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1 **Is long COVID a complement dysregulation disease?**

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7 *Running title:* Complement in long COVID.

8 *Keywords:* long COVID, complement, drugs.

9 *Abbreviations:* Coronavirus disease, COVID; severe-acute-respiratory-syndrome-
10 related coronavirus, SARS-CoV-2; post-acute sequelae of SARS-CoV-2 infection, PASC;
11 computed tomography, CT; terminal complement component, TCC; enzyme linked
12 immunosorbent assay, ELISA; complement component 2, C2; complement factor B, FB;
13 Complement component 5, C5; Complement component 7, C7; membrane attack complex,
14 MAC; thrombospondin-1, TSP-1; von Willebrand factor, vWF, complement factor D, FD;
15 complement component 3, C3.

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22 In a recent report in *Science*, Cervia-Hasler identified the complement system as the top
23 dysregulated biological pathway in long Coronavirus disease (COVID), thus implicating
24 complement in the disease process and raising the prospect of blocking complement
25 activation as a promising therapeutic intervention.¹ However, this finding has not been
26 independently validated, which emphasizes the need for data replication in order to draw a
27 reliable conclusion. In this brief commentary I seek to set this observation in context.

28 The legacy of COVID-19 extends far beyond the acute phase with ~20% of cases (in the UK
29 ~1.9 million people or 2.9% of the population) experiencing symptoms more than 12 weeks
30 after the acute infection, referred to as long COVID or post-acute sequelae of severe-acute-
31 respiratory-syndrome-related coronavirus (SARS-CoV-2) infection (post-acute sequelae of
32 SARS-CoV-2 infection; PASC). Typical symptoms include persistent fatigue, shortness of breath,
33 muscle pains and brain fog. Long COVID imposes a substantial burden on the economy, as
34 individuals grappling with the condition are often incapacitated, unable to work, and cannot
35 contribute fully to society. In our recent study, almost 90% of individuals with long COVID were
36 not in employment.² The long-term repercussions of long COVID may extend to future health
37 complications such as diabetes, cardiovascular disease, and dementia.^{1,2} Understanding
38 mechanisms to guide therapy is thus an urgent priority.

39 Persistent inflammation is a key feature in long COVID, perhaps a consequence of virus
40 persistence in endothelial cells. Increased levels of inflammatory cytokines along with C-
41 reactive protein and serum amyloid A were found in long COVID patients 6-9 months post
42 SARS-CoV-2 infection. In a systematic review including 22 studies, increased concentrations of
43 plasma interleukin-6 in plasma were consistently associated with long COVID.³ Conversely, the
44 presence of autoantibodies against inflammatory chemokines during the convalescent phase

45 correlated with improved outcome and a reduced likelihood of developing long COVID.
46 Activation of innate immune cells has also been identified as a contributing factor to lung
47 fibrosis and inflammation in a humanised mouse model of long COVID (reviewed in ref 2). All
48 these reports provide evidence of immune system dysregulation with accompanying
49 inflammation as crucial factors in long COVID, yet none pinpoint an obvious inflammatory
50 trigger or target for therapy.

51 Although complement, a key driver of inflammation, is strongly implicated in acute COVID-19,
52 its contribution to long COVID is underexplored. One recent study reported that levels of the
53 small complement component C4 (C4) fragment C4d were associated with chest computed
54 tomography (CT) changes at 3 months in long COVID patients and that increased levels of
55 complement activation products were present in plasma at 3 months and 1 year in long COVID
56 individuals compared to healthy controls.⁴ We identified a set of complement activation
57 markers in long COVID cases implicating the activation loop (complement small factor B (FB)
58 fragment (Ba), complement small complement component (C3) fragment (iC3b), complement
59 small complement C5 (C5) fragments (C5a)) and downstream terminal pathway (terminal
60 complement complex (TCC)), providing a diagnostic tool for long COVID.² Notably, we
61 previously showed that the amplification loop is dysregulated in acute COVID-19 subjects with
62 a single amplification loop-specific biomarker, Ba, being the best death predictor (reviewed in
63 2).

64 The study by Cervia-Hasler et al. comprised a longitudinal study of 268 serum samples
65 obtained from 39 healthy controls and 113 COVID-19 patients followed for up to one year
66 after acute SARS-CoV-2 infection. They utilised a non-targeted approach to identify the best
67 predictive biomarker(s) associated with long COVID by measuring >6500 proteins in these

68 serum samples using the SomaScan proteomics platform. At the 6-month follow-up, 40
69 patients exhibited long COVID symptoms. Machine learning and other computational tools
70 were applied to identify candidate biomarkers predictive of long COVID; these were then
71 validated in wet lab settings (using ELISA and mass spectrometry). Best hits in this analysis
72 included elevated levels of complement activation products (terminal complement complex
73 (TCC), complement small C3 fragment C3d) and complement proteins (complement
74 component C2 (C2), FB, complement component C5 (C5)); increased complement activity was
75 also noted. The most informative biomarkers were the complement component C7 (C7) and
76 C7 containing complexes, both markedly reduced in long COVID, implicating the terminal
77 complement pathway.¹ Whether the observed reduction in terminal pathway markers
78 reflected decreased synthesis or increased consumption secondary to membrane attack
79 complex (MAC) formation in tissues was not tested, although the latter might explain the
80 observed tissue damage. The authors suggest that monitoring C7 levels in acute COVID-19
81 cases could provide a predictive biomarker for long COVID, supporting prediction of disease
82 course and associated tissue damage. Regardless of the precise mechanism these analyses
83 provide compelling evidence of ongoing complement dysregulation in long COVID, a likely
84 driver of the observed persistent inflammation.

85 Our study implicated complement activation products Ba, iC3b, C5a and TCC as informative
86 biomarkers for long COVID,² while Cervia-Hasler et al. identified C7 and C7-containing
87 complexes as most relevant biomarkers of long COVID. Unfortunately, these markers are not
88 routinely measured in clinical laboratories and assays are poorly validated even in specialist
89 laboratories, a major limitation to their utilisation as potential disease biomarkers. If these
90 assays are to be used in assessment of long COVID, efforts are needed to further develop and
91 standardise them to allow their use in routine settings.

92 Cervia-Hasler and colleagues did seek further insight into the underlying pathological
93 mechanism. They showed correlation of complement dysregulation with markers of
94 thromboinflammation, a hallmark of long COVID; these included coagulation factor VIII,
95 thrombospondin-1 (TSP-1), von Willebrand factor (vWF), fibrinogen beta, factor XI, protein C,
96 and heparin cofactor II.¹ The observed link between complement dysregulation and
97 thromboinflammation confirms the importance of cross-talk between complement and
98 coagulation systems, a finding seen in other contexts; cross-talk is bidirectional, tightly
99 controlled, and crucial for driving the immune response, inflammation, and hemostasis.^{1,5} The
100 authors propose that after acute COVID-19 infection, localised activation of complement and
101 coagulation systems persists across various tissues in those who progress to long COVID. The
102 endothelial cell damage is mediated by complement terminal pathway complexes perturbing
103 cell membranes and leading to the release of thrombotic markers, including vWF and TSP1.
104 These in turn induce platelet activation, facilitate thrombin generation and promote
105 interactions between monocytes and platelets, resulting in microclot formation, a common
106 feature of long COVID. Accumulating vWF aggregates in turn activate the amplification loop
107 of complement resulting in the small complement component of C3 fragment C3b deposition
108 and sustaining local complement activation and inflammation.

109 Currently, there are no specific therapies available for long COVID; available treatments
110 primarily involve alleviating symptoms and rehabilitation. Some ongoing clinical trials are
111 exploring medications for specific symptoms, such as ivabradine for cardiac damage,
112 pirfenidone and inhaled interferon-1 for fibrotic lung injury, and leronlimab for inflammation
113 triggered by acute SAR-CoV-2 infection. The demonstration that complement dysregulation is
114 a core feature of long COVID highlights the potential for use of anti-complement drugs in
115 therapy of the condition. Therapeutic complement inhibition might break the vicious cycle of

116 complement activation and tissue damage and restore normal homeostasis. Several
117 complement inhibitors are already in the clinic,⁵ and could be repurposed for long COVID
118 therapy (Figure 1). Given the evidence implicating the amplification loop detailed above,
119 drugs targeting amplification such as iptacopan (targeting FB), danicopan (targeting
120 complement factor D (FD)) or pegcetacoplan (targeting C3) might be most effective. However,
121 targeting the terminal pathway, for example with the long established C5-blocking antibody
122 eculizumab, may also be beneficial. Anti-complement drugs used in trials for acute COVID-19
123 showed limited success with a single drug, the anti-C5a antibody vilobelimab, gaining limited
124 FDA approval.^{2,5} Long COVID is a very different disease, manifesting with low-grade
125 inflammation as opposed to the acute hyperinflammatory state typifying some cases of acute
126 COVID-19. Anti-complement therapies may prove to be more effective in this scenario,
127 particularly if used in conjunction with reliable complement biomarkers (e.g. complement
128 activation products; Ba, iC3b, TCC) to identify patients likely to benefit from the treatment. A
129 reliable biomarker would inform treatment response, particularly indicating a reduction in
130 complement activation. A proof-of-concept study or clinical trial utilising complement
131 inhibitors is needed to validate this hypothesis.

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148 **Figure 1. The complement cascade and complement drugs in clinics.** The complement system
149 is activated via the classical and lectin pathways and amplified by the amplification loop of the
150 alternative pathway. The classical pathway is initiated by complement component C1 (C1)
151 binding to antigen-antibody complexes. A small fragment of C1 C1s in the C1 complex cleaves
152 C4 and C2 to form the C3 convertase C4b2a. The lectin pathway is activated by mannose
153 binding lectin (MBL) or other lectins binding surface carbohydrates; attached (mannan binding
154 lectin serine proteases) MASPs are activated to cleave C4 and C2 to generate C4b2a. The C3
155 convertase cleaves C3 to C3b (a small fragment of C3) and C3a. C3b, C4b (a small fragment of
156 C4) and their degradation products are important opsonins. C3b also binds FB enabling its
157 cleavage by FD to form the C3bBb convertase that cleaves more C3 in a feedback cycle, the
158 alternative pathway amplification loop. Binding of a further C3b to either C3 convertase
159 creates a C5 convertase which cleaves C5 to initiate the terminal pathway culminating in
160 formation of the soluble (terminal complement complex; TCC) and membrane-inserted
161 (membrane attack complex; MAC) complexes. Complement small fragment of C3, C3a and
162 C5a, are anaphylatoxins that signal via their receptors to recruit immune cells. FDA (Food and

163 Drug Administration) approved complement drugs in clinics are shown in red. Numerous other
164 complement inhibitors are currently in development, undergoing preclinical and clinical trials,
165 with a focus on targeting diverse pathways. Figure created with BioRender (BioRender.com).

166

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Figure 1

