



Review

## SARS-CoV-2 and Plasma Hypercoagulability

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(Received 15 January 2021; accepted 14 June 2021; published online 28 June 2021)

Associate Editor Michael R. King oversaw the review of this article.

**Abstract**—Hypercoagulability has emerged as a prominent consequence of COVID-19. This presents challenges not only in the clinic, but also in thrombosis research. Health and safety considerations, the status of the blood and plasma supply, the infection status of individual donors, and the mechanisms by which SARS-CoV-2 activates coagulation are all of concern. In this review, we discuss these topics from the basic research perspective. As in other respiratory illnesses, blood and plasma from COVID-19 positive patients carries minimal to no risk of infection to practitioners or researchers. There are currently no special regulatory mandates directing individual donors (for research purposes), blood centers/services or vendors (for blood products for research) to test blood/plasma for SARS-CoV-2 or antibodies. We discuss current theories about how SARS-CoV-2 leads to hyper-coagulant state in severe cases of COVID-19. Our current understanding of the mechanisms behind COVID-19 associated thromboembolic events have centered around three different pathways: (1) direct activation of platelets, enhancing coagulation; (2) direct infection and indirect activation (e.g. cytokine storm) of endothelial cells by SARS-CoV-2, shifting endothelium from an anti-thrombotic to a pro-thrombotic state; and (3) direct activation of complement pathways, promoting thrombin generation. Further investigation on how SARS-CoV-2 affects thrombosis in COVID-19 patients may bring novel anti-thrombotic therapies to combat the disease.

**Keywords**—COVID-19, Thrombosis, Endothelial cells, Platelets, Complement.

### INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global pandemic, with

132 million reported cases and 2.87 million deaths to date (April 7, 2021) worldwide. Although the disease largely manifests in the clinic with severe respiratory symptoms, there is a rapidly expanding body of evidence of cardiovascular complications of the disease. Different surveys have found that pre-existing cardiovascular diseases were associated with the highest case fatality rate, over other comorbidities.<sup>48,75</sup> Elevated D-dimer levels in the blood, which indicates thrombus formation and fibrinolysis, is a predictive measure of poor patient prognosis and death<sup>50</sup>; disseminated intravascular coagulation (DIC) is another.<sup>31</sup> In fact, DIC has been observed in up to 71.4% of non-survivors.<sup>91</sup> Venous thromboemboli (VTE) are common, even among anticoagulated patients.<sup>53</sup> Treatment with anticoagulants, including unfractionated and low molecular weight heparin, is associated with positive patient outcomes. In large-cohort studies,<sup>65,90</sup> treatment with heparin increased survival in severe cases of COVID-19.

The study of blood and plasma from individuals who have/have had COVID-19 is of great research interest for the sake of understanding COVID-19 hypercoagulability alone. Currently, much characterization of the hypercoagulable state of COVID blood/plasma comes from clinical data, with limited *in vitro* studies into the phenomenon available. Additionally, the potential impact of SARS-CoV-2 (and antibodies) on blood/plasma transfusion and hematology research (especially in thrombosis and endothelial dysfunction) is also intriguing to cardiovascular researchers. In this review, we discuss the status of the blood and plasma supply and its implication in basic research; our current understanding of the thromboembolic events that occur in COVID-19; and potential future avenues of study.

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## BLOOD SUPPLY: CURRENT STATUS

In the area of thrombosis research, acquisition of fresh human whole blood and blood products (such as plasma) can come from various sources. These include directly collecting whole blood from healthy donors/volunteers (following Institutional Review Board-approved protocols for research purposes), or purchasing from local blood centers/services or commercial vendors. In all cases, understanding the infection status of the donor is critical. The safety considerations of a global pandemic necessarily impact the supply of blood and plasma in the country. Blood drives, for example, are rendered impossible (when shelter-in-place orders are in effect) or logistically difficult (when social distancing mandates are in effect). As a result, the supply of donated blood and plasma is reduced.<sup>13</sup> Further, the existing blood supply may be considered unsafe for medical and research use, as SARS-CoV-2 RNA has been found in the blood of symptomatic patients.<sup>7</sup> However, as of this writing, the US Centers for Disease Control (CDC) and Food and Drug Administration (FDA) do not require or recommend that blood donation centers test their blood supply for COVID-19. Existing requirements for blood donation are in place to prevent people sick with the disease from donating: donors are required to be in good health, and their temperature taken at the time of donation. The FDA suggests “instructing... individuals not to donate for at least 14 days after complete resolution of symptoms or the date of the positive diagnostic test, whichever period is longer.<sup>74</sup>” Donors are suggested (but not required) to report COVID-19 symptoms or positive COVID-19 diagnoses to the blood establishment, so their blood can be quarantined.

The stated reason why blood establishments aren't required to test blood for SARS-CoV-2 is that “Respiratory viruses, in general, are not known to be transmitted by blood transfusion.<sup>74</sup>” This rationale is generally borne out by the data for COVID-19; to date, there have been no recorded cases of transmission of COVID-19 *via* blood transfusion.<sup>8</sup> Vertical pregnant person-to-fetus transmission of COVID-19 has been observed,<sup>105</sup> but is not universal. Many infants born to COVID-19 positive individuals were negative for the virus.<sup>16</sup> Indeed, known cases of blood transfusion between a COVID-19 positive donor and a COVID-19 negative recipient did not result in subsequent infection of the recipient.<sup>17,47</sup> Additionally, the presence of viral RNA in blood products was not found to correlate to the presence of infectious virus in the blood.<sup>5</sup> In short, testing of blood products is not required because there is little to no evidence/risk of contracting COVID-19 *via* whole blood or plasma transfusion. This means,

however, that it is possible that the blood of individuals who are asymptomatic carriers of COVID-19, individuals who become symptomatic and who do not report it, and convalescent COVID-19 patients are in the general blood supply. For basic thrombosis research, knowing the status (healthy, asymptomatic, and recovered) of the individual may be critical, given the known (but not yet fully understood) thrombotic potential of COVID-19. This is especially critical considering that the time course of these effects is not yet fully understood. In some patients, pro-thrombotic states have persisted for as long as 4 months after discharge from the hospital.<sup>97</sup>

### *Convalescent Plasma*

There has been active recruitment of individuals who have had COVID-19 and have recovered to donate plasma that may be used in the treatment of severe COVID-19 cases.<sup>20</sup> Convalescent plasma (CP) has been used previously to treat viral infections, such as SARS-CoV-1, MERS-CoV, and Ebola.<sup>58</sup> Antibodies present in the plasma of patients who have recovered from these infections may confer acquired passive immunity to those who are currently fighting the infection, or prophylactically for individuals exposed. Though large-scale studies have concluded that COVID-19 CP is generally safe for patient use,<sup>40</sup> proof of its effectiveness in improving outcomes has been equivocal.<sup>39,49,70,80</sup> With the rising use of antiviral drugs such as Remdesivir, the use of CP has fallen out of favor for management of COVID-19, but it is still of use in the study of potential long-term vascular complications of SARS-CoV-2.

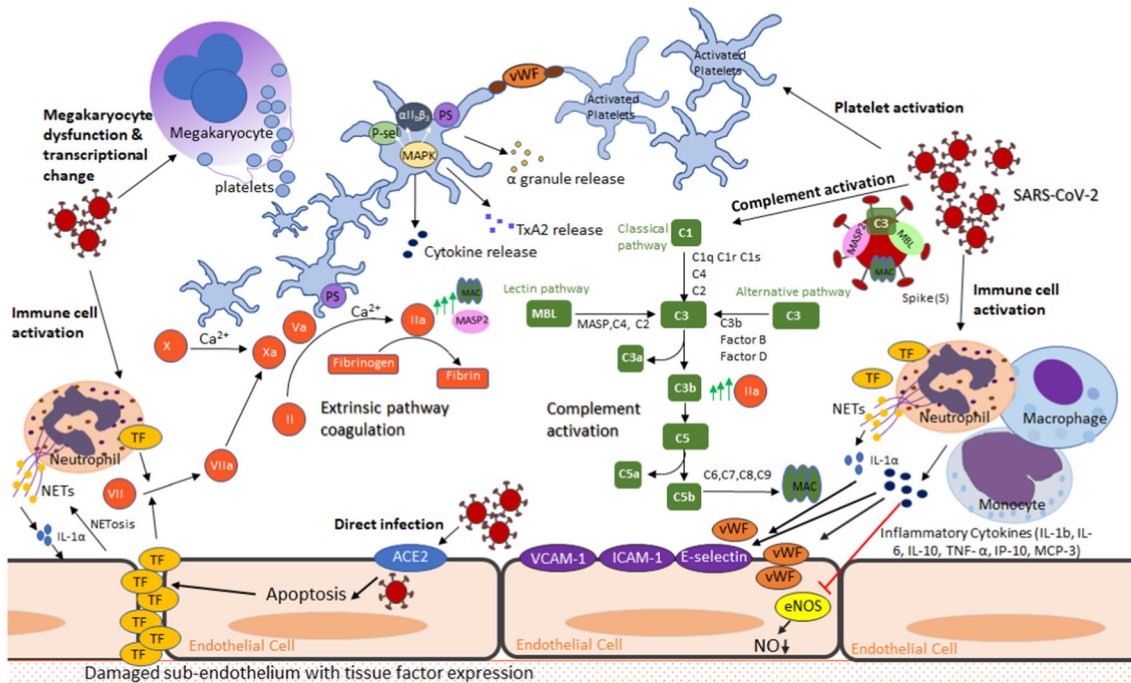
## THROMBOEMBOLIC EVENTS AND COVID-19: POSSIBLE MECHANISMS

### *Platelet Activation*

Platelets interact with both the immune system and the hemostatic system. Early and persistently through the pandemic, it was observed that COVID-19 patients exhibited thrombocytopenia. A meta-analysis study by Lippi, Plebani, and Henry found that severe cases of COVID-19 displayed drastically reduced platelet counts compared to less severe cases.<sup>51</sup> This thrombocytopenia was associated with hypercoagulability. Because of the substantial impact that COVID-19 has on the endothelium, and the well-established platelet responses towards endothelial dysfunction,<sup>83</sup> it was originally assumed that platelets were responding indirectly to endothelial dysfunction and leukocyte activation, rather than being directly impacted by the

virus. Endothelial cell dysfunction and apoptosis (caused by SARS-CoV-2) can lead to increased tissue factor activity and trigger extrinsic pathway coagulation (Fig. 1). Recently, however, evidence has emerged to challenge that assumption. Manne *et al.* found that platelets from COVID-19 patients displayed significant changes in mRNA gene expression, expressed higher levels of surface P-selectin, and were more prone to aggregation and adhesion.<sup>57</sup> Another study by Zaid *et al.* confirmed increased activation of platelets not just in severe COVID-19 cases, but in mild cases as well.<sup>104</sup> They also demonstrated that platelets themselves can release inflammatory cytokines, and that platelets from severe and non-severe COVID-19 patients were more prone to doing so than platelets from healthy donors. Zhang *et al.* provided evidence that this involves the mitogen-activated protein kinase (MAPK) pathway.<sup>106</sup> In platelets, activation of the MAPK pathway has been shown to lead to  $\alpha\text{IIb}\beta_3$  activation, thromboxane A2 and  $\alpha$ -granule release, phosphatidylserine exposure, and increased aggregation<sup>66</sup> (Fig. 1). Hottz *et al.* exposed normal platelets (obtained from healthy donors) to COVID-19 patient's plasma, and demonstrated COVID-19 plasma could directly activate non-COVID-19 platelets.<sup>38</sup> The mechanisms by which platelets can be impacted by the virus are not yet fully understood.

In addition to affecting platelets themselves, SARS-CoV-2 may also impact megakaryocytes, the cells that produce platelets. Recently, there have been a few studies that indicated megakaryocyte dysfunction in COVID-19. Increased pulmonary megakaryocyte presence, which may be associated with diffuse alveolar damage, has been observed in COVID-19 patients at autopsy.<sup>71,93</sup> Additionally, naked megakaryocyte nuclei presence (a sign of increased platelet production<sup>27</sup>) was increased in the bone marrow of a small cohort of COVID-19 patients.<sup>78</sup> Thrombocytopenia has been reported in severe SARS-CoV-2 infections.<sup>51,100,108</sup> As a pathophysiological response, the decreased platelet count should be countered by a feedback system to reflexively increase their number. This is supported by work demonstrating that during thrombocytopenia, the mRNA levels of thrombopoietin (the main stimulator of platelet release from megakaryocytes<sup>43</sup>) increase.<sup>87</sup> However, with severe SARS-CoV-2 infection, megakaryocytes may not be able to counteract the reduced platelet number, as some patients with increased megakaryocyte presence still experience low platelet counts.<sup>26,93</sup> Further, A multi-omics study published in December 2020<sup>9</sup> noted distinct changes in transcription of various genes in the megakaryocytes of COVID-19 positive patients compared to healthy controls, including increases in expression of genes that



**FIGURE 1.** SARS-CoV-2 can cause vascular endothelial cell inflammation (through direct infection and cytokines), and activate platelets, immune cells, coagulation pathways and complement pathways. MAPK mitogen-activated protein kinase pathway, TxA2 thromboxane A2, PS Phosphatidylserine, TF Tissue factor, MAC membrane attack complex, MBL Mannose-binding lectin, MASP2 MBL-associated serine protease 2, ↑↑↑ Indicates increased production or activation.



regulate integrin-mediated platelet adhesion and platelet aggregation.

### *Endothelial Dysfunction*

In severe cases of COVID-19, a hyperactive immune system and a procoagulant state often go hand in hand. Vascular endothelial cells (ECs) are major players in both of these systems,<sup>102</sup> and indeed, there is strong supporting evidence that the cardiovascular complications of COVID-19 are linked with endothelial activation and dysfunction. A healthy endothelium is anti-thrombotic in nature, but when dysfunctional, it can shift to a pro-coagulant state. There are several means by which it is believed SARS-CoV-2 may impact vascular ECs: direct viral damage to the tissue, and indirect inflammatory response.

Coronaviruses in general, and SARS-CoV-2 in particular, infect cells by way of angiotensin converting enzyme 2 (ACE2), a surface-bound protein that is abundant on ECs.<sup>37</sup> Varga *et al.* reported infection of SARS-CoV-2 in the endothelium of multiple organs,<sup>95</sup> although there is some debate about whether the microscopic images in that study had correctly identified viral particles or if the authors had mistaken the rough endoplasmic reticulum for SARS-CoV-2.<sup>33</sup> In a response to Goldsmith *et al.*,<sup>94</sup> Varga *et al.* noted that infection of ECs can occur through direct internalization of SARS-CoV-1,<sup>103</sup> which is similar in structure to SARS-CoV-2. Monteil *et al.* demonstrated direct SARS-CoV-2 infection of human ECs in engineered blood vessel organoids,<sup>62</sup> lending credence to the idea that direct infection may be occurring *in vivo*. Subsequently, Ackerman *et al.* also reported direct infection of the virus into the endothelium of alveolar microvasculature<sup>1</sup>; they also noted extensive microthrombi throughout the vasculature. The infection of ECs induces apoptosis, which can in turn contribute to thrombosis by increased tissue factor activity.<sup>2</sup> This infection may be treated by low molecular weight heparin (LMWH). Some human pathogens utilize heparan sulfate (HS) to help infect host cells.<sup>92</sup> The spike protein on SARS-CoV-2 has been shown to interact with HS and ACE2, and HS can promote SARS-CoV-2 binding to ACE2.<sup>18</sup> It seems promising that cellular infection of SARS-CoV-2 can be inhibited by heparin.<sup>63,89</sup> How SARS-CoV-2 directly causes endothelial dysfunction and apoptosis is summarized in Fig. 1.

Besides direct infection, SARS-CoV-2 can cause endothelial dysfunction indirectly. In a subgroup of about 5% of patients, late-stage COVID-19 resulted in multiple organ dysfunction, which, along with respiratory failure and septic shock, resulted in the deaths of about half of that group.<sup>82</sup> The mechanism largely

attributed to this catastrophic decline is cytokine storm. As illustrated in Fig. 1, when attacking the SARS-CoV-2 virus, immune cells such as macrophages, neutrophils, and monocytes can initiate an uncontrolled release of pro-inflammatory cytokines, which spread throughout the body.<sup>24</sup> The cytokines identified as participators in COVID-19-induced cytokine storm are interleukin (IL)1b, IL-6, IL-10, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon-induced protein (IP)-10, and MCP-3.<sup>98</sup> IL-6 levels, in particular, have been associated with poor prognosis.<sup>61</sup> These can result in an increased expression of intracellular adhesion molecules such as vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1, and E-selectin,<sup>32</sup> as well as releasing von Willebrand Factor (vWF).<sup>35</sup> Pro-inflammatory cytokines also downregulate the expression of endothelial nitric oxide synthase (eNOS),<sup>79,107</sup> reducing the bioavailability of nitric oxide (NO), a potent anticoagulant.<sup>6,69</sup> Currently, it is not clear how long these elevated cytokine levels persist, but a study by Bonny *et al.* found elevated cytokine levels in convalescent plasma (compared to plasma from healthy donors) up to 50 days post-diagnosis.<sup>10</sup>

SARS-CoV-2 can directly and indirectly cause vascular endothelial cell activation, and activated endothelial cells are pro-coagulant. Thus, injured endothelial cells in part mediate SARS-CoV-2-induced hypercoagulability. Further investigation is needed to achieve a better understanding of the associated cellular and molecular mechanisms.

### *Complement Activation*

Activation of the complement cascade has been observed in severe COVID-19 cases. In addition to immune responses, complement activation plays a role in thrombosis,<sup>3</sup> and is believed to be another driving force behind the hypercoagulant state observed in COVID-19 infections.<sup>19,29</sup> Complement is part of the innate immune system that can respond to viruses including SARS-CoV-1<sup>34</sup> that occurred between 2002 and 2003. Complement proteins work in multiple ways to clear pathogens, including opsonization (C3b), release of inflammatory cytokines C3a and C5a,<sup>59</sup> and formation of the membrane attack complex (MAC).<sup>14,72</sup> There is continuing evidence of the interaction between complement and the coagulation systems. MACs can cleave prothrombin (complement activating coagulation),<sup>99</sup> and activated thrombin can cleave C3 (coagulation activating complement).<sup>4</sup> The dysregulation of complement tied to thrombosis has been implicated in severe COVID-19 cases.<sup>15,76</sup>

There is growing evidence that the complement system is activated during severe COVID-19 cases (Fig. 1). It has been shown that there are increased levels of both C5a<sup>22</sup> and soluble C5b-9 (MAC) in the plasma of patients with severe COVID-19<sup>22,84</sup> along with increased neutrophil count and plasma fibrinogen levels.<sup>22</sup> Neutrophil extracellular traps (NETs) are a part of the immune reactions to microbes as they can release chromatin from the neutrophil to combat and capture pathogens.<sup>41</sup> NETs can kill pathogens with myeloperoxidase<sup>11</sup> and trigger extrinsic pathway coagulation *via* exposed tissue factor.<sup>86</sup> Endothelial cells activated by SARS-CoV-2 can trigger NETosis (the process of NETs release).<sup>36</sup> NETs can then further activate endothelial cells through IL-1 $\alpha$ ,<sup>30</sup> worsening the inflammatory reaction and causing further damage. Therefore, it was proposed that NETs have a role to play in SARS-CoV-2-induced inflammation.<sup>101,109</sup> This hypothesis was reinforced by the finding that neutrophils exposed to SARS-CoV-2-positive plasma were able to express activated tissue factor,<sup>84</sup> which can activate the extrinsic coagulation pathway.<sup>55</sup> Antibody-antigen complexes formed at the RBD-domain of the S-protein in the first 2 weeks of the primary immune response can trigger the activation of the classical complement system.<sup>25,77,81</sup> MAC, Mannose-binding-lectin (MBL), MBL-associated serine protease 2 (MASP2), C3 and C4d are among the proteins found colocalized to the SARS-CoV-2 nucleocapsid (N) protein and Spike (S) protein in lung samples of severe cases of COVID-19.<sup>54,56</sup> MASP2 is also known to activate prothrombin to thrombin.<sup>46</sup>

There is also evidence of raised C3a in SARS-CoV-2 positive plasma. In small cohorts of severe COVID-19 patients, C3a inhibition by AMY-101 (Amyndas Pharmaceuticals) can cause decreased IL-6 activity in plasma. Lowering IL-6 can reduce the amount of complement proteins and mannose binding proteins produced by the liver, thus decrease the risk of lung injury and in-hospital mortality.<sup>52</sup> AMY-101 can also inhibit tissue factor expression,<sup>84</sup> which may be beneficial to prevent thrombosis induced by COVID-19. However, simply inhibiting complement protein activation did not seem to have significant clinical outcomes in treating COVID-19. Downstream complement inhibition in human subjects with monoclonal antibody against C5 (eculizumab, Alexion Pharmaceuticals) did not have strong effects.<sup>60</sup> Inhibiting C5a using monoclonal antibody vilobelimab did not improve patient outcomes in a randomized study, although the incidence of pulmonary embolism declined.<sup>96</sup> Increased IL-6 levels during SARS-CoV-2 infection may stimulate the production of platelets. IL-6 has been shown to stimulate platelet production by upregulating thrombopoietin transcription in the liver

which then leads to platelet release from megakaryocytes (thrombocytosis).<sup>42,44</sup> The presence of thrombopoietin can also activate platelets in the presence of thrombin with increased levels of  $\alpha$ -granule secretion and aggregation.<sup>45</sup> Combined, these factors can all contribute to the hypercoagulable state experienced in patients with SARS-CoV-2 infection.

Platelets are a known mediator in complement activation and have roles in both vascular inflammation and thrombus formation. Platelets can activate C3 and C5 to their active forms C3a and C5a.<sup>23,67</sup> With the presence of tissue factor from neutrophils that can activate platelets, these activated platelets can in turn further activate C3 and C5 in a positive feedback loop. Consequently, there is increased D-dimer levels and DIC (disseminated intravascular coagulation) in COVID-19 patients. Although current plasma apheresis protocols do not induce complement activation or activate platelets during collection from healthy subjects,<sup>12,85</sup> recovered donors that are eligible to donate blood may be in a hypercoagulable state due to the lingering presence of residual activated complement proteins and inflammatory mediators.

## FUTURE AVENUES OF STUDY

With the vaccines already developed and administered in many countries, the end of COVID-19's grip on the world seems to be on the horizon. It will not be the last pandemic to emerge, though. In the last two decades, three different coronaviruses have sparked epidemics. By continuing to study the pathogenesis of SARS-CoV-2, we not only discover ways to better treat the disease, but we also prepare ourselves for what is ahead.

The potential platelet pathologies that may contribute to hypercoagulability in COVID-19 patients is an intriguing new avenue of study. Interaction between coronaviruses and platelets have not been well studied. Whether SARS-CoV-2 has the ability to enter platelets through ACE2 (as suggested by Ref. 106) or through some other means requires further exploration. Better understanding of the mechanisms behind platelet activation may open up the possibility of using anti-platelet therapies to combat severe coagulopathy (though this must always be carefully considered, as excessive bleeding is also possible).

Most research into the coagulopathy of COVID-19 is centered around severe cases, and for good reasons. However, there may be risk in asymptomatic and mild cases as well. Delayed thrombotic events have occurred in otherwise asymptomatic or mild cases.<sup>28,88</sup> The study by Zaid *et al.* demonstrated that platelets from even non-serious cases are more procoagulant than

healthy individuals. Expanding the research on coagulopathy to include comparison between serious, non-serious, and asymptomatic cases may help us understand the mechanisms behind these events. Studies on convalescent plasma may shed light on how long coagulopathy exists. There is also the evidence<sup>38,64</sup> that COVID-19 plasma itself can cause hypercoagulability in platelets, which requires validation, but may have implications on the use of such plasma in a research setting. Additionally, the mobilization of certain vaccines has brought new questions about coagulation to the forefront. The vaccine created by AstraZeneca/Oxford University, which has not been approved by the US FDA but has been approved by the European Medicines Agency (EMA) and the UK Medicines and Healthcare Products Regulatory Agency (MHRA), has been pulled by various EU nations due to a small number of patients developing blood clots after receiving the vaccine.<sup>21</sup> Both the EMA<sup>68</sup> and MHRA<sup>73</sup> have identified a possible link between these thrombotic events and the AstraZeneca/Oxford vaccine (while clarifying that the benefits of that vaccine far outweighed the risks). Though there seems to be similarities between these events and heparin-induced thrombocytopenia,<sup>68</sup> more research is required to truly understand the root cause.

### CONFLICT OF INTEREST

There is no conflict of interest for Elisabeth Steadman, Marina Fandaros, and Wei Yin.

### ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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