– *Catholic University of Sacred Heart of Rome, Rome, Italy.*
E-mail: marcello.candelli@policlinicogemelli.it

Keywords: immune thrombocythopenic purpura, SARS-CoV-2, vaccines

First published online 2 May 2021
doi: 10.1111/bjh.17508

References

1. Lee EJ, Cines DB, Gernsheimer T, Kessler C, Michel M, Tarantino MD, et al. Thrombocytopenia following Pfizer and Moderna SARS-CoV-2 vaccination. *Am J Hematol*. 2021;**96**:534–7.
2. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al.; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;**383**:2603–15.
3. Voysey M, Clemens SAC, Madhi S, Weckx LY, Folegatti PM, Aley PK, et al.; Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomized controlled trials in Brazil, South Africa, and the UK. *Lancet*. 2021;**397**:99–111.
4. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al.; COVE Study Group. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;**384**:403–16.
5. Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, et al. Interim results of a phase 1-2a trial of Ad26.COV2.S Covid-19 vaccine. *N Engl J Med*. 2021. Epub ahead of print. https://doi.org/10.1056/NEJMoa2034201
6. Othman M, Labelle A, Mazzetti I, Elbatarny HS, Lillicrap D, et al. Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance. *Blood*. 2007;**109**:2832–9.

Immune responses and therapeutic challenges in paediatric patients with new-onset acute myeloid leukaemia and concomitant COVID-19

Acute myeloid leukaemia (AML) is a medical emergency often presenting with hyperleucocytosis, coagulopathy and pulmonary infiltration necessitating emergent initiation of therapy. AML with concomitant severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection presents a unique challenge given the lack of evidence-based guidelines or historical experience. While cohort studies have shown early serological responses to SARS-CoV-2 in healthy adults,[1,2] little is known about the serological responses to infection in patients with AML and the impact of chemotherapy on this response. In the present study, we detail the clinical presentations, treatments, serological and virological responses, and outcomes of two adolescents who presented with AML and concurrent coronavirus disease 2019 (COVID-19).

Two adolescents presenting with AML and COVID-19 were enrolled on an Institutional Review Board-approved protocol to collect prospective/residual specimens used for SARS-CoV-2 serological and virological testing. Nasopharyngeal (NP) real-time reverse transcription polymerase chain reaction (RT-PCR), antibody testing by enzyme-linked immunosorbent assay (ELISA), live-virus focus reduction neutralisation assay-mNG, surface plasmon resonance (SPR) assay and viral genetic sequencing were performed (Data S1). Patient details, treatment and outcome data were abstracted from medical records.

Patient 1 was a 16-year-old Caucasian male with a history of classical Hodgkin lymphoma who presented with fever, cough and hyperleucocytosis [white blood count (WBC) 176 $\times$ 10^9/l]. His NP SARS-CoV-2 RT-PCR test was positive and peripheral blood flow cytometry (PBFC) confirmed a diagnosis of therapy-related AML. He received hydroxyurea, followed by cytarabine starting on hospital day (HD) 3. Treatment for COVID-19 included hydroxychloroquine, remdesivir and supplemental oxygen (Fig 1).

On HD4, the patient had detectable immunoglobulin (Ig) M and IgG antibodies to SARS-CoV-2 (Table I). The patient did not become lymphopenic throughout his COVID-19 course and maintained detectable binding and neutralising antibodies to SARS-CoV-2. SPR demonstrated that binding antibodies to the pre-fusion conformation of S, the receptor-binding domain (RBD) and S2 subunits all peaked by HD26. The patient had detectable IgM, IgA and IgG1 titres in the final sample on HD70 (Figure S1). He cleared the virus on HD16. Bone marrow (BM) on HD20 showed rare blasts on morphology in the setting of pancytopenia, with no disease detected by flow cytometry. He received additional chemotherapy with azacitidine and gemtuzumab starting on HD26. His treatment complications included bacteraemia and perirectal abscess with *Pseudomonas aeruginosa* and Epstein–Barr virus (EBV) viraemia resulting in multi-organ failure and death on HD74.

Patient 2 was a 19-year-old obese (body mass index of 32 kg/m^2), Hispanic male who presented with fever, cough, dyspnoea and hyperleucocytosis (WBC count 67 × 10^9/l). His NP SARS-CoV-2 RT-PCR test upon admission was positive and PBFC confirmed a diagnosis of AML. He began induction chemotherapy with cytarabine, daunorubicin and etoposide (ADE) on HD2 and he clinically deteriorated on HD4 requiring intubation and mechanical ventilation. For treatment of COVID-19, he received convalescent plasma (CP), remdesivir, tocilizumab and therapeutic plasma exchange (Fig 1).

The patient was lymphopenic by HD5 and showed no immune response to SARS-CoV-2 with absence of IgM antibodies, waning IgG (post CP) and undetectable neutralisation titres (Table I). Concurrently, the patient's NP RT-PCR remained positive with low cycle-threshold (Ct) values. Coinciding with haematological recovery on HD25, the patient demonstrated a serological response with rising IgM, IgG, neutralising antibody titres and SARS-CoV-2 RT-PCR Ct values until his first negative NP RT-PCR result on HD48. His SPR antibody profiling demonstrated class switching (Figure S1) and coincided with robust increases in binding and neutralising titres with haematological recovery. Genetic sequencing and phylogenetic analysis of his SARS-CoV-2 virus from saliva on HD23 indicated that the sequence clustered with clade 20B,[3] which was relatively uncommon in Georgia at the time (Figure S2). A total of 40 intra-host single nucleotide variants (iSNVs) were identified with little variability in S and envelope genes (Figure S3 and Table SI). On HD25 his bone marrow evaluation demonstrated complete remission, but he remained critically ill and on HD48 he suffered a fatal sudden cardiac arrest secondary to sepsis.

In the present report, we highlight both the therapeutic challenges in treating COVID-19 and AML concomitantly and describe the immune response in the setting of myelosuppressive chemotherapy. The viral clearance of the two patients negatively correlated with the intensity of chemotherapy given for each and likely contributed to the overall severity of COVID-19. Paediatric recommendations for the management of AML and COVID-19 are not well defined. Use of a 'mild' induction regimen (MAG) in nine patients with AML and COVID-19 in Brazil was recently described to have excellent outcomes.[4] While reduced-intensity regimens are a valid approach, they preclude response-based risk assignment in AML but warrant further investigation in the setting of concomitant COVID-19.

SARS-CoV-2 evolution within immunocompromised patients with prolonged virus replication has been described.[5,6] Although our investigation was limited to one time point, we did observe within-sample SARS-CoV-2 variants for Patient 2. The functional importance of these variants is unclear; however, future studies into the intra-host variation of SARS-CoV-2 genotypes are needed in the immunocompromised population. Furthermore, Ct values associated with RT-PCR testing for SARS-CoV-2 could provide indirect assessment of viral load.[7] Lower median Ct values in adults with cancer correlated with higher rates of mortality.[8] Patient 2's Ct values remained <25 until HD17 suggesting a high viral load. While PCR Ct values have limitations based on sample collection and instrumentation, serial measurements could be used for clinical correlation.[9]

Limitations of our present study include the small number of patients, which limits generalisability of the data. Co-existing factors such as underlying obesity and race may have contributed to a worse outcome in Patient 2.[10,11] In addition, absence of serological immunity at presentation and administration of CP in Patient 2 confounds interpretation of serological results. Investigation into early immune responses is warranted in this population as they may serve as prognostic indicators at the time of presentation.

In conclusion, our experience demonstrates that patients with AML and COVID-19 can mount immune responses to

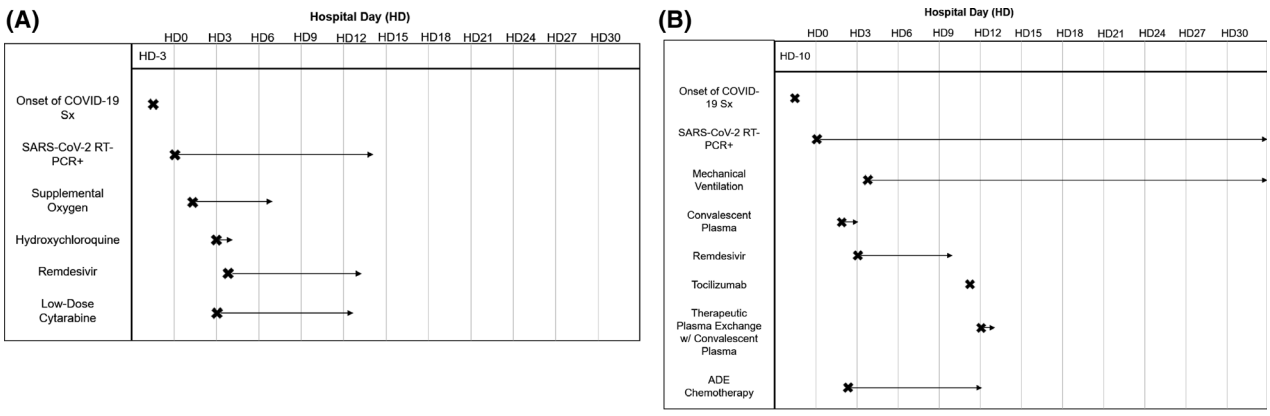

Fig 1. Significant clinical events in first 30 days of hospitalisation for Patient 1 (A) and Patient 2 (B). X, day of first positive sample or first administration; Arrow, duration of positivity or administration; RT-PCR, reverse transcription polymerase chain reaction; Sx, symptoms; ADE, cytarabine, daunorubicin, etoposide.

Table I. Laboratory results for Patient 1 (A) and Patient 2 (B).

Days from COVID-19 symptom onset	Hospital day	Absolute lymphocyte count, /μl	IgM antibody titre	IgG antibody titre	SARS-CoV-2 NeutAb	SARS-CoV-2 RT-PCR
(A)						
3	0	1763*	N/A	N/A	N/A	Positive
7	4	696*	804	1327	236	N/A
19	16	590	720	3436	193	Negative
29	26	880	467	4790	91	Negative
37	34	510	312	5621	85	N/A
45	42	770	179	3781	37	N/A

Days from COVID-19 symptom onset	Hospital day	Absolute lymphocyte count, /μl	IgM antibody titre	IgG antibody titre	SARS-CoV-2 NeutAb	SARS-CoV-2 RT-PCR	RT-PCR S protein Ct value	RT-PCR ORF1ab Ct value
(B)								
10	0	4496†	–	–		Positive	13·1	13·9
14	4	1330†	85‡	619§	10‡	Positive	17·7	18·2
22	12	0	85	487	10	Positive	N/A	N/A
27	17	0	85	200¶	10	Positive	14·6	15·5
35	25	770	3263	1349	559	Positive	29·2	36·9
41	31	1535	33562	26804	2763	Positive**	24·8	24·7
45	35	1506	33326	41520	4379	Positive††	33·4	32·4
58	48	1310	N/A	N/A	N/A	Negative	N/A	N/A

NeutAb, neutralising antibody; RT-PCR, reverse transcription polymerase chain reaction; Ct, cycle threshold; N/A, not available.

*>95% peripheral myeloblasts.

†>85% peripheral myeloblasts 90%.

‡Negative titre.

§Received convalescent plasma on hospital day 2 and 3.

¶Received convalescent plasma on hospital day 12 and 13.

**RT-PCR done on hospital day 33.

††RT-PCR done on hospital day 40.

SARS-CoV-2 even in the face of immune suppression by chemotherapy; however, the intensity of chemotherapy may play a role in the response. Longitudinal research is needed in this vulnerable population to better understand response to SARS-CoV-2 infection and now vaccination.

Acknowledgements

The authors gratefully acknowledge the clinical research coordinators who have contributed to patient enrolment, specimen collection, chart abstraction and data collection for this study including Laila Hussaini MPH, Lisa Macoy MSN, Felicia Glover and Amber Samuel. We thank Nadine Rouphael MD and the Emory Hope Clinic Laboratory for assisting with sample processing. We thank Robert Jerris PhD and the Children's Healthcare of Atlanta (CHOA) Research Laboratory for assistance with collection and processing of residual clinical samples. Lastly, we thank the patients and their families for consenting to donate their samples and share their data to further our understanding of COVID-19 and its impact on paediatric patients with cancer.

Author contributions

Pratik A. Patel designed the research study, performed the research, analysed the data and helped write the paper. Gabrielle Grubbs, Stacey A. Lapp and Venkata V. Edara performed the research and analysed the data. Christina A. Rostad, Evan J. Anderson and Surender Khurana designed the research study, analysed the data and helped write the paper. Claire L. Stokes and Melinda G. Pauly helped write the paper. Anne Piantadosi and Mehul S. Suthar performed the research, analysed the data and helped write the paper. Himalee S. Sabnis analysed the data and helped write the paper. All authors made substantial contributions to research design, or acquisition, analysis or interpretation of data, drafting the paper or revising it critically and approval of the submitted and final versions of the paper.

Conflict of interest

Christina A. Rostad's institution has received funds to conduct clinical research unrelated to this manuscript from BioFire Inc., MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Novavax, Sanofi-Pasteur, Micron, Janssen and Moderna. Christina A. Rostad is co-inventor on patented respiratory syncytial virus (RSV) vaccine technology unrelated to this manuscript, which has been licensed to Meissa Vaccines, Inc. Evan J. Anderson has received personal fees from AbbVie, Pfizer and Sanofi-Pasteur for consulting, and his institution receives funds to conduct clinical research unrelated to this manuscript from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Novavax, Sanofi-Pasteur, Micron, Janssen and Moderna. He also serves on a safety monitoring board for Sanofi-Pasteur and Kentucky BioProcessing, Inc. The other authors have no relevant conflicts of interest to disclose.

Funding

Mehul S. Suthar's work is supported in part by grants [National Institutes of Health (NIH) P51OD011132 and 1U54CA260563-01 to Emory University) from the National Institute of Allergy and Infectious Diseases (NIAID), NIH, by the Emory Executive Vice President for Health Affairs Synergy Fund award, the Pediatric Research Alliance Center for Childhood Infections and Vaccines and Children's Healthcare of Atlanta, COVID-Catalyst-I[3] Funds from the Woodruff Health Sciences Center and Emory School of Medicine, Woodruff Health Sciences Center 2020 COVID-19 CURE Award and the Vital Projects/Proteus funds.

Pratik A. Patel[1,2]
Stacey A. Lapp[3,4]
Gabrielle Grubbs[5]
Venkata V. Edara[3,4,6,7]
Christina A. Rostad[3,4]
Claire L. Stokes[1,2]
Melinda G. Pauly[1,2]
Evan J. Anderson[3,4,8]
Anne Piantadosi[8,9]
Mehul S. Suthar[3,4,6,7]
Surender Khurana[5]
Himalee S. Sabnis[1,2]

[1]Department of Pediatrics, Division of Hematology/Oncology/Bone Marrow Transplantation, Emory University School of Medicine, [2]Aflac Cancer & Blood Disorders Center, Children's Healthcare of Atlanta, [3]Department of Pediatrics, Division of Infectious Diseases, Emory University School of Medicine, [4]Center for Childhood Infections and Vaccines, Children's Healthcare of Atlanta, Atlanta, GA, [5]Division of Viral Products, Center for Biologics Evaluation and Research, FDA, Silver Spring, MD, [6]Emory Vaccine Center, Emory University School of Medicine, [7]Yerkes Primate Center, Emory University School of Medicine, [8]Department of Medicine, Emory University School of Medicine and [9]Department of Pathology, Emory University School of Medicine, Atlanta, GA, USA.
E-mail: hsabnis@emory.edu

Keywords: AML, COVID-19, induction chemotherapy, serology, children

First published online 7 June 2021
doi: 10.1111/bjh.17517

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Antibody kinetics in serum following SARS-CoV-2 infection.

Fig S2. Phylogenetic tree of SARS-CoV-2 sequence for Patient 2.

Fig S3. Scatter plot indicates the frequency of intra-host single nucleotide variants (iSNVs) across the SARS-CoV-2 genome.

Table SI. Intra-host single nucleotide variants (iSNV) of patient 2's SARS-CoV-2 sequence.

Data S1. Supplementary Information.

References

1. Suthar MS, Zimmerman MG, Kauffman RC, Mantus G, Linderman SL, Hudson WH, et al. Rapid generation of neutralizing antibody responses in COVID-19 patients. *Cell Rep Med*. 2020;**1**:100040.
2. Wang Y, Zhang LU, Sang L, Ye F, Ruan S, Zhong B, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. *J Clin Invest*. 2020;**130**:5235–44.
3. Nextclade [Internet]. Available from: https://clades.nextstrain.org. Accessed 13 January 2021
4. Lins MM, Costa JT, Wanderley AV, Seber A, Rocha C, Silva LN, et al. Successful outcomes of children simultaneously diagnosed with acute myeloid leukemia and Covid-19: the role of a mild chemotherapeutic induction regimen. *Blood*. 2020;**136**(Suppl 1):3–4.
5. Baang JH, Smith C, Mirabelli C, Valesano AL, Manthei DM, Bachman MA, et al. Prolonged severe acute respiratory syndrome coronavirus 2 replication in an immunocompromised patient. *J Infect Dis*. 2021;**223**:23–7.
6. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. *N Engl J Med*. 2020;**383**:2291–3.
7. Rao SN, Manissero D, Steele VR, Pareja J. A Narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. *Infect Dis Ther*. 2020;**9**:573–86.
8. Westblade LF, Brar G, Pinheiro LC, Paidoussis D, Rajan M, Martin P, et al. SARS-CoV-2 viral load predicts mortality in patients with and without cancer who are hospitalized with COVID-19. *Cancer Cell*. 2020;**38**:661–671.e2.
9. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis*. 2020;**39**:1059–61.
10. Hendren NS, de Lemos JA, Ayers C, Das SR, Rao A, Carter S, et al. Association of body mass index and age with morbidity and mortality in patients hospitalized with COVID-19. *Circulation*. 2021;**143**:135–44.
11. Poulson M, Neufeld M, Geary A, Kenzik K, Sanchez SE, Dechert T, et al. Intersectional disparities among hispanic groups in COVID-19 outcomes. *J Immigr Minor Health*. 2021;**23**:4–10.

AZD1222 vaccine-related coagulopathy and thrombocytopenia without thrombosis in a young female

The rollout of a vaccination programme against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been one of the biggest steps towards controlling the global COVID-19 pandemic, with four new vaccines currently authorised for use by the European Medicines Agency (EMA). Since February 2021, a preliminary report of cases of unusual thrombosis in the setting of concomitant thrombocytopenia following vaccination has emerged.[1] The authors describe nine patients in Germany and Austria who presented with clinical and serological features which mimic heparin-induced thrombocytopenia (HIT) and the name vaccine-induced prothrombotic immune thrombocytopenia (VIPIT) was initially proposed.[1] The term vaccine induced immune thrombotic thrombocytopenia (VITT) has since been also commonly used, with the condition being referred to as thrombosis with thrombocytopenia syndrome (TTS) by the CDC and FDA. Patients presented with symptomatic thrombosis, primarily cerebral sinus venous thrombosis (CSVT), and were predominantly young females. The EMA's Pharmacovigilance Risk Assessment Committee (PRAC) noted disproportionality for rare events such as disseminated intravascular coagulopathy (DIC), CSVT and haemorrhagic stroke, especially in younger patients aged 18–54.[2] They have since acknowledged a causal association between AZD1222 (COVID-19 Vaccine AstraZeneca) and acquired coagulopathy as of 7 April 2021.

We describe the first reported Irish case of VITT. A 35-year-old Caucasian woman presented 14 days after vaccination with AZD1222. She presented with a medical history of migraine, with no known risk factors for thrombosis.

Following vaccination, she described general myalgia and extreme fatigue, and noticed the onset of bruising and petechiae 10 days later. She attended the Emergency Department 14 days following vaccination. Her petechiae had resolved but she had persistent bruising. She described a headache which was slightly different to her known migraine headaches. She had no recent exposure to heparin.

Laboratory investigation revealed thrombocytopenia with a platelet count of 50×10^9/l, with a normal haemoglobin and white cell count. A coagulation screen showed a mildly prolonged PT and APTT at 12·7 s (normal: 9·6–11·8 s) and 31·3 s (normal 20·8–30·8 s), respectively. Fibrinogen was reduced at 1·16 g/l (normal 1·5–4 g/l) and the D-dimer was markedly elevated at 9·83 μg/ml (normal < 0·42 μg/ml). Examination of the peripheral blood smear showed no red cell fragmentation, no blasts and some atypical lymphocytes. A MR cerebral venogram was carried out which excluded underlying CSVT. Marrow aspirate revealed some reactive