BRIEF REPORT

Acquired Coagulopathy With Immune Checkpoint Inhibitors: An Underrecognized Association Between Inflammation and Coagulation

Jacinth J. Joseph, MD,[a,b] Arun Rajan, MD,[c,*] James L. Gulley, MD, PhD,[d] Sawa Ito, MD,[e,f] Craig M. Kessler, MD[g]

[a]Cellular Therapy Branch, National Heart, Lung, and Blood Institutes, Bethesda, Maryland
[b]Department of Hematology/Oncology, Medstar Washington Hospital Center, Georgetown University, Washington, District of Columbia
[c]Thoracic and Gastrointestinal Malignancies Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
[d]Genitourinary Malignancies Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
[e]Hematology and Oncology, University of Pittsburgh, Pittsburgh, Pennsylvania
[f]Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland
[g]Division of Hematology/Oncology, Lombardi Comprehensive Cancer Center, MedStar Georgetown University Hospital, Washington, District of Columbia

Received 3 March 2020; revised 22 April 2020; accepted 24 April 2020
Available online - 04 May 2020

ABSTRACT

Introduction: Immune-related adverse events affecting virtually every organ system have been described in individuals receiving immune checkpoint inhibitors. The spectrum of hematologic adverse effects is diverse and includes autoimmune cytopenias, hemolysis, or inhibition of coagulation factors. The interplay of inflammation and the coagulation cascade is complex, and immune checkpoint inhibitors can induce coagulopathy by disrupting the intricate link between these pathways.

Methods: We report acquired coagulopathy in two patients treated with the programmed death-ligand 1 antibodies, atezolizumab and avelumab, respectively. Clinical findings and results of extensive laboratory workup are reported. We hypothesize that cytokine release is a potential pathologic mechanism responsible for acquired coagulopathy.

Results: Symptoms included fever, fatigue, and disorientation in one patient and fever, myalgias, and skin rash in the other. Laboratory features included an abnormal coagulation profile; low fibrinogen levels; and elevated D-dimer, ferritin, and triglycerides. Treatment consisted of intravenous glucocorticoids in both cases and the use of fresh frozen plasma, cryoprecipitate, and clotting factor support in one patient.

Conclusions: Recognition of acquired coagulopathy as a complication of immunotherapy and its aggressive management are crucial to reduce morbidity and mortality associated with this condition.

Keywords: Immune checkpoint inhibitors; Immunotherapy; Acquired coagulopathy; Disseminated intravascular coagulation; Inflammatory coagulopathy

Introduction

With the increasing use of immunotherapy, a wide spectrum of adverse events caused by immune activation has been observed.[1] Hematologic complications are relatively uncommon in patients treated with immune

*Corresponding author.

Disclosure: The authors declare no conflict of interest.

Address for correspondence: Arun Rajan, MD, Thoracic and Gastrointestinal Malignancies Branch, National Cancer Institute, National Institutes of Health, 10 Center Drive, 10-CRC, Room 4-5330, Bethesda, MD 20892. E-mail: rajana@mail.nih.gov

Cite this article as: Joseph JJ, et al. Acquired Coagulopathy With Immune Checkpoint Inhibitors: An Underrecognized Association Between Inflammation and Coagulation. JTO Clin Res Rep 1:100049

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2020.100049

Table 1. Case 1: Key Laboratory Results Before and After Treatment With Atezolizumab

Laboratory Test (Normal Values)	Baseline	Day 11 (ER Visit)	Day 13 (Day of Admission)	Day 19 (After 3 Days of Pulse Steroids)	Day 23 (Discharge)	Day 29 (1-Week Follow-Up)	Day 50
Platelet count ($\times 10^9$/liter) (145-400)	282	155	9	53	103	188	297
Hemoglobin (g/dL) (11-14.5)	9.3	9.6	7.6	7.1	8	10	9.3
LDH (U/liter) (84-246)	–	–	514	417	324	353[a]	–
Haptoglobin (mg/dL) (31-200)	–	–	<8	<8	76	137[a]	–
PT (s) (11.8-14.6)	13.4	–	17.3	16.4	15.6	9.9	10
aPTT (s) (23.4-36.2)	29.8	–	39.2	27.3	27	22	26
Fibrinogen (mg/dL) (222-475)	–	–	215 167[b]	136	159	327	500
Ferritin (ng/mL) (5-148)	–	–	650[b]	426[c]	277	208	83
Absolute reticulocyte count (million/μL) (0.020-0.100)	–	–	0.067	0.090	0.273	–	–
Sodium (mmol/liter) (137-145)	135	126	128	139	140	130	136

[a]Day 25
[b]Day 17
[c]Day 20
aPTT, activated partial thromboplastin time; ER, emergency room; LDH, lactate dehydrogenase; PT, prothrombin time;

checkpoint inhibitors (ICIs).[2] The association between ICIs and coagulopathy is poorly understood. In a retrospective series of 83 patients with NSCLC receiving ICIs, thrombosis, hemorrhage, or a combination of the two complications developed in 10 patients.[3]

Here, we describe two patients with life-threatening coagulation disorders related to treatment with programmed death-ligand 1 (PD-L1) inhibitors and offer a possible explanation for the development of unmodulated coagulopathy owing to treatment-induced inflammation.

Case 1

A 77-year-old woman with recurrent NSCLC, hypertension, dyslipidemia, and coronary artery disease provided informed consent for treatment with atezolizumab 1200 mg intravenously (IV) once every 3 weeks. She was not using other pertinent medications concomitantly. Previous anticancer therapy consisting of weekly carboplatin and concurrent radiation therapy was completed 6 months earlier. Two days after receiving the first dose of atezolizumab, she started having fatigue, vertigo, confusion, nausea, and decreased oral intake. She was treated in the emergency room for dehydration and hyponatremia 11 days after treatment. Her platelet count had decreased to 155×10^9/liter from 282×10^9/liter before treatment.

A total of 13 days after receiving atezolizumab, she was disoriented, noncommunicative, and febrile. She was hospitalized and received antibiotics for suspected sepsis. Results of laboratory workup with reference ranges are illustrated in Table 1. Prolongation of prothrombin time and activated partial thromboplastin time along with low fibrinogen (215 mg/dL; normal range, 222–475 mg/dL) and elevated D-dimer (1.84 μg/mL; normal, $\leq$ 0.77 μg/mL) raised concerns of acquired coagulopathy. Furthermore, the platelet count was 9×10^9/liter, absolute reticulocyte count was 0.067 million/μL (normal range, 0.020–0.100 million/μL), and C-reactive protein, ferritin, and triglyceride levels were high (Table 1). On the basis of these results, a cytokine release syndrome (CRS) producing concurrent cytopenias and coagulopathy was considered.

Bone marrow biopsy revealed hemophagocytic histiocytes within a hypercellular marrow (70%). Further testing revealed serum interleukin (IL)-2 of 46 pg/mL (reference, $\leq$12 pg/mL), IL-1β of 0.2 pg/mL (reference, <1 pg/mL), IL-10 of 21.7 pg/mL (reference, <2 pg/mL), and tumor necrosis factor-alfa (TNF-α) of 14.5 pg/mL (reference, $\leq$2.8 pg/mL). The complement panel was normal, confirming the involvement of a non–complement-mediated pathway. A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity was less than 5% with

negative ADAMTS13 inhibitors, indicating a consumptive process other than thrombotic thrombocytopenic purpura. The infectious workup was negative. The hematologic picture was most consistent with disseminated intravascular coagulation (DIC) resulting in microangiopathic hemolysis. Brain magnetic resonance imaging revealed several scattered foci of acute ischemia in both cerebral hemispheres and posterior fossa, likely embolic in nature, also consistent with DIC. Evaluation for an embolic source was unrevealing. Workup for hyponatremia confirmed syndrome of inappropriate antidiuretic hormone. Creatinine remained at baseline throughout the illness. She had mild transaminitis; alanine aminotransferase and aspartate aminotransferase peaked at 76 IU/liter and 31 IU/liter, respectively, on day 14. Thyroid function was assessed during admission and found to be within normal limits. Adrenal function was not assessed at baseline.

The patient initially received 50 mg hydrocortisone IV once at admission on day 13 and dexamethasone 40 mg by mouth daily before being switched to methylprednisolone (on day 16, with suspicion for CRS) 1 gram IV daily for 4 days, resulting in an improvement of her platelet count. Methylprednisolone was tapered to 125 mg IV daily for 3 days before switching to prednisone 60 mg by mouth once daily followed by a gradual taper over 2 months. On discharge, her platelet count was 103×10^9/liter, fibrinogen 159 mg/dL, ferritin 277 ng/mL, and haptoglobin levels had normalized. Atezolizumab was permanently discontinued and 4 months later the patient elected for hospice care. The evolution of key laboratory parameters after treatment is illustrated in Table 1.

Case 2

A 42-year-old woman with recurrent Masaoka stage IVA, WHO subtype B2 thymoma provided informed consent for participation in a clinical trial (NCT03076554) of the anti–PD-L1 antibody, avelumab, and was treated at a dose of 10 mg/kg IV. Comorbidities included iron deficiency anemia, mild hyponatremia of uncertain cause and duration, and gluten and lactose intolerance. The patient was not using any medications on study entry. Seven days after receiving the first dose of avelumab, she began manifesting myalgias, weakness of bilateral lower extremities, erythematous maculopapular skin eruptions over upper extremities, conjunctivitis/blepharitis, and fever. Transaminitis was noted with alanine aminotransferase of 81 IU/liter and aspartate aminotransferase of 270 IU/liter on day 10. She was started on methylprednisolone 1 mg/kg/day IV on day 13 for the treatment of immune-related hepatitis. Hyponatremia steadily worsened after the start of treatment and further workup confirmed a diagnosis of syndrome of inappropriate antidiuretic hormone.

On day 13 postavelumab, hemoglobin was 7.6 g/dL (10 g/dL at baseline) and coagulation parameters included activated partial thromboplastin time of 53.6 seconds and prothrombin time of 15.7 seconds, both of which were corrected on mixed studies. Thrombin time was 21.7 seconds (normal, 13.2–20.5 s). Fibrinogen was low (164 mg/dL) and D-dimer was increased (7.11 μg/mL) on day 12; triglyceride level was 312 mg/dL on day 14. The direct antiglobulin test was positive when tested with anti-IgG and negative when tested with anti-complement antisera, consistent with the presence of autoantibodies against red cells. Assay of coagulation factors revealed factor II activity of 48%, factor IX 81%, factor X 65%, and factor VIII 99%. Absolute reticulocyte count was 0.018 K/μL (normal, 16.4–77.6 K/μL). Liver biopsy performed on day 16 (per protocol) for evaluation of treatment-related liver injury revealed no evidence of hemophagocytosis. Ferritin level peaked at 28,055 ng/mL on day 14. This marked elevation, in the absence of fulminant liver failure, raised suspicion for cytokine release with associated consumptive coagulopathy, consistent with DIC.

Liver biopsy was complicated by persistent bleeding, despite efforts to keep the fibrinogen level over 150 mg/dL with transfusion of fresh frozen plasma and cryoprecipitate. The patient received activated prothrombin concentrate, activated recombinant factor VII concentrate, and tranexamic acid and achieved adequate hemostasis. Further testing on posttreatment day 20 revealed elevated von Willebrand factor (VWF) antigen of 240 IU/dL with VWF activity greater than 240 IU/dL. The activity level was 43% for factor II, 88% for factor VII, 95% for factor IX, 60% for factor X, 75% for factor XI, and 92% for factor VIII. Factor XIII activity was normal. Plasminogen activator inhibitor type 1 was elevated at 98 IU/mL (normal, 3–86), pointing to an inflammatory state. Thromboelastogram was normal. She received her last factor support on day 24, after which fibrinogen remained above 150 mg/dL. There was no compromise of kidney function throughout her admission. On follow-up after 1 week, all parameters continued to improve. Treatment with avelumab was resumed uneventfully after 6 months. Key laboratory results are summarized in Table 2.

Discussion

The complex interactions between inflammatory and coagulation pathways are well described.[4,5] Inflammation is associated with reactive elevation of fibrinogen and C-reactive protein, which enhances plasminogen activator inhibitor-1 and promotes tissue factor

Table 2. Case 2: Selected Laboratory Results Before and After Administration of Avelumab

Laboratory Test (normal values)	Baseline	Day 7	Day 13	Day 16 (Liver Biopsy)	Day 24	Day 36 (Discharge)	Day 48
Platelet count (million/mm^3) (173-369)	256	221	134	157	174	98	254
Hemoglobin (g/dL) (11.3-15.7)	10	9	7.6	7.1	8.2	8.9 (transfused)	9.2
LDH (U/liter) (113-226)	–	–	1022	1604	1476	641[a]	229
Haptoglobin (mg/dL) (30-200)	–	–	153	–	–	<10[a]	86
PT (s) (11.6-15.2)	15.6	16.3	15.7	14.7	16.1	14.6	12.7
aPTT (s) (5.3-37.3)	35.6	40.1	53.6	45.7	33.6	29.8	28.2
Fibrinogen (mg/dL) (177-466)	–	–	164[b]	112	104	201	–
Factor II activity % (61-135)	–	–	48	43[c]	–	–	–
Ferritin (μg/liter) (13-150)	58	–	23683	17428	–	3643[d]	–
Absolute reticulocyte count (K/μL) (16.4-77.6)	–	–	0.018	–	–	0.029	–
Sodium mEq/liter (136-145)	128	125	125	127	138	132	133
ALT/AST IU/liter (0-33)/(0-32)	21/34	25/52	142/461	212/657	122/243	75/72	53/35

[a]Day 25
[b]Day 12
[c]Day 20
[d]Day 30
ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; PT, prothrombin time.

formation leading to impaired fibrinolysis and coagulopathy.[6,7] Other inflammatory cytokines also modulate the coagulation process. TNF-α decreases thrombomodulin, which inactivates protein C and effectively produces a procoagulant effect.[8] IL-6 releases the CD40 ligand, which induces the tissue factor, resulting in activation of the coagulation pathway, and also increases inflammatory cytokines.[9] IL-6 also increases the exposure of P-selectin on the platelet surface and inhibits ADAMTS13 activity of converting VWF multimers to smaller, less thrombogenic forms.[10] This perfect storm creates a procoagulant state.

Coagulopathy, in turn, exacerbates inflammation. Factor Xa, thrombin, and the tissue factor–factor VIIa complex can give rise to a proinflammatory state. Thrombin, in particular, can induce IL-6 production in endothelial cells.[11] Tissue–factor VIIa complex induces major histocompatibility antigen-II expression on macrophages.[12] The activated platelets release the CD40 ligand, which further releases inflammatory cytokines. In contrast, activated protein C and antithrombin-III have anti-inflammatory effects on mononuclear cells and granulocytes, separate from their anticoagulant activity. However, the levels of both are decreased in inflammatory states.[5] Endotoxin, IL-1β, and TNF-α down-regulate both thrombomodulin and endothelial cell protein C receptor, thereby decreasing the generation of activated protein C and suppressing its anti-inflammatory properties.[13] This interplay of inflammation and coagulation ultimately causes severe DIC.

ICIs can potentially induce secondary coagulopathy by disrupting this cell-based mechanism of hemostasis (Fig. 1). Acquired coagulopathy has also been described with other immunologic therapies such as CD19-specific chimeric antigen receptor–modified T cells (CAR-T) owing to the development of CRS.[14] Patients with higher degrees of CRS exhibit more severe coagulopathy, thus confirming a strong link between inflammation and coagulopathy.

Among our patients, the presence of CRS in the first patient is evidenced by elevated inflammatory markers, ferritin, and the presence of hemophagocytosis in the bone marrow. In the second patient, the presence of hepatic dysfunction, at the least, contributed to coagulopathy. In addition, ferritin was profoundly increased in the absence of fulminant hepatic failure. This points to marked inflammation and supports the notion that CRS indirectly induced acquired coagulopathy.

There is limited literature on the diagnosis and management of acquired coagulopathy in patients receiving immunotherapy. The imbalance between normal and pathologic hemostasis in the context of ICI therapy likely involves multiple, poorly understood mechanisms. Our experience with cancer-related

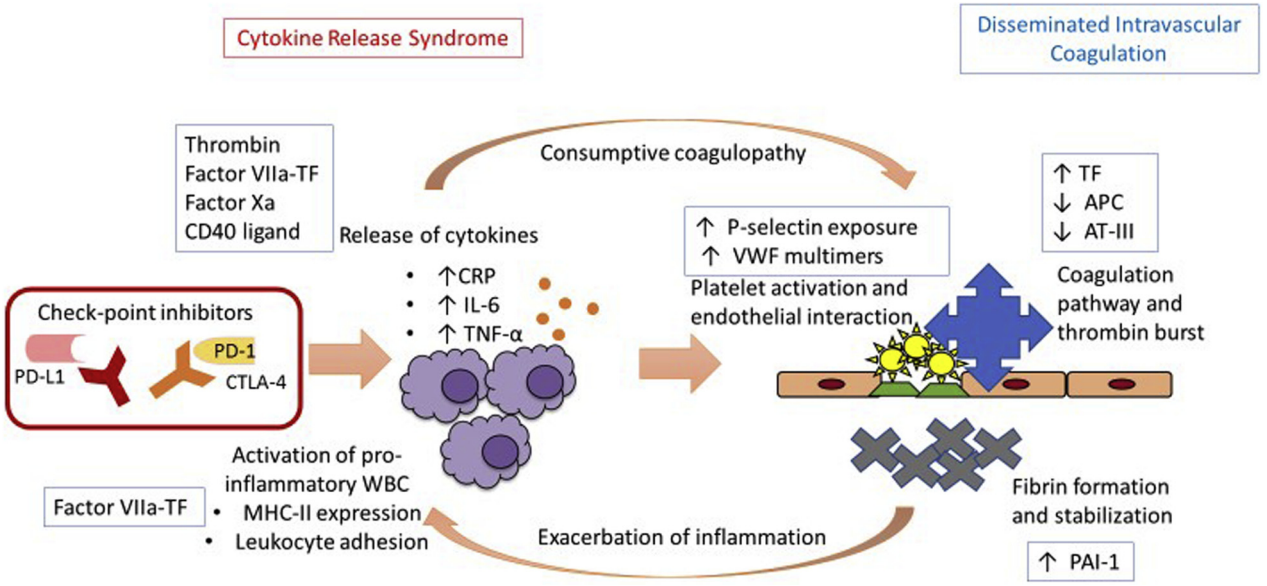

Figure 1. Disruption of cell-based hemostasis by ICIs. ICIs can lead to exacerbated immune response characterized (among other effects) by the following: (1) elevated CRP, which leads to increase in PAI-1; (2) increase in IL-6, leading to increased exposure of P-selectin on the platelet surface and larger VWF multimers; and (3) elevation of TNF-α, which, along with inflammation itself, leads to decrease AT-III and APC. Inflammatory cytokines IL-1, IL-6, and TNF-α lead to increased expression of tissue factor. The resultant coagulation, with an increase in factor Xa, tissue factor VIIa complex, and, notably, thrombin, along with the release of the CD40 ligand from activated platelets, leads to the release of cytokines, which then fuel the vicious cycle. APC, activated protein C; AT-III, antithrombin III, CRP, C-reactive protein; ICIs, immune checkpoint inhibitors; IL-1, interleukin-1; IL-6, interleukin-6; PAI-1, plasminogen activation inhibitor-1; PD-1, programmed cell death-protein 1; PD-L1, programmed death-ligand 1; TF, tumor factor; TNF-α: tumor necrosis factor-alpha; VWF, von Willebrand factor.

coagulopathy suggests that monocyte- or tumor cell–induced production of tissue factors plays a crucial role in creating hemostatic imbalance. Sato et al.[3] have also noted that disordered clinical hemostasis in patients receiving ICI therapy occurs soon after initiation of ICI therapy in patients with high tumor cell PD-L1 expression. Furthermore, tissue factor synthesis was highest in those patients whose peripherally circulating monocytes possessed high PD-L1 expression in vitro. These observations provide crucial clues for the development of diagnostic and therapeutic strategies for the management of ICI-induced coagulopathy and also provide an impetus for performing prospective clinical trials that include coagulation and cytokine testing to develop biomarkers of risk for acquired coagulopathy.

Our patients responded readily to corticosteroids and did not require other immunomodulating agents, such as tocilizumab (humanized monoclonal antibody against the IL-6 receptor). CRS has also been reported with nivolumab, with a clinical picture similar to the first patient, in which the patient responded to tocilizumab.[15] Further research is needed to identify optimal treatment strategies for ICI-induced coagulopathy. Physicians faced with a diagnostic and therapeutic dilemma related to CRS-induced coagulopathy can consider using existing guidelines for CRS, such as the CARTOX treatment algorithms that are widely used for the management of CAR-T cell therapy–associated toxicity.[16]

Conclusions

With the increased use of ICIs, a higher incidence of ICI-mediated coagulopathy is anticipated. Early recognition and treatment of acquired coagulopathy are crucial in mitigating morbidity and mortality in recipients of immunotherapy. Our experience also highlights the need for further research to uncover the pathophysiology of acquired coagulopathy in patients receiving immunotherapy and to identify biomarkers that can be used for the prompt diagnosis and management of immunotherapy-associated coagulopathy.

Acknowledgments

This research was supported in part by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health, and through a Cooperative Research and Development Agreement between the National Cancer Institute and EMD Serono. Drs. Joseph, Ito, and Kessler have designed and conceptualized this work. Drs. Joseph and Rajan have collected and assembled the data. Drs. Joseph, Rajan, Gulley, Ito, and Kessler performed data analysis

and interpretation. Drs. Joseph, Rajan, Gulley, Ito, and Kessler contributed to the manuscript writing and final approval. Informed consent was obtained from patients for publication of this case series.

References

1. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med*. 2018;378:158–168.
2. Delanoy N, Michot JM, Comont T, et al. Haematological immune-related adverse events induced by anti-PD-1 or anti-PD-L1 immunotherapy: a descriptive observational study. *Lancet Haematol*. 2019;6:e48–e57.
3. Sato R, Imamura K, Sakata S, et al. Disorder of coagulation-fibrinolysis system: an emerging toxicity of anti-PD-1/PD-L1 monoclonal antibodies. *J Clin Med*. 2019;8:762.
4. Levi M, Keller TT, van Gorp E, ten Cate H. Infection and inflammation and the coagulation system. *Cardiovasc Res*. 2003;60:26–39.
5. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol*. 2005;131:417–430.
6. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood*. 1993;82:513–520.
7. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation*. 2003;107:398–404.
8. Conway EM, Rosenberg RD. Tumor necrosis factor suppresses transcription of the thrombomodulin gene in endothelial cells. *Mol Cell Biol*. 1988;8:5588–5592.
9. Miller DL, Yaron R, Yellin MJ. CD40L-CD40 interactions regulate endothelial cell surface tissue factor and thrombomodulin expression. *J Leukoc Biol*. 1998;63:373–379.
10. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104:100–106.
11. Johnson K, Choi Y, DeGroot E, Samuels I, Creasey A, Aarden L. Potential mechanisms for a proinflammatory vascular cytokine response to coagulation activation. *J Immunol*. 1998;160:5130–5135.
12. Cunningham MA, Romas P, Hutchinson P, Holdsworth SR, Tipping PG. Tissue factor and factor VIIa receptor/ligand interactions induce proinflammatory effects in macrophages. *Blood*. 1999;94:3413–3420.
13. Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem*. 1994;269:26486–26491.
14. Hay KA, Hanafi LA, Li D, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood*. 2017;130:2295–2306.
15. Rotz SJ, Leino D, Szabo S, Mangino JL, Turpin BK, Pressey JG. Severe cytokine release syndrome in a patient receiving PD-1-directed therapy. *Pediatr Blood Cancer*. 2017;64:e26642.
16. Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol*. 2018;15:47–62.