Longitudinal Cytokine Profiling in Severe COVID-19 Patients on ECMO and Haemoadsorption

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GL, AC, and MS designed the study and collected the data; KD, PQ, and GG performed the

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cytokines analyses; KD and PQ performed the statistical analysis; MS wrote the first draft of the manuscript; GL, AC, and GG provided critical revisions of the manuscript. All authors gave critical comments on the manuscript.

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Dear Editor,

Early in the COVID-19 pandemic, the description of a cytokine storm associated with the most severe forms of the disease elicited consideration of anti-cytokine therapies. However, more recent data showed that inflammatory cytokine concentrations in patients with critical COVID-19 are markedly less than those reported in sepsis or acute respiratory distress syndrome (ARDS) unrelated to COVID-19 (1). In the most severe forms of COVID-19 which required extracorporeal membrane oxygenation (ECMO), concerns were raised about the potential harm of ECMO itself (2, 3) that may increase serum and bronchoalveolar lavage fluid interleukine-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations (4) while significantly higher mortality had been associated with higher serum IL-6 in COVID-19 patients (5). Besides, TNF-α and IL-8 concentrations rose rapidly during the first 2 hours of ECMO in a neonatal porcine ECMO model (6). Although estimated 60-day survival of ECMO-rescued patients with COVID-19 was similar to that of studies published in the past 2 years on ECMO for severe ARDS (7), the contribution of ECMO itself to the cytokine release syndrome observed in some COVID patients remains a matter of debate.

CytoSorb® (CytoSorbents Europe, Berlin, Germany) is a haemoadsorption cartridge containing polystyrene divinylbenzene beads coated with polyvinylpyrrolidone designed to remove cytokines from the blood. Small case series have suggested that cytokine removal with this device that can be simply connected to the ECMO circuit may improve the outcomes of severe ARDS or cardiac surgery patients (8).

In this context, we studied 22 COVID-19 ECMO patients. The first consecutive 11 patients were included prospectively with a CytoSorb® adsorber being combined to ECMO. During that period, 4 ECMO patients were not included because a pre-ECMO sample was not possible. Then, 11 non-contemporaneous ECMO patients without CytoSorb® (i.e control

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group) were subsequently included. All of these patients were included without any selection based on clinical criteria, elevated levels of IL-6, or other biomarkers of inflammation. Whole blood was collected before ECMO, 4 hours after ECMO start without CytoSorb® adsorber, and after 12 and 48 hours of CytoSorb® and ECMO or after 12 and 48 hours on ECMO alone in the control group, respectively. We used highly sensitive, classical or digital, multiplex enzyme-linked immunosorbent assay technologies, to directly analyze combined cytokine production profiles (IL-1β, IL-6, IL-8, IL-22, IL-10, IL-17A, IL-18, GM-CSF, IFN-α, IFN-γ, TNF-α, and IFN-β) in serum of patients at these different time points. Serum samples were also obtained at the same time points in 11 control patients (i.e COVID-19 patients on ECMO who did not receive CytoSorb®). We compared cytokine profiles in patients with ECMO and CytoSorb® (CytoSorb group) or ECMO alone (control group), between 1) before and after four hours on ECMO (i.e direct impact of the ECMO) and 2) four and 48 hours on ECMO. Comparisons were performed by using a Wilcoxon test for non-parametric variables (SPSS version 21.0, IBM Corp, 2012). The study was performed at the Pitie Salpetrière hospital and approved by the local ethical committee, Comité d'Ethique de la Recherche of Sorbonne University (#CER-SU-2020-21 and -31).

Twenty-two COVID-19 ECMO patients (11 with ECMO and CytoSorb®) were included: 16 males, median (range) age 49 (33-65), SAPS II 46 (17-92), and time between intubation and ECMO start of 3 (1-11) days. The 48-hour study period was completed for 8/11 patients with Cytosorb® and all control group patients, respectively. Pre-ECMO, only IL-1β was significantly lower in the Cytosorb group compared to the control group ($p=0.022$). Levels of IL-6, IL-8, and IL-18 were very high in all patients, and higher than serum concentrations of other cytokines tested. Importantly, serum cytokine levels, and specifically IL-6, did not increase after 4 hours on ECMO (Figure 1A and 1B). Furthermore, IL-10 and IFN-γ concentrations decreased after 48 hours of CytoSorb® treatment ($p=0.008$

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and p=0.02, respectively). IL-6 levels also decreased from baseline levels, although the difference did not reach statistical significance (p=0.08) (Figure 1B). Other cytokine-levels were not altered by haemoabsorption. However, IL-6, IL-8, and IL-10 concentrations also significantly decreased in the 48 hours following ECMO initiation in the 11 patients who did not receive CytoSorb® (Figure 1A). The eight 60-day survivors in the Cytosorb® group were on ECMO for 25 (6-56) days whereas the 7 survivors of the control group spent 20 (1-42) days on ECMO.

Our findings suggest that ECMO itself does not exacerbate cytokine release in COVID-19 patients, contrary to what was previously suggested (2). Our results also question the actual impact of the CytoSorb® treatment to decrease serum concentrations of IL-10, IFN-γ, and IL-6 in this context. Indeed, a prompt switch to "ultra-protective" mechanical ventilation aiming to markedly reduce tidal volume and the driving pressure (9, 10) or the spontaneous evolution of the disease may explain the significant decrease of IL-6, IL-8, and IL-10 also observed in controls in the 48 hours post-ECMO initiation (Figure 1A). However, cytokine adsorption was associated with a more pronounced decrease of serum IL-6 in a recent series of four COVID-19 patients on ECMO (11). While these preliminary results need confirmation in larger cohorts, it should be mentioned that the non-selective reduction of cytokines with the CytoSorb® absorber could lead to paradoxical effects. Indeed, decreasing cytokine levels of IL-10, which is considered to dampen inflammation (12), may exacerbate COVID-19 associated organ damage. On the other hand, the reduction of IFN-γ, the main driver of macrophage activation, could contribute to control haemophagocytic lymphohistocytosis-like features associated with organ damage in some severe cases of COVID-19 pneumonia.

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Our study has some limitations. First, the two groups were not randomly selected and the control group was non contemporaneous. Second, timing of initiation of Cytosorb® was heterogeneous. We cannot rule out that the cytokine profile/response with the Cytosorb® could have been different if we had selected patients with higher level of inflammation before ECMO or if we had protocolized the timing of the Cytosorb® initiation. Third, the number of patients included is small, which limits the interpretation and generalization of our results. The ongoing randomized, multicenter controlled trial evaluating cytokine adsorption in COVID-19 patients on ECMO (NCT04385771) may help to clarify whether this strategy improves the outcomes of these severe patients.

In conclusion, ECMO does not exacerbate cytokine release in COVID-19 patients whereas IL-6, IL-8, and IL-10 decrease after 48 hours on ECMO with ultra-protective mechanical ventilation. To what extent combining a Cytosorb® adsorber with ECMO could enhance the decrease of these cytokines and improve outcomes, warrants further investigations.

Disclosures:

Dr. Combes reports grants and personal fees from Getinge, personal fees from Baxter, personal fees from Xenios, outside the submitted work. Dr Schmidt reports lectures fees from Getinge, Drager, and Xenios, outside the submitted work. Other authors have no conflicts of interest to disclose.

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data mining and biobanking for this part of the Cimi COVID-19 effort focused on ECMO patients.

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Figure 1: Cytokine production profiles in COVID-19 patients A) before, after 4, 12, and 48 hours on ECMO alone (n=11), and B) before, after 4 hours on ECMO, 12, and 48 hours with the CytoSorb® adsorber combined with ECMO (n=11).

Symbols represent individual subjects; bars show the median. Statistical analyses were conducted using the Wilcoxon signed-rank test.

Comparisons were performed between before ECMO and after 4 hours on ECMO as well as between after ECMO and either 48 hours on ECMO (control group) or 48 hours on Cytosorb® (Cytosorb group)

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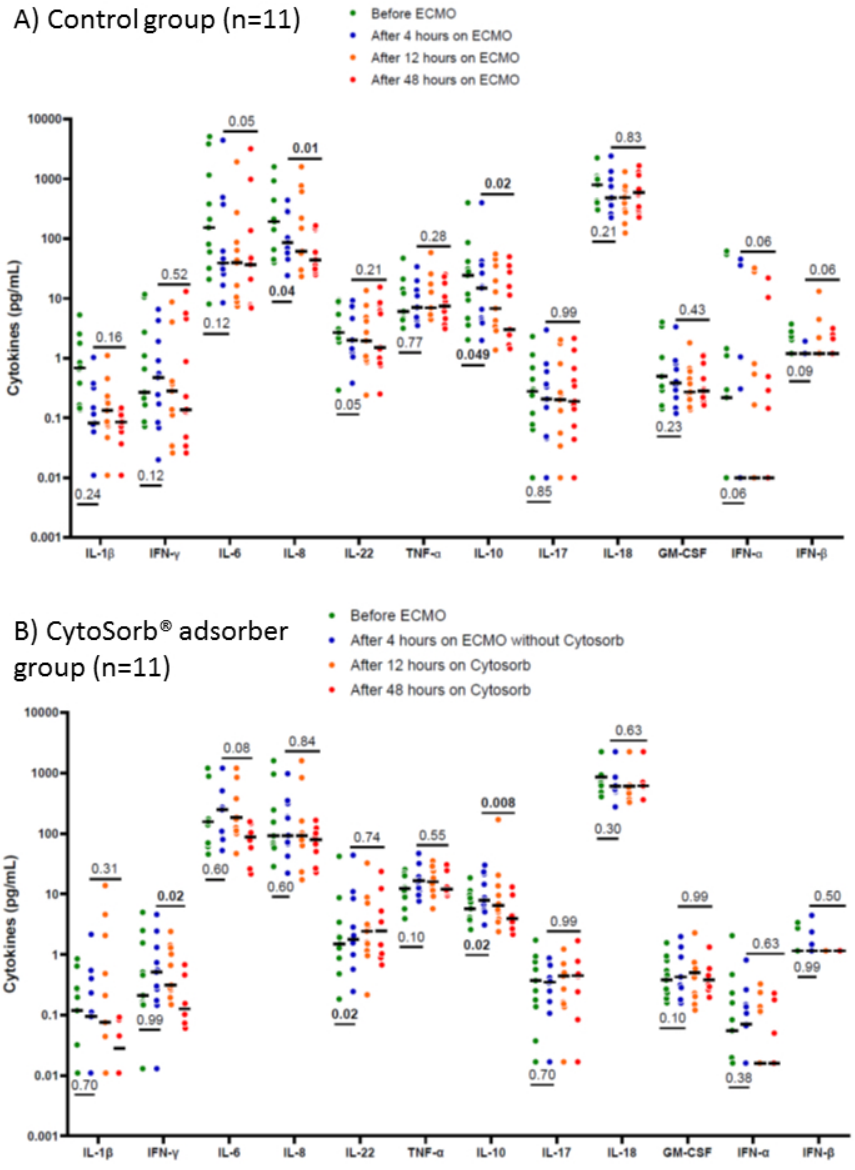

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