use was equally prevalent between the two phenotypes: hypoinflammatory 5/8 (61%) vs 19/31 (63%) hyperinflammatory (p = 0.99).

Comparison with a historical ARDS cohort

The entire HARP-2 cohort (n = 539) had a similar age range (median 54, IQR: 42 – 66) to the COVID-19 cohort. The median PaO_2/FiO_2 in HARP-2 was 15 kPa (11 – 21) compared to 18 kPa (15 – 21; p = 0.07) in the COVID-19 cohort. Median APACHE II score in HARP-2 (15, IQR: 11 -21) was significantly higher than the COVID-19 cohort (12, IQR: 10 – 16; p < 0.0001). **Table S3** (supplement p5) shows a comparison of the variables used to match the COVID-19 cohort with HARP-2

matched cohort (n = 39); these observations suggest good matching between the two cohorts. Differences in the baseline characteristics of the entire HARP-2 cohort, the HARP-2 matched cohort and COVID-19 cohort are presented in **Table S4**. APACHE II score was significantly higher in the HARP-2 matched cohort compared to the COVID-19 cohort ($p < 0.0001$; **Figure 3A**). There were no significant differences in levels of IL-6 between the two cohorts ($p = 0.35$; **Figure 3B**), whereas sTNFR-1 was significantly lower in the COVID-19 cohort ($p = 0.0258$; **Figure 3C**). Platelets ($p = 0.0068$; **Figure 3D**) were significantly higher in COVID-19. There was no significant difference in creatinine levels between HARP-2 matched and the COVID-19 cohorts (**Figure 3E**).

Despite the lower APACHE II score and similar PaO_2/FiO_2 ratio, percentage mortality at day 28 in the COVID-19 cohort (17/39, 44%) was significantly higher than HARP-2 (132/539, 24%; $p = 0.0128$) and HARP-2 matched (11/39, 28%), though this comparison was not statistically significant ($p = 0.16$). Using the Youden Index to assign class, the COVID-19 cohort had a smaller proportion of patients classified in the hyperinflammatory phenotype 8/39 (21%) compared to both the entire HARP-2 cohort 186/539 (35%) and HARP-2 matched 11/39 (28%; **Table 3**). Mortality at day 28 in the hypoinflammatory phenotype 12/31 (39%) in the COVID-19 cohort was higher than those observed in this phenotype in the two HARP-2 cohorts: whole cohort: 59/353 (17%); matched cohort: 6/28 (21%; **Table 3**). Interestingly, the mortality rate in the COVID-19 hypoinflammatory phenotype was comparable to the mortality rate in the hyperinflammatory phenotype in HARP-2 and HARP-2 matched (**Table 3**). In contrast, the hyperinflammatory phenotype in the COVID-19 cohort had mortality rates approaching 5/8 63%.

A sensitivity analysis was performed by excluding the patients from UCH and the findings were similar to those presented (data not shown).

Discussion

To our knowledge, this study is the first that has sought to identify the prevalence of previously described ARDS phenotypes in the COVID-19 ARDS population. The findings of this preliminary study of 39 patients with COVID-19 associated ARDS suggest that the prevalence of the hyperinflammatory phenotypes was low in the studied population (10% - 21%). Mortality rates were approximately 20% higher in the hyperinflammatory phenotype compared to the hypoinflammatory phenotype, in keeping with previous findings. However, whilst the magnitude of difference in mortality between the phenotypes was consistent, the mortality rate in both phenotypes was considerably higher compared to historical ARDS data. A second novel feature of the study was the use of a rapid point of care assay to quantify both IL-6 and sTNFR-1, both of which had similar or lower levels in the patients with COVID-19 ARDS compared to prior ARDS patients.

The hyperinflammatory phenotype of ARDS is associated with higher circulating levels of pro-inflammatory biomarkers such as IL-6, IL-8 and sTNFR-1 and lower levels of Protein C. Further, this phenotype is associated with increased evidence of multi-organ failure and shock.[8-11] The low prevalence of the hyperinflammatory phenotype in COVID-19 ARDS challenges the hypothesis of the "cytokine storm" in its pathogenesis and suggests that it may not be as

ubiquitous as purported and less frequently encountered than in ARDS secondary to other causes.

The high mortality rate in the hypoinflammatory phenotype in COVID-19 is a notable and novel finding of this study. In prior studies, mortality in the hypoinflammatory phenotype was approximately 20%.[8-11,15] The mortality in the hypoinflammatory phenotype in COVID-19, however, was nearly double. Coupled with the lower burden of systemic inflammatory responses, at least as measured by IL-6 and TNFr-1, the findings of higher mortality rates in COVID-19 suggests severity of pathogenesis not captured by these inflammatory biomarkers. The differences in mortality compared to pneumonia patients in the HARP-2 matched cohort, where the infective pathogen is more likely to be bacterial, may allude to the pathogenesis of SARS-CoV-2 and an absence of therapeutic options for source control in COVID-19 ARDS. A second factor to consider is whether attributable mortality in these patients differs. Whereas in non-COVID-19 ARDS multiorgan failure is frequently encountered as the attributable factor for death,[16] in COVID-19, reports suggest that a greater proportion of patients are dying due to respiratory failure,[13] a physiological abnormality that may be pathologically independent of systemic inflammation and subject to more localized injury to the lungs.

It is also worth noting that the APACHE II scores in the COVID-19 population were significantly lower compared to HARP-2 despite the higher mortality in the former. All COVID-19 patients in this study were managed in ICUs in surge capacity with relaxed nursing ratio and may, in part, explain this finding. Overwhelmed ICU capacity may have an impact on outcomes in COVID-19

and lower mortality rates have been reported in ICUs that have operated under more conventional conditions and staffing ratios in COVID-19 patients with similar APACHE II scores.[17,18] The relatively low APACHE II scores are also in keeping with those reported by the Intensive Care National Audit and Research Centre in 9777 patients admitted to the ICU in the National Health Service hospitals in the U.K., where the median APACHE II score in COVID-19 was 14 (IQR 11 – 18) and a mortality rate that was greater than 40% (ICNARC report on COVID-19 in critical care 12 June 2020). These consistent findings suggest that the APACHE II score may not be valid for prognostication in COVID-19. Taken together, the findings of the low APACHE II score and high mortality, suggest that alternative phenotyping approaches may be needed to identify biologically and clinically homogeneous clusters using novel biomarkers that may, in turn, enhance our understanding of pathogenesis and improve prognostication in COVID-19 ARDS.

One advantage of specifically studying the COVID-19 population is that the heterogeneity of aetiology, a common feature of non-COVID-ARDS, is largely negated. It is interesting to note that the prevalence of vasopressor-use at baseline was similar between the two phenotypes, whereas in prior studies, vasopressor-use was significantly higher in the hyperinflammatory phenotype. This may in part be explained by the fact that in prior studies the risk factor for ARDS differed between the phenotypes with sepsis predominantly featuring in the hyperinflammatory phenotype. In COVID-19, given the uniformity of aetiology, it may be that there are additional drivers of vasopressor-use that are disease-specific and extraneous to inflammatory phenotypes, such as cardiovascular complications.[19]

It is also known that aetiology is an important determinant of the signature of circulating biomarkers.[20] For example, indirect causes of lung injury, such as sepsis, are associated with higher levels of endothelial injury, whereas direct lung injury is associated with higher levels of markers of epithelial injury.[21] Biomarkers pertaining to severity of epithelial injury and cell death may be more informative in COVID-19 ARDS as the primary source of injury is presumed to be a viral pneumonitis. In two recent case series of autopsies of patients with severe COVID-19, the only common findings in all patients across both studies was diffuse alveolar damage.[22,23] Currently, however, this theory remains speculative, and it stands to reason that prior to phenotyping, we need to more comprehensively "type" COVID-19 and its biological signature using data, preferably from large-multinational collaboratives such as ISARIC 4C (https://isaric4c.net/).

Another strength of this study has been to demonstrate the logistical feasibility of rapid, point-of-care, phenotyping of patients in a busy ICU using a novel bioanalyser. Precision-based care has been a promising, yet elusive, opportunity in critical care medicine.[24] Whilst other specialties have the luxury of time, in the ICU any phenotype-based decisions need to be made rapidly. The time to undertake ELISA based assays have been prohibitive in the clinical implementation of biomarker-driven phenotypes.[20] Using this novel solid state-based analysing technology, we were able to classify patients into biomarker-driven phenotypes in under one hour from sample acquisition. The availability of such assays has important implications for future precision medicine studies in critical care.

Paradoxically, this strength is also a limitation of the study. The larger PHIND study, from which this COVID-19 subset was derived, was designed in order to further validate the above-mentioned POC platform. Currently, the POC platform has only been validated using stored plasma samples, and its performance using real samples from ICU patients is yet to be formally validated. Given this uncertainty, the findings of this study should be interpreted with caution. The clinically measured biomarker component of the model, namely bicarbonate, can often be informative of the validity of the distribution of the phenotypes. In a prior ARDS cohort, where the prevalence of hyperinflammatory phenotype was 37%, the mean serum bicarbonate level was 22 mmol/L ($\pm$ 6) compare to 27 mmol/L ($\pm$ 6) in the presented COVID-19 cohort.[11] This comparison suggests that the estimated prevalence of the hyperinflammatory phenotype between 10-20% in this cohort has face validity.

The key limitation of this study is the relatively small sample size. The even smaller number in the hyperinflammatory phenotype and the observed sample size imbalance when comparing phenotypes makes comparative statistics difficult to interpret, and differences between groups must be interpreted with caution. A further limitation of the study is that it is focused on baseline data only for phenotype classification. The natural progression of COVID-19 over time may lead to changing phenotypes and requires further studies. Another important limitation is that only circulating levels of two biomarkers were studied. Inflammatory markers may differ more substantively in the lungs. In addition, if a larger number of plasma inflammatory biomarkers were studied in a larger population, more distinct patterns of differences in the inflammatory response may have been detected. Further, we were unable to validate the biomarkers

quantified using the Multistat analyser against conventional enzyme-linked immunosorbent assays due to a lack of stored plasma samples in COVID-19 patients. Future studies of COVID-19 pneumonia, where feasible, should study the circulating and lung compartments simultaneously and over the course of COVID-19 critical illness.

In summary, in this small exploratory analysis of 39 patients, the prevalence of the hyperinflammatory phenotype in COVID-19 ARDS was lower compared to historical data. This finding suggests that, compared to non-COVID-19 ARDS the excessive mortality observed in COVID-19 ARDS is unlikely to be due to upregulation of the inflammatory pathways described by the parsimonious model. Finally, with the caveat that the findings require validation with LCA-derived phenotypes, the POC platform used to classify phenotypes at the bedside demonstrates the feasibility of conducting phenotype-informed trials in the ICU.

Research in Context

Evidence before the study: Two phenotypes of ARDS have consistently been identified in randomised controlled trials with divergent characteristics, clinical outcomes and treatment responses. The hyperinflammatory phenotypes had more severe plasma inflammatory responses and worse outcomes. It has been hypothesized that the "cytokine storm" is integral to the pathogenesis of severe COVID-19. The prevalence of this phenotype in COVID-19 related ARDS was unknown.

Added value of this study: Using a previously validated parsimonious model and a point-of-care biomarker analyser, in this preliminary report, we classified 39 patients with COVID-19 ARDS into hypo- and hyper-inflammatory phenotypes. In comparison to historical cohorts of ARDS, the prevalence of the hyper-inflammatory phenotype was lower, with mortality at day 28 higher in the hyper-inflammatory phenotype. Mortality in both phenotypes was higher compared to historical data.

Implications of all the available evidence: The findings of this exploratory study suggest that the hyperinflammatory phenotype of ARDS is less prevalent in COVID-19 than in previous ARDS cohorts, undermining the theory that "cytokine storm" is disproportionately characteristic of COVID-19. Future studies are needed to confirm these findings and to better understand the pathophysiology driving poor COVID-19 ARDS outcomes.

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Figures

Figure 1. Correlation matrix of the biomarkers measure at baseline in the COVID-19 ARDS cohort. Blue colour denotes positive correlation and red negative correlation. Increased size and darkness of the circles represents higher correlation. Coefficients are derived using the spearman's correlation test.

Figure 2. Differences in selected variables between the hyperinflammatory and hypoinflammatory phenotypes. Phenotypes were assigned using the Youden Index as the cut-off ($\geq$ 0.274). P-values represent the Wilcoxon-rank test. **Figure 2A:** Creatinine. **Figure 2B:** Lactate dehydrogenase. **Figure 2C:** Lymphocyte count.

Figure 3: Differences in key variables between the COVID-19 ARDS cohort and the HARP-2 Matched cohort. P-values represent the Wilcoxon-rank test. **Figure 3A:** APACHE-II score. **Figure 3B:** Interleukin-6. **Figure 3C:** Soluble tumour necrosis factor receptor-1. **Figure 3D:** Platelets **Figure 3E:** Creatinine.

Table 1. Baseline characteristics of the cohort. The cohort is stratified into groups of survivors and non-survivors. The P-value represent the Wilcoxon-rank test unless denoted otherwise.

	Total Population (n= 39)	Survivors (n= 22)	Non-survivors (n= 17)	P-value
Age (Years)	57 (52 – 61)	54 (45 – 57)	60 (56 – 64)	**0.0036**
Gender: Male	25 (64%)	11 (50%)	14 (82%)	**0.0490**[a]
Race				
White	19 (49%)	10 (45%)	9 (53%)	0.40 [a]
Asian	9 (23%)	4 (18%)	5 (29%)	
Black	4 (10%)	2 (9%)	2 (12%)	
Other	7 (18%)	6 (27%)	1 (6%)	
Diabetes Mellitus	9 (23%)	6 (27%)	3 (18%)	0.70 [a]
Hypertension	6 (15%)	2 (9%)	4 (24%)	0.37 [a]
Heart Rate (beats/min)	103 (81 – 142)	106 (84 – 153)	98 (79 – 130)	0.34
Mean Arterial Pressure (mmHg)	64 (61 – 72)	64 (61 – 69)	65 (61 – 72)	0.60
PaO_2/FiO_2 (kPa)	18 (15 – 21)	20 (17 – 24)	15 (11 – 18)	**0.0040**
Minute Ventilation (L/min)	10.5 (9.4 – 12.1)	10.2 (9.3 – 12.2)	10.8 (9.8 – 11.2)	0.60
Plateau Pressure ($cm.H_2O$)	31 (27 – 34)	30 (27 – 34)	31 (26 – 34)	0.82
PEEP ($cm.H_2O$)	12 (6 – 20)	13 (12 – 15)	12 (10 – 15)	0.37
Compliance ($mL/cm.H_2O$)	24 (20 – 28)	24 (21 – 28)	25 (20 – 29)	0.79
White Blood Cells ($x\ 10^9/L$)	10 (8 – 12)	8.6 (7.8 – 12)	10.4 (9.7 – 14.2)	0.25
Lymphocytes ($x\ 10^9/L$)	1 (0.6 – 1.1)	0.90 (0.6 – 1.1)	1 (0.6 – 1.4)	0.56
Platelets ($x\ 10^9/L$)	272 (213 – 330)	285 (236 – 332)	244 (177 – 319)	0.16
Albumin (g/L)	23 (20 – 26)	24 (20 – 27)	23 (20 – 25)	0.61
Bilirubin (μmol/L)	10 (6 – 23)	8 (6 – 12)	23 (9 – 40)	**0.0235**
Bicarbonate (mmol/L)	26 (24 – 30)	27 (24 – 31)	25 (23 – 27)	0.32
Creatinine (μmol/L)	84 (65 – 172)	74 (63 – 165)	94 (74 – 201)	0.19
Troponin (ng/L)	18 (5 – 37)	9 (5 – 21)	23 (12 – 58)	0.0549
Lactate Dehydrogenase (units/L)	458 (336 – 591)	439 (343 – 499)	530 (307 – 732)	0.24
Procalcitonin (ng/mL)	1.2 (0.4 – 2.9)	1.2 (0.3 – 2.9)	1.7 (0.9 – 7.1)	0.28
Fibrinogen (g/L)	6.6 (5.8 – 6.8)	6.4 (5.8 – 6.6)	6.6 (6.2 – 7.1)	0.0520
D-Dimer (ng/mL)	1622 (888 – 3742)	1089 (815 – 2262)	3730 (1604 – 5640)	**0.0187**
Ferritin (mcg/L)	1196 (421 – 2825)	806 (382 – 1613)	2178 (471 – 2947)	0.12
C-Reactive Protein (mg/L)	214 (154 – 320)	199 (145 – 322)	277 (205 – 293)	0.19
Interleukin-6 (pg/mL)	192 (112 – 556)	149 (84 – 270)	457 (192 – 1042)	**0.0048**
sTNFR-1 (pg/mL)	3150 (2455 – 4405)	2735 (2323 – 3705)	4200 (3030 – 4590)	**0.0197**
Vasopressor-use (baseline)	24 (62%)	14 (64%)	10 (59%)	0.99 [a]
Invasive Ventilation (baseline)	35 (90%)	21 (95%)	14 (82%)	0.44[a]
SOFA Score	6 (5 – 8)	6 (4 – 7)	7 (6 – 9)	0.09
APACHE II score	12 (10 – 16)	12 (10 – 15)	14 (11 – 16)	0.26

PEEP = Positive End-Expiratory Pressure. sTNFR-1 = Soluble Tumour Necrosis Factor-1. a = Fisher's exact test.

Table 2 Difference in baseline characteristics between the hypoinflammatory and hyperinflammatory phenotypes using a probability cut-off of ≥ 0.274 (Youden Index) to assign class. P-values represent the Wilcoxon rank test unless annotated otherwise.

	Hypoinflammatory (n = 31)	Hyperinflammatory (n = 8)	P-value
Age (Years)	57 (53 – 61)	57 (46 – 60)	0.55
Gender: Male	19 (63%)	6 (75%)	0.69 [a]
Race			
White	17 (55%)	2 (25%)	0.38 [a]
Asian	6 (19%)	3 (37%)	
Black	3 (10%)	1 (13%)	
Other	5 (16%)	2 (25%)	
Diabetes Mellitus	7 (23%)	2 (25%)	0.99 [a]
Hypertension	6 (19%)	0 (0%)	0.31 [a]
Heart Rate (beats/min)	98 (77 – 141)	104 (97 – 144)	0.44
Mean Arterial Pressure (mmHg)	64 (61 – 71)	70 (60 – 75)	0.64
PaO_2/FiO_2 (kPa)	18 (16 – 22)	17 (11 – 21)	0.27
Minute Ventilation (L/min)	10.2 (9.4 – 11.3)	10.6 (9.3 – 13.0)	0.75
Plateau Pressure ($cm.H_2O$)	31 (26 – 34)	31 (28 – 34)	0.98
PEEP ($cm.H_2O$)	12 (12 – 15)	12 (11 – 15)	0.83
Compliance ($mL/cm.H_2O$)	24 (20 – 28)	27 (21 – 29)	0.68
White Blood Cells ($x\ 10^9/L$)	9.9 (7.6 – 12.2)	10.6 (9.1 – 12.7)	0.30
Lymphocytes ($x\ 10^9/L$)	0.8 (0.6 – 1.1)	1.1 (1.0 – 1.4)	0.06
Platelets ($x\ 10^9/L$)	272 (216 – 314)	259 (197 – 314)	0.48
Albumin (g/L)	23 (20 – 27)	24 (22 – 25)	0.96
Bilirubin (μmol/L)	10 (6 – 21)	12 (8 – 28)	0.55
Creatinine (μmol/L)	78 (63 – 130)	216 (104 – 275)	**0.0217**
Troponin (ng/L)	18 (5 – 29)	23 (8 – 220)	0.34
Lactate Dehydrogenase (units/L)	439 (315 – 534)	597 (534 – 758)	**0.0392**
Procalcitonin (ng/mL)	0.9 (0.4 – 2.9)	2.6 (1.6 – 10.5)	0.14
Fibrinogen (g/L)	6.6 (6.0 – 6.8)	5.8 (5.4 – 6.8)	0.39
D-Dimer (ng/mL)	1601 (873 – 4081)	1643 (1126 – 3226)	0.91
Ferritin (mcg/L)	807 (422 – 1855)	2878 (1229 – 4225)	0.21
C-Reactive Protein (mg/L)	206 (145 – 304)	255 (145 – 348)	0.78
Vasopressor-use (baseline)	19 (61%)	5 (63%)	0.99 [a]
Invasive Ventilation (baseline)	28 (90%)	7 (87.5%)	0.76
SOFA Score	6 (5 – 8)	8 (6 – 10)	0.10
APACHE II score	12 (10 – 15)	17 (16 – 18)	**0.0223**
Mortality at Day 28	12 (39%)	5 (63%)	0.26[a]

PEEP = Positive End-Expiratory Pressure. sTNFR-1 = Soluble Tumour Necrosis Factor-1. a = Fisher's exact test.

Table 3. Comparison of mortality at day 28 between HARP-2, HARP-2 matched cohort, and the COVID-19 PHIND cohort. In HARP-2 and HARP-2 matched cohorts the phenotypes were derived from the original LCA studies. In COVID-19 the phenotypes were derived using the parsimonious model using a probability cut-off of 0.274 (Youden Index).

	Total Cohort		Hypoinflammatory		Hyperinflammatory	
	N	**Mortality**	**N**	**Mortality**	**N**	**Mortality**
HARP-2	539	132 (24%)	353 (65%)	59 (17%)	186 (35%)	73 (39%)
HARP-2 Matched	39	11 (28%)	28 (72%)	6 (21%)	11 (28%)	5 (45%)
COVID-19	39	17 (44%)	31 (79%)	12 (39%)	8 (21%)	5 (63%)

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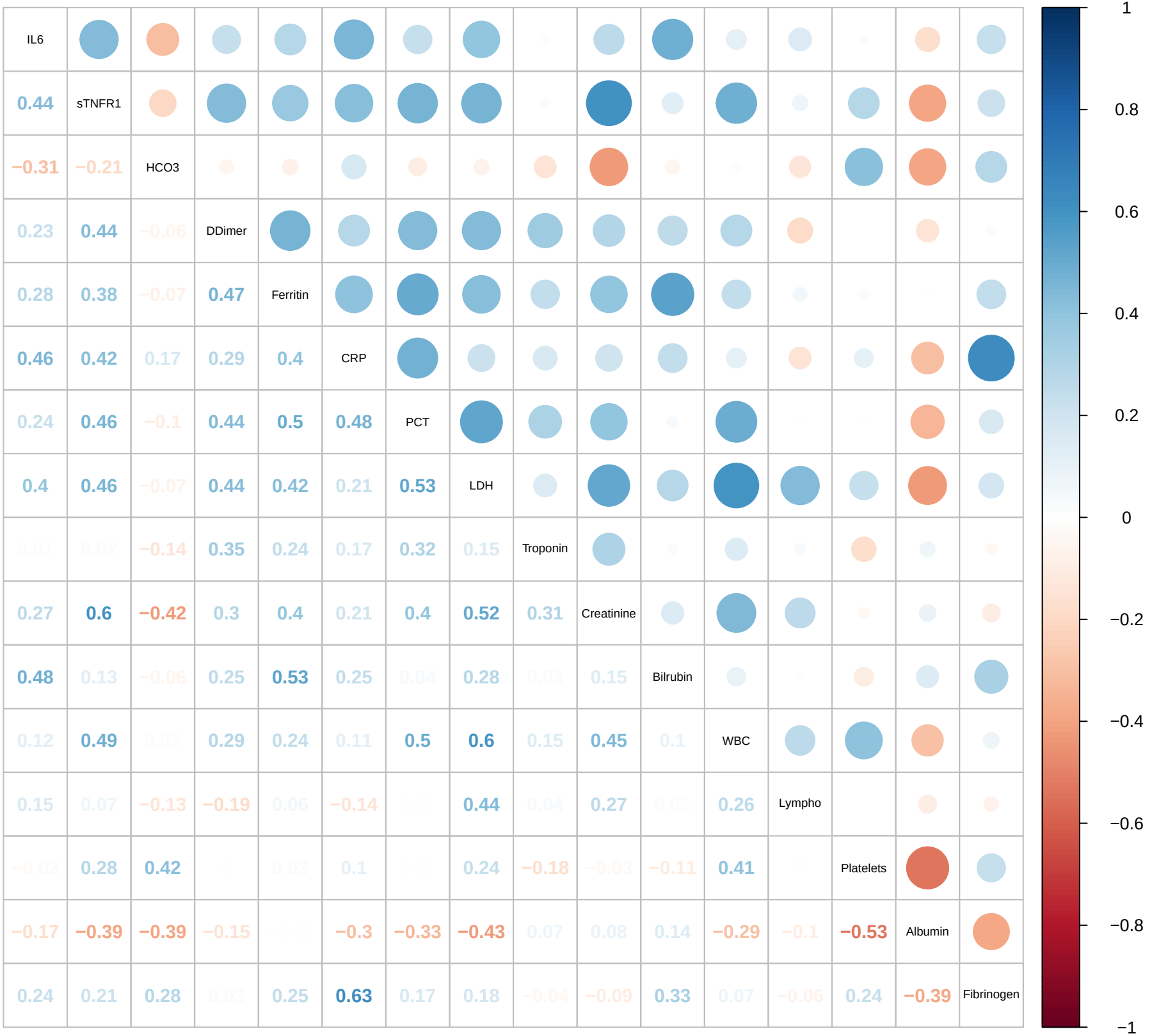
IL6
0.44 sTNFR1
−0.31 −0.21 HCO3
0.23 0.44 −0.06 DDimer
0.28 0.38 −0.07 0.47 Ferritin
0.46 0.42 0.17 0.29 0.4 CRP
0.24 0.46 −0.1 0.44 0.5 0.48 PCT
0.4 0.46 −0.07 0.44 0.42 0.21 0.53 LDH
0.01 0.02 −0.14 0.35 0.24 0.17 0.32 0.15 Troponin
0.27 0.6 −0.42 0.3 0.4 0.21 0.4 0.52 0.31 Creatinine
0.48 0.13 −0.06 0.25 0.53 0.25 0.04 0.28 0.03 0.15 Bilrubin
0.12 0.49 0.02 0.29 0.24 0.11 0.5 0.6 0.15 0.45 0.1 WBC
0.15 0.07 −0.13 −0.19 0.06 −0.14 0.03 0.44 0.04 0.27 0.02 0.26 Lympho
−0.02 0.28 0.42 0 0.03 0.1 0.03 0.24 −0.18 −0.03 −0.11 0.41 0 Platelets
−0.17 −0.39 −0.39 −0.15 0.07 −0.3 −0.33 −0.43 0.07 0.08 0.14 −0.29 −0.1 −0.53 Albumin
0.24 0.21 0.28 0.03 0.25 0.63 0.17 0.18 −0.04 −0.09 0.33 0.07 −0.06 0.24 −0.39 Fibrinogen
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

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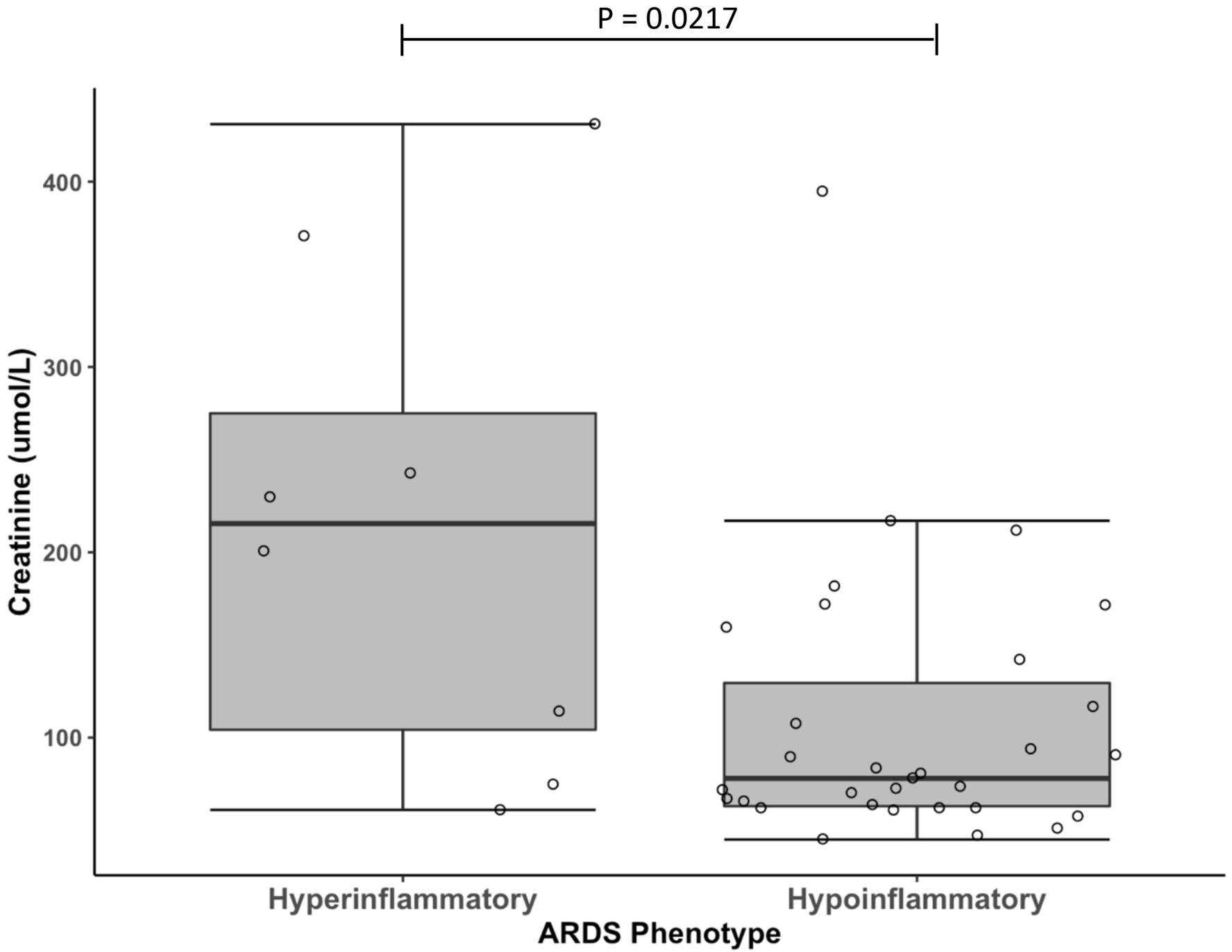
A

P = 0.0217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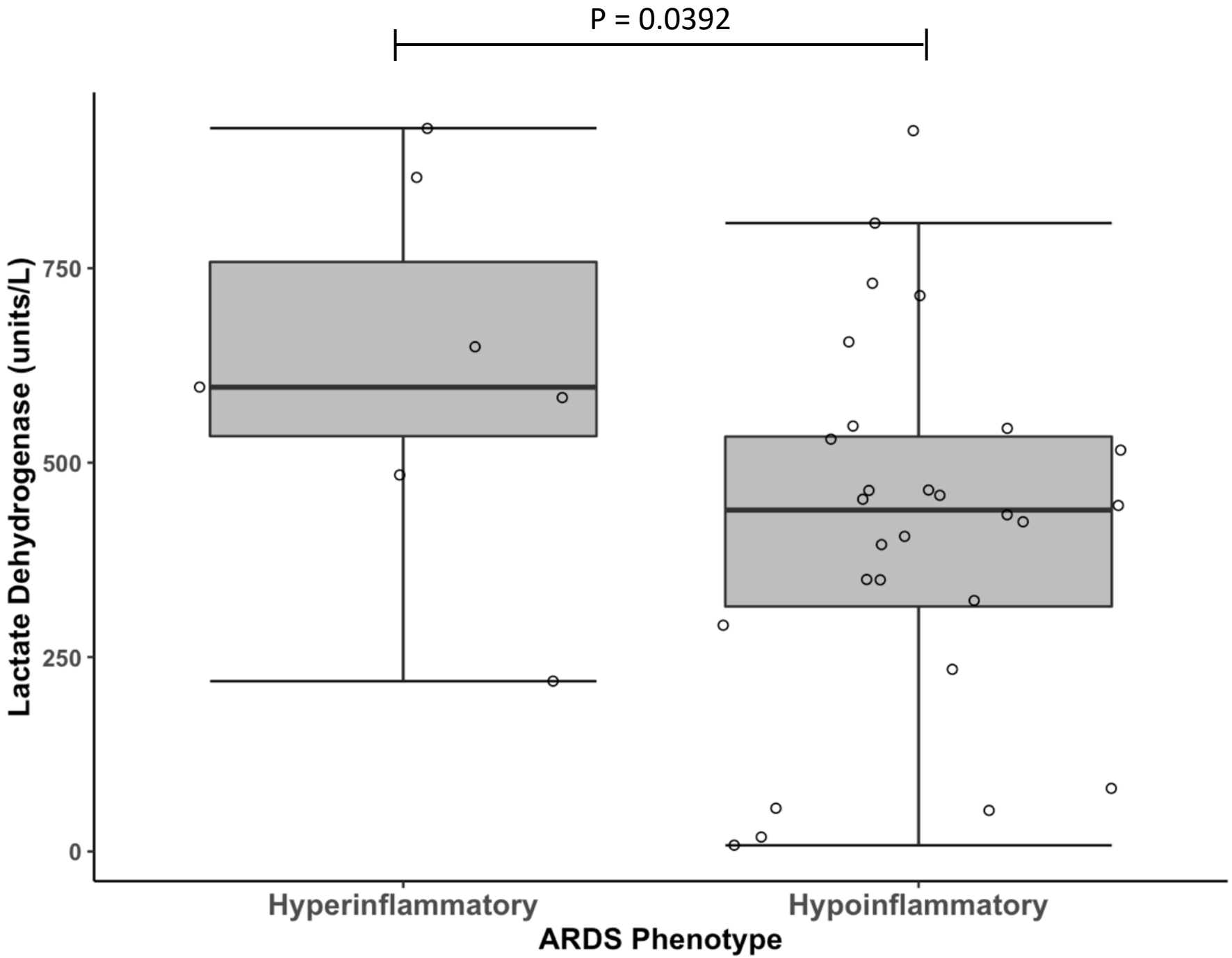
Creatinine (umol/L)
400
300
200
100
Hyperinflammatory
Hypoinflammatory
ARDS Phenotype

B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Lactate Dehydrogenase (units/L)
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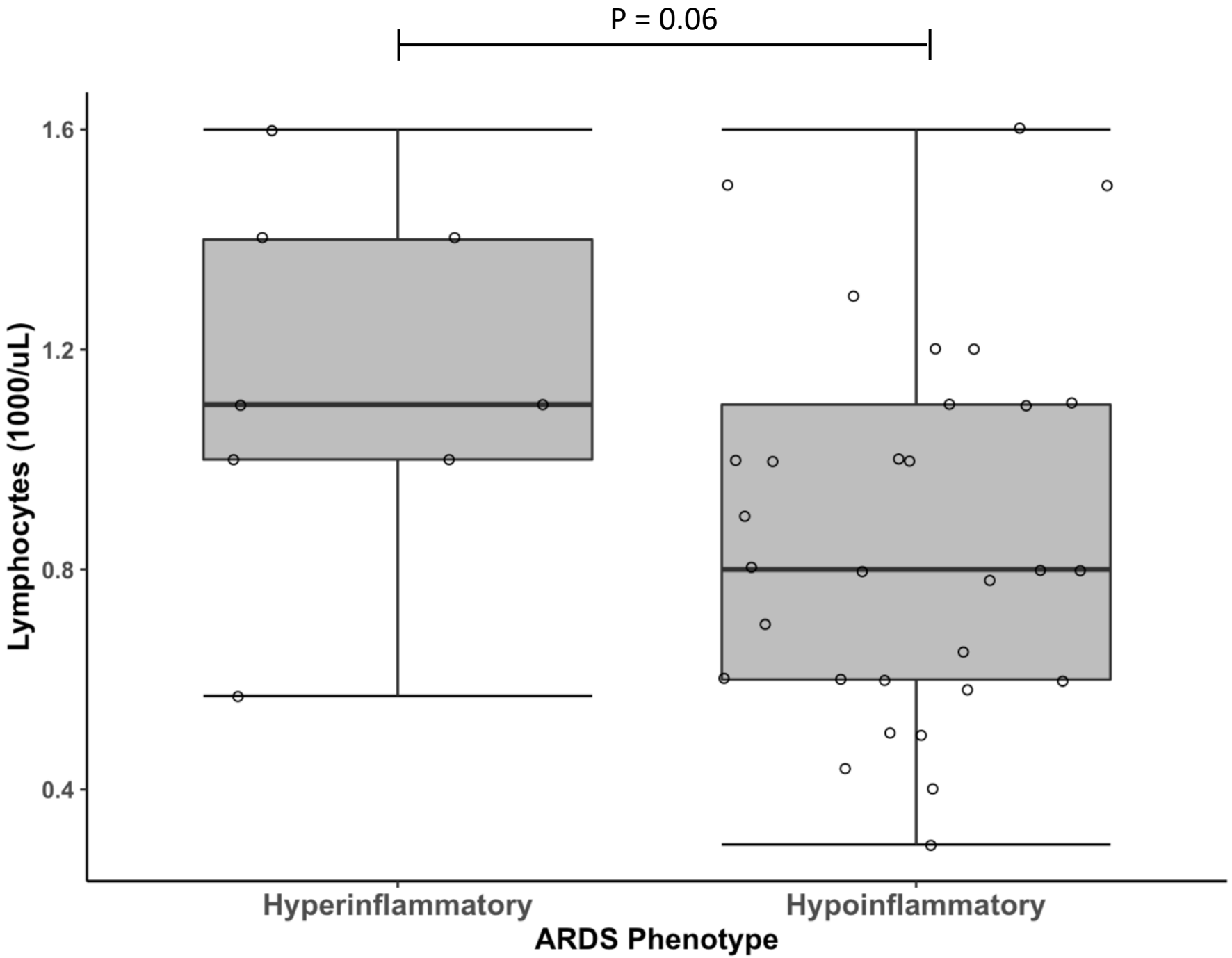
500
250
0
Hyperinflammatory
Hypoinflammatory
ARDS Phenotype

C


P = 0.06
1.6
1.2
0.8
0.4
Lymphocytes (1000/uL)
Hyperinflammatory
Hypoinflammatory
ARDS Phenotype

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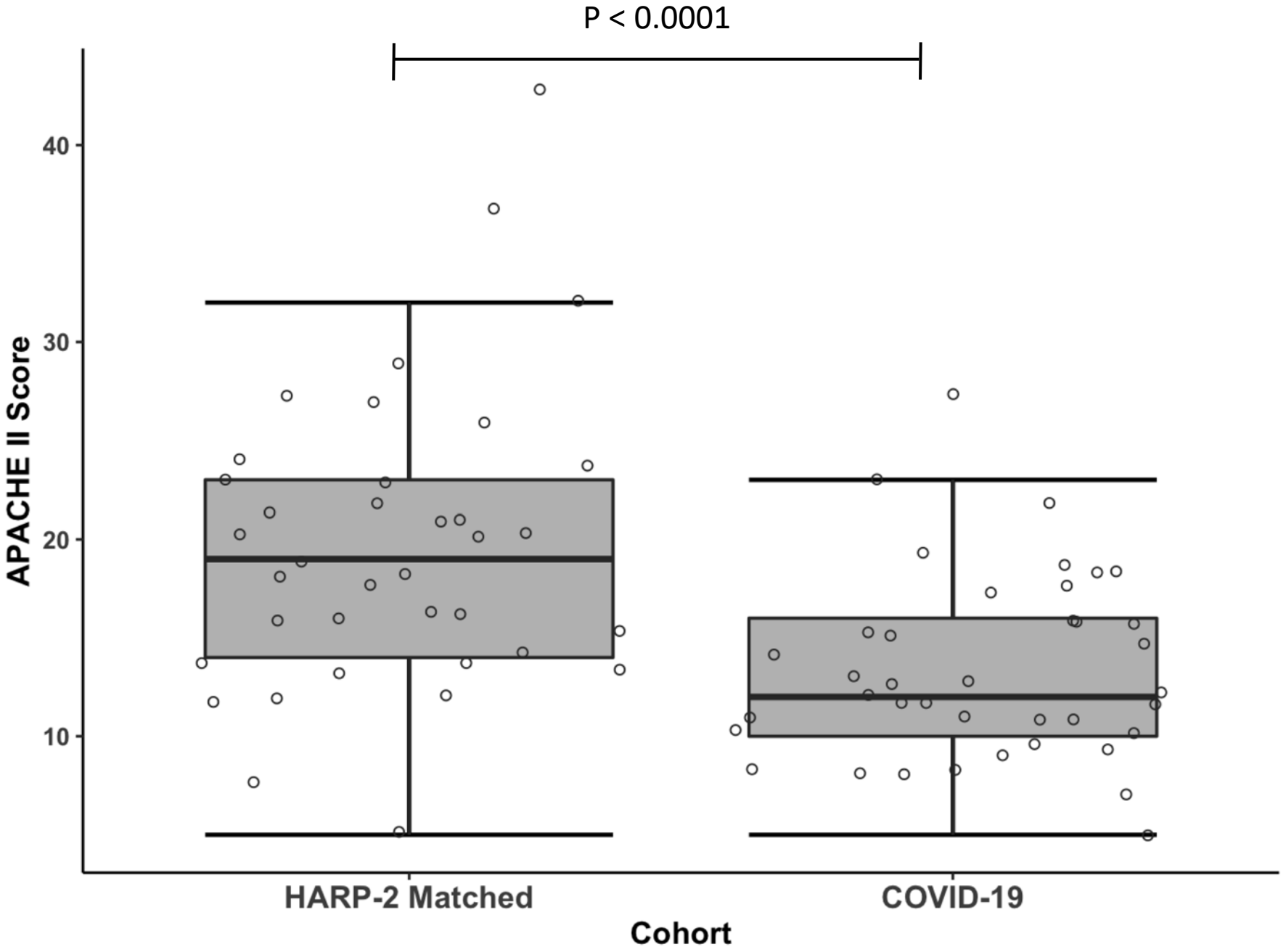
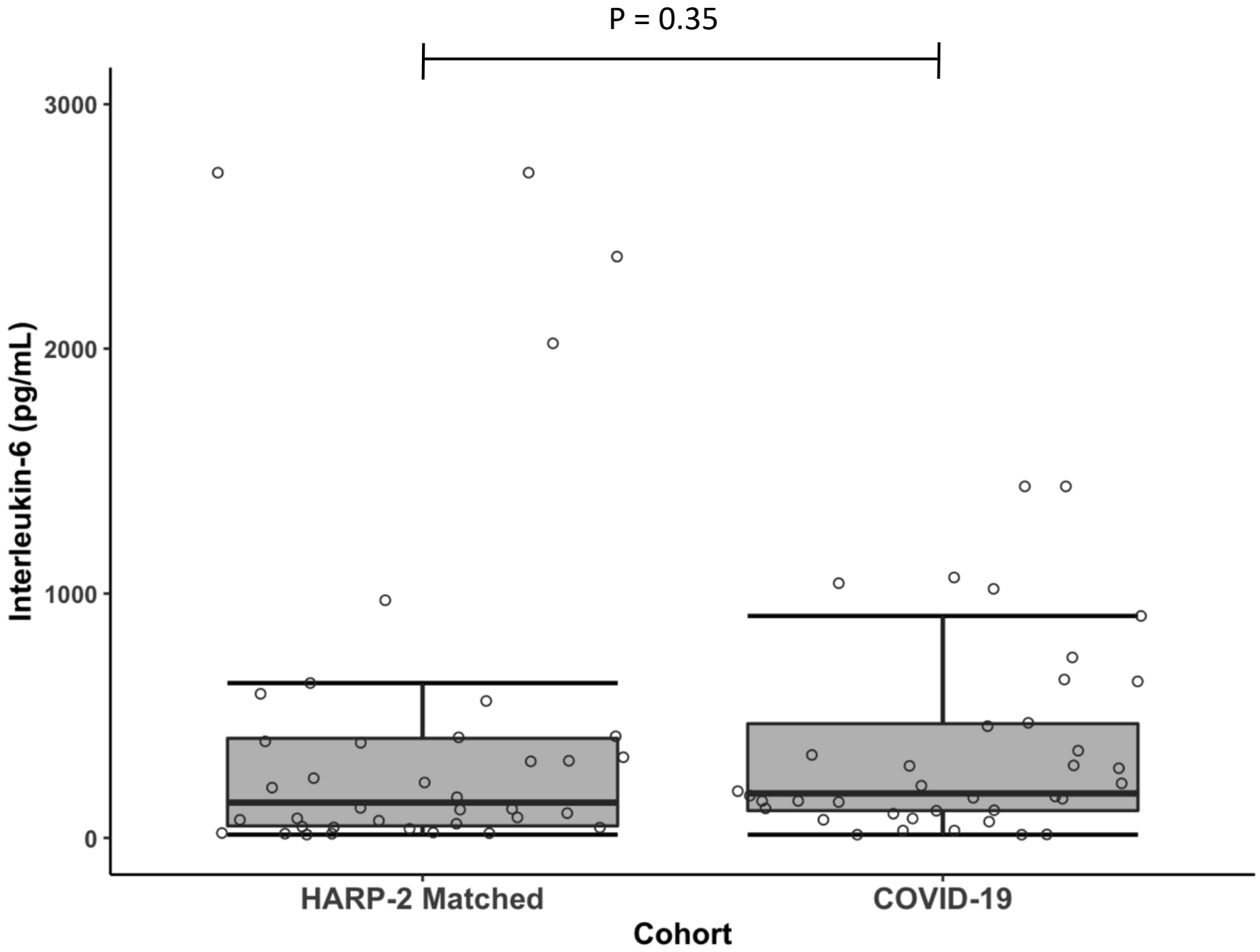
A


P < 0.0001
APACHE II Score
40
30
20
10
HARP-2 Matched
COVID-19
Cohort

B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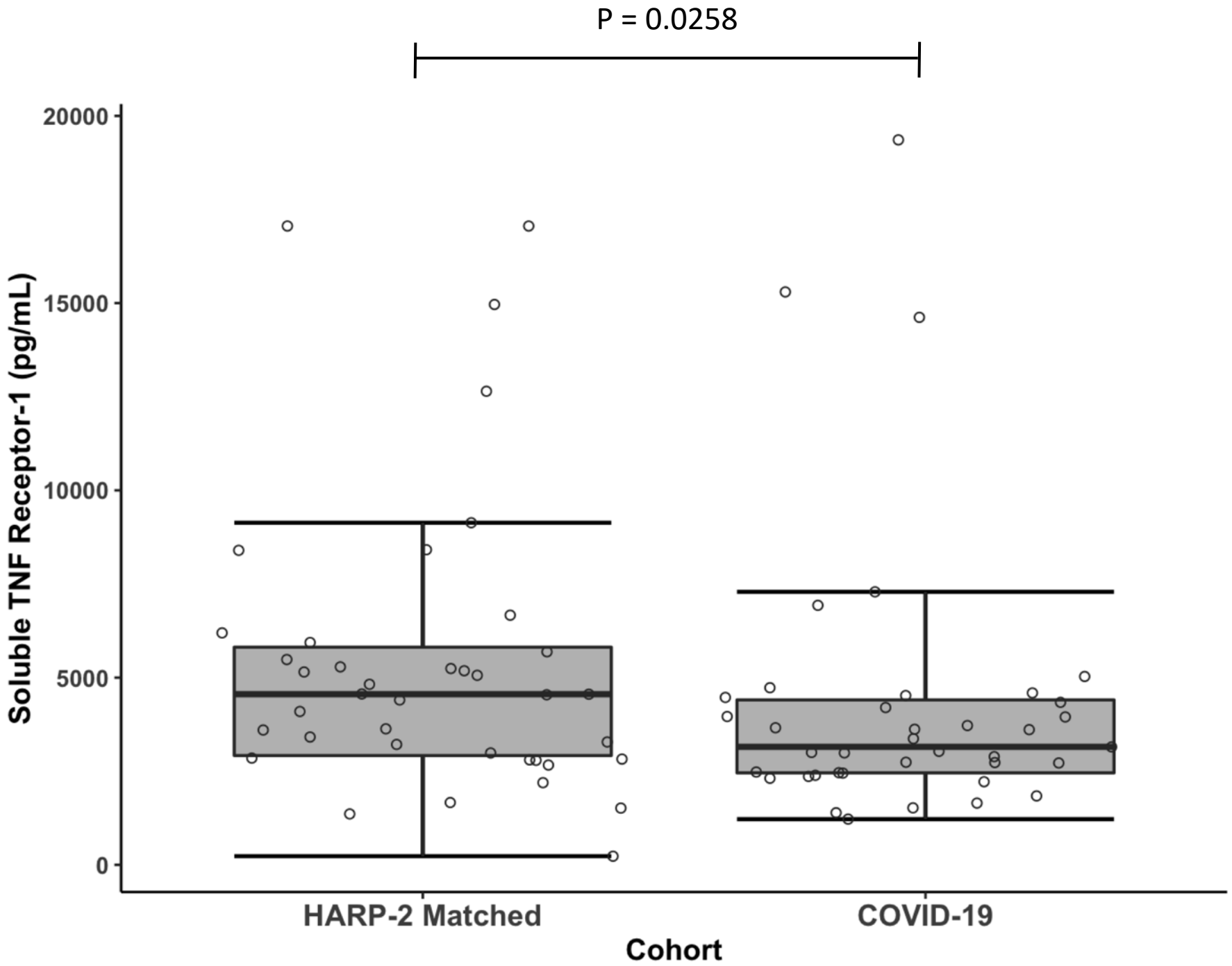
3000
2000
1000
0
Interleukin-6 (pg/mL)
HARP-2 Matched
COVID-19
Cohort

C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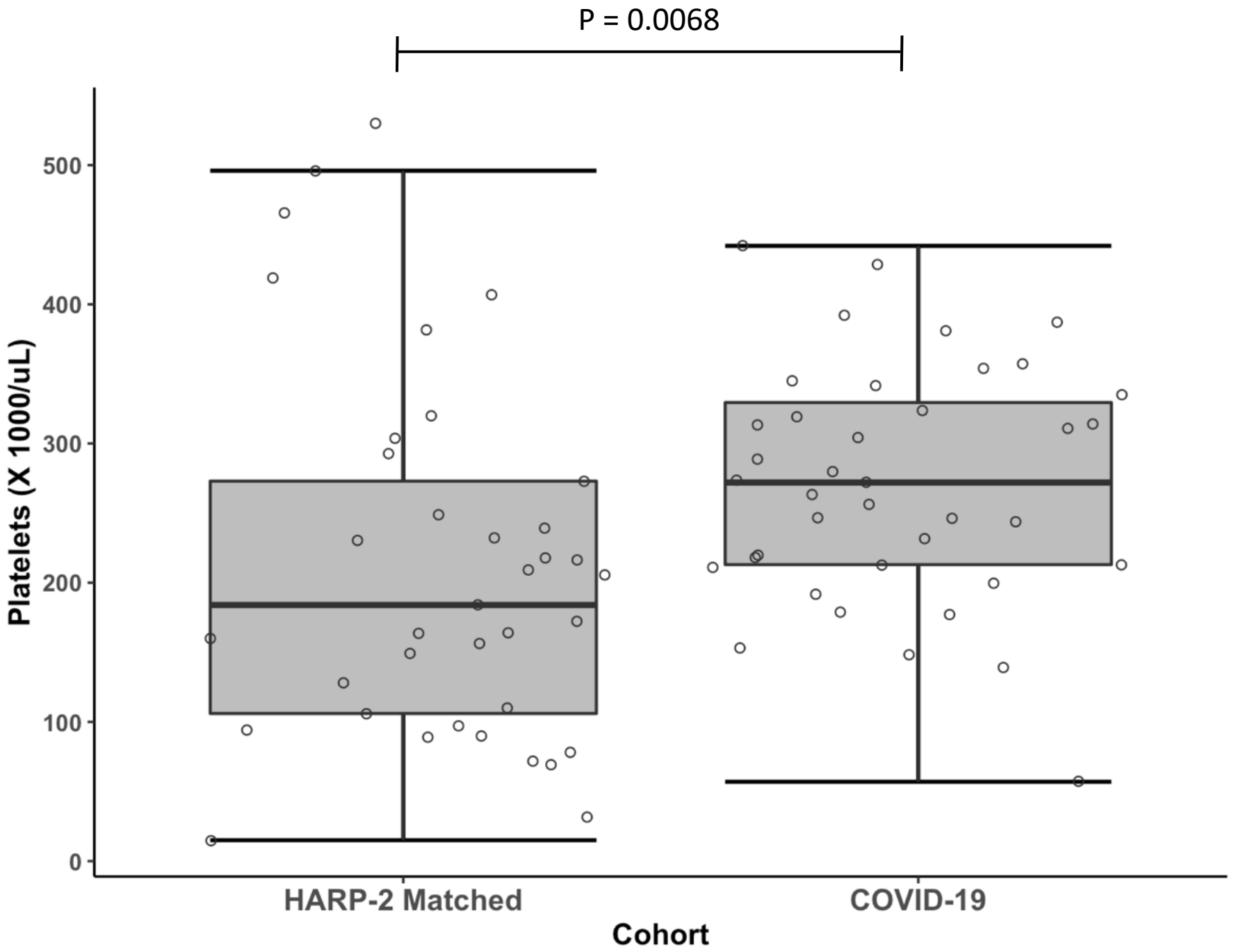
15000
10000
5000
0
Soluble TNF Receptor-1 (pg/mL)
HARP-2 Matched
COVID-19
Cohort

D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Platelets (X 1000/uL)
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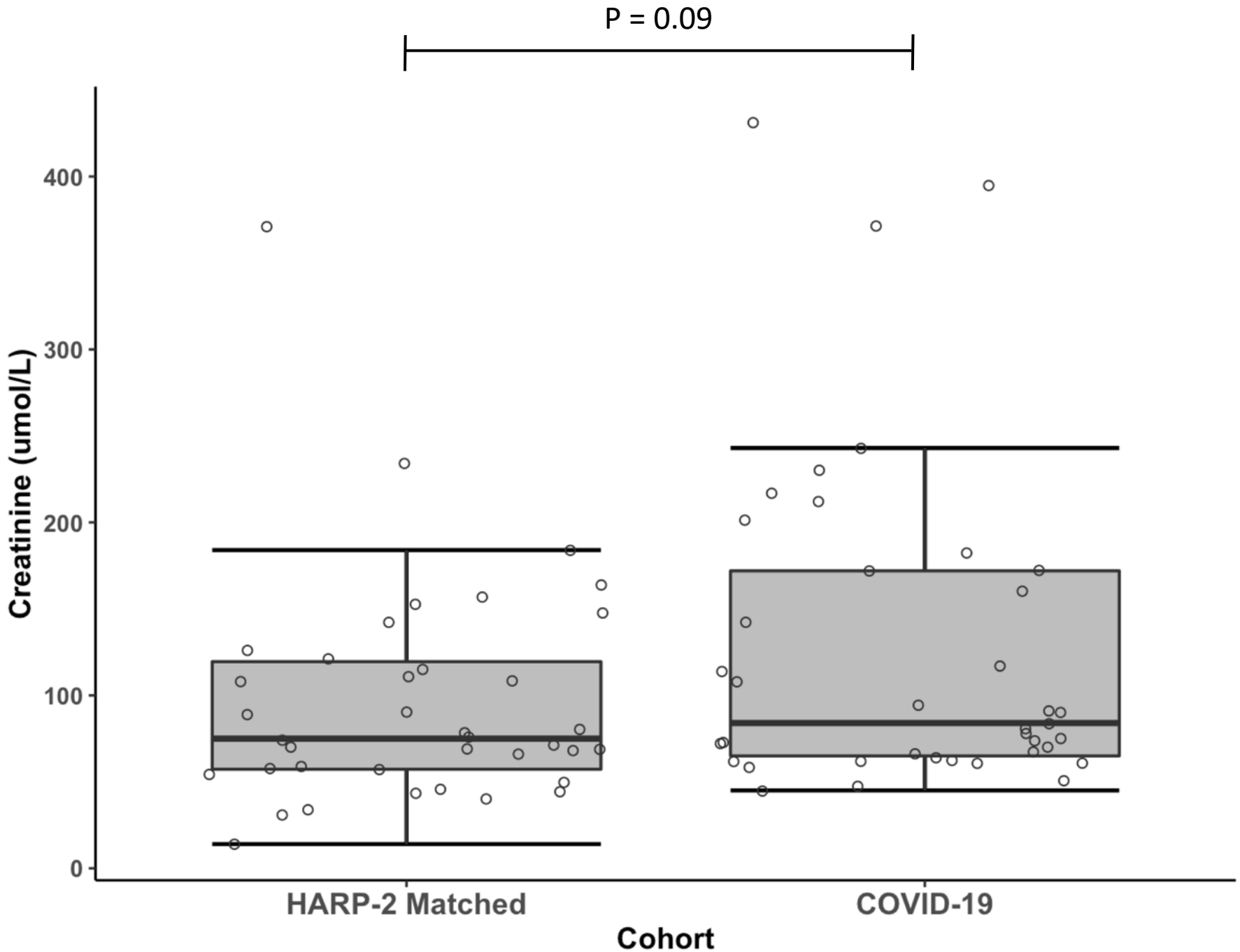
400
300
200
100
0
HARP-2 Matched
COVID-19
Cohort

E


P = 0.09
Creatinine (umol/L)
400
300
200
100
0
HARP-2 Matched
COVID-19
Cohort