Complement C5 inhibition in patients with COVID-19 - a promising target?

Since the report of the first cases in December 2019, infection with the severe acute respiratory coronavirus 2 (SARS-CoV-2) commonly referred as COVID-19 has become a worldwide pandemic.[1] In patients with COVID-19 infection, respiratory deterioration has been associated with not only increased viral loads in the lung but also with an inadequate and exaggerated immune response.[2] Preclinical data have demonstrated a role for complement activation in CoV-mediated disease. Gralinski *et al.*, found activation of the complement system in a mouse model of CoV.[3] In some patients with COVID-19, significant deposits of terminal complement components C5b-9 (membrane attack complex), C4d, and mannose binding lectin (MBL)-associated serine protease 2 (MASP-2) have been found in the microvasculature of different organs, consistent with sustained, systemic activation of the lectin- complement pathway.[5] MASP-2 mediated complement overactivation has also been reported in some patients by a Chinese group.[6]

We assessed complement activity in 103 patients with COVID-19 followed in the Saint-Louis hospital (Pneumology Unit, Infectious Disease Unit and Intensive Care Unit [ICU]). We used a validated routine complement hemolytic activity assay (reported as CH50) which consists of testing the capacity of patient plasma to lyse sheep erythrocytes coated with antibodies, C3, C4 and sC5b-9 circulating levels by nephelometry (Siemens) and ELISA (Quidel, San Diego, CA, USA), respectively, according to the manufacturers' instructions. We found that the levels of C3 and C4 were increased in 57.2 % (59 of 103) and 36.9 % (38 of 103) of patients, respectively (*data not shown*). Moreover, the level of circulating sC5b-9 was increased in 64% of the patients (66 of 103), highlighting the systemic C5 cleavage during Covid-19 infection (healthy controls median 160 ng/mL, range: 49-362 *vs.* Covid-19 patients median 344 ng/mL, range: 71-883, $P<0.0001$). We then classified the severity of COVID-19 patients as moderate (dyspnea requiring a maximum of 3 L/min of oxygen and no other organ failure), severe (respiratory distress requiring more than 3 L/min of oxygen and no other organ failure) and critical cases (respiratory failure requiring mechanical ventilation or high flow oxygen support, shock and/or other organ failure necessitating intensive care unit [ICU] care). The plasma levels of sC5b9 were significantly higher in the three groups of patients than in the heathy donors ($P<0.001$ in the three groups) and higher in the patients with critical disease than in moderate disease ($P=0.01$) (Figure 1). Altogether, these findings suggest that C5 activation might be associated with disease severity, even if we agree causality still needs to be investigated. Nevertheless, this result has to be taken with caution since sC5b-9 may not properly reflect the situation on a cellular level. Moreover, tissue biopsies were not performed in any of our patients and the presence or absence of complement deposition in the lungs or microvasculature could, thus not be assessed.

Taken together, however, these findings suggest that complement might be targeted for specific intervention. In China, two deteriorating patients were rescued using an anti-C5a monoclonal antibody.[6] In Italy, four patients with severe pneumoniae successfully recovered after treatment with eculizumab.[8] In the absence of proven effective therapy, we decided to treat COVID-19 patients with severe pneumonia with eculizumab on an emergency compassionate-use basis. Five patients with severe pneumonia requiring ≥ 5L/min of oxygen to maintain SpO_2 >97% (but not requiring ICU) and three patients with respiratory failure requiring mechanical ventilation and suffering from renal injury (defined by AKI ≥2 or requiring dialysis) and vasopressive drugs support thus received eculizumab off label on a compassionate-use basis. All patients had confirmed severe COVID-19 using specific RT-PCR (positive PCR on nasal swabs). Characteristics of the patients are detailed in Table 1. This report is based on data from patients who received

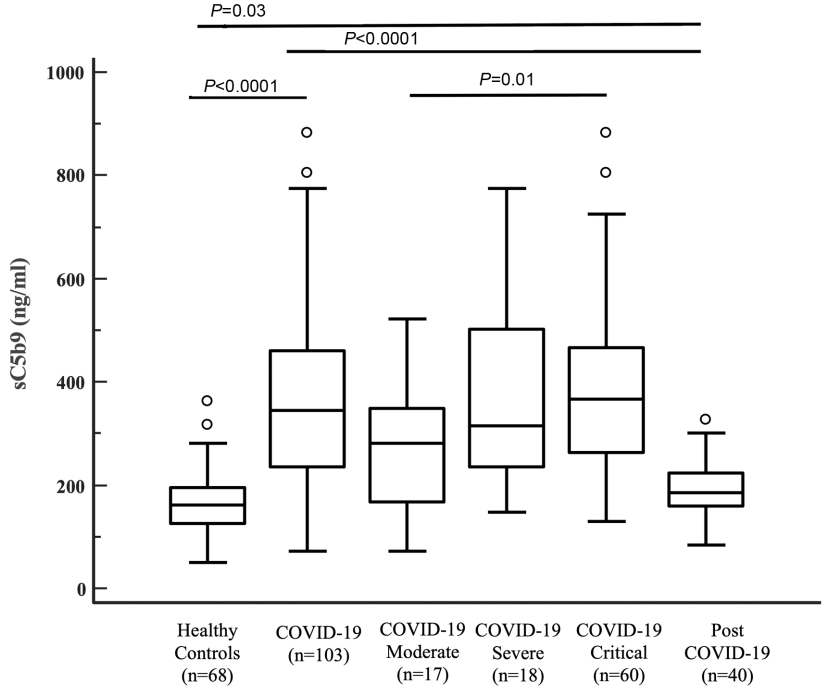

Figure 1. The level of C5b9 according to clinical severity. Plasma levels of sC5b-9 in healthy controls (n=68) and in patients with moderate (n=17), severe (n=18), critical COVID-19 (n=60), as well as patients with COVID-19 sampled at least 2 months after hospital discharge (n=40) are represented. Patients with COVID-19 were sampled during hospitalization up to 5 days after the admission. up to 5 days after the admission. The normal values of sC5b-9 are below 300 ng/mL. The plasma level of sC5b-9 was increased in 41 % (7 of 17), 50 % (9 of 18) and 68 % (41 of 60) of the patients with moderate, severe and critical disease. The median plasma levels of sC5b9 (Q1- Q3) in the patients with moderate, severe and critical disease were 281 ng/mL (range: 168-348), 314 ng/mL (range: 235-501) and 367 ng/mL (range: 262-467) respectively. The plasma levels of sC5b9 returned back to the normal range for the patients sampled 2 months after their discharge from hospital.

Table 1. Patients baseline characteristics, at time of Eculizumab initiation and during treatment.

	Patient#1	Patient#2	Patient#3	Patient#4	Patient#5	Patient#6	Patient#7	Patient#8
Age, years	65	58	31	73	58	56	64	47
Sex	M	F	M	M	M	F	F	M
Coexisting conditions	Multiple Myeloma Amyloidosis	7 months post AlloBMT (AML) High Blood Pressure Obesity Hypothyroidism	High Blood Pressure Takayasu disease	High Blood Pressure	High Blood Pressure Diabetes Obesity	High Blood Pressure Diabetes Obesity	High Blood Pressure Diabetes Obesity Asthma	None
Baseline characteristics at admission								
Symptoms	Fever, cough, dysgeusia, anosmia	Fever cough, dyspnea, nausea, vomiting	Dyspnea, throat pain	Fever, cough, dyspnea	Fever, cough	Fever, dyspnea	Fever, dyspnea	Fever, dyspnea
Time from first symptoms (days)	5	5	3	12	5	7	15	10
Oxygen supplementation to maintain SpO_2 ≥97% (L/mn)	5	5	9	5	15	15	4	6
Creatinine clearance (mL/mn)	24	49	115	79	On dialysis	20	40	109
C-reactive protein (mg/L)	96	195	90	187	137	221	NA	95
Characteristics at time of Eculizumab initiation								
Time from first symptoms to Eculizumab first injection (days)	6	6 10	3	13	8	9	17	
Oxygen supplementation to maintain SpO_2 ≥97% (L/minute)	5	6	6	6	Mechanical ventilation	Mechanical ventilation	Mechanical ventilation	High flow
Additional treatments	Enoxaparin 60 mg/day –	Enoxaparin 40 mg/day –	Enoxaparin 40 mg/day Dexamethasone	Enoxaparin 60 mg/day –	Unfractioned heparin Dexamethasone	Enoxaparin 60 mg/day Dexamethasone	Enoxaparin 60 mg/day Dexamethasone	Enoxaparin 40 mg/day –
Evolution under Eculizumab treatment								
sC5b9 (ng/mL) (at time Eculizumab first injection)	593	315	492	550	435	354	505	393
sC5b9 (ng/mL) (at day +1 post Eculizumab first injection)*	182	147	182	422	169	223	429	NA
sC5b9 (ng/mL) at time of last follow-up (days from the first Eculizumab injection)	388 (27)	329 (46)	390 (12)	305 (41)	415 (9)	271 (4)	252 (48)	290 (6)
CH50 and trough eculizumab concentration at day 1 and day 4 after the first Ecu injection	0 (49)/0 (271)	0 (30)/ 0 (<24)	22 (116)/ 28 (48)	36(174)/ 41 (65)	21 (118)/ 99 (<24)	22 (208)/ 26 (70)	18 (163)/ 0 (88)	NA (215)
Time from first injection to ambient air of low flow supplemental oxygen (≤2 L/minute) (days)	39	33	13	13	NA	NA	23	5
Therapeutic schedule and total number of Eculizumab injection	900 mg every 4 days (4)	900 mg every 4 days (5)	900 mg every 4 days (3)	1,200 mg every 4 days (3)	900 mg every 7 days (2)	1,200 mg every 4 days (1)	1,200 mg every 4 days (4)	1,200 mg every 4 days (2)
Status at last follow-up, location and length of stay in the hospital (days)	Alive discharged from hospital (40)	Alive discharged from hospital# (34)	Alive Home (13)	Alive Home (14)	Death (MOF) (13)	Death (PE) (6)	Alive discharged from hospital (25)	Alive Home (5)

Thrombosis history: none of the eight patients have previously presented thrombosis prior to the episode of COVID; patients 1 was diagnosed 10 days after Eculizumab injection with deep vein thrombosis and pulmonary embolism; the evolution was favorable under Eculizumab continuation and anticoagulation; patients 6 died 4 days after Eculizumab first injection (massive PE). Complement pathway activity monitoring: CH50 (screening hemolytic assay using sheep Erythocytes) is routinely used to monitor patients under Eculizumab; at day 1, all patients showed drastic diminution of CH50 below 20% of the normal value. AML: acute myeloid leukemia; alloBMT: allogeneic bone marrow transplantation; MOF: multi organ failure; NA: not available; PE: pulmonary Embolism. #BMT related bronchiolitis flaired 15 days after Eculizumab injection with a concomitant diagnosis of Parainfluenzae Virus infection; Dexamethasone was given intravenously at dose of 20 mg once daily from day 1 to day 5, and then 10 mg once daily from day 6 to day 10. *At day +1 after injection, seven patients with available samples showed a decreased of circulating levels of sC5b9. The median sC5b9 levels pre (463 ng/mL) and post (317 mg/mL) Eculizumab was significantly different (P=0.01) using non-parametric statistical analysis (Wilcoxon test). However, no correlation between the percentage reduction and response to Eculizumab was found.

eculizumab during the period from March 17, 2020 through May 19, 2020.

The first ICU patient (patient 1) was treated according to SOLIRIS® SmPC – dosing regimen of atypical Hemolytic and Uremic Syndrome (aHUS, induction period with 900 mg every week). He continues to be monitored closely with regard to the complement activity during follow-up according to our usual practice.[9] The level of free eculizumab in the plasma was assessed using in house ELISA as previously described.[9] At day 7, we observed a lack of complete inhibition of C5 with normal CH50 activity and undetectable free eculizumab circulating levels in patient 1. These findings suggest a much higher clearance of eculizumab in patients with COVID-19 than usually observed after a single injection in other diseases like aHUS and paroxysmal nocturnal hemoglobinuria.[9] Patients 2, 3 and 5 thus received 900 mg every 4 days allowing better but not optimal and prolonged complement blockade. We observed low eculizumab levels at day 4 (below 50 μg/mL in 2 of the 3 patients) with an efficient complement inhibition since day 1. The next four patients thus received three induction doses of 1,200 mg at day 1, 4 and 8, which appears satisfactory on a PK/PD standpoint (CH50 blocked before reinjection and residual free eculizumab upper to 50 μg/mL in all evaluable patients). Because of the complement blockade the patients received prophylactic antibiotics against meningococcal infection prior to initiating eculizumab treatment and were vaccinated when possible.

At time of eculizumab initiation, three patients were intubated, one received high flow oxygen and four patients were treated with standard oxygen support only. They all had elevated sC5b-9 circulating levels. Over a median follow-up of 19.5 days (range: 13-31 days) after receiving the first dose of eculizumab, six patients showed an improvement in the category of oxygen support, including one patient receiving mechanical ventilation who was subsequently extubated. At last follow-up, six patients had been discharged (day +5, +13, +13, +23, +34, +40 days after first eculizumab injection, respectively). Two patients who received invasive ventilation died (day +4 and +10 after the first eculizumab injection, respectively). Patient 5 presented a septic shock and multi organ failure while patient 6 was diagnosed with massive pulmonary embolism and cardiac arrest. In addition, patient 1 also presented severe thrombotic complications during evolution (deep venous thrombosis and pulmonary embolism). Those latter observations are in line with the recent findings suggesting that up to 30% of patients with severe COVID-19 infection develop life-threatening thrombotic complications.[10] Those considerations reinforce the recommendation to strictly apply thrombosis prophylaxis in COVID-19 patients,[11] also in the context of complement inhibition. While the relationship between thrombosis occurrence and complement activation is still unclear in patients with COVID 19, evidence is emerging that innate immunity contributes to the inflammatory storm that leads to respiratory failure in many patients with COVID-19. In particular, neutrophils-derived neutrophil extracellular traps (NET) which are extracellular webs of DNA, histones, microbicidal proteins, and oxidant enzymes that are released by neutrophils to corral infections have the potential to propagate inflammation and thrombosis if not properly regulated[12,13] as is the case in patients with COVID 19.[14] Excessive inflammation together with massive complement activation might thus participate in the predisposition of patients to a higher thrombotic risk.

Overall, we showed that the terminal pathway of the complement is overactivated in 64% of COVID-19 patients on admission to hospital. This may contribute to the severity of the disease. Naturally, it is difficult to draw any robust conclusion on the efficacy of complement blocking, firstly because of the small number and heterogeneity of our patients, and secondly in the absence of a control group.

However, all of our eight patients were particularly severe at the time of eculizumab initiation and six improved significantly. Even if further investigation is needed, our results suggest that the inhibition of one mechanism of COVID-induced organ damage may be an add-on treatment for this condition.

Our experience also highlights that eculizumab pharmacokinetics in COVID patients differ from reports in other complement-mediated diseases. In this particular disease, higher eculizumab doses and/or shorter intervals to ensure an efficient and sustained blockade seem required. The degree of complement activation at the start of therapy, possibly including C5b9 deposition in the tissue may be a significant determinant of eculizumab clearance and disease response. Controlled trials are timely to confirm the value of eculizumab in patients with COVID-19.

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The CORE group is detailed in Online Supplementary Appendix 1.

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