Numbers and phenotype of non-classical CD14$^{\text{dim}}$CD16$^+$ monocytes are predictors of adverse clinical outcome in patients with coronary artery disease and severe SARS-CoV-2 infection

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Short title: Monocytes predict outcome in COVID-19 patients

Keywords: Coronary artery disease; SARS-CoV-2 infection; respiratory failure; immuno-response; non-classical monocytes
Abstract

**Aims:** To elucidate the prognostic role of monocytes in the immune response of patients with coronary artery disease (CAD) at risk for life-threatening heart and lung injury as major complications of SARS-CoV-2 infection.

**Methods and Results:** From February to April 2020, we prospectively studied a cohort of 96 participants comprising 47 consecutive patients with CAD and acute SARS-CoV-2 infection (CAD+SARS-CoV-2), 19 CAD patients without infections, and 30 healthy controls. Clinical assessment included blood sampling, echocardiography, and electrocardiography within 12 hours of admission. Respiratory failure was stratified by the Horovitz Index (HI) as moderately/severely impaired when HI ≤ 200mmHg. The clinical endpoint (EP) was defined as HI ≤ 200mmHg with subsequent mechanical ventilation within a follow-up of 30 days.

The numbers of CD14\(^{dim}\)CD16\(^+\) non-classical monocytes in peripheral blood were remarkably low in CAD+SARS-CoV-2 compared to CAD patients without infection and healthy controls (p<0.0001). Moreover, these CD14\(^{dim}\)CD16 monocytes showed decreased expression of established markers of adhesion, migration, and T cell activation (CD54, CD62L, CX3CR1, CD80, HLA-DR). Decreased numbers of CD14\(^{dim}\)CD16\(^+\) monocytes were associated with the occurrence of EP. Kaplan-Meier curves illustrate that CAD+SARS-CoV-2 patients with numbers below the median of CD14\(^{dim}\)CD16\(^+\) monocytes (median 1443 cells/mL) reached EP significantly more often compared to patients with numbers above the median (log-rank 5.03, p=0.025).

**Conclusion:** Decreased numbers of CD14\(^{dim}\)CD16\(^+\) monocytes are associated with rapidly progressive respiratory failure in CAD+SARS-CoV-2 patients. Intensified risk assessments comprising monocyte sub- and phenotypes may help to identify patients at risk for respiratory failure.

**Translational Perspective**

Patients with coronary artery disease (CAD) are at risk of life-threatening heart and lung injury accelerated by the pro-inflammatory and pro-thrombotic immune response during SARS-CoV-2 infection. We found substantially low numbers of CD14\(^{dim}\)CD16\(^+\) non-classical monocytes with an
altered phenotype suggesting impaired migration behaviour and T cell activation capacity in peripheral blood of SARS-CoV-2 patients with CAD, compared to CAD patients without infection or healthy controls. Decreased numbers of CD14^{dim}CD16^{+} monocytes predicted rapidly progressive respiratory failure (Horovitz index \leq 200 mmHg) with subsequent mechanical ventilation. Therefore, early sub- and phenotyping of CD14^{dim}CD16^{+} monocytes using simple flow cytometry might predict worsening of respiratory failure at an early stage of SARS-CoV-2 infection in high-risk CAD patients, who require an extensive heart failure and anti-thrombotic therapy to improve their clinical outcome.
1. Introduction

Patients with coronary artery disease (CAD) are at risk to develop severe courses of SARS-CoV-2 infection comprising life-threatening heart and lung injury which are some of the most frequent complications according to the current assessment of the European Society of Cardiology.\textsuperscript{1, 2} SARS-CoV-2 infection leads to a pro-inflammatory and pro-thrombotic immune response which, in the presence of CAD, can often lead to acute coronary syndrome with subsequent impairment of left or right ventricular function.\textsuperscript{3, 4} An accompanying myocarditis caused by SARS-CoV-2 infection has also been described in the literature.\textsuperscript{5, 6} Viral myocarditis comprises a broad spectrum of symptoms ranging from asymptomatic to most severe clinical courses resulting in congestive heart failure and inflammatory cardiomyopathy with potentially life-threatening functional impairment of the ventricle and poor prognosis.\textsuperscript{3, 5, 7} Patients with CAD are also particularly at risk for acute right heart failure due to pre-existing right heart and diastolic dysfunction as well as elevated pulmonary artery pressure.\textsuperscript{1, 2} Right heart dysfunction is triggered by SARS-CoV-2 infection as pneumonic infiltrates and lung involvement, often associated with progressive respiratory failure and acute respiratory distress syndrome (ARDS), lead to an additional increase of pulmonary pressure and tricuspid regurgitation resulting in right heart overload and finally failure.\textsuperscript{3} Furthermore, patients are at risk to develop pulmonary embolisms due to infect-associated coagulopathy resulting in acute right heart failure and disseminated intravascular coagulation.\textsuperscript{4} These clinical scenarios explain the increased rate of organ failure, admissions to the intensive care unit (ICU) with rapidly progressive respiratory failure, and mortality in cardiovascular patients when SARS-CoV-2 infection occurs.\textsuperscript{1-3, 7}

Chronic alterations of inflammatory mediators, like C-reactive protein (CRP), pro-inflammatory cytokines, or adhesion molecules trigger atherogenesis and progression in CAD.\textsuperscript{8} Up-regulation of the involved cytokines and chemokines recruit inflammatory cells like monocytes to the vessel wall causing atherosclerotic lesions.\textsuperscript{8} Inflammatory cells like monocytes and macrophages have been implicated in many inflammatory heart diseases, e.g. CAD, myocardial infarction, myocarditis, and heart failure.\textsuperscript{8-10} In humans, three distinct monocyte subpopulations have been classified based on their surface receptor expression into classical (CD14\textsuperscript{+}CD16\textsuperscript{-}), intermediate (CD14\textsuperscript{+}CD16\textsuperscript{+}), and non-classical (CD14\textsuperscript{dim}CD16\textsuperscript{+}) monocytes.\textsuperscript{11, 12}
Classical monocytes secrete soluble mediators and differentiate into monocyte-derived dendritic cells (DCs) and therefore show a rather pro-inflammatory phenotype. Intermediate monocytes are specialized in antigen presentation, whereas non-classical monocytes are important for the anti-viral immune responses.\textsuperscript{11-13} Non-classical monocytes predominantly remain in the vascular system and migrate along the endothelium. This process is termed patrolling and is mediated by their expression of the adhesion-related receptor CX3CR1 among others.\textsuperscript{14, 15} An athero-protective role and also anti-inflammatory and pro-homeostatic effects of these patrolling non-classical monocytes have recently been suggested.\textsuperscript{14} During infections viral RNA and DNA is recognized by non-classical (CD14\textsuperscript{dim}CD16\textsuperscript{+}) monocytes via TLR7 expression leading to the production of TNF-\textalpha, IL-1\textbeta, and CCL3.\textsuperscript{14} In addition, in HIV-infected patients, a central role of TNF overproduction by non-classical monocytes has been proposed, indicating that they could be considered as key players in the immune hyperactivation of the disease.\textsuperscript{12} Moreover, in viraemic HIV-infected patients a pivotal role by TNF overproduction was shown for non-classical monocytes, indicating that they might be considered as a major actor in the immune hyperactivation of the disease.\textsuperscript{16} Furthermore, patients with HIV and subclinical atherosclerosis show altered expression of activation markers on non-classical monocytes.\textsuperscript{17}

Coronavirus disease 2019 (COVID-19) has spread rapidly worldwide and is associated with significant mortality, especially in risk groups with poor prognostic features, such as CAD.\textsuperscript{18} In hospitalized patients infected with SARS-CoV-2, the causative agent of COVID-19, pneumonia, sepsis, respiratory failure and ARDS are common complications.\textsuperscript{18-20} The pathophysiology of SARS-CoV-2 is characterized by an early production of proinflammatory cytokines (tumour necrosis factor (TNF), IL-6, and IL-1\textbeta) described as a cytokine storm, resulting in an increased risk of vascular hyperpermeability and, if long-lasting, multiorgan failure, and eventually death.\textsuperscript{20} This is mediated after entry of the coronavirus and the release of RNA as genomic material into the cell by activation of TLRs. The frequency of monocytes is reduced in patients with severe SARS-CoV-2 infection\textsuperscript{21}, however, the different monocyte subsets were not analysed in detail yet. On the other hand, increased frequencies of non-classical monocytes have been associated with the occurrence of acute coronary syndromes in the general population.\textsuperscript{22} Thus, we analysed numbers of monocyte subsets and their
surface marker expression in CAD patients with and without SARS-CoV-2 infection in order to determine prognostic markers for treatment options.
2. Methods

Study design, participants, and assessment of clinical parameters

From February to April 2020 we prospectively studied a consecutive cohort of 96 participants. Out of these, 47 consecutive patients with pre-existing CAD and acute SARS-CoV-2 infection (CAD+SARS-CoV-2) were admitted with progressive respiratory failure. N=19 patients with pre-existing stable CAD without any infections were matched for the group of CAD+SARS-CoV-2 patients. N=30 healthy participants served as controls. All patients underwent clinical and cardiac assessment including echocardiography, electrocardiography, concomitant medication, comorbidities, and blood sampling for routine laboratory parameters and phenotyping of monocytes within 12 hours of admission. SARS-CoV-2 infection was diagnosed by RNA detection from nasopharyngeal secretions with real-time reverse transcriptase polymerase chain reaction. CAD was determined by coronary angiography in all patients before hospital admission. CAD was defined as >25-50% diameter luminal stenosis of two or more coronary vessels or left main or proximal left anterior descending coronary artery stenosis >50%. Respiratory failure was stratified into four groups by the Horovitz Index (HI) as follows: normal HI > 300 mmHg, mildly impaired HI 201 - 300 mmHg, moderately impaired HI 101 - 200 mmHg, severely impaired ≤ 100 mmHg.23 All patients were admitted and treated at the Department of Cardiology and Angiology of the University Hospital of Tübingen, Germany.

Inclusion criteria of our study were an age older than 18 years and confirmed coronary artery disease with or without SARS-CoV-2 infection. Exclusion criteria were other viral or bacterial co-infections and cancer. The study was approved by the local ethics committee (240/2018BO2) and complies with the declaration of Helsinki and the good clinical practice guidelines on the approximation of the laws, regulations and administrative provisions of the member states relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. Written informed consent was obtained from every patient.

N terminal-pro-B-type natriuretic peptide (NT-pro-BNP, >300 ng/L), high sensitive troponine I (hs TNI, >37 ng/L) and C-reactive protein (CRP, >0.5 mg/dL) were classified as elevated laboratory markers of myocardial and inflammatory distress. Echocardiographic parameters included left and
right ventricular function, right ventricular dilatation, presence of tricuspid valve regurgitation, and pericardial effusion according to current guidelines.\textsuperscript{24,25}

**Clinical Follow-up**

The clinical endpoint (EP) was defined as rapidly progressive respiratory failure with indication to mechanical ventilation characterized by a moderately or severely impaired Horovitz Index (moderately impaired HI defined as 101 - 200 mmHg, severely impaired HI defined as $\leq 100$ mmHg) within a follow-up of 30 days. All patients were followed for 30 days after study inclusion for the occurrence of the primary EP.

**Flow Cytometry staining**

Blood samples of patients with coronary artery disease (including SARS-CoV-2 infection) and healthy human were collected in CPDA monovettes for flow cytometry staining in order to stain monocytes. 10 x RBC Lysis buffer (BioLegend) was diluted 1:10 in Millipore water and whole blood was lysed and incubated for 15 min at RT in the dark. After two washing steps with PBS containing 1 % FCS, 2 mM EDTA and 1 % sodium azide, cell count was adjusted to 3 x $10^6$ cells per well in a 96 well plate (Falcon). Unspecific antibody binding was blocked by cell incubation with human IgG (Sigma) for 20 min at 4 °C. First, extracellular staining with CX3CR1 PE/Dazzle antibody (clone 2A9-1, BioLegend) was performed for 1 h at 37 °C followed by staining with HLA-DR PerCP-Vio77 (clone REA805Miltenyi), CD54 PE (clone REA266, Miltenyi), CD3 BV510 (clone OKT3), CD15 BV510 (clone W603), CD19 BV510 (clone HLB19), CD20 BV510 (clone 2H7), CD56 BV510 (clone HCD56), CD14 FITC (clone M5E2), CD16 BV711 (clone 3G8), CD62L PE-Cy7 (clone DREG-56), CD80 BV786 (clone 2D10), and Zombie NIR (BioLegend) for 20 min at 4 °C and washed with PBS containing 1 % FCS, 2 mM EDTA and 1 % sodium azide. Next, cells were fixed overnight at 4°C with FoxP3 Staining Buffer Set (eBioscience) according to manufacturer’s instructions and acquired using the LSR Fortessa (BD Biosciences). Supplementary Figure 1 shows the gating strategy of human monocyte subsets. Data analysis was done with FlowJo software V.10.6.2 (Tree Star).
Histopathological and immunohistological analysis of heart and lung tissue

Specimens were gained from heart and lung tissue of healthy controls, CAD, and CAD+SARS-CoV-2 patients and fixed in 4 % buffered formaldehyde for immunohistology. Paraffin-embedded EMB were stained with hematoxylin/eosin (H&E) and analyzed by light microscopy.26, 27 Histological analysis followed the Dallas criteria complemented by immunohistology to assess ongoing inflammation. For immunohistological staining, tissue sections were treated with an avidin–biotin–immunoperoxidase method (Vectastain-Elite ABC Kit, Vector, Burlingame, CA, USA), in combination with the following monoclonal antibodies: anti-CD68 (macrophages; DAKO, Hamburg, Germany), anti-CD14 (Santa Cruz Biotechnology, USA), and anti-CD16 (Santa Cruz Biotechnology, USA).

Statistical analysis

We determined clinical and laboratory baseline characteristics in relation to measured monocyte phenotypes, marker expression, and clinical outcome. Continuous, not normally distributed variables are expressed as median and interquartile range (IQR) and were compared using Mann–Whitney U test for two group comparison and Kruskal-Wallis test for three group comparison, where applicable. Continuous parameters were dichotomized at established cut-off values if necessary. Categorical data are presented as total numbers and proportions and were analysed by chi-squared test. Correlation analysis was performed by Spearman rank correlation coefficient r. Survival curves of patients grouped by pre-specified variables were calculated by Kaplan-Meier analyses and compared using the log-rank test. Cox proportional-hazards regression analysis was carried out for multivariable analysis to assess the association of risk factors with the primary endpoint. The risk for endpoint occurrence is presented as a hazard ratio (HR) with 95% confidence interval (CI). Comparisons were considered statistically significant if two-sided p-value was <0·05. Statistical analysis of all participants was performed using SPSS Statistics Version 26 (SPSS, Inc.) and GraphPad Prism Version 8.4.0 (GraphPad Software).
Results

Demographic and clinical characteristics of patients with coronary artery disease and SARS-CoV-2 infection

We prospectively studied a consecutive cohort of 96 participants from February to April 2020. 47 out of 96 patients with pre-existing CAD and acute SARS-CoV-2 infection (CAD + SARS-CoV-2) were admitted with progressive respiratory symptoms. 19 patients with pre-existing stable CAD without any infections were matched to CAD + SARS-CoV-2 patients, while 30 healthy participants served as controls. Baseline characteristics and demographics of the overall cohort are given in Table 1. The median age of the population was 62 (IQR 48–79) years; 44 (45.8%) patients were men. Of the 47 CAD+SARS-CoV-2 patients, 14 (29.8%) were admitted to the intensive care unit (ICU) due to progressive respiratory, circulatory, or multiorgan failure, as they required vasopressor therapy or high-flow oxygen to correct hypoxaemia (Table 2). 11 out of 14 (78.6%) patients showed rapidly progressive respiratory failure defined by a Horovitz Index (HI) of ≤ 200 mmHg and required mechanical ventilation (Table 3). This was the pre-defined clinical endpoint (EP) during a follow-up of 30 days.

Patients with SARS-CoV-2 infection show significantly lower numbers of CD14^{dim}CD16^{+} monocytes in peripheral blood

We analysed subtypes of monocytes in an intensified risk assessment of patients with CAD and SARS-CoV-2 infection to elucidate their prognostic impact during the cause of the disease. Therefore, we stratified monocytes in peripheral blood by their expression of CD14 and CD16 into classical CD14^{+}CD16^{-}, intermediate CD14^{+}CD16^{+} and non-classical CD14^{dim}CD16^{+} subtypes using flow cytometry. We found no difference in the numbers of classical monocytes in patients with CAD (median 227550, IQR [143400–115800]) compared to CAD + SARS-CoV-2 (median 223380, IQR [115800–329600]) and healthy controls (median 291745, IQR [236228–408465]), whereas significantly less classical monocytes were detected in CAD + SARS-CoV-2 patients compared to healthy controls (p=0.018 for overall comparison in Kruskal-Wallis test, CAD vs CAD + SARS-CoV-2 p=0.99, CAD vs healthy control p=0.18, healthy control vs CAD + SARS-CoV-2 p=0.017) (Fig.
In contrast, slightly increased numbers of intermediate monocytes were observed in CAD (median 31030, IQR [23100–45540]) compared to healthy controls (median 22600, IQR [15645–36735], CAD vs healthy control p=0.22) whereas these numbers were similar to healthy controls in CAD + SARS-CoV-2 patients (median 23230, IQR [7840–34560], p=0.99, CAD vs CAD + SARS-CoV-2 p=0.048) (Fig. 1A). Most interestingly, the numbers of non-classical monocytes were substantially reduced by 82 or 75% in CAD + SARS-CoV-2 patients (median 1443, IQR [649–4266]) compared to CAD patients (median 27820, IQR [18600–50840]) and healthy controls (median 21180, IQR [12788–34185]), respectively (p<0.0001 for overall comparison in Kruskal-Wallis test, CAD vs CAD + SARS-CoV-2 p<0.0001, CAD vs healthy control p=0.63, healthy control vs CAD + SARS-CoV-2 p<0.0001) (Fig. 1A). Spearman rank correlation analysis revealed an inverse, yet significant association of CD14<sup>dim</sup>CD16<sup>+</sup> non-classical monocytes with CRP levels in the overall cohort of healthy controls, CAD patients, and CAD + SARS-CoV-2 patients (r=-0.674 and p<0.0001). In subgroup analysis of the CAD + SARS-CoV-2 patients the observed association remained significant with r=-0.373 and p= 0.010 (data not shown).

As current literature suggested gender-specific immune response in patients with SARS-CoV-2 infection, we stratified our data by gender to detect differences between men and women in our cohort. 28-30

Interestingly, there were no statistically relevant, gender-specific differences regarding monocyte subtypes in the overall cohort and in the comparison of controls vs CAD vs CAD + SARS-CoV-2 patients (supplementary Fig. 2A-C).

**CAD patients with SARS-CoV-2 infection admitted to ICU show the lowest numbers of CD14<sup>dim</sup>CD16<sup>+</sup> monocytes**

Analysis of CAD + SARS-CoV-2 patients admitted to ICU (here referred to as ICU patients) (median 712, IQR [161.3–2769]) showed significantly less CD14<sup>dim</sup>CD16<sup>+</sup> monocytes compared to non-ICU patients (median 2150, IQR [879–4878], p=0.03), whereas no differences were found for CD14<sup>+</sup> CD16<sup>−</sup> and CD14<sup>+</sup>CD16<sup>+</sup> monocytes (p=0.29 and p=0.30, respectively) (Fig. 1B). Gender-specific
subgroup analysis showed similar results for female and male ICU patients compared to non-ICU patients (supplementary Fig. 3A, B).

Moreover, the lowest number of CD14<sup>dim</sup>CD16<sup>+</sup> monocytes was detected in ICU patients with progressive respiratory failure, an HI ≤ 200 mmHg, and mechanical ventilation (here referred to as ICU + HI ≤ 200 mmHg; median 388, IQR [129–858]) compared to ICU + HI > 200 mmHg (median 2324, IQR [917.5–4577]), p=0.0017) (Fig. 1C). ICU + HI ≤ 200 mmHg patients (median 6020, IQR [1980–7840]) showed also lower numbers of CD14<sup>+</sup>CD16<sup>+</sup> monocytes compared to ICU + HI > 200 mmHg patients (median 2324, IQR [125–4577]), p=0.001) (Fig. 1C). When further stratified by gender, there were no statistically relevant differences between men and women in the group of patients with HI ≤ 200 mmHg compared to HI > 200 mmHg (supplementary Fig. 3C, D).

**Remaining CD14<sup>dim</sup>CD16<sup>+</sup> monocytes in peripheral blood of CAD + SARS-CoV-2 patients are functionally impaired**

We further assessed the expression of activation markers on CD14<sup>dim</sup>CD16<sup>+</sup> monocytes important for adhesion, migration and T cell activation like CD54, CD62L, CX3CR1, CD80 and HLA-DR. CD54 and CD62L mediate adhesion and trans-endothelial migration. The expression of CD54 and the frequency of CD62L<sup>+</sup>CD14<sup>dim</sup>CD16<sup>+</sup> monocytes was significantly increased by two- and eight-fold on CAD patients (CD54: median 16117, IQR [14349–18633], CD62L: median 0.12, IQR [0.073–0.17]) compared to healthy controls (CD54: median 7262, IQR [6298–8775], CD62L: median 0.0046, IQR [0.0033–0.0103]), CD54: p<0.0001, CD62L: p<0.0001), respectively (Fig. 2A-B). Interestingly, this increase was absent in CAD + SARS-CoV-2 patients (CD54: median 8153, IQR [2115–11299], CD62L: median 0.0068, IQR [0.0015–0.014], CAD versus CAD + SARS-CoV-2 CD54: p<0.0001, CD62L: p<0.0001) (Fig. 2A-B), indicating impaired migration of the remaining CD14<sup>dim</sup>CD16<sup>+</sup> monocyte to the site of tissue injury. Moreover, CX3CR1, binding fraktaline, which mediates tissue extravasation, was less expressed on CD14<sup>dim</sup>CD16<sup>+</sup> monocytes in CAD + SARS-CoV-2 patients (median 20365, IQR [11990–32958]) compared to CAD patients (median 41732, IQR [36812–46084], p<0.0001), and to healthy controls (median 40150 IQR [36089–43352], p<0.0001) (Fig. 2C). In addition, HLA-DR expression was reduced on CD14<sup>dim</sup>CD16<sup>+</sup> monocytes in CAD (median 25458,
IQR [21512–26573]) and CAD + SARS-CoV-2 (median 25580, IQR [17327–32643]) patients compared to healthy controls (median 36128, IQR [28089–45615]), CAD versus healthy controls: p<0.0001, CAD + SARS-CoV-2 versus healthy controls: p<0.0001) (Fig. 2D), while CD80 expression was four-fold higher in CAD patients (median 2240, IQR [1946–2609]) compared to healthy controls (median 589, IQR [458–719], p<0.0001) (Fig. 2E). Similarly as for CD54 and CD62L described above, this increase in CD80 expression on CD14"dim"CD16" monocytes was absent in CAD + SARS-CoV-2 patients (median 503, IQR [391–489], p<0.0001) (Fig. 2E). These data suggest impaired migration behaviour and T cell activation capacity of the remaining CD14"dim"CD16" monocytes.

Subgroup analysis of ICU patients reveals an impaired activation phenotype of CD14"dim"CD16" monocytes in peripheral blood

In subgroup analysis of ICU patients, assessing the markers described above, we detected an even lower expression of HLA-DR on and even less numbers of CD62L"CD14"dim"CD16" monocytes compared to non-ICU patients (HLA-DR: ICU patients median 2456, IQR [1221–11368] versus non-ICU patients median 14349, IQR [5274–27282], p=0.015), CD62L"CD14"dim"CD16" monocytes: ICU patients median 0.0017, IQR [0.00049–0.00659] versus non-ICU patients median 0.00823, IQR [0.00269–0.014], p=0.019, respectively) (Fig. 2B, D). CD54, CD80 and CX3CR1 were reduced for trend but not statistically significant on CD14"dim"CD16" monocytes (CD54: p=0.12, CD80: p=0.22, and CX3CR1: p=0.11) (Fig. 2A, C, E). Analysis of ICU + HI ≤ 200 mmHg compared to ICU + HI > 200 mmHg patients revealed no difference in CD54 and CD80 expression on CD14"dim"CD16" monocytes (CD54: p=0.07, CD80: p=0.84) (Fig. 2A, E). In contrast, the frequency of CD62L" and the expression of HLA-DR and CX3CR1 on CD14"dim"CD16" monocytes were significantly reduced in ICU + HI ≤ 200 mmHg patients (CD62L: median 0.0014, IQR [0.00045–0.0022], HLA-DR: median 2650, IQR [1000–9707], CX3CR1: median 11455, IQR [8913–15967] vs ICU + HI > 200 mmHg CD62L: 0.0075, IQR [0.0032–0.14], HLA-DR: median 14316, IQR [4400–26766], CX3CR1: median 25008, IQR [14425–33356]; p=0.003, p=0.012, p=0.005, respectively) (Fig. 2B-D).

Immunohistochemistry of heart and lung tissue of CAD patients with SARS-CoV-2 infection detects increased infiltration of CD68", CD14", and CD16" positive inflammatory cells
In a subgroup of patients we performed histological and immunohistochemical analysis of heart and lung tissue (Figure 3). Representative heart tissue sections from healthy controls, CAD, and CAD+SARS-CoV-2 patients (n=4 in each group) were stained with anti-CD68, anti-CD14, and anti-CD16. We identified distinct expression patterns of these CD68+, CD14+, and CD16+ inflammatory cells infiltrating the myocardium to maintain local inflammation in CAD+SARS-CoV-2 compared to healthy controls and CAD patients (Figure 3 A). Interestingly, we could detect a dramatically increased number of CD68+, CD14+, and CD16+ cells in CAD+SARS-CoV-2 patients compared to healthy controls and CAD patients (Figure 3 A). These findings suggest that non-classical monocytes and macrophages migrate to affected myocardial tissue and maintain local inflammation in SARS-CoV-2 related myocarditis (Fig. 3 A).

Analysis of affected lung tissue showed similar results. Here, we found substantially higher numbers of CD68+, CD14+, and CD16+ inflammatory cells in CAD+SARS-CoV-2 patients compared to healthy controls (Fig. 3 B).

**Low numbers of CD14^{dim}CD16^{+} monocytes predict outcome in CAD patients with SARS-CoV-2 infection**

In order to identify prognostic markers for CAD patients with SARS-CoV-2 infection indicating progressive respiratory failure we elucidated the role of CD14^{dim}CD16^{+} monocytes during a follow-up of 30 days. 11/47 CAD + SARS-CoV-2 patients (23.4%) reached the primary endpoint defined as the occurrence of a Horovitz index ≤ 200 mmHg and mechanical ventilation. For Kaplan-Meier analysis the number of patients was divided into two groups based on the median of the non-classical monocyte count. This revealed that significantly more patients with EP were below the cut-off value (median 1443 cells/mL). 41.7% ICU + HI > 200 mmHg patients showed a cell number below the median, while this was the case for 81.8% ICU + HI ≤ 200 mmHg patients (p=0.02 (Fig. 4A) indicating rapidly progressive respiratory failure. Kaplan–Meier curves illustrate that CAD + SARS-CoV-2 patients with low numbers of CD14^{dim}CD16^{+} non-classical monocytes reached the clinical endpoint significantly more often compared to patients with cell counts above the median (log-rank 5.03, p=0.025) (Fig. 4B). Remarkably, considering the median number of CD14^{dim}CD16^{+} monocytes from ICU patients in
relation to all CAD + SARS-CoV-2 patients, Kaplan-Meier analysis revealed that patients with a CD14^{dim}CD16^{+} monocyte cell count below the median of 388 cell/mL develop most frequently respiratory failure (log-rank 14.18, p<0.0001) (Fig. 4C). Additionally, we performed gender-specific Kaplan-Meier analysis (Supplementary Fig. 4A, B). Even though low numbers of non-classical monocytes could predict the occurrence of the clinical endpoint in women by trend, this was not statistically significant compared to men (men: log-rank, 1.797, p=0.180, supplementary Fig. 4A; women: log-rank 3.78, p=0.052, supplementary Fig. 4B). We also performed cox regression analysis for the occurrence of progressive respiratory failure defined by a Horovitz index of ≤ 200 mmHg. Cox regression analysis taken into account for the pre-defined confounding factors age, gender, and oral anticoagulation use identified CD14^{dim}CD16^{+} non-classical monocyte count to remain the only independent predictor for the primary endpoint (HR 5.10; CI 1.08 – 24.19, p=0.040, supplementary table 1). We performed another cox regression analysis to correct for several comorbidities and confounders including age, gender, oral anticoagulation use, arterial hypertension, hyperlipidemia, diabetes mellitus, and chronic kidney disease. Cox regression analysis identified CD14^{dim}CD16^{+} non-classical monocyte count and hyperlipidemia as independent predictors for the occurrence of the primary endpoint (CD14^{dim}CD16^{+} non-classical monocyte count: HR 0.18; CI 0.03 – 0.90, p=0.037; hyperlipidemia: HR 6.29; CI 1.17 – 33.93, p=0.032, supplementary table 2).

Discussion

In contrast to other studies on COVID-19^{18, 19} we focussed on patients with pre-existing coronary artery disease, which are prone to progressive heart and lung failure when suffering from SARS-CoV-2 infection.{1} To our knowledge, this is the first study to show that the clinical outcome of CAD + SARS-CoV-2 patients is associated with reduced numbers of CD14^{dim}CD16^{+} monocytes in peripheral blood. Our data are in accordance with reduced numbers of monocytes (not dissected into the three subsets) in hospitalized patients with SARS-CoV-2 infection compared to healthy controls^{21} and a study from Gatti et al. showing reduced frequencies of non-classical and intermediate monocyte subsets in patients with severe SARS-CoV-2 infection.^{31} On the contrary, one study addressing the role of intermediate CD14^{+}CD16^{+} monocytes in peripheral blood of SARS-CoV-2 infected patients,
showed an increased frequency of this subsets in peripheral blood and even higher frequencies in severe pulmonary syndrome patients from ICU.\textsuperscript{32} However, we cannot compare our data showing reduced numbers of CD14\(^+\)CD16\(^+\) monocytes in CAD + SARS-CoV-2 patients compared to CAD patients with this study of Zhou et al.\textsuperscript{32} as CAD patients are characterized by doubling of intermediate monocytes which is even associated with cardiovascular outcomes.\textsuperscript{33} Zhou et al. do not give information about comorbidities of the cohort, especially not of presence or absence of CAD.\textsuperscript{32}

We showed that in infected CAD patients with progressive respiratory failure not only the numbers of monocytes were substantially reduced, but also their function was impaired. More precise, these patients showed less expression of adhesion and activation markers on non-classical monocytes when compared to CAD patients without infection. This was surprising as SARS-CoV-2 infection results in elevated levels of inflammatory mediators, a so-called cytokine storm, e.g. TNF, MCP-1, MIP-1\(a\), and IL-6 described in the plasma of patients.\textsuperscript{5, 18, 20} This should increase monocyte numbers and also their activation markers, which we did not observe in our cohort. Most likely these findings are reflected by monocyte invasion of CD14\(^{dim}\)CD16\(^+\) into injured tissue via the adhesion molecules CD62L and CX3CR1\textsuperscript{34} where they differentiate into macrophages. Our analysis of affected heart and lung tissue showed increased numbers of CD68\(^+\), CD14\(^+\), and CD16\(^+\) inflammatory cells in CAD+SARS-CoV-2 patients. These CD68\(^+\), CD14\(^+\), and CD16\(^+\) monocytes and macrophages infiltrate the myocardium in SARS-CoV-2 related myocarditis and the affected lungs during progressive respiratory failure. These findings support our hypothesis that non-classical monocytes may migrate from the blood to the lungs and the myocardium in CAD+SARS-CoV-2 patients during the course of the disease and maintain systemic and local inflammation. Monocyte migration to the site of inflammation in affected heart and lung tissue might lead to reduced numbers of non-classical monocytes in peripheral blood with impaired surface marker expression that we observed in our analysis.\textsuperscript{35} Moreover, since CAD patients characteristically show increased numbers and activation of non-classical monocytes\textsuperscript{34}, it was unexpected that patients with CAD and SARS-CoV-2 infection showed an opposite phenotype with drastically decreased numbers and activation markers. Therefore, we speculate that in the context of SARS-CoV-2 infection, these monocytes migrate from the blood into the affected tissue, where they promote and maintain local inflammation and tissue damage. This could be the causal link for the low
number of remaining peripheral CD14^{dim}CD16^{+} monocytes. This extraordinary finding is reflected in the clinical course and is even of prognostic importance. CAD patients with low CD14^{dim}CD16^{+} monocyte counts show more often rapidly progressive respiratory failure with HI ≤ 200 mmHg and mechanical ventilation at an early stage of the infection as demonstrated by the Kaplan-Meier curves. Thus, we propose the additional determination of the monocyte phenotype as a simple and new tool which could improve routine diagnostics at the beginning of the infection.3,36 Thus, we speculate that sequestrated, activated non-classical monocytes promote the localized inflammatory response of affected lung tissue. This is of great clinical importance as the decreased number of CD14^{dim}CD16^{+} non-classical monocytes with their impaired phenotype are specifically found in CAD + SARS-CoV-2 infected patients, but not in CAD patients, which is mirrored in the severity of the respiratory failure. Since there is currently no causal therapy for the infection, it is essential to detect and treat prognostically relevant comorbidities at an early stage according to current guidelines in order to prevent a fatal course triggered by the infection. CAD patients with pre-existing burden of cardiac and vascular dysfunction might benefit from intensified heart failure and anti-thrombotic therapy before progression of respiratory failure occurs.4

In conclusion, decreased numbers of CD14^{dim}CD16^{+} non-classical monocytes are associated with adverse clinical outcome of CAD + SARS-CoV-2 patients and might serve as a prognostic marker to predict worsening of respiratory failure. An early phenotyping of monocytes using simple flow cytometry analysis may help to identify patients at risk requiring a more intensified heart failure and anti-thrombotic therapy in the course of the infection, especially in patients with pre-existing coronary artery disease.
Funding

This work was supported by the German Research Foundation (DFG) – Project number 374031971–TRR 240 and by the Ministry of Science, Research and the Arts of the State of Baden-Württemberg (COVID-19 Funding). The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication. Karin Klingel received funding by Deutsche Herztiftung.

Author contributions

K.A.L.M., M.P.G. and S.E.A. conception and design of the study, interpretation of data, drafting of the manuscript and revising it critically for important intellectual content; C.L., performed experimental analysis, data acquisition and interpretation; M.G., S.P., S.G., B.L. performed experimental analysis; Á.P.-U., P.J. data acquisition and interpretation; K.P.K.: interpretation of data, revising manuscript critically for important intellectual content; T.C., D.R. interpretation of data, revising manuscript critically for important intellectual content. K.K. and H.B. provided and analyzed heart and lung tissue and performed histological and immunohistochemical stainings and analysis.

Conflict of interest

None of the authors has any conflict of interest to declare. Our study complies with the Declaration of Helsinki, our locally appointed ethics committee has approved the research protocol, and informed consent has been obtained from all participants.

Data Availability Statement

For original data, please contact Karin Anne Lydia Mueller, k.mueller@med.uni-tuebingen.de.
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Tables

Table 1. Baseline characteristics of the overall cohort (n = 96)

Table 2. Baseline characteristics of patient population stratified by the admission to intensive care unit (ICU)

Table 3. Baseline characteristics of patient population stratified by the occurrence of the clinical endpoint (progressive respiratory failure defined by Horovitz Index HI < 200mmHg and mechanical ventilation)

Figure Legends

Figure 1: Reduced numbers of CD14^{dim}CD16^{+} monocytes in CAD + SARS-CoV-2 infected patients

Whole blood human CD14^{+}CD16^{+}, CD14^{+}CD16^{+} and CD14^{dim}CD16^{+} monocytes were analyzed by flow cytometry from (A) healthy controls (n=30), CAD patients (n=19) and CAD + SARS-CoV-2 infected patients (n=47), (B) CAD + SARS-CoV-2 infected patients stratified by ICU admission (no ICU n=33, ICU n=14) and (C) ICU patients with rapidly progressive respiratory failure (Horovitz index HI ≤ 200 mmHg) requiring mechanical ventilation (HI > 200 mmHg; n=36, HI ≤ 200 mmHg n=11). Graphs show the number of CD14^{+}CD16^{+}, CD14^{+}CD16^{+} and CD14^{dim}CD16^{+} monocytes (median with interquartile range [IQR]). Kruskal-Wallis or Mann-Whitney test were performed for three or two group comparison, respectively. For this analysis a p-value ≤ 0.050 was considered significant, indicated by *, p-value ≤ 0.010 indicated by **, p-value ≤ 0.001 indicated by *** and p-value ≤ 0.0001 indicated by ****.

Figure 2: Reduced expression of activation markers on CD14^{dim}CD16^{+} monocytes in CAD + SARS-CoV-2 infected patients

(A) CD54, (B) CD62L, (C) CX3CR1, (D) HLA-DR, and (E) CD80 surface marker expression was evaluated by flow cytometry on CD14^{dim}CD16^{+} monocytes in whole blood from (left panel) healthy controls (n=30), CAD patients (n=19), and CAD + SARS-CoV-2 infected patients (n=47), (middle panel) CAD + SARS-CoV-2 infected patients stratified by ICU admission (no ICU n=33, ICU n=14),
and (right panel) ICU patients with rapidly progressive respiratory failure requiring mechanical ventilation (HI > 200 mmHg; n=36, HI ≤ 200 mmHg n=11). Graphs show the median fluorescence of the indicated marker on CD14^dimCD16^+ monocytes as dot plots (median with interquartile range (IQR)). Kruskal-Wallis or Mann-Whitney test were performed for three or two group comparison, respectively. For this analysis a p-value ≤ 0.050 was considered significant, indicated by *, p-value ≤ 0.010 indicated by **, p-value ≤ 0.001 indicated by *** and p-value ≤ 0.0001 indicated by ****.

**Figure 3: Histopathological and immunohistological findings in the heart and lung tissue of patients with coronary artery disease and severe SARS-CoV-2 infection.**

(A) Representative myocardial tissue sections from patients with coronary artery disease with and without acute SARS-CoV-2 infection and controls without any heart or lung disease. We analyzed n=4 patients in each group and performed histological and immunohistochemical stainings with hematoxylin/eosin (H&E), anti-CD68, anti-CD14 and anti-CD16 antibodies as described in the method section.

The left panel illustrates the findings in normal myocardial tissue of a patient without any heart disease. There is no detection of inflammatory cells within the myocardium.

The middle panel depicts heart tissue of a patient with coronary artery disease. Immunohistochemical stainings for the detection of CD68, CD14 and CD16 show few positive cells indicated by the arrows but no relevant local inflammation.

The right panel shows heart tissue of a patient with coronary artery disease and acute SARS-CoV-2 infection with progressive respiratory failure. Immunohistochemical staining detects dramatically increased numbers of CD68^+, CD14^+ and CD16^+ cells (indicated by arrows). Presence of higher numbers of infiltrating inflammatory cells is a characteristic finding of acute myocardial inflammation during viral infection like SARS-CoV-2 related myocarditis.

(B) Representative lung tissue sections from patients with coronary artery disease with acute SARS-CoV-2 infection and controls without any heart or lung disease. Histological and
immunohistochemical stainings were performed from n=4 patients in each group with hematoxylin/eosin (H&E), anti-CD68, anti-CD14 and anti-CD16 antibodies as described in the method section.

The left panel shows the findings in normal lung tissue from a patient without any heart and lung disease. There is no detection of inflammatory cells within the lung tissue.

The right panel depicts lung tissue of a patient with coronary artery disease and acute SARS-CoV-2 infection. Immunohistochemical staining detects increased numbers of CD68+, CD14+ and CD16+ cells (indicated by arrows). This finding suggests that inflamed lung tissue is characterized by the presence of CD68+ macrophages and CD14+ and CD16+ monocytes which migrated into the lung tissue to trigger and maintain local tissue inflammation during progressive respiratory failure due to SARS-CoV-2 infection.

**Figure 4: Low numbers of CD14dimCD16+ monocytes are associated with rapidly progressive respiratory failure in CAD + SARS-CoV-2 patients**

(A) The number of patients was divided into 2 groups based on the median of the non-classical monocyte count (median 1443 cells/mL). Graph represents the frequency of patients with a CD14dimCD16+ monocyte cell count below the median stratified by HI ≤ 200 mmHg. Chi-square test was performed for categorical variables. For this analysis a p-value ≤ 0.050 was considered significant. (B) Kaplan-Meier curves illustrate the occurrence of the clinical study endpoint stratified by CD14dimCD16+ monocyte count of all CAD + SARS-CoV-2 patients (median 1443 cell/mL). During a follow-up of 30 days 11/47 patients (23.4%) reached the primary endpoint. The primary study endpoint was defined as rapidly progressive respiratory failure with a Horovitz index ≤ 200 mmHg and mechanical ventilation. Nine out of 11 patients (81.8%) showed a CD14dimCD16++ monocyte count below the median of 1443 cells/mL (log-rank 5.03, p=0.025). (C) Kaplan-Meier curves illustrate the occurrence of the clinical study endpoint stratified by CD14dimCD16+ monocyte count of all ICU patients (median 388 cell/mL). Six out of 11 patients (54.5%) showed a CD14dimCD16++ monocyte count below the median of 388 cells/mL (log-rank 14.18, p<0.0001).
| Parameters                          | All Patients, N=96 | CAD+SARS-CoV-2, N=47 | CAD, N=19 | Control, N=30 | p-value  |
|-----------------------------------|--------------------|----------------------|-----------|---------------|----------|
| **Clinical characteristics**      |                    |                      |           |               |          |
| Age, y                            | 62 (48.79)         | 79 (59.82)           | 70 (62.77)| 39 (28.53)    | <0.001   |
| Male                              | 44 (45.8)          | 24 (51.1)            | 8 (42.1)  | 12 (40.0)     | 0.596    |
| BMI (kg/m²)                       | 25.5 (23.28)       | 27.8 (25.1-31.8)     | 25.9 (23.6-27.9) | 23 (22.27.3) | 0.001    |
| Fever                             | 31 (32.3)          | 31 (65.9)            | 0 (0)     | 0 (0)         | <0.001   |
| hs TNI > 37 (ng/L)                | 8 (8.3)            | 8 (17)               | 0 (0)     | 0 (0)         | 0.171    |
| NT-pro BNP > 300 (ng/L)           | 27 (28.1)          | 27 (57.4)            | 0 (0)     | 0 (0)         | <0.001   |
| CRP > 0.5 ml/dL                   | 53 (55.2)          | 44 (93.6)            | 6 (31.6)  | 3 (10.0)      | <0.001   |
| **Cardiovascular risk factors**   |                    |                      |           |               |          |
| Arterial hypertension             | 53 (55.2)          | 37 (78.7)            | 15 (78.9) | 1 (3.3)       | <0.001   |
| Dyslipidemia                      | 36 (37.5)          | 24 (51.1)            | 11 (57.9) | 1 (3.3)       | <0.001   |
| Diabetes mellitus                 | 14 (14.6)          | 12 (25.5)            | 1 (5.3)   | 1 (3.3)       | 0.012    |
| Current smokers                   | 8 (8.3)            | 1 (2.1)              | 3 (15.8)  | 4 (13.3)      | 0.100    |
| Atrial fibrillation               | 16 (16.8)          | 10 (21.3)            | 6 (31.6)  | 0 (0)         | 0.007    |
| Chronic kidney disease            | 7 (7.3)            | 7 (14.9)             | 0 (0)     | 0 (0)         | 0.02     |
| **Parameters of echocardiography**|                    |                      |           |               |          |
| Left ventricular ejection fraction, % | 60 (60-60)    | 60 (60-60)           | 60 (60-60) | 60 (60-60) | 0.872    |
| Right ventricular dilatation      | 15 (15.6)          | 15 (31.9)            | 0 (0)     | 0 (0)         | 0.036    |
| Right ventricular function (TAPSE in mm) | 21 (15.3-26.7)  | 20 (14.9-25.1)       | 26 (20.3-31.7) | -            | 0.104    |
| Tricuspid regurgitation >1        | 9 (9.3)            | 6 (12.8)             | 3 (15.8)  | 0 (0)         | 0.205    |
| Pericardial effusion              | 19 (19.8)          | 19 (40.4)            | 0 (0)     | 0 (0)         | 0.010    |
| Pleural effusion                  | 12 (12.5)          | 11 (23.4)            | 1 (5.3)   | 0 (0)         | 0.713    |
| PAPsys (mmHg)                     | 25 (20.3-33)       | 25 (20-30)           | 26 (22.5-38) | -            | 0.327    |
| **Concomitant cardiac medication at study entry** |                    |                      |           |               |          |
| Oral anticoagulation              | 12 (12.5)          | 5 (10.6)             | 7 (36.8)  | 0 (0)         | 0.001    |
| ACE-I or ARB                      | 41 (42.7)          | 30 (63.8)            | 11 (57.9) | 0 (0)         | 0.036    |
| Aldosterone inhibitors            | 7 (7.3)            | 6 (12.8)             | 1 (5.3)   | 0 (0)         | 0.085    |
| Laboratory parameters and biomarkers                  | Data (µg/dL)                        | Data (mg/dL)                        | Data (mL/m²)                        | Data (ng/mL)                       | Data (µg/dL)        |
|------------------------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|--------------------|
| Diuretics                                            | 22 (22.9)                           | 19 (40.4)                           | 3 (15.8)                            | 0 (0)                               | <0.001             |
| Calcium channel blockers                             | 18 (18.8)                           | 13 (27.7)                           | 5 (26.3)                            | 0 (0)                               | 0.005              |
| Beta blockers                                        | 25 (26)                             | 18 (38.3)                           | 7 (36.8)                            | 0 (0)                               | <0.001             |
| Statins                                              | 26 (27.1)                           | 20 (42.6)                           | 6 (31.6)                            | 0 (0)                               | <0.001             |
| ASA                                                  | 18 (5.3)                            | 13 (27.7)                           | 5 (26.3)                            | 0 (0)                               | 0.005              |
| P2Y12 inhibitors                                     | 2 (2.1)                             | 1 (2.1)                             | 1 (5.3)                             | 0 (0)                               | 0.464              |
| Parameters of electrocardiography                    |                                     |                                     |                                     |                                     |                    |
| Heart Rate (bpm)                                     | 76 (65-83)                          | 77 (69-88)                          | 69 (64-82)                          | 61 (58-74)                          | 0.057              |
| Heart Rhythm                                         |                                     |                                     |                                     |                                     |                    |
| - Sinus rhythm                                       | 46 (49)                             | 29 (61.7)                           | 17 (89.5)                           | 0 (0)                               |                    |
| - Atrial fibrillation                                | 7 (7.3)                             | 6 (12.8)                            | 1 (5.3)                             | 0 (0)                               | 0.463              |
| - Pacemaker with ventricular pacing                  | 4 (4.2)                             | 3 (6.4)                             | 1 (5.3)                             | 0 (0)                               |                    |
| Laboratory parameters and biomarkers                  |                                     |                                     |                                     |                                     |                    |
| Leukocytes (1000/µL)                                 | 6805 (5112.5-8152.5)                | 6880 (4190-7650)                    | 7660 (5940-9000)                    | 6890 (6000-7670)                   | 0.051              |
| Lymphocytes (1000/µL)                                | 920 (660.-1730)                     | 690 (590-970)                       | 1540 (1370-2050)                    | 2110 (1545-2425)                   | <0.001             |
| Hb (g/dL)                                            | 13.4 (11.8-14.1)                    | 13 (11.2-13.8)                      | 13.9 (12.9-14.6)                    | 13.9 (13-14.2)                      | 0.025              |
| Platelets (1000/µL)                                  | 211.5 (152.5-285.5)                 | 169 (142-243)                       | 240.5 (203.8-301.5)                 | 252 (218-305)                       | 0.002              |
| INR (%)                                              | 1.1 (1-1.1)                         | 1.1 (1-1.1)                         | 1 (1-1)                             | 1 (1-1)                             | 0.136              |
| PTT (s)                                              | 24 (22.75-28)                       | 24 (22-28)                          | 25 (23-33.5)                        | 24 (23-24.5)                        | 0.335              |
| D-Dimer (µg/dL)                                      | 0.9 (0.6-1.7)                       | 0.9 (0.7-1.7)                       | 0.3 (0.3-0.3)                       | 0.2 (0.2-0.2)                       | 0.099              |
| Creatinine (mg/dL)                                   | 0.8 (0.7-1)                         | 0.9 (0.7-1.2)                       | 0.8 (0.6-0.8)                       | 0.7 (0.7-0.8)                       | 0.035              |
| GFR-MDRD (mL/min)                                    | 80.1 (69.3.-100.5)                  | 75.5 (58.3-98.9)                    | 84.6 (74.1-99.7)                    | 90.1 (80.9-119.3)                   | 0.012              |
| Sodium (mmol/L)                                      | 139 (136-140)                       | 136 (134-140)                       | 140 (138.8-142.3)                   | 140 (139-142)                       | <0.001             |
| CRP (mg/dL)                                          | 1.5 (0.4-9.63)                      | 4 (1.4-12.2)                        | 0.3 (0.1-1.2)                       | 0.1 (0-0.5)                         | <0.001             |
| PCT (ng/mL)                                          | 0.1 (0.1-0.2)                       | 0.1 (0.1-0.2)                       | -                                   | -                                   | -                 |
| IL-6                                                 | 19.9 19.9 (10.2-43.5)               | 13.5 (5.2-27.3)                     | 26.9 (10.2-59.8)                    | 31.9 (16.3-50.1)                    | 0.013              |
| TNF-α                                                | 55.5 (28.8-149.6)                   | 143.6 (37.5-362.6)                  | 62.3 (43.1-116.4)                   | 27.9 (21.5-55.5)                    | <0.001             |
| hs TNI (ng/dL)                                       | 8 (3-19.5)                          | 10 (3-25)                           | 4.0 (3-6.5)                         | 3 (3-3)                             | 0.068              |
| NT-pro-BNP (ng/L)                                    | 364 (98-3068)                       | 411 (132-3422)                      | 330 (280-1035)                      | 84 (44.3-168)                       | 0.030              |
| Measure | Intensive Care | Horovitz Index (mmHg) |
|---------|----------------|-----------------------|
| CK (U/L) | 113 (63-198) | - 300 mmHg          | 79 (82.3)                  |
|         | 122 (66-250) | - 201 - 300 mmHg     | 6 (6.3)                    |
|         | 74 (52.5-128) | - 101 - 200 mmHg     | 6 (6.3)                    |
|         | 99 (71-165.5) | ≤ 100 mmHg           | 5 (5.2)                    |
| AST (U/L) | 22 (16-38)  | > 300 mmHg           | 30 (63.8)                  |
|         | 35 (20-43.5) | 201 - 300 mmHg       | 6 (12.8)                   |
|         | 16 (13-20.5) | 101 - 200 mmHg       | 6 (12.8)                   |
|         | 19 (15.5-25) | ≤ 100 mmHg           | 5 (10.6)                   |
| ALT (U/L) | 24 (17.5-36) | - greater than 300 mmHg | 0.065                     |
|         | 27 (19-38)   | - 201 - 300 mmHg     | 0.001                     |
|         | 20 (14.8-28) | - 101 - 200 mmHg     | 0.001                     |
|         | 22.2 (16.5-30.8) | ≤ 100 mmHg           | 0.001                     |
| LDH (U/L) | 218 (188.5-285.8) | > 300 mmHg           | 0.065                     |
|         | 263.5 (210.3-332.5) | 201 - 300 mmHg       | 0.001                     |
|         | 195.5 (176-210.8) | 101 - 200 mmHg       | 0.001                     |
|         | 162.5 (147.3-227.3) | ≤ 100 mmHg           | 0.001                     |
| HbA1c (%) | 6 (5.6-6.3)  | Bacterial co infection | 4 (4.2)                   |
|         | 6.2 (6-6.4)  | Acute kidney injury | 2 (2.1)                    |
|         | 5.6 (5.4-6)   | Acute hepatic injury | 3 (3.1)                   |
|         | 5.4 (5-6)     | Vasopressors | 6 (6.3)                    |
|         | - | Lactate (mmol/L) | 1.4 (0.9-1.9)               |
|         | - | pH               | 7.4 (7.4-7.5)               |
| Values are given as numbers (n) and percentage (%) or are given as median and interquartile range (IQR). ACE - Angiotensin Converting Enzyme, Afib - atrial fibrillation, ALT – alanine amino-tranferase, ARB - Angiotensin II Receptor Blockers, ASA – Acetylsalicylic acid , AST – aspartate-aminotransferase, BMI – body mass index, CAD – coronary artery disease, CK – creatinine kinase, CRP – C-reactive protein, GFR-MDRD – glomerular filtration rate, Hb – hemoglobin, hs TNI - High sensitive Troponin I, INR – international normalized ratio, LDH – lactate dehydrogenase, NT-pro-BNP – N-terminal pro- brain natriuretic peptide, Pap Sys – pulmonary arterial pressure systolic, PCT – procalcitonin, PTT – partial thromboplastin time, SARS-CoV-2 – severe acute respiratory syndrome coronavirus-2 |
| Parameters                                      | All Patients, N=47 | No ICU, N=33   | ICU, N=14    | p-value |
|------------------------------------------------|--------------------|----------------|--------------|---------|
| **Clinical characteristics**                   |                    |                |              |         |
| Age, y                                         | 79 (59-82)         | 75 (56-81)     | 79 (67.8-84.5)| 0.264   |
| Male                                           | 24 (51.1)          | 16 (48.5)      | 8 (57.1)     | 0.587   |
| BMI (kg/m²)                                    | 27.8 (25.1-31.8)   | 27.5 (25.2-31.9)| 28.7 (25-33) | 0.644   |
| Fever                                          | 31 (65.9)          | 22 (66.7)      | 9 (64.3)     | 0.867   |
| hs TNI > 37 (ng/L)                             | 8 (17)             | 3 (9.1)        | 5 (37.7)     | **0.034**|
| NT-pro BNP > 300 (ng/L)                        | 27 (57.4)          | 15 (45.5)      | 12 (85.7)    | **0.011**|
| CRP > 0.5 ml/dL                                | 44 (93.6)          | 30 (90.9)      | 14 (100)     | 0.244   |
| **Cardiovascular risk factors**                |                    |                |              |         |
| Arterial hypertension                          | 37 (78.7)          | 24 (72.7)      | 13 (92.9)    | 0.123   |
| Dyslipidemia                                   | 24 (51.1)          | 18 (54.5)      | 6 (42.9)     | 0.608   |
| Diabetes mellitus                              | 12 (25.5)          | 8 (24.2)       | 4 (28.6)     | 0.756   |
| Current smokers                                | 1 (2.1)            | 1 (3)          | 0 (0)        | 0.526   |
| Atrial fibrillation                            | 10 (21.3)          | 6 (18.8)       | 4 (28.6)     | 0.457   |
| Chronic kidney disease                         | 7 (14.9)           | 3 (9.1)        | 4 (28.6)     | 0.086   |
| **Parameters of echocardiography**             |                    |                |              |         |
| Left ventricular ejection fraction, %          | 60 (60-60)         | 60 (60-60)     | 60 (60-60)   | 0.575   |
| Right ventricular dilatation                   | 15 (31.9)          | 10 (30.3)      | 5 (35.7)     | 0.714   |
| Right ventricular function (TAPSE in mm)       | 20 (14.9-25.1)     | 20 (14.7-25.3) | 25 (21.3-28.7)| 0.304   |
| Tricuspid regurgitation >1                     | 6 (12.8)           | 4 (12.1)       | 2 (14.3)     | 0.152   |
| Pericardial effusion                           | 19 (40.4)          | 14 (42.4)      | 5 (35.7)     | 0.370   |
| Pleural effusion                               | 11 (23.4)          | 8 (24.2)       | 3 (21.4)     | 0.933   |
| PAPsys (mmHg)                                  | 25 (20-30)         | 24 (20-30)     | 30 (23.8-37.8)| 0.081   |
| **Concomitant cardiac medication at study entry**|                    |                |              |         |
| Oral anticoagulation                           | 5 (10.6)           | 3 (9.1)        | 2 (14.3)     | 0.407   |
| ACE-I or ARB                                   | 9 (19.1)           | 7 (21.2)       | 2 (14.3)     | 0.541   |
| Aldosterone inhibitors                         | 22 (46.8)          | 14 (42.4)      | 8 (57.1)     | 0.176   |
### Diuretics
- 6 (12.8)
- 4 (12.1)
- 2 (14.3)
- 0.720

### Calcium channel blockers
- 19 (40.4)
- 14 (42.4)
- 5 (35.7)
- 0.901

### Beta blockers
- 13 (27.7)
- 10 (30.3)
- 3 (21.4)
- 0.686

### Statins
- 18 (38.3)
- 13 (39.4)
- 5 (35.7)
- 0.950

### ASA
- 20 (42.6)
- 15 (45.5)
- 5 (35.7)
- 0.757

### P2Y12 inhibitors
- 13 (27.7)
- 11 (33.5)
- 2 (14.3)
- 0.252

### Parameters of electrocardiography

| Heart Rate (bpm) | 77 (69.5-87.8) | 76 (69.5-86.5) | 77 (68.9-88) | 0.817 |
|------------------|----------------|----------------|--------------|------|
| Heart Rhythm     |                |                |              |      |
| - Sinus rhythm   | 29 (61.7)      | 20 (60.6)      | 9 (64.3)     |      |
| - Atrial fibrillation | 6 (12.8) | 3 (9.1)      | 3 (21.4)     | 0.672 |
| - Pacemaker with ventricular pacing | 3 (6.4) | 2 (6.1) | 1 (7.1) |      |

### Laboratory parameters and biomarkers

| Leukocytes (1000/µL) | 60,80 (41,990-76,500) | 56,70 (41,570-73,750) | 69,05 (42,600-92,655) | 0.285 |
|----------------------|-----------------------|-----------------------|-----------------------|------|
| Lymphocytes (1000/µL) | 690 (590-790)        | 810 (635-1200)        | 585 (500-775.5)        | 0.008 |
| Hb (g/dL)            | 13 (11.2-13.8)       | 13 (11.7-13.8)       | 12.5 (9.4-14.4)        | 0.609 |
| Platelets (1000/µL)  | 169 (142-243)        | 173 (142.5-244)      | 163 (120.8-253.8)      | 0.545 |
| INR (%)              | 1.1 (1-1.1)          | 1.1 (1-1.1)          | 1.1 (1-1.1)            | 0.647 |
| PTT (s)              | 24 (22-28)           | 24 (22-26.5)         | 26.5 (23-31)           | 0.092 |
| D-Dimer (µg/dL)      | 0.9 (0.7-1.7)        | 0.8 (0.5-1.1)        | 1.7 (1-2.7)            | 0.003 |
| Creatinine (mg/dL)   | 0.9 (0.7-1.2)        | 0.8 (0.7-1)          | 1 (0.6-1.5)            | 0.520 |
| GFR-MDRD (mL/min)    | 75.5 (58.3-98.9)     | 79 (59.9-99.7)       | 73 (40.7-96.8)         | 0.471 |
| Sodium (mmol/L)      | 136 (134-140)        | 136 (132.5-139.5)    | 137 (134.8-140)        | 0.726 |
| CRP (mg/dL)          | 4.1 (1.4-12.2)       | 2.3 (1.2-6)          | 12.3 (9.5-21.3)        | <0.001 |
| PCT (ng/mL)          | 0.1 (0.1-0.2)        | 0.1 (0.1-0.2)        | 0.1 (0.1-0.7)          | 0.004 |
| IL-6                 | 13.5 (5.2-27.3)      | 15.2 (5.5-29.4)      | 11.7 (4.2-19.5)        | 0.366 |
| TNF-α                | 143.6 (37.5-362.6)   | 59.7 (32.3-211.5)    | 259.9 (146.8-911.5)    | 0.019 |
| hs TNI (ng/dL)       | 10 (3-25)            | 7 (2-17)             | 20 (12-105.8)          | 0.010 |
| NT-pro-BNP (ng/L)    | 411 (132-3422)       | 309 (92-826.5)       | 2328 (639.5-12168.5)   | 0.005 |
| CK (U/L)             | 122 (66-250)         | 121 (64-251)         | 181.5 (115.8-244.3)    | 0.329 |
Values are given as numbers (n) and percentage (%) or are given as median and interquartile range (IQR). Continuous, not normally distributed variables are expressed as median and IQR and were compared using Mann–Whitney U test for two group comparison and Kruskal-Wallis test for three group comparison, where applicable. Continuous parameters were dichotomized at established cut-off values if necessary. Categorical data are presented as total numbers and proportions and were analysed by chi-squared test. Comparisons were considered statistically significant if two-sided p-value was <0.05.

|                  |       |       |      |            |
|------------------|-------|-------|------|------------|
|                  | 35 (20-43.5) | 32 (20-42) | 40 (24-62.3) | 0.073      |
| AST (U/L)        |       |       |      |            |
| ALT (U/L)        | 27 (19-38)  | 28 (19.5-39.5) | 21.5 (17.8-34) | 0.306      |
| LDH (U/L)        | 263.5 (210.3-332.5) | 235.5 (193.3-304.3) | 327.5 (269.3-405.3) | **0.003**  |
| Hba1c (%)        | 6.2 (6-6.4)  | 6.1 (6-6.4)  | 6.3 (6.05-6.5) | 0.325      |
| Lactate (mmol/L) | 1.4 (0.9-1.9) | 1.3 (0.9-1.8)  | 1.6 (0.9-2.3)  | 0.590      |
| pH               | 7.4 (7.4-7.5) | 7.4 (7.4-7.5) | 7.4 (7.4-7.5) | 0.803      |

### Intensive Care

|                          |       |       |      |            |
|--------------------------|-------|-------|------|------------|
| Bacterial co infection   | 4 (8.5) | 0 (0)  | 4 (28.6) | **0.004**  |
| Acute kidney injury      | 2 (4.3) | 0 (0)  | 2 (14.3) | **0.024**  |
| Acute hepatic injury     | 3 (6.4) | 2 (6.1) | 1 (7.1)  | 0.882      |
| Vasopressors             | 6 (12.8) | 1 (3)  | 5 (35.7) | **0.001**  |

### Horovitz Index (mmHg)

|                          |       |       |      |            |
|--------------------------|-------|-------|------|------------|
| > 300 mmHg               | 30 (63.8) | 26 (78.8) | 4 (28.6) |            |
| 201 - 300 mmHg           | 6 (12.8)  | 6 (18.2) | 0 (0)   |            |
| 101 - 200 mmHg           | 6 (12.8) | 1 (3)  | 5 (35.7) |            |
| ≤ 100 mmHg               | 5 (10.6)  | 0 (0)  | 5 (35.7) |            |

Values are given as numbers (n) and percentage (%) or are given as median and interquartile range (IQR). Continuous, not normally distributed variables are expressed as median and IQR and were compared using Mann–Whitney U test for two group comparison and Kruskal-Wallis test for three group comparison, where applicable. Continuous parameters were dichotomized at established cut-off values if necessary. Categorical data are presented as total numbers and proportions and were analysed by chi-squared test. Comparisons were considered statistically significant if two-sided p-value was <0.05. ACE - Angiotensin Converting Enzyme, Afib - atrial fibrillation, ALT – alanine aminotransferase, ARB - Angiotensin II Receptor Blockers, ASA – Acetylsalicylic acid, AST – aspartate-aminotransferase, BMI – body mass index, CAD – coronary artery disease, CK – creatinine kinase, CRP – C-reactive protein, GFR-MDRD – glomerular filtration rate, Hb – hemoglobin, hs TNI - High sensitive Troponin I, INR – international normalized ratio, LDH – lactate dehydrogenase, NT-pro-BNP – N-terminal pro- brain natriuretic peptide, Pap Sys – pulmonary arterial pressure systolic, PCT – procalcitonin, PTT – partial thromboplastin time, SARS-CoV-2 – severe acute respiratory syndrome coronavirus-2.
Table 3. Baseline characteristics of patient population stratified by the occurrence of the clinical endpoint (progressive respiratory failure defined by Horovitz Index HI < 200mmHg and mechanical ventilation)

| Parameters                          | All Patients, N=47 | HI > 200mmHg, N=36 | HI ≤ 200mmHg, N=11 | p-Value |
|-------------------------------------|--------------------|--------------------|--------------------|---------|
| **Clinical characteristics**        |                    |                    |                    |         |
| Age, y                              | 79 (59-82)         | 77 (55.5-81)       | 79 (68-86)         | 0.170   |
| Male                                | 24 (51.1)          | 18 (50)            | 6 (54.5)           | 0.792   |
| BMI (kg/m²)                         | 27.8 (25.1-31.8)   | 27.8 (25.2-31.9)   | 27.7 (24.8-33.8)   | 0.959   |
| Fever                               | 31 (67.4)          | 25 (69.4)          | 6 (54.5)           | 0.573   |
| hs TNI > 37 (ng/L)                  | 8 (17)             | 4 (11.1)           | 4 (36.4)           | 0.064   |
| NT-pro BNP > 300 (ng/L)             | 27 (57.4)          | 17 (47.2)          | 10 (90.9)          | 0.010   |
| CRP > 0.5 ml/dL                     | 44 (93.6)          | 33 (91.7)          | 11 (100)           | 0.322   |
| **Cardiovascular risk factors**     |                    |                    |                    |         |
| Arterial hypertension               | 37 (78.7)          | 27 (75)            | 10 (90.9)          | 0.259   |
| Dyslipidemia                        | 24 (51.1)          | 20 (55.6)          | 4 (36.4)           | 0.229   |
| Diabetes mellitus                   | 12 (25.5)          | 9 (25)             | 3 (27.3)           | 0.880   |
| Current smokers                     | 1 (2.1)            | 0 (0)              | 1 (9.1)            | 0.571   |
| Atrial fibrillation                 | 10 (21.3)          | 6 (16.7)           | 4 (36.4)           | 0.178   |
| Chronic kidney disease              | 7 (14.9)           | 5 (13.8)           | 2 (18.2)           | 0.726   |
| **Parameters of echocardiography**  |                    |                    |                    |         |
| Left ventricular ejection fraction, %| 60 (60-60)        | 60 (60-60)         | 60 (60-60)         | 0.176   |
| Right ventricular dilatation        | 15 (31.9)          | 11 (30.6)          | 4 (36.4)           | 0.229   |
| Right ventricular function (TAPSE in mm) | 20 (14.9-25.1)   | 20.5 (18-25.3)     | 19 (19-19)         | 0.524   |
| Tricuspid regurgitation >1          | 6 (12.8)           | 4 (11.1)           | 2 (18.2)           | 0.494   |
| Pericardial effusion                | 19 (40.4)          | 13 (36.1)          | 6 (54.5)           | 0.162   |
| Pleural effusion                    | 11 (23.4)          | 8 (22.2)           | 3 (27.3)           | 0.765   |
| PAPsys (mmHg)                       | 25 (20-30)         | 24 (20-30)         | 30 (24.8-47.3)     | 0.053   |
| **Concomitant cardiac medication at study entry** | | | | |
| Oral anticoagulation                | 2 (4.3)            | 2 (5.6)            | 0 (0)              | 0.869   |
| ACE-I or ARB                        | 30 (63.8)          | 24 (66.7)          | 6 (54.5)           | 0.464   |
### Laboratory parameters and biomarkers

| Parameter                        | Mean (Range)       | Median (Range)    | 95% CI (Range)     | p-value |
|----------------------------------|--------------------|-------------------|--------------------|---------|
| Aldosterone inhibitors          | 6 (12.8)           | 4 (11.1)          | 2 (18.2)           | 0.300   |
| Diuretics                        | 19 (40.4)          | 16 (44.4)         | 3 (27.3)           | 0.720   |
| Calcium channel blockers         | 13 (27.7)          | 10 (27.7)         | 3 (27.3)           | 0.586   |
| Beta blockers                    | 18 (38.3)          | 14 (38.9)         | 4 (36.4)           | 0.563   |
| Statins                          | 20 (42.6)          | 18 (50)           | 2 (18.2)           | 0.199   |
| ASA                              | 13 (27.7)          | 12 (33.3)         | 1 (9.1)            | 0.243   |
| P2Y12 inhibitors                 | 1 (2.1)            | 1 (2.8)           | 0 (0)              | 0.633   |
| **Parameters of electrocardiography** |                   |                   |                    |         |
| **Heart Rhythm**                 |                    |                   |                    |         |
| Heart Rhythm                     | 77 (69.5-87.8)     | 77 (71-86.8)      | 74.5 (62.8-97.5)   | 0.595   |
| - Sinus rhythm                   | 29 (61.7)          | 22 (61.1)         | 7 (63.6)           |         |
| - Atrial fibrillation            | 6 (12.8)           | 4 (11.1)          | 2 (18.2)           | 0.861   |
| - Pacemaker with ventricular pacing | 3 (6.4)            | 1 (2.8)           | 2 (18.2)           |         |
| **Laboratory parameters and biomarkers** |                   |                   |                    |         |
| Leukocytes (1000/µL)             | 6080 (4190-7650)   | 5410 (4095-7637.5)| 6840 (5980-8690)   | 0.315   |
| Lymphocytes (1000/µL)            | 690 (590-970)      | 795 (632.5-1130)  | 580 (410-680)      | **0.007** |
| Hb (g/dL)                        | 13 (11.2-13.8)     | 13 (11.2-13.7)    | 13.3 (11.2-15.5)   | 0.407   |
| Platelets (1000/µL)              | 169 (142-243)      | 171 (137.5-241.8) | 164 (143-334)      | 0.821   |
| INR (%)                          | 1.1 (1-1.1)        | 1.1 (1-1.1)       | 1.1 (1-1.1)        | 0.820   |
| PTT (s)                          | 24 (22-28)         | 24 (22-27.8)      | 26 (23-31)         | 0.265   |
| D-Dimer (µg/dL)                  | 0.9 (0.7-1.7)      | 0.8 (0.5-1.1)     | 1.6 (0.9-2.6)      | **0.034** |
| Creatinine (mg/dL)               | 0.9 (0.7-1.2)      | 0.9 (0.7-1.2)     | 1 (0.6-1.4)        | 0.791   |
| GFR-MDRD (ml/m²)                 | 75.5 (58.3-98.9)   | 77.2 (58.6-96.9)  | 73.9 (48.3-100.6)  | 0.860   |
| Sodium (mmol/L)                  | 136 (134-140)      | 136 (135-139.8)   | 136 (132-140)      | 0.752   |
| CRP (mg/dL)                      | 4 (1.4-12.2)       | 2.6 (1.2-8)       | 12.2 (10.6-18.3)   | **0.001** |
| PCT (ng/mL)                      | 0.1 (0.1-0.2)      | 0.1 (0-0.2)       | 0.1 (0.1-0.2)      | **0.045** |
| IL-6                             | 13.5 (5.2-27.3)    | 13.4 (5.9-28.6)   | 11.7 (3-44.3)      | 0.690   |
| TNF-α                            | 143.6 (37.5-362.6) | 61.7 (32.1-220.7) | 272.5 (176.2-766.7)| **0.011** |
| hs TNI (ng/dL)                   | 10 (3-25)          | 7.5 (2-18.5)      | 22 (13-101)        | **0.010** |
Values are given as numbers (n) and percentage (%) or are given as median and interquartile range (IQR). Continuous, not normally distributed variables are expressed as median and IQR and were compared using Mann–Whitney U test for two group comparison and Kruskal-Wallis test for three group comparison, where applicable. Continuous parameters were dichotomized at established cut-off values if necessary. Categorical data are presented as total numbers and proportions and were analysed by chi-squared test. Comparisons were considered statistically significant if two-sided p-value was <0.05.

**ACE** – Angiotensin Converting Enzyme, **Afib** – atrial fibrillation, **ALT** – alanine aminotransferase, **ARB** – Angiotensin II Receptor Blockers, **ASA** – Acetylsalicylic acid, **AST** – aspartate-aminotransferase, **BMI** – body mass index, **CAD** – coronary artery disease, **CK** – creatinine kinase, **CRP** – C-reactive protein, **GFR-MDRD** – glomerular filtration rate, **Hb** – hemoglobin, **Hs TNI** – High sensitive Troponin I, **INR** – international normalized ratio, **LDH** – lactate dehydrogenase, **NT-pro-BNP** – N-Terminal pro-brain natriuretic peptide, **Pap Sys** – pulmonary arterial pressure systolic, **PCT** – procalcitonin, **PTT** – partial thromboplastin time, **SARS-CoV-2** – severe acute respiratory syndrome coronavirus-2

|          | Median (IQR)       | Median (IQR)       | Median (IQR)       | p-value |
|----------|--------------------|--------------------|--------------------|---------|
| NT-pro-BNP (ng/L) | 411 (132-3422)     | 316 (94.3-2511)   | 1588 (749-8845)    | 0.018   |
| CK (U/L) | 122 (66-250)       | 119 (65.3-249.5)  | 192 (116-266)      | 0.269   |
| AST (U/L) | 35 (20-43.5)      | 32.5 (20-40.3)    | 43 (25-70)         | 0.025   |
| ALT (U/L) | 27 (19-38)         | 27 (19.3-38.8)    | 26 (18-37)         | 0.669   |
| LDH (U/L) | 263.5 (210.3-332.5)| 242 (197-309)     | 367 (279-445)      | 0.002   |
| HbA1c (%) | 6.2 (6-6.4)        | 6.1 (5.9-6.4)     | 6.3 (6.2-6.5)      | 0.331   |
| Lactate (mmol/L) | 1.4 (0.9-1.9)   | 1.5 (0.9-1.8)     | 1.3 (0.9-2.3)      | 0.836   |
| pH       | 7.4 (7.4-7.5)      | 7.4 (7.4-7.5)     | 7.4 (7.4-7.5)      | 0.941   |

### Intensive Care

| Condition                          | Median (IQR) | Median (IQR) | Median (IQR) | p-value |
|------------------------------------|--------------|--------------|--------------|---------|
| Bacterial co infection             | 4 (8.5)      | 0 (0)        | 4 (36.4)     | 0.009   |
| Acute kidney injury                | 2 (4.3)      | 0 (0)        | 2 (18.2)     | 0.038   |
| Acute hepatic injury               | 3 (6.4)      | 2 (5.6)      | 1 (9.1)      | 1.000   |
| Vasopressors                       | 6 (12.8)     | 1 (2.8)      | 5 (45.5)     | 0.004   |

| Horovitz Index (mmHg)              | Median (IQR) | Median (IQR) | Median (IQR) | p-value |
|------------------------------------|--------------|--------------|--------------|---------|
| - > 300 mmHg                       | 30 (63.8)    | 30 (83.3)    | 0 (0)        |         |
| - 201 - 300 mmHg                   | 6 (12.8)     | 6 (16.7)     | 0 (0)        | <0.001  |
| - 101 - 200 mmHg                   | 6 (12.8)     | 0 (0)        | 6 (54.5)     |         |
| - ≤ 100 mmHg                       | 5 (10.6)     | 0 (0)        | 5 (45.5)     |         |
Figure 2

A

CD4 (median) on non-classical CD4+CD161-

Control CAD CAD+ SARS-CoV-2

B

CD2 (median) on non-classical CD4+CD161-

C

CD3 (median) on non-classical CD4+CD161-

D

HLA-DR (median) on non-classical CD4+CD161-

E

CD80 (median) on non-classical CD4+CD161-

Control CAD CAD+ SARS-CoV-2

No ICU ICU

Hb > 200 mmHg Hb < 200 mmHg
Numbers and phenotype of non-classical CD14$^{\text{dim}}$CD16$^+$ monocytes are predictors of adverse clinical outcome in patients with coronary artery disease and severe SARS-CoV-2 infection.