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### ► To cite this version:

Christian Devaux, Laurence Camoin-Jau, Jean-Louis Mege, Didier Raoult. Can hydroxychloroquine be protective against COVID-19-associated thrombotic events ?. *Journal of Microbiology, Immunology and Infection*, 2021, 54 (1), pp.37-45. 10.1016/j.jmii.2020.12.010 . hal-03281965

**HAL Id: hal-03281965**

**<https://amu.hal.science/hal-03281965>**

Submitted on 10 Mar 2023

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**Title: Can hydroxychloroquine be protective against COVID-19-associated thrombotic events ?**

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Abstract length: 122 words; Manuscript length: 2852 words ; Figure: 1

**Keywords:** COVID-19; thrombosis; antiphospholipid antibodies; hydroxychloroquine

30 **Abstract**

31 Although SARS-CoV-2 is considered a lung-tropic virus, severe COVID-19 is not just a viral  
32 pulmonary infection, clinically it is a multi-organ pathology with major coagulation  
33 abnormalities and thromboembolism events. Recently, antiphospholipid (aPL) antibodies  
34 were found increased in a large number of COVID-19 patients. Elevated aPL have been well  
35 documented in antiphospholipid syndrome (APS), a systemic autoimmune disorder  
36 characterized by recurrent venous or arterial thrombosis and/or obstetrical morbidity. Among  
37 treatment regimen of APS, hydroxychloroquine (HCQ) is one of the molecules proposed in  
38 the primary prevention of thrombosis and obstetrical morbidity in those patients. Due to its  
39 antithrombotic properties documented in APS therapy, HCQ could be considered a good  
40 candidate for the prevention of thrombotic events in COVID-19 patients in association with  
41 anticoagulant and its repurposing deserves further evaluation.

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## 56 **Introduction**

57 The 4-aminoquinoline drug hydroxychloroquine (HCQ) belong to the same molecular family  
58 than chloroquine (CQ), an amine acidotropic form of natural quinine that was synthesised in  
59 the early 1930s and emerged approximately 70 years ago as a substitute for quinine against  
60 malaria. HCQ was reported to be as active as CQ against *Plasmodium falciparum* and less  
61 toxic, allowing long periods of high dose therapy with very good tolerance <sup>1</sup>. Moreover, HCQ  
62 has been widely used for many years in the therapy of autoimmune diseases as it acts as  
63 immune modulator interfering with lysosomal activity and autophagy, modulating signaling  
64 pathways (such as inhibition of Toll-like receptor signaling in dendritic cells, phospholipase  
65 A2 activity and arachidonic acid production in platelets, nitric oxide production by endothelial  
66 cells, antiphospholipid- $\beta$ 2 glycoprotein complexes on monocytes surfaces, inhibiting  
67 cytokines production by T lymphocytes) and transcriptional activity <sup>2-4</sup>. The antithrombotic  
68 properties of HCQ was described as far as 1975 <sup>5</sup>. In the context of autoimmune disease  
69 (SLE), HCQ inhibits stimulated platelets at the arachidonic acid pathway and thromboxane 2  
70 generation in activated platelets (an activator of platelets aggregation), which is associated  
71 with decreased circulating levels of endothelin-1, and allows improvement of endothelial  
72 function <sup>3, 6-8</sup>. Interestingly, using network-based approach to prediction and population-based  
73 validation of *in silico* drug repurposing it was found that the Healthcare registry data for 220  
74 million people showed that HCQ intake (a series of 37,795 patients receiving HCQ) was  
75 associated with a lower risk for coronary artery disease <sup>9</sup>.

76 The main purpose of this review is to summarize the evidence supporting a potential  
77 beneficial role of HCQ in the prevention of thrombosis in patients with antiphospholipid  
78 syndrome and discuss the possible repurposing of this molecule as an additional member of  
79 the therapeutic arsenal in association with classical antithrombotic drugs used for the  
80 prevention of the obstructive thrombo-inflammatory syndrome associated with severe forms  
81 of COVID-19.

82

## 83 **The pathophysiology of COVID-19**

84 An outbreak caused by a novel human coronavirus, severe acute respiratory syndrome  
85 coronavirus 2 (SARS-CoV-2) was first described in Wuhan in December 2019 <sup>10-12</sup>. During  
86 the past nine months the SARS-CoV-2 has spread worldwide and was responsible for severe  
87 COVID-19 disease forms characterized by cytokine storm, acute respiratory distress

88 syndrome (ARDS), and severe thrombotic events leading to multiple organ dysfunction  
89 syndrome (MODS), and high risk of fatal evolution <sup>11,13-14</sup>. To date (one year after the first  
90 outbreak in China), SARS-CoV-2 has been responsible for more than 1.57 million deaths  
91 among about 68.95 million of infected people (<https://coronavirus.jhu.edu/map.html>), and  
92 these numbers are still raising daily.

93 It is currently admitted that COVID-19 pathogenesis is mainly characterized by production of  
94 pro-inflammatory cytokines including IL-6, a key contributor in the development of cytokine  
95 storm associated with microvascular injury, obstructive thrombo-inflammatory syndrome  
96 which represent the primary causes of lethality <sup>15,16</sup>, with an estimated fatality rate of 2,27%.  
97 The lack of specific treatment for COVID-19 led all clinical teams to try to speed up the  
98 implementation of therapeutic strategies by carrying out drug repurposing. HCQ which was  
99 known to inhibit the replication of several coronavirus *in vitro*, was reported to inhibit SARS-  
100 CoV-2 *in vitro* <sup>17-20</sup>. This antiviral activity likely occurs through several mechanisms and  
101 cellular targets <sup>21-22</sup>. By February 2020, the first report from China on the clinical efficacy of  
102 chloroquine phosphate in treatment of COVID-19 associated pneumonia was submitted for  
103 publication <sup>23</sup>. Considering the possible benefit of this molecule as both antiviral agent and  
104 immunomodulator compound, it was tempting to evaluate its potential in the prevention  
105 and/or treatment of COVID-19 <sup>24-28</sup>. This therapeutic approach has lead to an impassioned  
106 global debate on the promises and pitfalls of HCQ in COVID-19, in both clinical and  
107 scientific communities. By mid-July 2020, among 2,654 registered clinical trials from 43  
108 countries aimed at testing the capacity of compound at preventing severe COVID-19, 239  
109 included HCQ treatment or prophylaxis while 82 addressed chloroquine efficiency  
110 (Clinicaltrials.gov). Still today, the debate remains fierce between those who consider HCQ to  
111 save lives, those who find no significant impact on COVID-19 progression and those who still  
112 claim that HCQ is toxic <sup>29-34</sup>. This does not make it possible to conclude whether HCQ is  
113 beneficial or not, it simply indicates that the case series and treatment protocols are different  
114 and that meta-analysis algorithms are either unsuitable or misused.

115 As knowledge about the disease grew it became evident that one of the major issues to be  
116 addressed in severe COVID-19 was that of thrombosis <sup>35,36</sup>. The use of anticoagulants was  
117 considered as a major therapy to reduce the harmful circle of inflammation-coagulation  
118 observed in patients with a severe form of COVID-19 <sup>37,38</sup>. Antithrombotic therapy improved  
119 COVID-19 patients outcomes <sup>39</sup>.

120 *In vitro*, HCQ was found to induce attenuation of human aortic endothelial cells activation  
121 upon exposure to proinflammatory cytokine TNF $\alpha$  by reducing of VCAM and IL-1 $\beta$   
122 production <sup>9</sup>. The ectonucleoside triphosphate diphosphohydrolase I/CD39, present on  
123 endothelial cells and circulating blood cells such as leukocytes, neutrophils, T- and B-  
124 lymphocytes and macrophages is a known interface between vascular inflammation and  
125 thrombosis through regulation of ATP, ADP and AMP levels <sup>40</sup>. Mice lacking CD39  
126 expression have marked fibrin deposit in pulmonary and cardiac tissues <sup>41</sup>. IL-6 and other  
127 cytokines are increased in COVID-19 patients <sup>38, 42-43</sup>, and the profound inflammatory state of  
128 the patients can be characterized by high levels of C reactive protein (CRP) and fibrinogen.  
129 These observations led to the adoption of the anti-IL6 receptor monoclonal antibody  
130 tocilizumab for the treatment of pneumonia-associated to cytokine storm <sup>44, 45</sup>. It is also  
131 known that blocking IL-6 increases the frequency of CD39+ Treg cells <sup>46</sup>.  
132 In severe COVID-19, patients with thrombosis have significantly higher blood levels of  
133 markers of neutrophil extracellular traps (NETs), neutrophil activation (calprotectin, cell-free  
134 DNA) and D-dimers <sup>47</sup>. SARS-CoV-2 induces functional changes in platelet. Platelet  
135 hyperreactivity may contribute to thrombotic events through increased platelet-platelet and  
136 platelet-leukocytes interactions <sup>48</sup>. Moreover, antiphospholipid (aPL) antibodies were  
137 recently found increased in a large number of COVID-19 patients <sup>49-54</sup>. Higher aPL antibodies  
138 were associated with neutrophil hyperactivity including the release of NETs <sup>55</sup>. Recently, it  
139 was reported that increased count of CD15<sup>+</sup>CD16<sup>+</sup> neutrophils is a COVID-19 signature <sup>56</sup>.

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#### 141 **Thrombotic disease in severe COVID-19 patients**

142 Coagulation abnormalities are common in COVID-19 patients. Degree of elevation of  
143 fibrinogen and D-Dimers is correlated to the severity of the disease and elevated D-dimer  
144 upon admission and during course of disease is associated with increased mortality <sup>57-61</sup>.  
145 Clinical evidence indicated that COVID-19 is associated with an increase risk of thrombotic  
146 events leading to sustained activation of the clotting, generally venous thromboembolism  
147 (VTE) almost assuredly underdiagnosed, due to the difficulty of the performing blood vessels  
148 investigation when patients are under isolated care and the fact that the D-dimer level is  
149 already high.  
150 However, 20-30 % of acute pulmonary embolisms were reported in critically patients <sup>15, 54, 62-</sup>  
151 <sup>65</sup>. Large vessel occlusion stroke was also described <sup>66, 67</sup>. While diagnosis of disseminated  
152 intravascular coagulopathy (DIC) has been mainly discussed in severe COVID-19, this

153 diagnosis is limited to end stage of COVID-19. Yet, this disorder is not a typical DIC  
154 fibrinogen levels are often high and platelets are rarely reduced. It is more similar to  
155 complement mediated thrombotic microangiopathy (TMA) syndromes, that is involve  
156 disorders of complement. Mediators of TMA syndromes overlap with those released in  
157 cytokine storm <sup>68, 69</sup>. Interestingly, the presence of platelet-fibrin microthrombi in small  
158 arterial vessels of lung tissues was reported in 87% (33/38) of patient who have died with  
159 COVID-19 and for whom pulmonary post-mortem examination was requested <sup>70</sup>. For the  
160 patients who have had symptomatic forms of the disease, delayed pulmonary fibrosis may be  
161 found in a relatively important proportion of COVID-19 patients once they have healed <sup>37</sup>.  
162 These observations allowed proposing a new pulmonary vasculopathy named pulmonary  
163 intravascular coagulopathy (PIC) <sup>43, 71</sup>. Moreover, microangiopathic vessel occlusions and  
164 endothelium damages was described in kidney <sup>72</sup>. Elevated plasma von Willebrand factor  
165 (VWF) concentrations in COVID-19 patients, a factor mainly biosynthesized by activated  
166 endothelial cells, was reported <sup>73-75</sup>. Deposits of complement components C5b-9, C4d in the  
167 microvasculature of lung and skin was also reported <sup>76</sup>. These observations would be in favor  
168 of complement activation which participates to microvascular injury.

169 Also, COVID-19 induced micro and microvasculature dysfunction. The cytokine storm  
170 observed plays a determining role in this immunoinflammatory thrombosis and in endothelial  
171 damages. All of these mechanisms, whose kinetics remains unclarified today, might explain  
172 the occurrence of various thromboembolic events and multiples organ dysfunction observed  
173 in critical COVID-19 patients.

174 In parallel to these observations, it is important to underline the fact that a high prevalence of  
175 antiphospholipid (aPL) antibodies has been observed in critical COVID-19 patients and is  
176 reminiscent of a clinical scenario of antiphospholipid syndrome (APS). Among aPL  
177 antibodies, Lupus anticoagulant (LA) are more associated to thrombotic events. In agreement  
178 with Helms' study <sup>54</sup>, it was demonstrated that 85 % of critically COVID-19 patients  
179 presented Lupus anticoagulant <sup>77</sup>. Recently, our group described a prevalence of 62% and  
180 25% of LA respectively in hospitalized and ambulatory patients (Camoin-Jau et al.,  
181 Submitted). This high prevalence could be linked to cytokine storm and dysimmunity. To the  
182 best of our knowledge, the role of Lupus anticoagulant in the occurrence of thrombotic event  
183 is not yet demonstrated. High levels of aPL- $\beta$ 2GPI antibodies where also recently found in a  
184 large number of COVID-19 patients <sup>52, 78</sup>. Regarding clinical events described during COVID-

185 19 infection and biological abnormalities, Cavalli and colleagues <sup>79</sup>, suggested that a  
186 secondary form of APS is present in COVID-19 patients.

187

### 188 **Lesson from the Antiphospholipid syndrome (APS)**

189 Mainly associated with systemic lupus erythematosus (SLE) and other autoimmune diseases  
190 (such as rheumatoid arthritis, dermatomyositis, systemic scleritis, Sjögren's syndrome), the  
191 antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by  
192 recurrent venous or arterial thrombosis with or without pregnancy morbidity in the presence  
193 of persistent antiphospholipid (aPL) autoantibodies including the lupus anticoagulant (LA) or,  
194 anticardiolipin (aCL) or anti-beta 2glycoprotein (anti $\beta$ 2-GPI) autoantibodies. In 53% of  
195 patients it exists alone as primary APS (PAPS) <sup>80, 81</sup>. The presence of antibodies directed  
196 against CL and/or  $\beta$ 2-GPI has been observed in a percentage of healthy individuals without  
197 clinical symptoms of APS ranging from 4.5 to 5.5% <sup>82, 83</sup>. There is still controversy about  
198 whether pharmacologic primary thromboprophylaxis is indicated in asymptomatic carriers of  
199 aPL antibodies <sup>84</sup>.

200 Many clinical trials have been focused on the benefit of aspirin on prevention of thrombotic  
201 events in APS patients. Actually, thromboprophylaxis with aspirin is proposed only for  
202 asymptomatic aPL carriers with high risk profile, especially in the presence of other  
203 thrombotic risk factors <sup>85, 86</sup>. Low molecular weight heparin are proposed in all aPL carriers in  
204 high-risk situations, such as surgery, prolonged immobilization and the puerperium. Usually  
205 APS patient receive oral anticoagulant <sup>87-89</sup>, and/or low-dose aspirin <sup>90</sup>.

206 By opposite, HCQ (grade 1B recommendation) and low-dose aspirin are proposed in SLE  
207 with LA or isolated persistent aCL <sup>86</sup>. Indeed, several studies suggest that beside its anti-  
208 inflammatory effects, HCQ could play a role in lowering antiphospholipid titers and  
209 potentially be anti-thrombotic through several mechanisms involving inhibition of platelet  
210 adhesion, intravascular aggregation of red blood cells, interactions between platelets and  
211 coagulation factors, and binding of antiphospholipid (aPL) antibodies phospholipid surface <sup>91-  
212 93</sup>. In antiphospholipid syndrome (APS) an important risk of thrombosis relapse should be  
213 considered <sup>94</sup>. HCQ benefit to protect against thromboembolism was described more than 30-  
214 years ago, with reported reduction of erythrocyte aggregation *in vitro* and thrombus size <sup>95, 96</sup>.  
215 *In vitro*, CQ at 1 mM inhibit both ADP-stimulated platelets aggregation and thrombin-  
216 stimulated platelets aggregation <sup>97</sup>. Jancinova and colleagues hypothesized that CQ, as a



217 cationic amphiphilic molecule, might inhibit platelets aggregation either through interaction  
218 with membrane phospholipids, membrane receptor such as ADP receptor, induce the  
219 displacement of membrane-bound calcium, or pass membrane and directly act on platelets  
220 phospholipase A2, phospholipase C or calmodulin, or the production of aggregating-  
221 amplifying substances such as histamine, serotonin and adenine nucleotides. HCQ  
222 administration to patients was found to significantly reduce the thrombus size and duration <sup>98</sup>.  
223 Similar observations were reported using mouse models of APS in which administration of  
224 HCQ limited aPL binding on target cells decreased pro-inflammation, and reduced the size  
225 and duration of the thrombus <sup>98, 99</sup>. Miranda and colleagues <sup>99</sup>, found that HCQ increased the  
226 p-eNOS/e-NOS ratio leading to an improvement in the production of NO. A protective effect  
227 of HCQ against thrombosis in asymptomatic aPL-positive APS individuals was reported <sup>100</sup>.  
228 In the LUMINA observational cohort of 442 SLE patients HCQ was found  
229 thromboprotective <sup>101</sup>. These encouraging preliminary reports lead to the organization of an  
230 international consortium (APS ACTION) with the aim to set up a randomized controlled trial  
231 of HCQ in the primary thrombosis prevention of persistently aPL-positive but thrombosis free  
232 patients without other systemic autoimmune diseases <sup>102</sup>; they had the objective to investigate  
233 a cohort of 1,000 patients age 18-60 years, without pharmaceutical support, but 6-years only  
234 20 patients had been included in the cohort and the trial was stopped <sup>103</sup>. However, there are  
235 evidences that HCQ could be beneficial to APS patients. Among asymptomatic aPL-positive  
236 patients with SLE, primary prophylaxis with HCQ appears to reduce the frequency of  
237 thrombotic events <sup>104</sup>. HCQ could reduce subclinical atherosclerosis and its use may provide  
238 survival benefit <sup>105</sup>. HCQ was found to protect patients against both venous and arterial  
239 thromboses <sup>106</sup>. HCQ is considered to have several antithrombotic effects <sup>107-110</sup>. Rand and  
240 colleagues <sup>108, 109</sup>, demonstrated that HCQ reduces the binding of aPL autoantibodies/ $\beta$ 2GPI  
241 complexes (responsible for the thrombotic effect) to phospholipid bilayers, and protect the  
242 anticoagulant annexinA5 (AnxA5) from disruption by aPL autoantibodies. Interestingly, HCQ  
243 blocks platelet aggregation and adhesion and improves cholesterol profiles <sup>111</sup>. Schmidt-  
244 Tanguy and colleagues <sup>112</sup>, reported that in patients with clinical history of venous thrombosis  
245 (one or two episodes) 6/20 patients treated with oral anticoagulants (fludione) alone showed  
246 recurrent venous thromboses whereas no recurrent venous thromboses (0/20) in patients who  
247 received HCQ (400 mg daily) in addition to oral anticoagulants (fludione). The long term  
248 administration of HCQ to PAPS patients reduced aPL antibodies <sup>92</sup>. HCQ also partially  
249 reverse the aPL-induced impaired trophoblast migration <sup>113</sup>. This is consistent with the  
250 observation that addition of HCQ to conventional treatment may be associated with a

251 reduction of first-trimester miscarriages in pregnant patients <sup>114</sup>.

252 Trials are still ongoing. The HYPATIA multicenter trial will examine the use of HCQ versus  
253 placebo in aPL-positive women planning to conceive <sup>115</sup>. A more recent study, HIBISCUS,  
254 plans to examine the effect of HCQ on secondary thrombosis and APS-related morbidity in  
255 PAPS <sup>116</sup>. HCQ was also reported to decrease LDL cholesterol and serum glucose levels in  
256 PAPS patients likely contributing to reduce the risk of thrombosis <sup>117</sup>. Recently, a pilot open  
257 label randomized prospective study on the use of HCQ (200 mg daily for patients weighing  
258 below 60 Kg and 400 mg daily for those weighing above 60 Kg) for prevention of thrombosis  
259 in 50 patients with PAPS concluded to the efficiency of the treatment with a decreased  
260 incidence of thrombosis associated to a reduction in aPL titers <sup>118</sup>.

261

262 **Anti-thrombotic effect of HCQ in COVID-19 ?**

263 **HCQ as a possible treatment of thrombotic events observed in COVID-19?**

264 Right at the center of the debate on the use of HCQ as a prophylaxis and treatment of  
265 COVID-19 and lack of proper design of many trials, the idea that treatment of COVID-19  
266 patients by HCQ might reduce the thrombotic events observed in severe forms of the disease  
267 has emerged <sup>119</sup>.

268 The vascular endothelium functions as an integral barrier, separating blood from the  
269 subendothelial tissue compartments. It maintains its integrity and blood fluidity by acting as  
270 an anticoagulant through suppression of platelets activation and induction of fibrinolysis.  
271 Anti-SARS-CoV-2 defense mechanisms are likely to activate a tissue aggressive immune  
272 response including exaggerated IL-6 production that drives inflammatory reactions and the so  
273 called "cytokine storm" that increases tissue damage. Coagulopathy and vasculopathy walls  
274 following a pro-inflammatory process result in rapid activation of mechanisms aimed at  
275 leading to local damage repair, immune cells accumulation to prevent infection, platelets  
276 aggregation for primary and secondary hemostasis.

277 Thrombosis is common during critical illness especially in the oldest patients who had  
278 preexisting cardiovascular disease and it was reported in COVID-19 patients <sup>49, 52, 54</sup>. There is  
279 currently a debate whether or not the number of patients who experience arterial thrombotic  
280 events in COVID-19 is higher rate compared to critically ill patients without SARS-CoV-2

281 carriage <sup>120</sup>. Whatever the answer, COVID-19 appears to induce a hypercoagulable state with  
282 elevated fibrinogen and the fact remains that clinicians must prevent and/or treat thrombosis.  
283 In patients with previous thrombosis attributable to APS, anticoagulation has formed the  
284 cornerstone of treatment. Inflammation in the presence of aPL, is associated with an increased  
285 thrombotic risk, that requires rapid treatment with anticoagulant to reduce mortality. This is  
286 very similar to what is observed in COVID-19 (inflammation and thrombosis), which  
287 suggests that in this pathology also taking HCQ could have a beneficial effect against  
288 thrombotic events and preliminary investigation of such therapy is under evaluation <sup>121</sup>.

289

## 290 **Conclusion**

291 If the therapeutic use of HCQ in COVID-19 is still the subject of passionate debate in the  
292 infectious disease community, rheumatologists have successfully used it for a long time for  
293 the prevention of pro-inflammatory process and the thrombosis, two pathological processes  
294 frequently encountered in severe cases of COVID-19. When the HCQ/azithromycin was  
295 initiated in Marseille IHU Méditerranée infection for the treatment of people who were found  
296 positive for SARS-CoV-2, the main objective of this protocol was to take advantage of both  
297 the antiviral and immunosuppressive properties of HCQ <sup>122</sup>. Yet, we might hypothesize that the  
298 low mortality of COVID-19 patients treated in Marseille IHU with HCQ (fatality rate  
299 estimated 0.4% to be compared to 2.27% worldwide) could be also due to a protective effect  
300 of HCQ against thrombosis when used in association with a well-chosen anticoagulant. This  
301 will require further evaluation.

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310 **Funding**

311 This work was supported by the French Government under the « Investissements d’avenir »  
312 (Investments for the Future) program managed by the Agence Nationale de la Recherche  
313 (French ANR: National Agency for Research), (reference: Méditerranée Infection 10-IAHU-  
314 03), the Région Provence Alpes Côte d’Azur and European funding FEDER PRIMI

315

316 **Authorship**

317 All authors contributed to the design of the study and conceived the manuscript. CD and  
318 LCJ wrote the paper. DR obtained the funding for this study. All authors reviewed and  
319 approved the final version of the manuscript

320

321 **Declaration of Competing Interest**

322 CD declares a link of interest with the Sanofi pharmaceutical company which markets  
323 hydroxychloroquine. The other authors (LCJ, JLM, and DR) declare that they have no  
324 competing interests.

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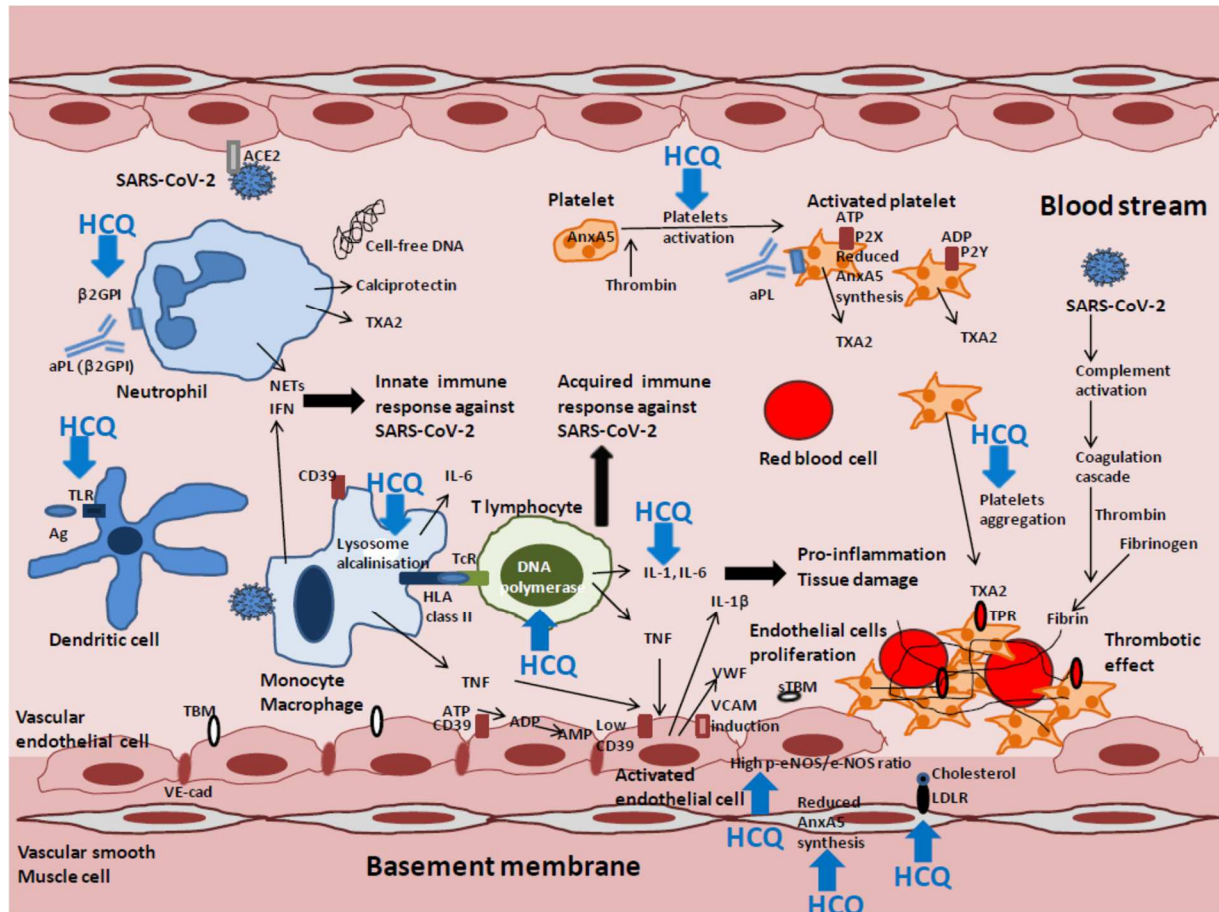
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334 **Figure 1:** Possible mechanisms of action of hydroxychloroquine (HCQ) against COVID-19-  
335 associated thrombosis. The vascular endothelium functions as an integral barrier through  
336 myriad mechanisms including VE-cadherin, and maintains blood fluidity by acting as an  
337 anticoagulant through suppression of platelets activation and induction of fibrinolysis,  
338 mechanism including heparan sulfate proteoglycans and CD39. During SARS-CoV-2  
339 infection, innate and acquired immune defense mechanisms are activated (including the  
340 cytokine storm IL-1, IL-2, IL-6, IL-8, IL-17, TNF $\alpha$ ) that provokes tissue damage in the lung  
341 parenchyma and the immediately adjacent bronchial alveolar lymphoid tissue and disruption  
342 of blood vessel walls. The endothelial cells express the angiotensin I converting enzyme 2  
343 (ACE2) molecule that act as cell-surface-receptor that facilitates SARS-CoV-2 entry into  
344 these cells. When activated by proinflammatory cytokines, or neutrophil extracellular traps,  
345 endothelial cells produce von Willebrand factor that retains platelets and leucocytes to the  
346 vessel wall and activates coagulation systems resulting in rapid activation of mechanism  
347 aimed at leading to local damage reparation, immune cells accumulation to prevent infection,  
348 platelets aggregation for primary and secondary hemostasis. The hyper-reaction set up in  
349 response to vascular damage, can influence a propensity toward local vascular micro-  
350 thrombosis. COVID-19 patients suffer from prominent alveolar oedema, intra-alveolar  
351 proteinosis, cell infiltration including lymphocytes apoptosis of virally-infected pneumocytes,  
352 fibrin deposition. HCQ treatment of COVID-19 patient is likely to reduce pro-inflammation  
353 and vascular micro-thrombosis due to its multiple actions (the expected mechanisms of action  
354 of HCQ to counteract pro-inflammation and thrombosis are indicated by a blue arrow and  
355 HCQ) in addition to reduce the patient viral load. aPL: antiphospholipids; IL-6: interleukin-6;  
356 TLR: toll like receptor; Ag : antigen; IFN: interferon; TNF: tumor necrosis factor;  
357 CD39/ENTPD1: Ectonucleoside triphosphate diphosphohydrolase-1 (also known as or P2  
358 receptors: P2X receptors are ion channels that open upon binding of ATP; P2Y receptors  
359 mediate cellular response to purine and pyrimidine such as ATP, ADP, UTP; in physiological  
360 conditions CD39 catalyze the reduction of ATP and ADP pool to AMP and CD73 transform  
361 AMP to adenosine whereas nucleotides released during cell activation/injury bind to P2  
362 receptors to activate thrombo-inflammatory programs); NETs: neutrophil extracellular traps;  
363 TXA2: Thromboxane A2 (induce platelets aggregation); AnxA5: annexinA5 (or annexin V or  
364 anchorin CII; anticoagulant, interact with phospholipids); TPR : thromboxane A2 prostanoid  
365 receptor: VE-cad: VE-cadherin; TBM: thrombomodulin prevents thrombosis; upon  
366 endothelial cell activation a soluble form of TBM (sTBM) is released in plasma further  
367 promoting procoagulant mechanisms. VWF: von Willebrand factor; Fibrin: fibrin is formed

368 from blood plasma fibrinogen (produced in the liver) by the action of thrombin; red thrombus  
 369 is composed of erythrocytes enmeshed in a fibrin network; LDLR: Low density lipoprotein  
 370 receptor (bind LDL/cholesterol).



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382 **References**

- 383 1. Wellems TE, Plowe CV. Chloroquine-resistant malaria . *J Infect Dis*, 2001; **184**: 770-776.
- 384 2. Xourgia E, Tektonidou MG. Type I interferon gene expression in antiphospholipid  
385 syndrome : pathogenetic, clinical and therapeutic implications. *J Autoimmun* 2019; **104**:  
386 102311
- 387 3. Schrezenmeier E, Dorner T. Mechanisms of action of hydroxychloroquine and chloroquine:  
388 implications for rheumatology. *Nat Rev Rheumatol* 2020; **16**: 155-166
- 389 4. Vitte J, Moïse M, Mezouar S, Bailo Diallo A, Boumaza A, Mege JL, Desnues B. Immune  
390 modulation as a therapeutic option during the SARS-CoV-2 outbreak: the case for  
391 antimalarial aminoquinolones. *Front Immunol* 2020a; **11**: 2159
- 392 5. Genton E, Gent M, Hirsh J, Harker LA. Platelet-inhibiting drug in the prevention of  
393 clinical thrombotic disease (third of three parts). *N Engl J Med* 1975; **293**: 1296-1300
- 394 6. Nosal R, Jancinova V, Petrikova M. Chloroquine inhibits stimulates platelets at the  
395 arachidonic acid pathway. *Thromb Res* 1995; **77**: 531-542
- 396 7. Rahman R, Murthi P, Singh H, Gurusinghe S, Mockler JC, Lim R, et al. The effects of  
397 hydroxychloroquine on endothelial dysfunction. *Pregnancy Hypertens* 2016; **6**: 259-262
- 398 8. Li R, Lin H, Ye Y, Xiao Y, Xu S, Wang J, et al. Attenuation of antimalarial agent  
399 hydroxychloroquine on TNF-alpha-induced endothelial inflammation. *Int Immunopharmacol*  
400 2018; **63**: 261-269.
- 401 9. Cheng F, Desai RJ, Handy DE, Wang R, Schneeweiss S, Barabasi AL, et al. Network-  
402 based approach to prediction and population-based validation of in silico drug repurposing.  
403 *Nat Commun* 2018; **9**: 2691
- 404 10. Lu R, Zhao X, Li J, Niu P, Yang B, Wu W, et al. Genomic characterization and  
405 epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.  
406 *Lancet* , 2020; **395**: 565-574
- 407 11. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from  
408 Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**:727–733
- 409 12. Frutos R, Lopez Roig M, Serra-Cobo J, Devaux CA. COVID-19: The conjunction of  
410 events leading to the coronavirus pandemic and lessons to learn for futur threats. *Front Med*  
411 2020; **7**: 223
- 412 13. Huang C, Wang Y, Li X, Ren PL, Zhao J, Hu Y, et al. Clinical features of patients  
413 infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**(10223): 497-506
- 414 14. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y et al. Dysregulation of immune response  
415 in patients with COVID-19 in Wuhan, China. *Clin Infect Dis* 2020; **71**(15): 762-768

- 416 15. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Miller MCA et al.  
417 Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb*  
418 *Haemost* 2020; **18**(8): 1995-2002
- 419 16. AbdelMassih AF, Kamel A, Mishriky F, Ismail HA, El Qadi L, Malak L, et al. It is  
420 infection or rather vascular inflammation? Game-changer insights and recommendations from  
421 patterns of multi-organ involvement and affected subgroups in COVID-19. *Cardiovasc.*  
422 *Endocrinol. Metab.* 2020; **9**(3): 110-120.
- 423 17. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral  
424 infections: an old drug against today's diseases? *Lancet Inf Dis* 2003; **3**: 722-727
- 425 18. Kearney JE. Chloroquine as a potential treatment and and prevention measure for the  
426 2019 novel Coronavirus: A review. Preprint 17 march 2020, not peer-reviewed. doi:  
427 10.20944/preprints202003.0275.v1
- 428 19. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M. et al. Remdesivir and chloroquine  
429 effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*  
430 2020; **30**: 269-271.
- 431 20. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H et al. Hydroxychloroquine, a less toxic  
432 derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell*  
433 *Discovery* 2020; **6**: 16
- 434 21. Hu TY, Frieman M, Wolfram J. Insights from nanomedicine in chloroquine efficacy  
435 against COVID-19. *Nature Nanotech* 2020; [epub ahead of print]. doi:10.1038/s41565-020-  
436 0674-9
- 437 22. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of  
438 chloroquine against coronavirus: what to expect for COVID-19, *Int J Antimicrob Agents*  
439 2020a; **55**: 105938
- 440 23. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent  
441 efficacy in treatment of COVID-19 associated pneumlonia in clinicla studies. *Biosci Trends*  
442 2020; [epub ahead of print] doi: 10.1038/s41422-020-0282-0
- 443 24. Li L, Li R, Wu Z, Yang X, Zhao M, Liu J, Chen D. Therapeutic strategies for critically ill  
444 patients with COVID-19. *Ann Intens Care* 2020; **10**: 45
- 445 25. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M., et al.  
446 Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label  
447 non-randomized clinical trial. *Int J Antimicrob Agents* 2020; **56**(1): 105949
- 448 26. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and  
449 microbiological effect of a combination of hydroxychloroquine and azithromycin in 80  
450 COVID-19 patients with at least a six-day follow-up: a pilot observational study. *Travel Med*  
451 *Infect Dis* 2020; **49**: 215219.



- 452 27. Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amrane S. et al. Early treatment  
453 of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of  
454 1061 cases in Marseille, France. *Travel Med Inf Dis* 2020; **35**: 101738
- 455 28. Lagier JC, Million M, Gautret P, Colson P, Cortaredona S, Giraud-Gatineau A, et al.  
456 Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and  
457 other regimens in Marseille, France. A retrospective analysis. *Travel Med Infect Dis* 2020; **36**:  
458 101791
- 459 29. Million M, Gautret P, Colson P, Roussel Y, Dubourg G, Fenollar F. Breaking news: the  
460 efficacy of Chloroquine derivatives in COVID-19: a meta-analysis on the first available  
461 reports. *IHU Méditerranée infection* 2020. Preprint not peer-reviewed, IHU prepublication  
462 site on COVID-19
- 463 30. Sarma P, Kaur H, Kumar H, Mahendru D, Avli P, Bhattacharyya A, et al. Virological and  
464 clinical cure in COVID-19 patients treated with hydroxychloroquine. A systematic review and  
465 meta-analysis. *J Med Virol* (2020) [Epub ahead of print] doi:10/1002:jmv.25898
- 466 31. Singh AK, Singh A, Singh R, Misra A. Hydroxychloroquine in patients with COVID-19.  
467 A systematic review and meta-analysis. *Diabetes Metab Syndr* 2020; **14**(4): 589-596
- 468 32. Fiolet T, Guihur A, Rebeaud M, Mulot M, Peiffer-Smadja N, Mahamat-Saleh Y. Effect of  
469 hydroxychloroquine with or without azithromycin on the mortality of COVID-19 patients: a  
470 systematic review and meta-analysis. *Clin Microbiol Inf* 2020. [epub ahead of print] doi:  
471 10.1016/j.cmi.2020.08.022.
- 472 33. Maisonnasse P, Guedj J, Contreras V, Behillil S, Solas C, Marlin R, et al.  
473 Hydroxychloroquine use against SARS-CoV-2 infection in non-human primates. *Nature*  
474 2020; [epub ahead of print]. doi: 10.1038/s41586-020-2558-4
- 475 34. The RECOVERY Collaborative Group. Effect of hydroxychloroquine in hospitalized  
476 patients with COVID-19. *N Engl J Med* 2020; **383**: 2030-2040
- 477 35. Devaux CA, Rolain JM, Raoult D. ACE2 receptor polymorphism : susceptibility to  
478 SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. *J*  
479 *Microbiol Immunol Infect* 2020; **53**(3): 425-435
- 480 36. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at  
481 increased risk for COVID-19 infection? *Lancet Resp Med* 2020; **8**: e21
- 482 37. Wang J, Wang BJ, Yang JC, Wang MY, Chen C, Luo GX et al. Research advances in the  
483 mechanism of pulmonary fibrosis induced by Coronavirus disease 2019 and the  
484 corresponding therapeutic measures [Article in Chinese]. *Zhonghua Shao Shang Za Zhi* 2020;  
485 **36**(8):691-697.
- 486 38. Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine storm in COVID-19: the current  
487 evidence and treatment strategies. *Front Immunol.* (2020) [Epub ahead of print]  
488 doi:10.3389/fimmu.2020.01708

- 489 39. Zhai ZC, Chen Y, Gerotziafas G, Zhang Z, Wan J, et al. Prevention and Treatment of  
490 Venous Thromboembolism Associated with Coronavirus Disease 2019 Infection: A  
491 Consensus Statement before Guidelines. *Thromb Haemost* 2020; **120**(6): 937–948
- 492 40. Kanthi YM, Sutton NR, Pinsky DJ. CD39: interface between vascular thrombosis and  
493 inflammation. *Curr Atheroscler Rep* 2014; **16**(7): 425
- 494 41. Enjyoji K, Sevigny J, Lin Y, Frenette PS, Christie PD, Esch JS, et al. Targeted disruption  
495 of cd39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation.  
496 *Nat Med* 1999; **5**(9):1010-1017
- 497 42. Grifoni E, Valoriani A, Cei F, Lamanna R, Gelli AMG, Ciambotti B, et al. Interleukin-6  
498 as prognosticator in patients with COVID-19. *J Infect* 2020; **81**(3): 452-482.
- 499 43. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The role of cytokines including  
500 interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome like  
501 disease. *Autoimmunity Rev* 2020; [epub ahead of print] doi:10.1016/j.autrev.2020.102537
- 502 44. Xu X, Han M, Li T, Sun W, Wang D, Fu B et al. Effective treatment of severe COVID-19  
503 patients with tocilizumab. *Proc Natl Acad Sci USA* 2020; **117**(20) :10970-10975
- 504 45. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe  
505 COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality.  
506 *Int J Antimicrob Agents* 2020; **55**(2): 105954
- 507 46. Thiolat A, Semerano L, Pers YM, Biton J, Lemeiter D, Portales P, et al. Interleukin-6  
508 receptor blockade enhances CD39+ regulatory T cell development in rheumatoid arthritis  
509 and in experimental arthritis. *Arthritis Rheumatol* 2014; **66**(2): 273-283
- 510 47. Zuo Y, Zuo M, Yalavarthi S, Gockman K, Madison JA, Shi H, Woodard W, Lezak SP,  
511 Lugogo NL, Knight JS, Kanthi Y. Neutrophil extracellular traps and thrombosis in COVID-  
512 19. *MedRxiv* (2020) , preprint not peer-reviewed. doi: 10.1101/2020.04.30.20086736
- 513 48. Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben CJ, et al. Platelet  
514 gene expression and function in COVID-19 Patients. *Blood* 2020; [epub ahead of print]. doi:  
515 10.1182/blood.2020007214
- 516 49. Harzallah I, Debliquis A, Drenou B. Lupus anticoagulant is frequent in patients with  
517 Covid-19. *J Thromb Haemost* ;2020; [epub ahead of print]. doi: 10.1111/jth.14867
- 518 50. Xiao M, Zhang Y, Zhang S, Qin X, Xia P, Cao W et al. Brief report: Anti-phospholipid  
519 antibodies in critically ill patients with coronavirus disease 2019 (COVID-19) . *Arthritis*  
520 *Rheumatol* 2020; [Epub ahead of print]. doi:10.1002/art.41425
- 521 51. Amezcua-Guerra LM, Rojas-Velasco G, Brianza-Padilla M, Vazquez-Rangel A,  
522 Marquez-Velasco R, Baranda-Tovar F, et al. Presence of antiphospholipid antibodies in  
523 COVID-19: a case series study . *Ann Rheum Dis* 2020; published online. doi:  
524 10.1136/annrheumdis-2020-218100

- 525 52. Devreese KMJ, Linskens EA, Benoit D, Peperstraete H. Antiphospholipid antibodies in  
526 patients with COVID-19: a relevant observation? *J Thromb Haemost* 2020; **18**: 2191-2201
- 527 53. Bertin D, Brodovitch A, Beziane A, Hug S, Bouamri A, Mege JL, Bardin N. Anti-  
528 cardiolipin IgG autoantibodies are an independent risk factor of COVID-19 severity.  
529 *Arthritis Rheumatol* 2020; [epub ahead of print]. doi: 10.1002/art.41409
- 530 54. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High  
531 risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective  
532 cohort study. *Intensive Care Med* 2020; **4**: 1-10
- 533 55. Zuo Y, Estes SK, Gandhi AA, Yalavarthi S, Ali RA, Shi H, et al. Prothrombotic  
534 antiphospholipid antibodies in COVID-19. MedRxiv (2020b) , preprint not peer-reviewed.  
535 doi: 10.1101/2020.06.15.20131607
- 536 56. Vitte J, Bailo Diallo A, Boumaza A, Lopez A, Michel M, Allardet-Servent J, Mezouar S,  
537 et al. A granulocytic signature identifies COVID-19 and its severity. *J Infect Dis* 2020b; in  
538 press, JID-70456R1
- 539 57. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and  
540 anticoagulation. *Blood* 2020; **135**(23). [epub ahead of print]. doi: 10.1182/blood.2020006000
- 541 58. Lippi G, Favaloro EJ. D-dimer is Associated with Severity of Coronavirus Disease 2019:  
542 A Pooled Analysis. *Thromb Haemost* 2020; **120**(5): 876-878
- 543 59. Sakka M, Connors JM, Hékimian G, Martin-Toutain I, Crichi B, Colmegna I, et al.,  
544 Association between D-dimer levels and mortality in patients with coronavirus disease 2019  
545 (COVID-19: a systematic review and pooled analysis. *J Med Vasc* 2020; **45**(5): 268-274
- 546 60. Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z, Zhang Z. D-dimer levels on admission to  
547 predict in-hospital mortality in patients with Covid-19. *J Thrombos Heamost* 2020; **18**(6):  
548 1324-1329
- 549 61. Leonard-Lorant I, Delabranche X, Severac F, Helms J, Pauzet C, Collange O, et al. Acute  
550 Pulmonary Embolism in COVID-19 Patients on CT Angiography and Relationship to D-  
551 Dimer Levels. *Radiology* 2020; [epub ahead of print]. doi:10.1148/radiol.2020201561
- 552 62. Faggiano P, Bonelli A, Paris S, Milesi G, Bisegna S, Bernardi N et al. Acute pulmonary  
553 embolism in COVID-19 disease: Preliminary report on seven patients. *Int J Cardiol* 2020;  
554 **313**. [epub ahead of print]. doi: 10.1016/j.ijcard.2020.04.028
- 555 63. Zhang C, Shen L, Le KJ, Pan MM, Kong LC, Gu ZC, et al. Thromboembolism in  
556 Hospitalized Coronavirus Disease 2019 Patients: A Systematic Review and Meta-Analysis.  
557 *Front Cardiovascular Med* 2020; **7**: article 151 2020
- 558 64. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassale F, et al. Pulmonary  
559 embolism in patients with COVID-19. *Circulation* 2020; **142**:184-186

- 560 65. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et  
561 al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19.  
562 *Thromb Res* 2020; **191**: 145-147
- 563 66. Majidi S, Fifi JT, Ladner TR, Lara-Reyna J, Yaeger KA; Yim B, et al. Emergent large  
564 vessel occlusion stroke during New York city's COVID-19 outbreak. *Stroke* 2020; **51**(9):  
565 2656-2663
- 566 67. Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP et al. Large-vessel stroke  
567 as a presenting feature of Covid-19 in young. *N Engl J Med* 2020; **382**:e60
- 568 68. Campbell CM, Kahwash R. Will complement inhibition be the new target in treating  
569 COVID-19 related systemic thrombosis ? *Circulation* 2020; **141**: 1739-1741 .
- 570 69. Merrill JT, Erkan D, Winakur J, James JA. Emerging evidence of a COVID-19 thrombotic  
571 syndrome has treatment implications . *Nature Rev Rheum* 2020; [epub ahead of print]. doi:  
572 10.1038/s41584-020-0474-5
- 573 70. Carsana L, Sanzagni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, et al. (2020).  
574 Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-  
575 centre descriptive study. *Lancet* 2020; published online. doi:10.1016/S1473-3099(20)30434-5
- 576 71. Marietta M, Coluccio V, Luppi M. COVID-19, coagulopathy and venous  
577 thromboembolism: more questions than answers. *Int Emerg Med* 2020; **11**: 1-13
- 578 72. Su H, Yang M, Wan C, Yi LX, Tang F, Zhu HY, et al. Renal histopathological analysis of  
579 26 postmortem findings of patients with COVID-19 in China. *Kidney Int* 2020; **98**: 219–227
- 580 73. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy  
581 in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study.  
582 *Lancet Haematol* 2020. [epub ahead of print]. doi: 10.1016/S2352-3026(20)30216-30217
- 583 74. Libby P, Lüscher T. COVID-19 is, in the end, an endothelial disease. *Europ Heart J* 2020;  
584 **4**: 3038-3044
- 585 75. O'Sullivan JM, Mc Gonagle D, Ward SE, Preston RJS, O'Donnell JS. *Lancet* 2020; **7**:  
586 e553-e555
- 587 76. Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, Baxter-Stoltzfus A,  
588 Laurence J. Complement associated microvascular injury and thrombosis in the pathogenesis  
589 of severe COVID-19 infection: a report of five cases. *Transl Res* 2020; **220**: 1-13
- 590 77. Siguret V, Voicu S, Neuwirth M, Delrue M, Gayat E, Stépanian A, Mégarbane B. Are  
591 antiphospholipid antibodies associated with thrombotic complications in critically ill COVID-  
592 19 patients ? *Thromb Res* 2020; **195**:74-76
- 593 78. Borghi MO, Beltagy A, Garrafa E, Curreli D, Cecchini G, Bodio C, et al. Anti-  
594 phospholipid antibodies in COVID-19 are different from those detectable in the anti-

595 phospholipid syndrome. *MedRxiv* Preprint July 16, 2020, not peer-reviewed.  
596 doi:10.1101/2020.06.17.20134114

597 79. Cavalli E, Bramanti A, Ciurleo R, Tchorbanov AI, Giordano A, Fagone P et al.  
598 Entangling COVID-19 associated thrombosis into a secondary antiphospholipid antibody  
599 syndrome: diagnostic and therapeutic perspectives (Review). *Int J Mol Med* 2020; **46**: 903-  
600 912

601 80. Cervera R, Serrano R, Pons-Estel GJ, Cervera R, Espinosa G, Shoenfeld Y, et al. Morbidity  
602 and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre  
603 prospective study of 1000 patients. *Ann Rheum Dis* 2015; **74**: 1011- 1018

604 81. Martirosyan A, Aminov R, Manukyan G. Environmental triggers of autoreactive  
605 responses: induction of antiphospholipid antibody formation. *Front Immunol* 2019; **10**: 1609

606 82. Catoggio C, Alvarez-Uria A, Fernandez PL, Cervera R, Espinosa G. Catastrophic  
607 antiphospholipid syndrome triggered by fulminant disseminated herpes simplex infection in a  
608 patient with systemic lupus erythematosus. *Lupus* 2012; **21**: 1359-1361

609 83. Mendoza-Pinto C, Garcia-Carrasco M, Cervera R. Role of infectious disease in the  
610 antiphospholipid syndrome (including its catastrophic variant). *Curr Rheumatol Rep* 2018; **20**:  
611 62

612 84. Giron-Gonzalez JA, Garcia del Rio E, Rodriguez C, Rodríguez- Martorell J, Serrano A.  
613 Antiphospholipid syndrome and asymptomatic carriers of antiphospholipid antibody: prospec-  
614 tive analysis of 404 individuals. *J Rheumatol* 2004; **31**:1560–1567

615 85. Ruiz-Irastorza G, Egurbide MV, Ugalde J. et al. High impact of antiphospholipid  
616 syndrome on irreversible organ damage and survival of patients with systemic lupus  
617 erythematosus. *Arch Intern Med* 2004; **164**(1): 77-82

618 86. Ruiz-Irastorza G, Cuadrado MJ, Ruiz-Arruza I, Brey R, Crowther M, R Derksen, et al.  
619 Evidence-based recommendations for the prevention and long-term management of  
620 thrombosis in antiphospholipid antibody-positive patients: Report of a Task Force at the 13th  
621 International Congress on Antiphospholipid Antibodies. *Lupus* 2011; **20**: 206

622 87. Cohen H, Hunt BJ, Efthymiou M, Arachchillage DRJ, Mackie IJ, Clawson S, et al.  
623 Rivaroxaban versus warfarin to treat patients with thrombotic antiphospholipid syndrome,  
624 with or without systemic lupus erythematosus (RAPS): a randomised, controlled, open-label,  
625 phase 2/3, non-inferiority trial. *Lancet Haematol* 2016; **3**: e426–e436

626 88. Resseguier AS, Pereira B, Rieu V, Le Guenno G, Grobost V, Ruivard M. Direct oral  
627 anticoagulants: an alternative treatment for thrombotic antiphospholipid syndrome? *Lupus*  
628 2017; **26**: 1297–1303.

629 89. Pengo V, Denas G, Zoppellaro G, Jose SP, Hoxha A, Ruffatti A, et al. Rivaroxaban vs  
630 warfarin in high-risk patients with antiphospholipid syndrome. *Blood* 2018; **132**: 1365–1371

- 631 90. Tektonidou MG, Andreoli L, Limper M, Amoura Z, Cervera R, Costedoat-Chalumeau N,  
632 et al. EULAR recommendations for the management of antiphospholipid syndrom in adults.  
633 *Ann Rheum Dis* 2019; **78**: 1296-1304
- 634 91. Belizna C. Hydroxychloroquine as an anti-thrombotic in antiphospholipid syndrome.  
635 *Autoimmun Rev* 2015; **14**: 358-362
- 636 92. Nuri, E, Taraborelli, M, Andreoli, L, Tonello M, Gerosa M, Calligaro A, et al. Long-term  
637 use of hydroxychloroquine reduces antiphospholipid antibodies levels in patients with  
638 primary antiphospholipid syndrome. *Immunol Res* 2017; **65**: 17–24.
- 639 93. Wang TF, Lim W. What is the role of hydroxychloroquine in reducing thrombotic risk in  
640 patients with antiphospholipid antibodies? *Hematology* 2016; **1**: 714-716
- 641 94. Pengo V, Ruffatti A, Legnani C, Gresele P, Barcellona D, Erba N, Testa S, Marongiu F,  
642 Bison E, Denas G, Banzato A, Padayattil JS, Lliceto S. Clinical course of high-risk patients  
643 diagnosed with antiphospholipid syndrome. *J Thromb Haemost* 2010; **8**: 237-242
- 644 95. Wallace DJ. Does hydroxychloroquine sulfate prevent clot formation in systemic lupus  
645 erythematosus ? *Arthritis Rheum* 1987; **30**: 1435-1436
- 646 96. Loudon JR. Hydroxychloroquine and postoperative thromboembolism after total hip  
647 replacement. *Am J Med* 1988; **85**: 57-61.
- 648 97. Jancinova V, Nosal R, Petrikova M. On the inhibitory effect of chloroquine on blood  
649 platelet aggregation. *Thromb Res* 1994; **74**: 495-504
- 650 98. Edwards MH, Pierangeli S, Liu X, Barker JH, Anderson G, Harris EN.  
651 Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice.  
652 *Circulation* 1997; **96**: 4380-4384
- 653 99. Miranda S, Billoir P, Damian L, Thiebault PA, Schapman D, Le Besnerais M, et al.  
654 Hydroxychloroquine reverses the prothrombotic state in a mouse model of antiphospholipid  
655 syndrome: role of reduced inflammation and endothelial dysfunction. *PLoS One* 2019; **14**(3):  
656 e0212614
- 657 100. Erkan D, Yazici Y, Peterson MG, Sammaritano L, Lockshin MD. A cross-sectional  
658 study of clinical thrombotic risk factors and preventive treatments in antiphospholipid  
659 syndrome. *Rheumatology* 2002; **41**: 924-929
- 660 101. Ho KT, Ahn CW, Alarco GS, Baethge BA, Tan FK, Roseman J, et al. Systemic lupus  
661 erythematosus in a multicentric cohort (LUMINA): XXVIII. Factor predictive of thrombotic  
662 event. *Rheumatology* 2005; **44**: 1303-1307
- 663 102. Erkan D, Lockshin. APS ACTION-AntiPhospholipid Syndrome Alliance for Clinical  
664 Trials and InternatiOnal Network . *Lupus* 2012; **21**: 695-698

- 665 103. Erkan D, Unlu O, Sciascia S, Belmont HM, Ware Branch D, Cuadrado MJ, et al.  
666 Hydroxychloroquine in the primary thrombosis prophylaxis of antiphospholipid antibody  
667 positive patients without systemic autoimmune disease. *Lupus* 2018; **27**: 399-406
- 668 104. Metjian A, Lim W. ASH evidence-based guidelines: should asymptomatic patients with  
669 antiphospholipid antibodies receive primary prophylaxis to prevent thrombosis? *Hematology*  
670 *Am Soc Hematol Educ Program* 2009; 247-249.
- 671 105. Ruiz-Irastorza G, Egurbide MV, Pijoan JI, Garmendia M, Villar I, Martinez-Berriotxo  
672 A. et al. Effect of antimalarials on thrombosis and survival in patients with systemic lupus  
673 erythematosus. *Lupus* 2006; **15**: 577-583
- 674 106. Jung H, Bobba R, Su J, Shariati-Sarabi Z, Gladman DD, Urowitz M, et al. The protective  
675 effect of antimalarial drug on thrombovascular events in systemic lupus erythematosus.  
676 *Arthritis Rheum* 2010; **62**: 863-868
- 677 107. Espinola RG, Pierangeli SS, Gharavi AE, Harris EN. Hydroxychloroquine reverse  
678 platelet activation induced by human IgG antiphospholipid antibodies. *Thromb Haemost*  
679 2002; **87**: 518-522.
- 680 108. Rand JH, Wu XX, Quinn AS, Chen PP, Hathcock JJ, Taatjes DJ. Hydroxychloroquine  
681 directly reduces the binding of antiphospholipid antibody-beta2-glycoprotein I complexes to  
682 phospholipid bilayers. *Blood* 2008; **112**: 1687-1695
- 683 109. Rand JH, Wu XX, Quinn AS, Ashton AW, Chen PP, Hathcock JJ, Andree HA, Taatjes  
684 DJ. Hydroxychloroquine protect the annexin A5 anticoagulant shield from disruption by  
685 antiphospholipid antibodies: evidence for a novel effect for an old anti-malarial drug. *Blood*  
686 2010; **115**: 2292-2299
- 687 110. Petri M. Use of hydroxychloroquine to prevent thrombosis in systemic lupus  
688 erythematosus and in antiphospholipid antibody-positive patients. *Curr Rheumatol Rep* 2011;  
689 **13**: 77-80.
- 690 111. Al-Homood IA. Thrombosis in systemic lupus erythematosus: a review article. *ISRN*  
691 *Rheumatol* 2012; **2012**:428269
- 692 112. Schmidt-Tanguy A, Voswinkel J, Henrion D, Subra JF, Loufrani LL, Rohmer V, Ifrah N,  
693 Belizna C. Antithrombotic effects of hydroxychloroquine in primary antiphospholipid  
694 syndrome patients. *Int Soc Thromb Haemost* 2013; **11**(10): 1927-1929
- 695 113. Albert CR, Schlesinger WJ, Viall CA, Mulla MJ, Brosens JJ, Chamley LW, Abrahams  
696 VM. Effect of hydroxychloroquine on antiphospholipid antibody-induced changes in first  
697 trimester trophoblast function. *Am J Reprod Immunol* 2014; **71**(2): 154-164
- 698 114. Ruffatti A, Tonello M, Hoxha A, Sciascia S, Cuadrado MJ, Latino JO, et al. Effect of  
699 additional treatments combined with conventional therapies in pregnant patients with high-  
700 risk antiphospholipid syndrome: a multicentre study. *Thromb Haemost* 2018; **118**: 639-648

701 115. Schreiber K, Breen K, Cohen H, Jacobsen S, Middeldorp S, Pavord S, et al.  
702 Hydroxychloroquine to improve Pregnancy outcome in women with Antiphospholipid  
703 Antibodies (HYPATIA) protocol: a multinational randomized controlled trial of  
704 hydroxychloroquine versus placebo in addition to standard treatment in pregnant women with  
705 antiphospholipid syndrome or antibodies. *Semin Thromb Hemost* 2017; **43**: 562–571

706 116. Belizna C, Pregnolato F, Abad S, Alijotas-Reig J, Amital H, Amoura Z, et al.  
707 HIBISCUS: hydroxychloroquine for the secondary prevention of thrombotic and obstetrical  
708 events in primary antiphospholipid syndrome. *Autoimmun Rev* 2018; **17**: 1153–1168.

709 117. Mangolin S, Mazetto BM, Annichino-Bizzacchi JM, Andrade Orsi F. Study of the role of  
710 hydroxychloroquine in immunomodulation and control of hypercoagulability in patients  
711 diagnosed with primary antiphospholipid syndrome. *Blood* 2019; **134**: 3682

712 118. Kravvariti E, Koutsogianni A, Samoli E, Sfrikakis PP, Tektonidou MG. The effect of  
713 hydroxychloroquine on thrombosis prevention and antiphospholipid syndrome: a pilot open  
714 label randomized prospective study. *Autoimmun Rev* 2020; **19**(4):102491

715 119. Pal A, Pawar A, Goswami K, Sharma P, Prasad R. Hydroxychloroquine and COVID-19:  
716 a cellular and molecular biology based update. *Indian J Clin Biochem* 2020; **35**: 274-284

717 120. Connell NT, Battinelli EM, Connors JM. Coagulopathy of COVID-19 and  
718 antiphospholipid antibodies. *J Thromb Haemost* (2020) 00: 1-2

719 121. Mathian A, Mahevas M, Rohmer J, Roumier M, Cohen-Aubart F, Amador-Borrero B, et  
720 al. Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with  
721 systematic lupus erythematosus under long-term treatment with hydroxychloroquine. *Ann*  
722 *Rheum Dis* 2020; **79**(6): 837-839

723 122. Gautret P, Million M, Kaplanski G, Camoin-Jau L, Colson P, Fenollar F. et al. Natural  
724 history of COVID-19 and therapeutic options. *Exp Rev Clin Immunol* 2020. in press.

725  
726