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Acquired Thrombotic Thrombocytopenic Purpura: A Rare Coincidence after COVID-19 mRNA Vaccine?

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There have been reports of de novo or relapse of acquired thrombotic thrombocytopenic purpura (aTTP) after coronavirus disease 2019 (COVID-19) messenger ribonucleic acid (mRNA) vaccinations.[1–6] We therefore present our own experience, and describe three patients with symptoms, or diagnosis, of aTTP within 28 days following mRNA COVID-19 vaccinations. We also attempted to establish causality between administration of mRNA vaccine and aTTP.

Our methods can be summarized as follows: (1) Anti-SARS-CoV-2 QuantiVac ELISA (IgG) (Euroimmun Medizinische Labordiagnostika AG, Germany, from here on referred to as Euroimmun) was employed to detect the concentration of immunoglobulin G (IgG) antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) S1 domain of the spike protein. Additional controls (plasma of a TTP patient pre-COVID [2016] and convalescent plasma from a donor recovered from SARS-CoV-2 infection) were used as additional negative and positive controls, respectively. (2) ADAMTS-13 (a disintegrin-like and metalloproteinase with a thrombospondin type 1 motif, member 13) activity and human autoantibodies (IgG) in serum or plasma against ADAMTS-13 were measured using Tecan Infinite F200 Pro microplate reader for fluorescent resonance energy transfer [FRET]-vWF73, and enzyme-linked immunosorbent assay (ELISA) (Technozym Fluorogenic ELISA kit, Technozym ADAMTS-13 INH ELISA kit). (3) The reagents used to demonstrate cross-reactivity to both ADAMTS-13 and SARS-CoV-2 Spike S1 protein include: Technozym ADAMTS-13 INH ELISA kit, biotinylated recombinant SARS-CoV-2 Spike S1 protein (Val16-Pro681, R&D Systems, Abingdon, UK). Microtiter plates coated with a recombinant form of ADAMTS-13 protease (Technozym) were incubated with the patients' diluted plasma or serum samples. The wells were washed and incubated with biotinylated recombinant SARS-CoV-2 Spike S1 protein at 1,000 ng/mL (R&D Systems), a concentration previously shown to be optimized for binding activity based on manufacturer's recommendation.[7,8] Wells were washed followed by avidin peroxidase (Sigma-Aldrich, Poole, UK) and development with tetramethylbenzidine (TMB) substrate (Sigma-Aldrich). Additional controls employed to confirm assay specificity: (1) *Negative Control 1*: plasma from a previous TTP patient collected in 2016, thus predates SARS-CoV-2 emergence and mRNA COVID-19 vaccines and lacking the necessary anti-Spike S1 antibody. (2) *Negative Control 2*: A rheumatoid arthritis patient's plasma collected in 1995 (pre-SARS and COVID-19). (3) *Negative Control 3*: precoated ELISA well with recombinant form of ADAMTS-13 protease and all reagents except the plasma sample. (4) *Positive control 1*: biotinylated recombinant SARS-CoV-2 Spike S1 protein immobilized onto ELISA wells, with addition of avidin-peroxidase and TMB substrate, to confirm all components were in working order. (5) *Positive control 2*: to confirm the activity of the recombinant SARS-CoV-2 Spike S1 protein in our assay, a direct ELISA was performed using anti-human IgG (Sigma-Aldrich) as capture antigen, then incubated with convalescent plasma from a donor who has recovered from SARS-CoV-2 infection, followed

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by biotinylated recombinant SARS-CoV-2 Spike S1 protein at 1,000 ng/mL as detecting reagent to confirm its binding activity. As a second method to validate our data to demonstrate antibody cross-reactivity, we employed the in vitro *diagnostic* approved Euroimmun SARS-CoV-2 Spike S1 ELISA wells precoated with recombinant SARS-CoV-2 Spike S1 protein as capture antigen to detect any potential IgG cobinding to both SARS-CoV-2 Spike S1 and ADAMTS-13 proteins. Undiluted plasma from patients 1 to 3 (as well as a 2016 patient with known TTP as additional control) were incubated in wells from Euroimmun SARS-CoV-2 Spike S1 ELISA, followed by detection with anti-ADAMTS-13 conjugate (based on Technozym ADAMTS-13 INH ELISA).

Case 1: A 39 years old female presented with symptomatic anemia and fever, 28 days after receiving the first dose of BNT162b2 (Pfizer/BioNTech) mRNA COVID-19 vaccine. Her hemoglobin (Hb) was 67 g/L, platelet 9×10^9/L, and reticulocyte count 4% (0.5–2.3%). There were schistocytes in the peripheral blood film with hemolysis, while her coagulation profiles were normal. A negative anti-platelet factor 4 (PF4) assay by ELISA (Asserachrom HPIA IgG ELISA, Stago, France) excluded vaccine-induced immune thrombotic thrombocytopenia (VITT).[9] Her PLASMIC score was 7, indicating high risk of severe ADAMTS-13 deficiency. aTTP was confirmed with ADAMTS-13 activity 0.06 IU/mL ($\geq$ 0.65) and autoantibodies to ADAMTS-13 > 92 U/mL ($\leq$ 15). A whole-body computed tomography scan showed no evidence of malignancy, but features of pulmonary hypertension. Her antinuclear antibody (ANA) was > 640 titer (normal < 80). She responded well initially to prednisolone and daily plasmapheresis; aspirin was commenced on day 5 at a platelet count of 115×10^9/L with normalization of bilirubin and lactate dehydrogenase (LDH) by day 6. However, on day 7, her platelet count dropped acutely to 11×10^9/L with recurrence of hemolysis. Rituximab was administered. She subsequently developed chest discomfort with type 1 respiratory failure. Acute myocardial infarction, pulmonary embolism, pulmonary hemorrhage, and infections were ruled out but she unfortunately deceased on day 8 after admission.

Case 2: A 49 years old female, with a 16 years' history of systemic lupus erythematosus (SLE) presented with generalized weakness and altered mental state, 9 days after receiving the second dose of mRNA-1273 (Moderna) COVID-19 vaccine. One-month prior to hospitalization, she stopped her regular SLE treatment (prednisolone and mycophenolate mofetil) on her own accord. Her admission Hb was 68 g/L, reticulocyte count 8.4%, and platelet count 19×10^9/L with normal coagulation profile, though hemolysis was evident. Her lupus anticoagulant (LA), anticardiolipin, and anti-double-stranded deoxyribonucleic acid (dsDNA) antibodies were negative and her complement proteins 3 and 4 were normal. Magnetic resonance imaging (MRI) brain with venogram showed no acute cerebral infarct or venous sinus thrombosis. She was initially treated for active SLE with corticosteroids. Subsequently, a peripheral blood film showing schistocytes, and high PLASMIC score of 7, prompted investigations with ADAMTS-13 activity (0.05 IU/mL) and autoantibodies to ADAMTS-13 (16.2 U/mL), and confirmed the diagnosis of aTTP. She responded well to seven daily plasmapheresis and rituximab, with normalization of LDH, Hb, and platelet counts. Her Hb and platelet counts remained normal with tapering dose of prednisolone and mycophenolate mofetil as maintenance treatment of SLE.

Case 3: A 58 years old male was diagnosed with SLE and Sjogren's syndrome and was treated with hydroxychloroquine and prednisolone but was lost to follow-up in 2020. He experienced headaches after both doses of the BNT162b2 mRNA COVID-19 vaccine. Four days prior to admission (10 weeks post second dose of vaccine), the headaches intensified and he became drowsy. On the day of admission, he presented with fever, altered mental state, and left-sided weakness. An MRI brain showed multiple acute to subacute infarcts. His initial Hb was 49 g/L, platelet count 16×10^9/L, and reticulocyte count 6.3%, respectively. Blood film showed schistocytes and there was hemolysis. Creatinine was elevated at 230 µmol/L (60–105 µmol/L) and he developed a myocardial infarction (troponin 2658 ng/L [0–18]). Coagulation profiles were normal and VITT was excluded by negative anti-PF4 assay. His LA and anti-dsDNA were negative, but ANA was > 640 titer (< 80) (speckled pattern), anti-Ro (SSA) 193 RU/mL (0–20), and anti-La (SSB) > 200 RU/mL (0–20). The PLASMIC score was 5, indicating intermediate risk of severe ADAMTS-13 deficiency. aTTP was subsequently confirmed with ADAMTS-13 activity of 0.02 IU/mL and elevated autoantibodies to ADAMTS-13 at 138 U/mL. A diagnosis of SLE flare was made and hydroxychloroquine was commenced along with corticosteroids, daily plasmapheresis, and rituximab for his aTTP. He responded rapidly with resolution of neurological deficits within 48 hours of admission. LDH normalized by day 5, creatinine by day 6, and platelet count reached $> 50 \times 10^9$/L on day 7. He required 22 daily plasmaphereses for his platelet count to recover (153×10^9/L) on day 34.

Of note, positive IgG neutralizing antibodies against the SARS-CoV-2 S1 domain of the spike antigen at 832.7, 1028.3, and 700 binding antibody units (BAU)/mL were demonstrated in patients 1 to 3, respectively (cutoff > 35.2 BAU/mL). ADAMTS-13 inhibitor through IgG activity was also demonstrated in all patients. However, there was no cobinding with recombinant SARS-CoV-2 Spike S1 protein (similar optical density readings as the negative controls and blanks; ►**Table 1**). In the validation experiment, all readings were also negative (►**Table 2**), demonstrating that while these samples have significant levels of IgG antibodies against SARS-CoV-2 Spike S1 protein, no cross-binding was observed to ADAMTS-13 proteins based on a lack of anti-ADAMTS-13 conjugate interaction.

In evaluating severe thrombocytopenia with recent history of mRNA COVID-19 vaccination, aTTP should be considered. Here, we present three patients who developed aTTP after mRNA COVID-19 vaccinations. We hypothesize that vaccine-induced molecular mimicry and cross-reactivity in genetically susceptible hosts may be responsible. All three patients had significant levels of neutralizing IgG antibodies against the SARS-CoV-2 Spike S1 protein after COVID-19 vaccinations. However, no cross-reactivity was demonstrated between their autoantibodies to ADAMTS-13 and the SARS-CoV-2 S1 Spike

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Table 1 Detection of antibodies against the ADAMTS-13 and Spike S1 antigen of SARS-CoV-2

Sample	Plasma/ Serum	Dilution	Optical density
Patient 1 pretreatment[a]	Plasma	1:50	0.026
Patient 1 pretreatment[a]	Plasma	1:100	0.028
Patient 1 posttreatment[b]	Plasma	1:50	0.024
Patient 1 posttreatment[b]	Plasma	1:100	0.021
Negative control 1 (TTP patient 2016)	Plasma	1:50	0.030
Negative control 1 (TTP patient 2016)	Plasma	1:100	0.029
Negative control 2 (RA patient 1995)	Plasma	1:50	0.029
Negative control 3 (reagents only)	–	–	0.027
Positive control	–	–	2.02
Blank	–	–	0.028
Patient 2 pretreatment	Plasma	1:50	0.002
Patient 2 pretreatment	Plasma	1:100	0.007
Patient 2 pretreatment	Serum	1:50	0.001
Patient 3 pretreatment	Plasma	1:50	0.001
Patient 3 pretreatment	Plasma	1:100	0.002
Patient 3 pretreatment	Serum	1:50	–0.003
Negative control 1 (TTP patient 2016)	Plasma	1:50	0.003
Negative control 3 (reagents only)	–	–	0.022
Positive control	–	–	2.096

Abbreviations: ADAMTS-13, a disintegrin-like and metalloproteinase with a thrombospondin type 1 motif, member 13; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TTP, thrombotic thrombocytopenic purpura.
[a]Plasma sample at diagnosis prior to treatment.
[b]Plasma sample posttreatment and prior to demise.

Table 2 Detection of IgG cobinding to both COVID Spike S1 and ADAMTS-13 proteins

Optical density	Sample
2.165	Euroimmun positive control
0.023	Patient 1 pretreatment
0.024	Patient 1 posttreatment
0.037	Patient 2 pretreatment
0.036	Patient 2 pretreatment
0.021	Patient 3
0.032	TTP patient (2016)
0.035	Blank

Abbreviations: ADAMTS-13, a disintegrin-like and metalloproteinase with a thrombospondin type 1 motif, member 13; IgG, immunoglobulin; TTP, thrombotic thrombocytopenic purpura.
Note: All readings except for the Euroimmun positive control were negative (within 2.5 standard deviations of the optical density of the Blank).

protein binding from two different commercial suppliers. The diagnoses of aTTP may be a coincidence, but patient 1 may have had an undiagnosed autoimmune condition based on her high ANA titer which may increase the propensity for developing aTTP (as in patients 2 and 3). It is not known if COVID-19 vaccines can modify the immune system triggering aTTP. We also cannot exclude cross-reactivity with other vaccine components stimulating the production of ADAMTS-13 autoantibodies, resulting in aTTP. Vaccination remains one of the most important armamentariums to save lives from COVID-19. Patients with autoimmune diseases, especially those on immunosuppressants, should be encouraged to be vaccinated as they are at higher risk of developing severe SARS-CoV-2 infections. Finally, it is paramount that treatment for autoimmune conditions is continued, as treatment noncompliance may place those with underlying SLE, at risk of developing aTTP.

Authors' Contributions

A.C.Y.T. acquired the clinical data and wrote the manuscript. B.P.L.L. and C.L.L.S. designed and performed the experiments. X.R. Lim provided the essential reagents. G.C.Y.L., H.S.H., and Y.W.L. collected the patient samples and acquired the clinical data. A.C.Y.T., B.P.L.L., and X.R.L. interpreted and analyzed the data. K.H.O. supervised the study and critically reviewed and revised the manuscript. B.E.F. and D.C. critically reviewed the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

None declared.

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