TRANSFUSION MEDICINE

Case report

Blood donations affect disease management in a case of warm autoimmune hemolytic anemia

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INTRODUCTION

Warm autoimmune hemolytic anemia (AIHA) is the most prevalent type of AIHA and is characterized by the destruction of red blood cells by warm autoantibodies optimally reactive at 37°C[1]. Current therapeutic interventions for warm AIHA include corticosteroids, rituximab, and surgical splenectomy. Many patients, however, cannot be treated with these therapies and may experience severe side effects[2]. In order to provide effective and definitive therapy for patients with primary warm AIHA, close collaboration between clinicians and laboratories may be needed[3]. Here, we describe how appropriate and timely laboratory practices led to proper therapeutic management, particularly when combined with the prompt recognition of the patient as a regular repeat blood donor.

CASE REPORT

In May 2021, a 23-year-old female presented to the Emergency Department with a sudden onset of myalgia, severe headache and heavy sweating. The patient had joined a hiking group and traveled to the tropical area of Taiwan for 5 days before the development of symptoms (days –12 to –7). Routine clinical chemistry tests revealed anemia (hemoglobin, 6.4 g/dL) with elevated C-reactive protein (5.19 mg/dL) and alanine aminotransferase (61 U/L), despite normal body temperature and other parameters. As the patient had a previous history of dengue fever, dengue Ag/IgM was screened and appeared negative. A polymerase chain reaction nasopharyngeal swab for severe acute respiratory syndrome coronavirus 2 was negative. The patient opted for watchful waiting as the active surveillance program for suspected coronavirus disease. Five days later (day 0), the patient returned to the Emergency Department and reported fatigue, loss of appetite and a brief loss of consciousness. She had pale lips and yellow discoloration of the sclera and skin. Laboratory findings showed total bilirubin 3.94 mg/dL, direct bilirubin 1.21 mg/dL, and lactate dehydrogenase 407 U/L. Notably, the patient was a healthy 6-month regular repeat serologically typed blood donor ($A^+C^+c'^-D^+e^+Jka^+Jkb^-M^+Mia^-$) who had made seven previous donations (**Figure 1**) without adverse reaction in the recipients recorded by the transfusion services. As the patient had no prior transfusion and her hemoglobin value was lower than 6 g/dL, she was given two units (500 mL) of matched, leukocyte-depleted packed red blood cells[4] and was admitted to hospital, after which her hemoglobin increased to 5.6 g/dL with no transfusion complications throughout the procedure. As the patient experienced mild sodium depletion (133 mmol/L), twice daily infusions of normal saline (1,000 mL/day) were prescribed for 3 days.

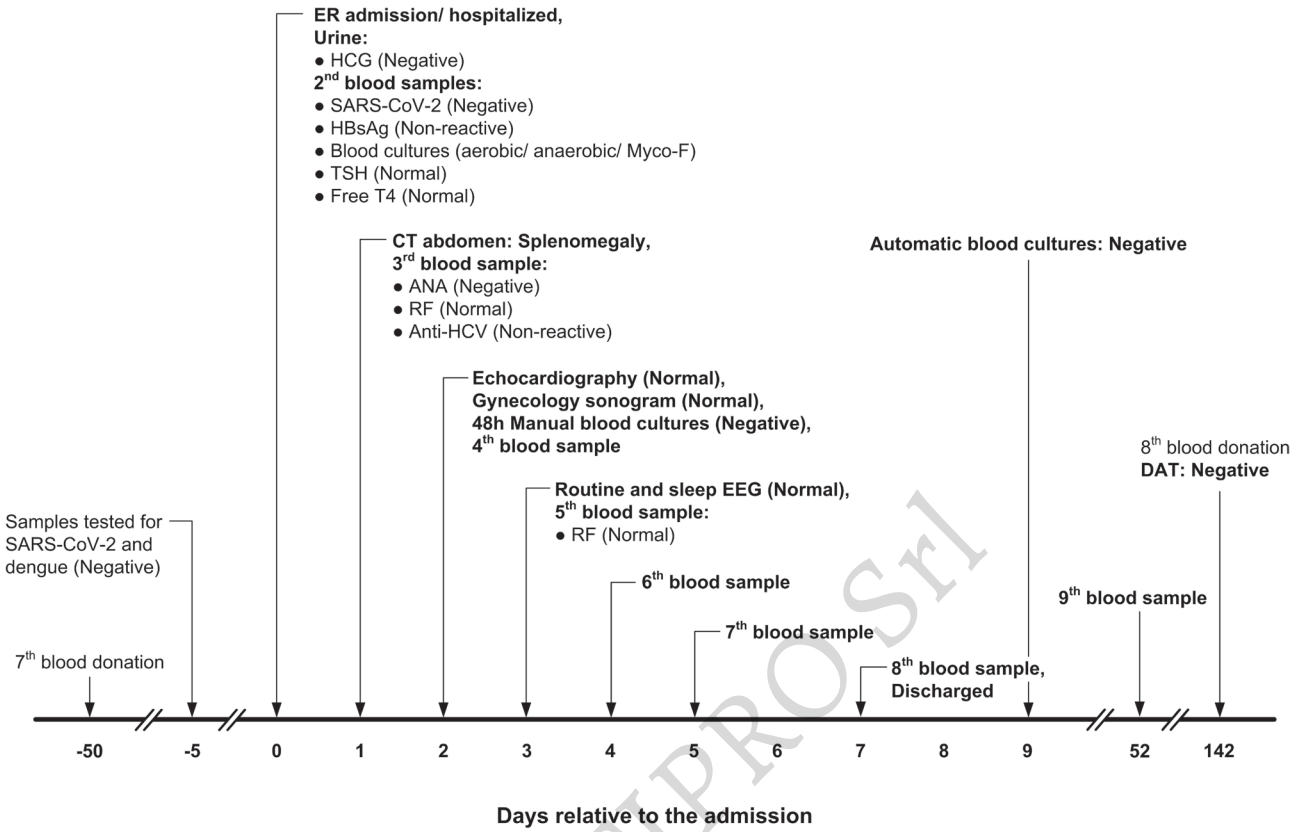

Figure 1 - Study timeline for laboratory results
ANA: anti-nuclear antibody; CT: computed tomography; DAT: direct antiglobulin test; EEG: electrencephalography; ER: emergency room; Free T4: free thyroxine; HCG: human chorionic gonadotropin; HCV: hepatitis C virus; RF: rheumatoid factor; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; TSH: thyroid stimulating hormone. The Institutional Review Board of the National Defense Medical Center/Tri-Service General Hospital approved the study protocol (2-105-05-071), and the subject who participated in this study was treated in compliance with the Helsinki Declaration on research involving human subjects.

Polyspecific direct antiglobulin tests (DAT) were repeatedly strongly positive (4+) and monospecific DAT yielded similar results (IgG:4^+, 3^+ and C3d:1^+); no cold agglutinins were detected. Drug-induced AIHA was excluded because the acid eluate from the patient's red blood cells showed even stronger (4+) reactivities against normal phenotyped red blood cells. Serology and multiplex polymerase chain reaction analysis for human immunodeficiency virus, hepatitis B and C viruses, human T-cell lymphotropic virus and parvovirus B19 was non-reactive. On the patient's second day of admission, her reticulocyte count was 17.8% (normal range: 0.5~1.5%), her haptoglobin level was less than 0.0781 g/L and antinuclear antibody, rheumatoid factor and thyroid function were not reactive or abnormal. The chest radiograph, computed tomography of the abdomen, routine and two-dimensional color Doppler echocardiography were all clear and normal, excluding pregnancy, endocarditis, pulmonary hypertension, and perivalvular leakage. Other investigations for possible infections, such as twice-daily body temperature, blood cultures and urinalysis, were likewise normal or negative. Her hemoglobin level increased gradually from 4.8 to 7.2 g/dL within a week without medication based on the patient's spontaneous improvement during hospitalization and her reluctance to take steroids. She was then discharged with a hemoglobin of 7.2 (day 7) and 10.8 g/dL follow-up (day 52) (**Figure 1**). Residual plasma samples from the date of admission, discharge and follow-up were investigated for the presence of

Table I - *Hematological findings and pro-inflammatory signals at various times relative to the date of admission (day 0)*

Characteristics	Day (-5)	Day 0	Day 5	Day 7	Day 52
Hematological findings					
Hematocrit (%)	18.5	14.1	21.6	21.9	32.2
Hemoglobin (g/dL)	6.4	4.8	6.8	7.2	10.8
White blood cell count (10^9/L)	7.0	15.2	5.7	6.0	5.4
Mean corpuscular volume (fL)	94.9	100	108.5	107.9	97.9
Platelet count (10^9/L)	262	380	258	258	301
Red blood cell count (10^{12}/L)	1.95	1.41	1.99	2.03	3.29
Differential count (%)					
Neutrophils	80.7	88.4	81.0	81.3	76.1
Lymphocytes	8.6	5.3	8.0	10.5	16.5
Monocytes	9.9	5.8	5.0	7.0	5.9
Eosinophils	0.4	0.2	2.0	1.0	1.1
Basophils	0.4	0.3	0.0	0.2	0.4
Pro-inflammatory signals					
Lipopolysaccharide (ng/mL)	N/A	8.5	N/A	1.2	<0.005
Interleukin-1β (pg/mL)	N/A	235.3	N/A	250.7	218.6
Interleukin-17 (pg/mL)	N/A	25.0	N/A	32.0	18.0
TNF-α (pg/mL)	N/A	5.4	N/A	4.9	5.2

N/A: not available; TNF-α: tumor necrosis factor-alpha.

inflammatory mediators, lipopolysaccharide (LPS), interleukin-1β, interleukin-17 and tumor necrosis factor-α. It was found that only plasma LPS (8.5, 1.2 and <0.005 ng/mL) appeared in parallel with the emergence of symptoms (**Table I**). At a follow-up after 142 days, the patient resumed her donor career with normal serum markers of hemolysis and a negative DAT (**Figure 1**).

DISCUSSION

In humans, intense exercise and heat stress have been demonstrated to increase intestinal permeability[5], allowing bacteria or even LPS to enter the systemic circulation and thereby elevate LPS concentrations in the blood. In line with these findings, the plasma LPS concentration of a healthy individual ranges from 0 to 0.2 ng/mL and can be as high as 2 to 10 ng/mL in patients with intestinal permeability disorders or other critical illnesses[6]. In the case presented here, the LPS levels were 8.5 ng/mL on day 0 and 1.2 ng/mL on day 7, displaying a temporal relationship with hemoglobin concentrations (**Table I**). After eliminating possible causes of warm AIHA, together with the presence of heat stress in the high temperature (>30°C)/high humidity (>75%) environment 2 days prior to the first visit to the Emergency Department and given that the pathological responses were analyzed over a specified time course, we hypothesized that heat stress-induced endotoxemia could have been the trigger for this particular case of AIHA.

The current hypothesis contradicts the historical theory that most cases of warm AIHA are secondary to molecular mimicry or dysregulated lymphocyte homeostasis; however, there is presumably great clinical heterogeneity of AIHA, from fully recovered, compensated to rapidly evolving fatal scenarios[7], which may be comparable to the murine models, suggesting that similar pathways might be involved in the activation of autoimmunity. Self-limiting murine AIHA was reported previously[8] in which the expansion and activation of innate B-1 B cells by LPS correlated directly with the onset and severity of AIHA in autoantibody transgenic mice. Subsequent repeated exposure of B-1 cells to red blood cells initiated their apoptotic death, leading to completely cured AIHA[9]. Compelling evidence from animal studies also suggests that the effect of LPS-mediated inflammation can be overcome by exogenous adenine at levels as low

as 0.1 mM[10]. The fact that adenine is normally present in blood components at a concentration around 2 mM, for anticoagulant/ preservative purposes, provides another rationale for transfusion therapies.

Given the value of close collaboration between clinicians and laboratories[3] and the broad spectrum of immunological controversies regarding AIHA, it is worth bearing in mind that a patient's extended phenotype and related history could be obtained from the blood services and that AIHA may be relieved completely even without the use of corticosteroids as the first line of therapy. Early identification of such patients could help to optimize treatment decisions.

AUTHORSHIP CONTRIBUTIONS

C-SL and JYW contributed equally to this manuscript as co-first Authors.

All Authors contributed to writing the manuscript and approved its final version.

The Authors declare no conflicts of interest.

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