Antiphospholipid antibodies and COVID-19 thrombotic vasculopathy: one swallow does not make a summer

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The high morbidity and mortality of COVID-19 have been associated with the thrombotic microangiopathy described in the patients in addition to the increased prevalence of thrombosis affecting medium/large arterial and venous vessels.[1 2] Initial reports demonstrating prolonged activated partial thromboplastin times (aPTT) and positivity for antiphospholipid antibody (aPL) assays raised the issue of whether common pathogenic mechanisms were shared by the antiphospholipid antibody syndrome (APS) and COVID-19.[3 4] In particular, the systemic thrombotic microangiopathy and the increased circulating levels of proinflammatory cytokines underlined the similarity between catastrophic APS (CAPS) and COVID-19.[5 6]

The similarities between APS/CAPS and COVID-19 are even more complex and intriguing as summarised in table 1. A proinflammatory environment that includes the activation of the complement system has been reported in all these conditions, although at different degrees. The involvement of several cell types playing a role in the coagulation cascade, such as platelets, monocytes and neutrophils, has been described which is closely associated with the proinflammatory and prothrombotic phenotypes.[7 8] In particular, an endothelial perturbation is generally thought to be a common denominator in these diseases and several authors described it with the term 'endothelitis' in the COVID-19.[9–11]

Both proinflammatory cytokine (eg, interleukin-6) and complement activation products (ie, C5a and C5b9) were thought to play a role in mediating the endothelitis together with a direct effect of SARS-CoV-2 on the endothelium.[10–12] However, the SARS-CoV-2 endothelial tropism is still a matter of debate despite the presence of the entry molecule (ie, ACE2) on the endothelial surfaces.[13] So, it is not surprising that additional potential mediators of endothelial perturbation have been suggested. In particular, aPL came into the limelight because of their well-known ability to bind and activate endothelium in the APS.[14]

aPL can be formally identified by functional PL-dependent coagulation assay (ie, the so-called lupus anticoagulant (LA) test) and by solid phase methods that detect antibodies against beta2 glycoprotein I (β2GPI) (ie, anticardiolipin and anti-β2GPI assays) or prothrombin complexed with phosphatidylserine (ie, aPS/PT). These two last families of autoantibodies are responsible for the large majority of the positive LA.[14] The papers reporting positive aPL tests in patients with COVID-19 are quite heterogeneous regarding frequency and biochemical characteristics of these autoantibodies; in particular, their clinical impact on the disease did not emerge in a recent meta-analysis and systematic review.[15]

Some variables can affect the reproducibility of the functional LA assay, and specific caveats have been underlined by the international scientific societies to avoid misinterpretation. For example, concomitant anticoagulant therapy (eg, heparin) and systemic inflammation with high C-reactive protein plasma levels are well-known factors that can produce LA false-positive results.[16 17] On the other hand, aPL solid phase tests are not affected by anticoagulant therapy or inflammation mediators.

The positivity for LA in the absence of anti-β2GPI and aPS/PT is usually considered of low diagnostic and prognostic value in the setting of APS and systemic autoimmune rheumatic diseases.[18 19] Likewise, the high frequency of isolated positive LA (and prolonged aPTT) in most of the published COVID-19 papers casts doubts on the true presence of thrombophilic aPL in line with the general assumption that the association between aPL and thrombosis is doubtful in most of the COVID-19 series already published.[15]

Nevertheless, SARS-CoV-2 itself can be responsible for aPL production as reported in other viral and non-viral infections.[20] Moreover, the occurrence of concomitant infections in moderate/severe COVID-19 may contribute to aPL production as well. In line with the above-mentioned facts, the paper by Trahtemberg *et al*[21] correctly did not check for LA and raised the issue of the right pathological control group including in the study a series of intensive care unit (ICU) patients without SARS-CoV-2 infection but potentially susceptible to the usual comorbidities occurring in ICU patients. The study did not find any significant difference in the presence of a large panel of aPL between ICU patients with and without COVID-19. Such an approach further supports the conclusion that aPL does not seem to be the main player in the COVID-19 thrombophilic microangiopathy. The authors reported an association between aPL serology and more severe disease that, however, was independent of the COVID-19 status.

Moreover, additional findings are supporting the idea that aPL in COVID-19 may represent bystander rather than pathogenic autoantibodies. In fact, there is evidence that this aPL is transient, usually at medium/low titre and frequently of the IgM isotype only.[15] Moreover, the β2GPI-dependent aPL was not directed against the domain (D)1 immune-dominant epitope of the molecule but frequently against D4,5.[15 22] This profile is diametrically

Table 1 Pathogenic pathways reported in APS, CAPS and COVID-19

Pathogenic paths	APS	CAPS	COVID-19
Thrombotic microangiopathy	+/–	++	++
EC perturbation	+	++	++
Complement activation	+	+	++
NETosis	+/–	?	++
Proinflammatory cytokines	+/–	++	++
Impaired fibrinolysis[29 30]	+	?	++
aPL	++	++	+/–

aPL, antiphospholipid antibody; APS, antiphospholipid antibody syndrome; CAPS, catastrophic APS; EC, endothelial cell.

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opposite to the persistent high-titre IgG against β2GPI D1 historically reported in autoimmune APS. High-titre anti-β2GPI D1 IgG has been closely associated with the vascular manifestations of the syndrome, was found in human tissue samples affected by APS thrombosis and displayed a thrombogenic effect in animal models at variance with aPL directed against other domains of the molecule.[14 23 24] Altogether, these findings are in line with the lack of a sound association between aPL and thrombosis reported in the majority of the studies,[15] and in the paper by Trahtemberg *et al*. In the same paper, the use of a solid phase assay that was suggested as a surrogate tool for LA further ruled out the presence of aPL theoretically responsible for LA and/or prolonged aPTT.[21]

It is important to keep in mind that patients with COVID-19 suffer from an acute form of systemic inflammation with complement activation, both responsible for endothelial perturbation.[8 9 11] In a similar situation, there is evidence that β2GPI can accumulate on the activated endothelium at high density, being much more available to the anti-β2GPI antibodies and ultimately favouring their pathogenic effect.[25] A comparable condition in which low titres of aPL can cause substantial damage was reported in obstetric APS, where high quantities of β2GPI are physiologically expressed in the placenta.[26] Therefore, while transitory low-titre aPL is likely to be clinically irrelevant in patients with COVID-19 as in other infections, their detection in a disease characterised by a strong inflammatory phenotype raises the issue of whether or not these antibodies may increase the ultimate thrombophilic risk and justify a prophylactic treatment. Accordingly, we could speculate that aPL may affect the clinical severity of the inflammatory disease in ICU patients regardless of the COVID-19 status as shown by Trahtemberg *et al*.[21]

While the use of prophylactic or therapeutic heparin therapy is widely accepted during the acute phase of the disease, this is still debated during the recovery period or even in the post-COVID-19 follow-up.[2] Until aPL positive, the patients can theoretically be at higher risk for thrombosis recurrences, and a prophylactic treatment be considered. Unfortunately, we do not have either large follow-up studies evaluating aPL-positive patients with COVID-19 or the best prophylactic regime for such kinds of patients.

If the hypothesis that SARS-CoV-2 is linked with an immune response against PL-binding proteins is true, then the other side of the coin should be represented by the risk of clinical manifestations or the increase in aPL titres in patients suffering from full-blown APS and concomitant SARS-CoV-2 infection. Besides few anecdotical case reports, there is no evidence that this is the case.[27 28]

The use of aPL test in patients with COVID-19 should be taken into consideration in the real life but critically assessed to avoid overinterpretation. For example, as previously discussed, the aPL characterisation in terms of persistence over time, isotype, titre and antigen specificity may help in discriminating between bystander antibodies and pathogenic ones. It is more difficult to draw definite conclusions from a clinical point of view: whether or not the aPL positivity can have a clinical significance to justify a specific treatment in the context of a disease characterised by the production of inflammatory mediators (eg, cytokines, complement activation products) potentially able to downregulate the threshold for endothelial activation.

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