Identification of two specific transcriptomic clusters of COVID-19 acute respiratory distress syndrome patients with different immune profiles and different outcomes

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Transcriptomic clustering of patients with ARDS due to COVID-19 identified different immune profiles and outcomes. This demonstrates heterogeneity among COVID-19 ARDS patients and may help to establish personalised therapies. https://bit.ly/3h61sCj

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the respiratory illness COVID-19 (coronavirus disease 2019). The virus was first identified in December 2019 in Wuhan, China and has since then spread globally, resulting in the ongoing SARS-CoV-2 pandemic, causing more than 615 million confirmed cases of infection (https://covid19.who.int/). Although the largest proportion of SARS-CoV-2 infections in humans is characterised by a mild course of disease, about 5% to 20% of patients are hospitalised with COVID-19 due to a more severe course of disease, and require admission to the intensive care unit for diffuse lung infiltrates and severe hypoxaemia [1]. This in turn can lead to the development of acute respiratory distress syndrome (ARDS). Multiple and heterogenous factors are known to cause ARDS, which is pathophysiologically characterised by acute diffuse damage to the alveolar–capillary barrier of the lung, resulting in overflooding of the alveolar space as well as severely limited gas exchange [2, 3]. Among the contributing factors to ARDS are surfactant dysfunction along with a reduction of the capacity of alveolar type I and II (ATI and ATII) epithelial cells to absorb excess fluid from the airspaces into the lung interstitium. Normally, fluid is eliminated from the lung interstitium *via* lymphatic capillaries by vectorial ion transport. The hypoxaemia seen in ARDS results from ventilation/perfusion mismatch and increased shunt perfusion, clinically detected by bilateral opacities on chest radiographs, associated with a decrease in lung compliance and increases in venous admixture of insufficiently oxygenated blood and physiological dead space. Also, acute exudative and proliferative phases are often followed by barrier repair and/or by lung fibrosis.

The duration of individual cases of ARDS varies and, as noted above, the respective clinical courses can range from mild to severe. ARDS can thus be regarded as a heterogenous syndrome, rather than a homogenous disease state. This substantial heterogeneity within the general ARDS population has most likely contributed to the failure of many experimental therapies for ARDS over the past decades, as well as in recent large clinical trials, despite promising preclinical data [4, 5]. There have been many attempts over the past years to identify meaningful subgroups in ARDS that likely respond differently to treatment among the very heterogenous population with ARDS. The clinical parameters used for this potential sub-phenotyping of ARDS patients have included cause of ARDS, time-course of ARDS, degree of hypoxaemia, arterial oxygen tension to inspiratory oxygen fraction ratio, dead space fraction, ventilatory ratio, driving pressure and radiographic pattern [3, 4]. Plasma protein biomarkers were found to be of particular interest with respect to their prognostic value, such as those of 1) epithelial injury, *e.g.* the soluble receptor for advanced glycation end products (sRAGE) and surfactant protein-D (SP-D),

2) endothelial injury, *e.g.* angiopoietin-2 (Ang-2) and intercellular adhesion molecule-1 (ICAM-1), 3) systemic inflammation (interleukin (IL)-6, IL-8, soluble tumour necrosis factor (TNF) receptor-1, IL-18), and 4) disordered coagulation (plasminogen activator inhibitor-1, protein C). Calfee *et al.* [6] identified two distinct sub-phenotypes of ARDS in their respective ARDS patient class analysis combining enrolled patients' clinical and biological data: the hyperinflammatory sub-phenotype (phenotype 2) was characterised by higher plasma concentrations of inflammatory biomarkers, a higher prevalence of vasopressor use, lower serum bicarbonate concentrations, and a higher prevalence of sepsis than phenotype 1. Patients categorised as phenotype 2 also had higher mortality and fewer ventilator-free days and organ failure-free days in both cohorts than did those in phenotype 1. Calfee and co-workers subsequently confirmed their respective sub-phenotype categorisation analysis method in several independent ARDS cohorts [7, 8]. In an independent approach, Bos *et al.* [9] identified two identical sub-phenotypes using hierarchical clustering, thereby further confirming the concept of hyper- and hypo-inflammatory phenotypes in ARDS.

COVID-19 ARDS can be regarded as a homogenous clinical entity when considering its initial cause, as well as the general pathophysiology of lung injury it entails. Viral infection of bronchial epithelial and ATII cells is thought to occur *via* the respective SARS-CoV-2 host receptor angiotensin converting enzyme-2 (ACE2) upon invasion of the virus into the bronchoalveolar system. However, human lungs were shown to possess limited permissiveness for SARS-CoV-2 due to limited ACE2 expression [10]. Interactions between epithelial and immune cells have henceforward been associated with severe courses of disease, particularly those involving CCR1, CCR2 and CCR5 [11]. Also, Nouailles *et al.* [12] demonstrated that endothelial cells develop an inflammatory phenotype despite not being infected, thus contributing to the recruitment of immune cells into the alveolar compartment. Intense recruitment and hyper-activation of neutrophils and monocytes has been linked with COVID-19 ARDS [13], as well as the specific transcriptional programming of neutrophils leading to enhanced neutrophil extracellular trap formation [14], coagulation [15], and immunothrombosis [16]. Michalick *et al.* [17] demonstrated that endothelial barrier failure is triggered by barrier-disruptive mediators released from the infected airspace epithelium, as well as by the consecutive immune response factors released into the bloodstream. SARS-CoV-2 possibly triggers profibrotic macrophage responses early in the inflammatory cascade, resulting in alveolar collapse and, ultimately, pronounced fibroproliferation [18, 19].

Intriguingly, individual clinical features of patients with COVID-19 ARDS may substantially differ from each other, despite homogenous underlying causes and comparatively uniform pathophysiology: while one patient may already be improving after a mild case of COVID-19 ARDS, the health status of another patient may be rapidly deteriorating, while a third patient might develop an advanced fibrotic stable state. Moreover, the extent of a respective contribution of a specific patho-mechanism to the observed severity of the resulting disease course may substantially differ between individuals. Thus, using a combination of clinical and biological parameters for sub-phenotyping of patients with COVID-19 ARDS may be advantageous in that it not only enables prediction of disease outcome more accurately than with single markers alone, but also increases anticipated success of individual treatment approaches. The ability of a biomarker panel to predict host response more accurately in patients with severe lung disease, to enable higher precision in clinical decision making, was convincingly demonstrated by Husain *et al.* [20] in this journal. Moreover, Messner *et al.* [21] noted as early as 2020 that it was most likely a specific combination of inflammation, coagulation, complement activation and lung barrier failure whose complex interaction determined the severity of COVID-19 disease progression. This suggests that ideal therapeutic approaches should be directed against more than one of these conditions. It is therefore important to note that COVID-19 therapies currently employed routinely have only one target, this being either the virus, or the inflammatory response, or coagulation. In this context, administration of monoclonal antibodies, antivirals, steroids [22] inhibitors of IL-1 [23], IL-6 [24], Janus kinase 1/2 [25] and granulocyte-macrophage colony-stimulating factor [26], as well as therapeutic anticoagulants [27], have been shown to markedly improve disease progression and outcome. Nonetheless, mortality of critically ill patients with COVID-19 remains markedly high.

In this issue of the *European Respiratory Journal*, López-Martínez *et al.* [28] report the identification of two transcriptomic clusters of critically ill COVID-19 with different immune profiles, as well as a cluster specific effect of steroids in either decreasing lymphocyte activation, or in promoting B cell activation, in both groups respectively. These findings make a case for the presence of different underlying pathogenetic mechanisms in severe COVID-19, potentially of use for improving risk stratification in these patients, as well as for better identification of those specific profiles which could potentially benefit from personalised treatments alleviating inflammatory responses. The transcriptomic cluster with worse outcome is termed the COVID-19 transcriptomic profile (CTP) 1 and is characterised by an interferon-driven response and

CD4+ T lymphocyte activation. Furthermore, López-Martínez *et al*. [28] show steroids to downregulate genes involved in lymphocyte activation, but to upregulate the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway in the CTP1 cluster. It has been previously shown that the JAK/STAT pathway can be activated by IL-6 and is related to mortality in patients with COVID-19 [29, 30]. These findings suggest a possible reason for the complementary effect of a combination therapy with steroids and a JAK/IL-6 inhibitor in suppressing the excessive inflammation response, resulting in better outcomes for patients in the CTP1 cluster [24, 25]. Another cluster termed CTP2 shows improved outcome and is characterised by B cell and regulatory T cell (Treg) activation and upregulation of immune checkpoints such as B cell lymphoma 2 (BCL2) and lymphocyte-activation gene 3 (LAG3), as well as that of the immunoglobulin and TNF superfamilies. Interestingly, findings of this study corroborate previous reports of Treg of having a beneficial effect on patient outcome [31, 32]. Treg plays an essential role in maintaining immune homeostasis, thus restraining excessive inflammation response. Previous reports have indicated that the balance in maintaining immune homeostasis between suppressive function of Treg and the antiviral immune responses is crucial for overcoming SARS-CoV-2 infection.

Patients' health status and general condition have been shown to deteriorate when the above-mentioned homeostasis is disrupted [33, 34]. Excessive Treg activity was demonstrated to result in virus persistence, as well as in more production of pro-inflammatory pathogen- and damage-associated molecular patterns, and in exacerbated inflammation. Conversely, insufficient Treg activity was shown to contribute to an aggressive inflammatory response including a cytokine storm, resulting in tissue damage and ARDS. Therefore, Treg status and respective levels of its constituents may be essential targets for future therapeutic strategies to improve outcome in patients with COVID-19 [34]. Upregulation of immune checkpoints, particularly those of co-inhibitory molecule, can either be an activation or an immune-paralysis marker of lymphocytes [35, 36]. Programmed cell death 1 (PD-1) is well-known as an immune-paralysis marker, indicative of immune cell exhaustion. However, previous studies have shown that the proportion of IFN-γ-producing SARS-CoV-2-specific $CD8^+$ T cells is higher in $PD\text{-}1^+$ cells than in $PD\text{-}1^-$ cells, indicating that SARS-CoV-2-specific $CD8^+$ T cells with high expression of a co-inhibitory immune checkpoint molecule are not exhausted, but functional [37]. Thus, assessment of immune checkpoints is complex, and immune cell functions need to be considered. The findings by López-Martínez *et al*. [28] provide important insights on future challenges in the field of virus-induced immune cell activation. Of note, samples for analysis in their study were collected in 2020 when the current (2022) treatment strategies for patients with COVID-19 ARDS were not yet established. Therefore, transcriptomic cluster analysis comparing responders and non-responders to the current standard therapy (antiviral agent plus steroids combined with a JAK/IL-6 inhibitor) would be helpful to improve and upgrade current treatment strategy. Moreover, investigation whether immunomodulation therapy using only steroids is important to determine whether this might be sufficient for patients assigned to CTP2 to prevent secondary infections due to unnecessary immunosuppressive therapy.

ARDS has been well-known as a pathological condition with heterogeneous subgroupings. This study by López-Martínez *et al*. [28] demonstrates that COVID-19 ARDS is also a heterogeneous phenomenon, albeit having a common causative pathogen, namely SARS-CoV-2. Importantly, this study reveals two specific transcriptomic clusters of COVID-19 ARDS patients with different immune profiles and different outcomes. Biomarkers available in the current clinical setting are not sufficient to enable classification of the specific therapies for respective patients. In the future, more clinical trials, such as those of -omics analyses of the López-Martínez *et al*. [28] study discussed above, are needed to develop more effective and individualised treatments improving COVID-19 ARDS outcome. Thus, the challenge continues to develop respective personalised therapies and rapid diagnosis methods for a more precise and comprehensive assessment of immunopathological status to improve outcome in COVID-19 ARDS patients.

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inhibiting the expression of Ang-2; DE 102020116249.9, camostat/niclosamide cotreatment in SARS-CoV-2 infected human lung cells; PCT/EP2021/075627, new medical use of cystic fibrosis transmembrane conductance regulator (CFTR) modulators; outside the submitted work.

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