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Anti-complement C5 therapy with eculizumab in three cases of critical COVID-19

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ABSTRACT

Respiratory failure and acute kidney injury (AKI) are associated with high mortality in SARS-CoV-2-associated Coronavirus disease 2019 (COVID-19). These manifestations are linked to a hypercoaguable, pro-inflammatory state with persistent, systemic complement activation. Three critical COVID-19 patients recalcitrant to multiple interventions had skin biopsies documenting deposition of the terminal complement component C5b-9, the lectin complement pathway enzyme MASP2, and C4d in microvascular endothelium. Administration of anti-C5 monoclonal antibody eculizumab led to a marked decline in D-dimers and neutrophil counts in all three cases, and normalization of liver functions and creatinine in two. One patient with severe heart failure and AKI had a complete remission. The other two individuals had partial remissions, one with resolution of his AKI but ultimately succumbing to respiratory failure, and another with a significant decline in FiO$_2$ requirements, but persistent renal failure. In conclusion, anti-complement therapy may be beneficial in at least some patients with critical COVID-19.

1. Introduction

Progressive respiratory failure and acute kidney injury (AKI) are associated with the highest risk of mortality in SARS-CoV-2-associated Coronavirus disease 2019 (COVID-19) [1–3]. Organ dysfunction is linked to a systemic microvascular thrombosis in the context of complement activation and a persistent inflammatory state [4]. Increasing D-dimers and high levels of pro-inflammatory cytokines, particularly interleukin (IL)-6 and C-reactive protein (CRP), are significant prognostic indicators of in-hospital mortality in COVID-19 [3–6]. In terms of the complement cascade, early COVID-19 lung involvement is characterized by a thrombogenic vasculopathy with prominent deposits of terminal complement component C5b-9, C4d, and the lectin pathway of complement enzyme mannose binding lectin (MBL)-associated serine protease (MASP)2 [4]. This pattern of pulmonary vascular damage with complement deposition is also a feature of the cutaneous [4] and renal

[7] microvasculature in severe COVID-19, findings consistent with systemic complement activation.

Neither initial nor peak viral loads of SARS-CoV-2 in saliva or nasopharyngeal samples distinguish mild from severe or critical COVID-19, nor do they correlate with known co-morbidities for COVID-19 severity such as diabetes mellitus and obesity [8]. These data suggest that, possibly apart from initiation of SARS-CoV-2 antiviral drugs very early after infection, such agents alone will be insufficient to control COVID-19 progression. Indeed, infection of wild-type mice with mouse-adapted SARS-CoV led to marked weight loss and respiratory dysfunction, and this was mitigated by genetic knock-out of complement C3, despite equivalent viral loads in the lungs of both types of mouse [9]. Similarly, complement blockade with an anti-C5a reagent in the MERS-CoV human DPP4 transgenic mouse model led to decreased disease severity [10]. In summary, while complement does not appear to play a major role in controlling replication of coronaviruses, it may

have a critical role in their pathogenicity.

Our group [4], and others [11,12], have proposed that complement blockade might be of benefit in severe COVID-19. Very recently, four patients with COVID-19 characterized by respiratory failure were reported to have a complete recovery following administration of the anti-C5 humanized monoclonal antibody (mAb) eculizumab [13]. However, these patients did not have laboratory findings which correlate with extended intensive care unit hospitalization or increased mortality, did not have an AKI, and one of the four had normal D-dimer levels, the others minimally elevated values. A report of a moderate COVID-19 patient treated with the cyclic peptide inhibitor of complement C3, AMY-101, is also of interest, but again this did not involve a severe or critical case [14]. We describe three patients with critical COVID-19 who experienced complete or partial remissions following eculizumab administration.

2. Methods

2.1. Patient population

These cases represent the first three patients who met the criteria described below in consideration of the use of anti-complement therapy. They were among the first 14 consecutive cases considered over a six week period. Cutaneous biopsies were requested by ICU staff responsible for the care of those 14 patients in order to assess for possible intervention with anti-complement therapy, based on the fact that clinical conditions were deteriorating despite use of corticosteroids, hydroxychloroquine, the antiviral remdesivir, and the anti-IL-6 receptor mAb tocilizumab, and reports of sustained complement activation in critical COVID-19 [4]. The coding system for these three patients—Cases 5, 6 and 9–corresponds to a dermatopathologic study of those patients pre-eculizumab treatment, along with 11 other severe or critical cases (submitted for publication).

All three patients had a respiratory tract sample positive for SARS-CoV-2 in a reverse transcriptase-polymerase chain reaction assay and a diagnosis of critical COVID-19, based on a requirement for extended mechanical ventilator support and presence of an AKI. AKI was defined as an increase in serum creatinine by ≥0.3 mg/dL within 48 h or an increase in serum creatinine to ≥1.5 times baseline within the prior seven days. In addition, they all had persistent increases in D-dimers despite therapeutic anticoagulation. Tissue evidence of systemic complement activation was documented by vascular deposition of C5b-9, C4d, and MASP2 in normal-appearing deltoid skin.

2.2. Patient medications

On hospital admission, all three patients were given hydroxychloroquine, dosed as 600 mg every 12 h for one day, then 400 mg every 12 h for 4 days, for a total of 5 days. Tocilizumab was initiated and dosed as described in the Case reports (below). Cases 6 and 9 also received remdesivir, 5 mg/kg intravenously daily for 10 days, with the timing of drug initiation decided by the treating physician. Intermittent moderate doses of steroids were used at variable intervals in all three patients. Anticoagulation regimens, as outlined in the Case reports and illustrated in Fig. 1A-C, consisted of: prophylactic doses of enoxaparin (0.5 mg/kg body weight subcutaneously daily) or unfractionated heparin (5000 U subcutaneously two or three times per day); intermediate dose enoxaparin (0.5 mg/kg twice per day); therapeutic enoxaparin, targeting peak anti-Xa levels of 0.4–0.5 IU/ml; or therapeutic heparin (intravenous, targeting anti-Xa levels of 0.4–0.7 IU/ml).

2.3. Immunohistochemical (IHC) studies

IHC assessment for the deposition of C5b-9, MASP2, and C4d via a diaminobenzidene (DAB) technique utilized commercial antibodies (Agilent, Santa Clara CA, M077701–5 for C5b-9 and C4d, and Sigma, St.

Louis, MO, HPA029313 for MASP2) applied to formalin-fixed, paraffin-embedded sections of skin. Heat-mediated antigen retrieval with Tris-EDTA buffer (pH = 9, epitope retrieval solution 2) was performed for 20 min, followed by incubation with each antibody for 15 min. Details have been described elsewhere [4]. Hematoxylin and eosin counterstain was used and mounted with Leica Micromount.

2.4. Laboratory findings consistent with critical COVID-19

Peak lactate dehydrogenase (LDH) levels ≥550 U/L and absolute neutrophil counts (ANC) ≥11.6/mm^3 are predictive of ICU admission and in-hospital mortality for COVID-19 [3,6], and these levels were seen in all three cases (Fig. 1A-C). Characteristics pre-disposing to, or indicative of, a hypercoaguable state in COVID-19, include evidence of liver damage by elevation of aspartate transaminase (AST) [15], and skeletal muscle damage assessed by creatine kinase (CK) [16], and these were also present in all three cases (Fig. 1A-C and Case reports). No patient had disseminated intravascular coagulation per International Society on Thrombosis and Hemostasis diagnostic criteria, scoring systems relying on SOFA (sequential organ system failure assessment), or elevation of the International Normalized Ratio in the setting of thrombocytopenia.

3. Case presentations

3.1. Case 5

A 40 year-old female, overweight (body mass index (BMI) 26.3) but with no past medical history except for recurrent migraine headaches, was brought to the Emergency Department after 2 weeks of fever, cough, and increasing dyspnea. She had a total body rash, most prevalent on the chest. She was hypoxemic, with diffuse bilateral patchy airspace opacities on chest x-ray, and was intubated. Overnight she developed cardiogenic shock, with a left ventricular ejection fraction (LVEF) of 10–15%, an electrocardiogram consistent with an anterior wall myocardial infarction, and a troponin I which increased overnight from < 0.3 to 15 ng/ml, and required vasopressor support. She was treated with hydroxychloroquine and prophylactic anticoagulation with enoxaparin. Her LDH was 663 U/L (nl. 118–230 U/L) and her ANC was 7.0/mm^3 (nl. 1.5–8.0/mm^3) on admission. The following day the ANC rose to 12.3/mm^3 and her D-dimer was markedly elevated at 1196 ng/ml (nl. 0-229 ng/ml) (Fig. 1A). CK levels were elevated to 478 U/L (nl. 34–145 U/L). There was no evidence of anti-phospholipid antibodies. Her C-reactive protein (CRP) was 5.5 ng/ml (nl. ≤0.9 ng/ml) and, recognizing the importance of positive feedback loops between CRP and IL-6 to the persistence of a pro-inflammatory state [17], she was given a 400 mg intravenous infusion of tocilizumab on day 2. Her anticoagulation was changed to therapeutic continuous infusion heparin for 3 days, followed by therapeutic enoxaparin. These maneuvers were accompanied by a transient decline in D-dimer (Fig. 1A).

D-dimer levels rose 4 days later, concurrent with elevations in AST and serum creatinine and an increase in FiO$_2$ requirement (Fig. 1A). A skin biopsy of a livedoid rash on a lower extremity showed luminal fibrin thrombi in deeper-seated venules associated with prominent granular deposits of C5b-9, MASP2, and C4d throughout the microvasculature of both thrombosed and normal-appearing vessels (data not shown). In addition, significant deposits of C5b-9, C4d, and MASP2 were observed within otherwise unremarkable vessels of a normal-appearing deltoid skin biopsy performed at the same time (Fig. 2, Case 5). The patient was vaccinated against *Neisseria meningitides*, maintained on antibiotics sufficient for prophylaxis against meningococcal disease, and 900 mg of eculizumab was administered intravenously. By the next day there was a significant fall in D-dimers, ANC, AST, and creatinine (Fig. 1A).

Within the next two days, vasopressor medications were discontinued, and by hospital day 10 she was successfully weaned from

ventilator support. Platelet counts, in the high normal range throughout her hospitalization (380's x $10^3/\mu L$; nl. 150–450 × $10^3/\mu L$), declined to the low 200's x $10^3/\mu L$ by the start of the third dose of eculizumab. Serum C3 and C4 complement levels were within normal limits (90-180 mg/dL and 12–36 mg/dL, respectively) throughout her hospital course. Her total hemolytic complement (CH50) levels were markedly suppressed on admission, at 0 CAE Units (nl. 60–144 CAE Units). This was thought secondary to consumption in tissues. Additional 1200 mg doses of eculizumab were given on days 13 and 20 (Fig. 1A). CH50 levels remained < 10 CAE Units throughout her hospitalization, as would be anticipated if suppression of C5 activation by eculizumab was achieved.

She was discharged to a rehabilitation facility on hospital day 22 on intermediate dose enoxaparin. Her livedoid rashes had resolved. Her echocardiogram-assessed LVEF at that time was 41%, and her cardiac MRI LVEF was 50%. She is now at home.

3.2. Case 6

A 28 year-old male with class III obesity (BMI 46.8) but otherwise no past medical history except for seasonal allergies presented to the Emergency Department with a 9 day history of a dry cough and a 6 day history of fever, myalgias, diarrhea, and increasing dyspnea on exertion. He was admitted, placed on O_2 per nasal cannula, and treated with hydroxychloroquine and prophylactic doses of enoxaparin. He became progressively more dyspenic and required mechanical ventilation on hospital day 6. At that time he had a spike in D-dimer levels from 366 to > 12,000bg/ml (Fig. 1B), with marked increases in ANC, LDH, and AST (Fig. 1B) shortly thereafter. CK rose to 912 U/L. Given elevated CRP levels to 32.4 mg/dL, and development of an AKI, an 800 mg intravenous infusion of tocilizumab was administered and remdesivir initiated on day 6, along with a change in anticoagulation regimen to therapeutic enoxaparin.

By day 14 there was a stabilization of his D-dimers and decline in LDH, but this was followed by a dramatic rise in ANC and AST, and a continued rise in serum creatinine (Fig. 1B). Platelet counts were in the low 300's x $10^3/\mu L$, and remained within the mid-normal range until late in his hospital course when a reactive thrombocytosis was noted. A biopsy of normal-appearing deltoid skin revealed significant microvascular deposits of C5b-9, C4d, and MASP2 (Fig. 2, Case 6) within otherwise unremarkable appearing dermal blood vessels. Serum C3 and C4 levels were within normal limits, as was CH50 (87 CAE Units). He was vaccinated for *N. meningitides*, maintained on prophylactic antibiotics, and given a 900 mg intravenous infusion of eculizumab on day 14. His CH50 declined the next day to 5 CAE Units and his serum creatinine normalized within 5 days (Fig. 1B). However, a *Clostridium difficile* infection developed during his prolonged treatment with antibiotics, used in concern for an occult infection in the face of persistent pneumothoraxes, including vancomycin, meropenem, and piperacillin, and therapy was revised to include levofloxacin. FiO_2 was maintained at 100% from days 10 through 38. FiO_2 requirements thereafter lessened (Fig. 1B) but, in view of persistent infections, he was not considered a candidate for additional doses of eculizumab. He expired in the 14th week of hospitalization.

3.3. Case 9

A 62 year-old male with class I obesity (BMI 30.3), a past medical history of hypertension, gastroesophageal reflux disease, and cutaneous melanoma, presented with a 9 day history of a dry cough, fever, chills, and progressive dyspnea. He was admitted, placed on O_2 per nasal cannula, and treated with hydroxychloroquine, prophylactic doses of enoxaparin, and remdesivir. He became progressively more dyspenic, requiring mechanical ventilation and vasopressor support on hospital day 4. At that time he had an acute spike in D-dimers from 500 to > 16,000 ng/ml (Fig. 1C), with increases in LDH and AST (Fig. 1C), and a

CK of 381 U/ml. His platelet counts, about 120 × $10^3/\mu L$ on admission, rose to 211 × $10^3/\mu L$ at the time of his D-dimer spike, and have fluctuated from 250 to 400 × $10^3/\mu L$ since then.

His CRP was 29.1 mg/dL and his IL-6 level was markedly elevated at 285 pg/ml (nl. ≤5 pg/ml). On day 7 he was given a 400 mg intravenous infusion of tocilizumab. On day 9 his anticoagulation regimen was changed to therapeutic enoxaparin. Evaluation for anti-phospholipid antibodies showed only a mild elevation in IgG (17.7 GPL, nl. 0–14 GPL) and IgM (13.2 MPL, nl. 0–12 MPL) anti-cardiolipin levels, with a normal β2GP1.

Over the next 7 days his D-dimers declined to about 4000 ng/ml, but his ANC rose markedly, paralleled by a rise in LDH and serum creatinine (Fig. 1C). On day 11 he developed atrial fibrillation and was started on amiodarone. An acral livedoid rash was noted but not biopsied. A biopsy of normal-appearing deltoid skin showed significant deposition of C5b-9, C4d, and MASP2 (Fig. 2, Case 9) within blood vessels throughout the dermis. Serum complement levels were elevated: C3, 180 mg/dL; C4, 32.4 mg/dL; CH50 192 ACE Units. He was vaccinated for *N. meningitides*, maintained on prophylactic antibiotics, and given a 900 mg intravenous infusion of eculizumab on day 21, followed by 1200 mg doses on days 28 and 42. His course has been complicated by *Pseudomonas aeruginosa* sepsis and *P. aeruginosa* and *Candida albicans* lung abscesses.

D-dimer levels have stabilized at about 1800 ng/ml, and his ANC and LDH have normalized. His atrial fibrillation and acral livedoid rashes have resolved. His FiO_2 requirements have markedly decreased from peaks of 100% to 45% with low positive end-expiratory pressure. He remains in non-oliguric renal failure and is on intermittent hemodialysis, but is being prepared for discharge to a rehabilitation service.

4. Discussion and conclusions

Administration of the anti-C5 monoclonal antibody eculizumab led to a rapid and marked decline in biomarkers for systemic clotting and inflammation in all three of our critical COVID-19 patients, accompanied by resolution of livedoid rashes, indicative of a systemic thrombotic vasculopathy [4], in the two cases receiving multiple doses of eculizumab. There was also variable improvement in functions of the lung, heart and kidney, with a complete response in one patient. This is noteworthy as eculizumab was instituted only after failure of these critically ill individuals to respond to multiple interventions, including steroids, remdesivir, and tociluzimab.

Advanced COVID-19 is characterized by a hyper-inflammatory and hypercoaguable state. This is accompanied by systemic thrombotic vascular injury and activation of complement cascades [4]. However, the sequence of these events, and the precise mechanisms of their induction, is unclear. SARS-CoV-2 may directly activate the LP of complement through binding to MBL via its glycoprotein envelope spikes, as has been documented for SARS-CoV [9], and suggested by the demonstration by our group of CoV-2 envelope spike protein binding to MASP2 [4]. Multiple positive feedback loops involving complement receptor-mediated inflammatory cytokine release and interactions among leukocytes, platelets, and endothelial cells have been documented in a variety of pathologic settings [18]. Significant deposits of complement components C5b-9, C4d, and MASP2 in the pulmonary, renal, and cutaneous microvasculature of SARS-CoV-2-infected patients [4,7], accompanied by microthrombosis, are consistent with systemic activation of complement and related pathology. Complement components C3a and C5a activate platelets, and can initiate the coagulation cascade with thrombin generation via a variety of mechanisms, including tissue factor expression elicited by pro-inflammatory cytokines from monocytes and endothelial cells [19,20].

Although complement levels were within the normal range or only slightly elevated in our cases, this may represent consumption of complement proteins at sites of tissue injury, as is classic for certain acute autoimmune disorders [21]. Indeed, total hemolytic complement

A

Case 5

B

Case 6
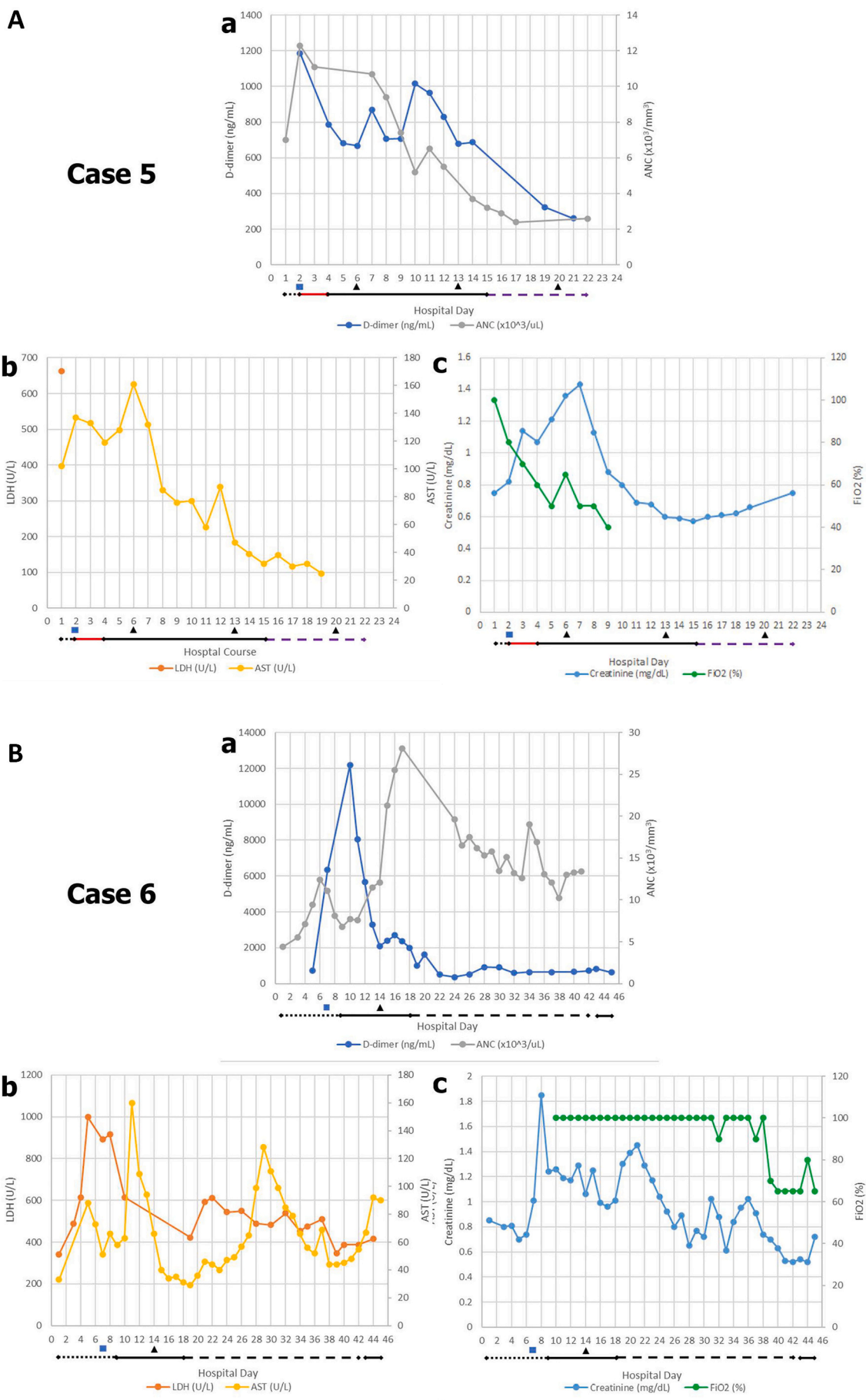
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Fig. 1. Impact of anti-complement therapy with eculizumab on laboratory values associated with progressive disease and markers of organ function in three patients with critical COVID-19. Three patients with SARS-CoV2 infection and critical disease, all involving respiratory failure and acute kidney injury, were followed from the time of hospitalization (day 1). Days of tocilizumab and eculizumab infusions are indicated by the squares and arrows, respectively. Anticoagulation: therapeutic intravenous heparin, red solid line; prophylactic heparin, red dotted line; prophylactic enoxaparin (0.5 mg/kg body weight, once daily), black dotted line; intermediate dose enoxaparin (0.5 mg/kg body weight twice daily), black dashed line; therapeutic dose enoxaparin (1 mg/kg body weight twice daily), black solid line. A: Case 5. B: Case 6. C: Case 9. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
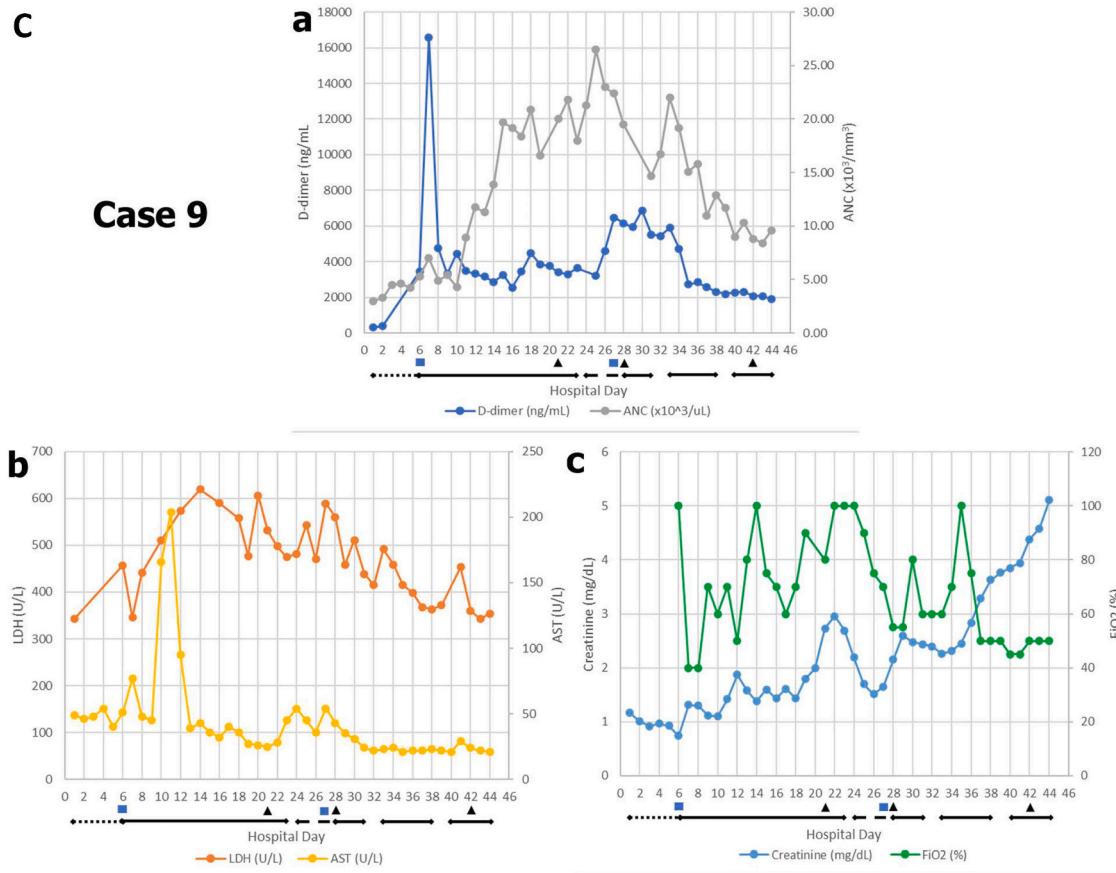

Fig. 1. (*continued*)

levels for Case 5 were 0, despite extensive deposition of C5b-9, C4d, and MASP2 in her microvasculature. Plasma levels of C3a and C5a would more accurately reflect complement-driven disease activity than standard measures of serum C3 and C4 [21]. C5a and soluble C5b-9 are elevated in plasmas of patients with severe COVID-19 [22]. One caveat in the interpretation of plasma levels of complement components is variations in clearance, with soluble C5b-9 less rapidly cleared than other factors.

Our cases extend the observations on use of eculizumab and an anti-C3 agent in much less severe COVID-19 cases [13,14]. As complement and inflammatory cytokines can interact in positive feedback loops [4,23], dual intervention with eculizumab and the JAK1/2 inhibitor ruxolitinib was proposed, and clinical improvement using this combination in seven cases of COVID-19 recently reported [24]. However, the moderate FiO$_2$ requirements (median 50%), minimal elevation in D-dimers (median 138 ng/ml), and absence of AKI in these patients [24] suggest the need for evaluation of such strategies in more severe/critical cases.

There are limitations to attributing the responses observed in our cases to eculizumab. First, a small number of patients were studied, and a complete response obtained in only one of the two cases receiving multiple doses of eculizumab. Second, delayed effects of other therapeutics are possible. A prominent impact from remdesivir is unlikely, given its lack of effect on mortality in at least one large study in severe

COVID-19 [25]. All three patients also received tocilizumab. IL-6 inhibition was unlikely to have influenced Cases 5 or 6, where only a single dose was given and little change in ANC or serum creatinine was noted, but it could have influenced responses in Case 9. For example, tocilizumab can interfere with complement signaling pathways to suppress C5aR1 expression [26], and therefore may have additive or synergistic effects with anti-complement therapies. Third, the doses of eculizumab administered initially, 900 mg, which are standard in the treatment of atypical hemolytic-uremic syndrome (aHUS) [27], as well as its increase to 1200 mg in subsequent infusions, may have been inadequate. Based on pharmacokinetic data in patients with severe SARS-CoV-2 infection that were unavailable to us at the time, higher doses and more frequent administration are now recommended (Alexion Pharmaceuticals, Inc., personal communication). Finally, the possibility that the new *C. difficile* infection in Case 6, and the persistent of pseudomonas infections in Case 9, were related to use of an anti-complement therapy must be considered. However, these individuals had several risk factors for such infections, including prolonged mechanical ventilation and immune suppression related to tocilizumab. In addition, these microorganisms have not been recognized during eculizumab treatment of adults with aHUS [27].

In conclusion, examining the variety of responses in terms of organ function and biomarkers for inflammation and coagulation to anti-complement therapy in our cases, and those recently reported by
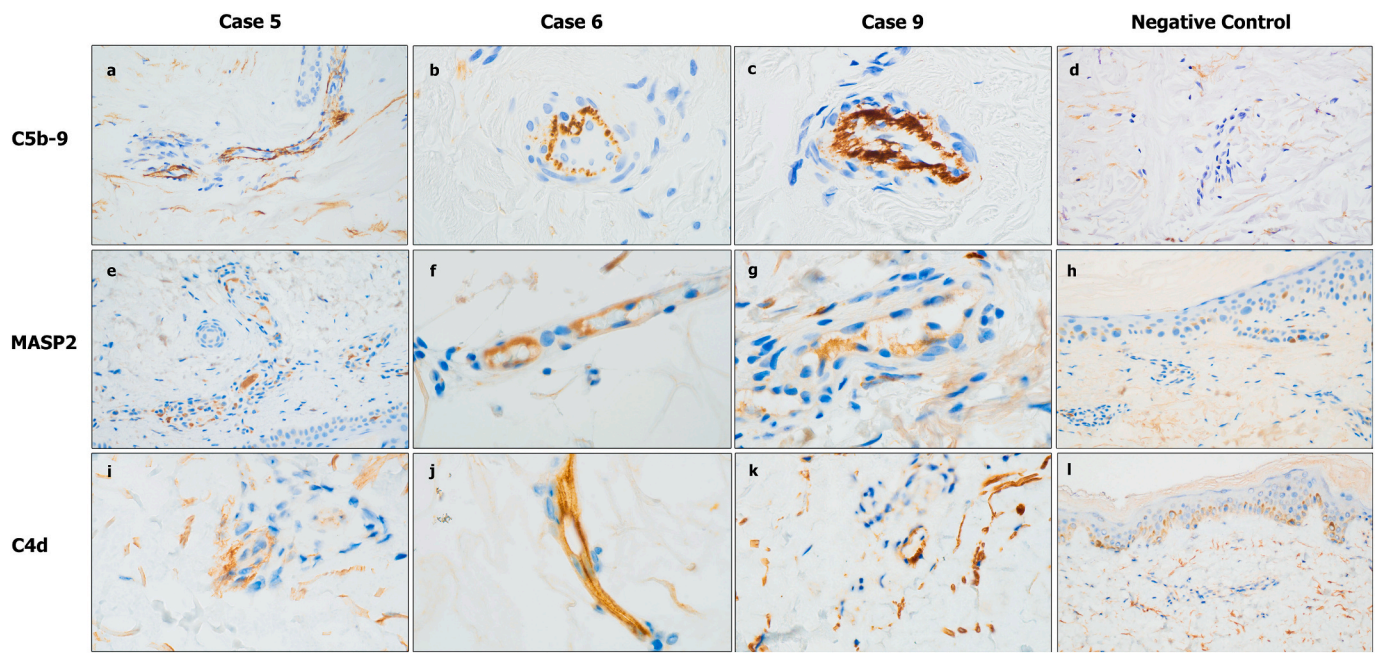
Fig. 2. Immunohistochemical analysis of complement deposition in normal-appearing deltoid skin biopsies of three severe COVID-19 patients. Significant deposits of C5b-9, MASP2, and C4d on endothelial cells are seen within the dermis of normal-appearing skin in all three cases. ,j). In control samples there is no significant vascular staining, though low-intensity, non-specific background staining of elastic fibers may be evident.(Diaminobenzidene stain: 400x for a,d,e,k and 1000x for b,c,e,f.)

others, should help establish criteria for clinical trials with anti-complement agents in critical COVID-19, and open the possibility for earlier intervention than at the end-stages of the disease.

Disclosure statement

Contributors

JL, JJM, and CM conceived this treatment. JH performed the skin biopsies. CM directed and interpreted the light microscopic and immunohistochemical studies. JL, MS, AR, E.J.S., DM, and EMH were involved in the treatment of these patients. All authors contributed to the writing and review of the manuscript.

Acknowledgments

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