AROTEC News Update:
Antiphospholipid antibodies and COVID-19 May 2021

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The enthusiastic team at AROTEC would like to share its second news update on publications related to COVID-19 infections, this time on the potential impact of antiphospholipid antibodies, against **Beta-2 Glycoprotein 1 (β2GP1)** in particular, as a predictive biomarker in COVID-19 patients. In addition, these articles demonstrate the now well-known connection between viruses and the triggering of autoimmunity.

Recent studies have created a meaningful debate on whether antiphospholipid antibodies are promising biomarkers for the prediction of severe events in critically ill patients with COVID-19 or just bystanders in a molecular mimicry event. A key observation has been thromboembolic incidence giving rise to the scenario of induced Antiphospholipid Syndrome (APS) leading to catastrophic APS.

The initial report on this topic came as early as April 2020 from a hospital in Wuhan, China:

Zhang Y *et al*. (Apr. 2020) Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. N Engl J Med. 2020;382(17) https://doi.org/10.1056/NEJMc2007575

- The authors were the first to report on three critically ill patients with **thrombotic events** and **Antiphospholipid Antibodies**.
- All patients showed Anticardiolipin IgA and **anti-ß-2-Glycoprotein-1 IgA / IgG**.
- From the literature the authors indicate that these antibodies can arise transiently with critical illness and various infections.
- The **presence of these antibodies may rarely lead to thrombotic events** that are difficult to differentiate from other causes of multifocal thrombosis in critically patients.

This report was quickly followed by a case study from the US:

Sung J & Anjum S (Jun. 2020) Coronavirus Disease 2019 (COVID-19) Infection Associated With Antiphospholipid Antibodies and Four-Extremity Deep Vein thrombosis in a Previously Healthy Female. Cureus. 2020;12(6) https://doi.org/10.7759/cureus.8408

- The authors reported on a case of a previously healthy 49-year-old female who was admitted to the hospital for COVID-19 pneumonia. It was known that SARS-CoV-2 has been associated with coagulopathy.
- Doppler later showed this case had extensive deep vein thrombosis (DVT) in all four extremities. This was accompanied by a steep rise in D-dimer levels and positive **antiphospholipid antibodies (APLA)** on further testing. Anti-Cardiolipin antibodies were elevated.
- This was an early case report describing **APLA-associated DVT** in a patient with COVID-19 pneumonia.
- Transient elevation of APLA from the viral illness may play a role in thrombosis associated with COVID-19.
- The authors advised there should be a low threshold to consider thromboembolism and anticoagulation in hospitalized COVID-19 patients with elevated D-dimer levels.

While the former observations were based on very few patients describing the phenomenon, further studies had a closer look at aAPL subgroups, persistency and thrombotic event association:

Vlachoyiannopoulos P *et al.* (Epub Jun. 2020) Autoantibodies related to systemic autoimmune rheumatic diseases in severely ill patients with COVID-19. Ann Rheum Dis 2020;79:1661–1663 https://doi.org/10.1136/annrheumdis-2020-218009

- 29 unselected severely ill COVID-19 patients with positive SARS-CoV-2 PCR were tested for antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), extractable nuclear antigens (ENA), anti-dsDNA, anti-Cardiolipin (IgG/IgM), anti-ß2GPI (IgG/IgM) and anti-cyclic citrullinated peptide (CCP).
- None of the patients had a history of systemic autoimmune rheumatic disease. Overall, **68.7%** of patients were positive for some kind of systemic autoantibody. With **34.5%** each, most prominent were antinuclear and **anti-ß2GPI** antibodies.
- In severely ill patients, innate immune hyperactivity could cause a **cytokine storm leading to autoinflammatory and/ or autoimmune mechanisms**. Interleukin-6, for example, can drive such mechanisms, probably via pre-existing natural B cell clones or molecular mimicry.

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Borghi M. O. *et al.* (Preprint Jun. 2020) Anti-Phospholipid Antibodies in COVID-19 Are Different From Those Detectable in the Anti-Phospholipid Syndrome Front. Immunol. 11:584241 https://doi.org/10.3389/fimmu.2020.584241

- The authors tested 122 sera of patients suffering from severe COVID-19. Of them, 16 displayed major thrombotic events. The objective was to evaluate the prevalence and the clinical association of anti-phospholipid antibodies (aPLs) in a large cohort of COVID-19 patients, and to characterize the epitope specificity of anti-ß2GPI antibodies.
- As heparin influences Lupus coagulant levels, the investigation focused on anticardiolipin (aCL) and anti-beta-2-glycoprotein I (aβ2GPI).
- The authors found aPL show a low prevalence in COVID-19 patients and are not associated with major thrombotic events: aPL was mainly directed against **β2GP1 (15.6%)** but at low titer.
- Further investigation on specific domain reactivity showed the same amount of sera reacting against **D1 and D4-5 of β2GP1**, indicating **non APS** like antibodies.

The article above shows the importance of an intact native conformation and epitope presentation of the ß2GPI antigen - features best ensured by AROTEC's dedicated preparation procedures.

Devreese K. *et al.* (Epub Jul. 2020) Antiphospholipid antibodies in patients with COVID-19: a relevant observation? J Thromb Haemost. 2020;18(9):2191-2201 https://doi.org/10.1111/jth.14994

- This study followed 31 confirmed COVID-19 patients admitted to the Intensive Care Unit.
- Antiphospholipid antibodies (aPL) were measured at one time point, with part of the positive patients retested after one month. The aim was to report all criteria aPL, including LAC (Lupus anticoagulant), aCL (Anti-Cardiolipin) and aβ2GPI (Anti-ß2-glycoprotein I) antibodies, the latter with their isotype and titer.
- While the incidence of aPL in this cohort was high with 74% of patients positive for at least one criterion aPL, the majority of patients showed a low risk profile: 16 single LAC positives, one sample with single aCL, one sample with double positivity, three samples with LAC and aCL positivity.
- The authors concluded, after focusing on current APS criteria which include IgG/IgM anti-phospholipid antibodies only, plus confirmation after re-testing at least three months, that single **Lupus coagulant positivity** is most often **transient** and **not clearly related to thrombotic complications**.

Xiao M et al. (Epub Oct. 2020) Brief Report: Anti-phospholipid antibodies in critically ill patients with Coronavirus Disease 2019 (COVID-19). Arthritis Rheumatol. 2020;72(12):1998-2004 https://doi.org/10.1002/art.41425

- In this study **66 critically ill and 13 non-critically ill patients** with COVID-19 were tested for anti-cardiolipin, anti-β2-glycoprotein 1 (IgG, IgM, and IgA) plus anti-domain 1 IgG by the chemiluminescence assay and anti-phosphatidylserine/prothrombin IgM and IgG by ELISA.
- Antibodies were detected in 47.0% of critically ill patients, but not in patients with non-critical conditions.
- **IgA aβ2GP1** was the most common antibody, present in **28.8%** of patients. A minority of patients (**3%**) showed reactivity against **β2GP1-Domain1.** Antibodies emerge around 35-39 days post-disease onset.
- Dynamic analysis revealed 4 patterns based on **persistence or transient** appearance.
- Patients with multiple antibodies displayed significantly higher incidence **of cerebral infarction**.
- Antibodies may trigger in **genetically predisposed COVID-19 patients development of "COVID-19-induced-APS-like-syndrome"**.

Even adding some functionality into these findings:

Zuo Y et al. (Epub Nov. 2020) Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. Sci Transl Med. 2020;12(570) https://doi.org/10.1126/scitranslmed.abd3876

- In **52%** (or in **30%** using more stringent cutoff) of serum samples from 172 patients hospitalized with COVID-19 showed **Antiphospholipid autoantibodies (aPLs)**.
- Titers of anti-Cardiolipin IgM correlated well with variables like C-reactive protein, D-dimer, Calprotectin as well as Myeloperoxidase–DNA complexes in serum as a marker of neutrophil extracellular traps.
- In functional assays **the authors show a link between IgG fractions of Covid-19 patient sera and neutrophil extracellular trap release plus accelerated in vivo thrombosis** in a mouse model.

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However, in summary the outcome and interpretation of results gives a heterogenous picture of the situation, leading to unifying concepts as proposed on behalf of the APS-COVID-19 Study Group/European Forum on Antiphospholipid Antibodies.

Serrano M. *et al.* (Epub Mar 2021) Beta-2-Glycoprotein-I Deficiency Could Precipitate an Antiphospholipid Syndrome-like Prothrombotic Situation in Patients With Coronavirus Disease 2019. ACR Open Rheumatol. 2021;3(4):267-276 https://doi.org/10.1002/acr2.11245

- In this study 474 adults with severe acute Covid-19 were tested on Antiphospholipid antibodies (aPLs) plus β2GP1 (Beta-2-glycoprotein I) plasma level.
- 21% of patients showed aPL, while **15% with aβ2GP1-IgA** at highest prevalence.
- aPL occurrence was not significantly related to survival, thrombosis or ventilation failure.
- Patients with **significant lower level of β2GP1** were associated with **ventilatory failure**.
- Discussion: Although IgA does not form circulating immune complexes (needed for thrombotic events), **low β2GP1 level reduce apoptotic bodies clearance, increasing their proinflammatory and prothrombotic activity**. Postulation: a synergy between autoantibody neutralisation, higher consumption due to infection plus genetically induced lower production of β2GP1 could be a common pathogenetic mechanism of thrombus formation in COVID-19 and APS.
- The study found no differences were observed in the COVID-19 evolution between aPL-positive and aPL-negative patients. Functional β2GPI deficiency could trigger a clinical process similar to that seen in APS but in the absence of aPLs.

A concise and up-to-date review on the topic of aPL, APS and Covid-19 can be found here:

Tung M. L. *et al.* (Feb 2021) Review: Anti-phospholipid syndrome and COVID-19 thrombosis: connecting the dots. Rheumatol Adv Pract. 2021;5(1) https://doi:10.1093/rap/rkaa081

- This paper is a recent review of APS in COVID-19 patients.
- There is an unusually high prevalence of thromboembolic events in COVID-19 patients, involving both the arterial and the venous circulation.
- Clinical and pathological features of COVID-19 thrombosis resemble APS.
- Acquired APS, via molecular mimicry/neoepitope formation and endothelial dysfunction, could plausibly explain thrombogenesis in COVID-19.
- The pathogenesis of APS remains elusive owing to the marked heterogeneity in clinical manifestations.

Whether you already use Beta-2 Glycoprotein 1 (β2GP1), have plans to conduct dedicated research on APS and COVID-19 or develop an IVD test based on β2GP1 AROTEC will be happy to work with you: we have the product in our portfolio – and AROTEC's ß2GPI has the complete and native conformation consisting of all domains/required epitopes (a crucial consideration for assay designs), thus offering a perfect antigen for development projects - and we have the people and mindset to collaborate with you to make your projects come to fruition. If you'd like to discuss these findings with your AROTEC representative feel free to contact us:

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Product Name/Antigen	Code	Type	Quantity	Source
Beta-2-Glycoprotein 1	ATG01-10	Antigen	1.0 mg	Human Plasma

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