SCIEPublish

Case Report

Immunohematological Investigation of Fatal Hemolysis in Long-Duration Autoimmune Hemolytic Anemia with Anti-S and Anti-Wr^a Alloantibodies: A Case Report

Hanyu Ge [1], Qiming Ying [1], Kedi Dong [1], Danni Song [1], Shilin Hu [1] and Dingfeng Lv [1,2,*]

[1] Department of Blood Transfusion, the First Affiliated Hospital of Ningbo University, Ningbo 315099, China; fyygehanyu@nbu.edu.cn (H.G.); 329198934@qq.com (Q.Y.); 17826865361@163.com (K.D.); fysongdanni@nbu.edu.cn (D.S.); 15968110331@163.com (S.H.)

[2] School of Medicine, Ningbo University, Ningbo 315099, China

* Corresponding author. E-mail: fyylvdingfeng@nbu.edu.cn (D.L.)

Received: 28 April 2024; Accepted: 13 June 2024; Available online: 30 June 2024

ABSTRACT: Autoimmune hemolytic anemia (AIHA) is a serious condition characterized by the severe hemolysis caused by increased levels of autoantibodies that specifically attack erythrocytes. Autoantibodies present difficulties in identifying alloantibodies, increasing the likelihood of hemolytic transfusion reactions (HTRs). In this report, we presented a rare and severe case of fatal HTRs caused by alloantibodies known as anti-S and anti-Wr^a in a female patient who had been suffering from hemolytic anemia for four decades. The patient's blood group type showed a significant inconsistency, later verified as AB CcDee by serologic tests. The antibody identification revealed unique patterns of anti-S and anti-Wr^a antibodies, with a noticeable disparity in agglutination intensity rated as 3+. Consequently, her erythrocytes were subjected to a freeze-thaw process, and the eluate showed robust pan-reactivity. The patient's MNS and Diego blood systems were analyzed using genetic sequencing, which confirmed the absence of the S or Wr^a antigen. However, the presence of a weakly positive S antigen in the serological result indicated the possibility of transfusion of leukocyte-poor red blood cells (LPRs) that carry the S antigen. As a result, the patient started receiving LPRs with the same phenotype, while avoiding S and Wr^a antigens. In addition, we evaluated the patient's state and discussed the appropriate blood transfusion protocol for AIHA in this case. Medical practitioners must possess expertise in identifying autoantibodies and alloantibodies in AIHA and devising a suitable blood transfusion plan.

Keywords: Autoimmune hemolytic anemia; Fatal hemolysis; Anti-S antibody; Anti-Wr^a antibody; Genotyping

1. Introduction

Autoimmune hemolytic anemia (AIHA) is a complex disorder in which autoantibodies attack the patient's own red blood cells (RBCs), leading to a reduced lifespan of erythrocytes. Chronic AIHA often necessitates multiple blood transfusions, which can result in the production of numerous alloantibodies and increase the risk of hemolytic transfusion reactions (HTRs). The simultaneous occurrence of both HTRs mediated by anti-S and anti-Wr^a alloantibodies is an uncommon complication of AIHAs. Yu et al. reported a 9.99% frequency of S antigen in Chinese populations [1]. The presence of the anti-S antibody, typically an IgG antibody, carries the potential to induce HTRs and severe hemolytic illness in the fetus and newborn [2,3]. Additionally, the Wr^a antigen has a prevalence of 0.01% in Chinese populations, while the anti-Wr^a antibody is one of the most common naturally occurring antibodies. It is detectable in healthy individuals and exhibits a notably high frequency in AIHA cases [4–6]. Because of the limited availability of commercial panel cells and the high cost of corresponding reagent antibodies, the Wr^a antigen and antibody are infrequently detected, thereby increasing the likelihood of HTRs [7–9]. Here we reported a case in which fatal hemolysis occurred post-transfusion in an AIHA patient with concurrent anti-S and anti-Wr^a alloantibodies.

https://doi.org/10.70322/BG20240110007

2. Case Presentation

A 57-year-old Chinese female with a 40-year history of hemolytic anemia presented with symptoms of cough, fatigue, and abdominal distension that had been occurring for the past 20 days. Upon admission to the local hospital, her hemoglobin level was critically low at 37 g/L (Figure 1). Her total bilirubin was 197.2 μmol/L, with direct bilirubin at 25.6 μmol/L and indirect bilirubin at 171.6 μmol/L. An abdominal plain scan showed substantial enlargement of the spleen (splenomegaly). Due to concerns regarding the effectiveness of the treatment, she was directed to our hospital's emergency department after being administered 2 units of leukocyte-poor red blood cells (LPRs).

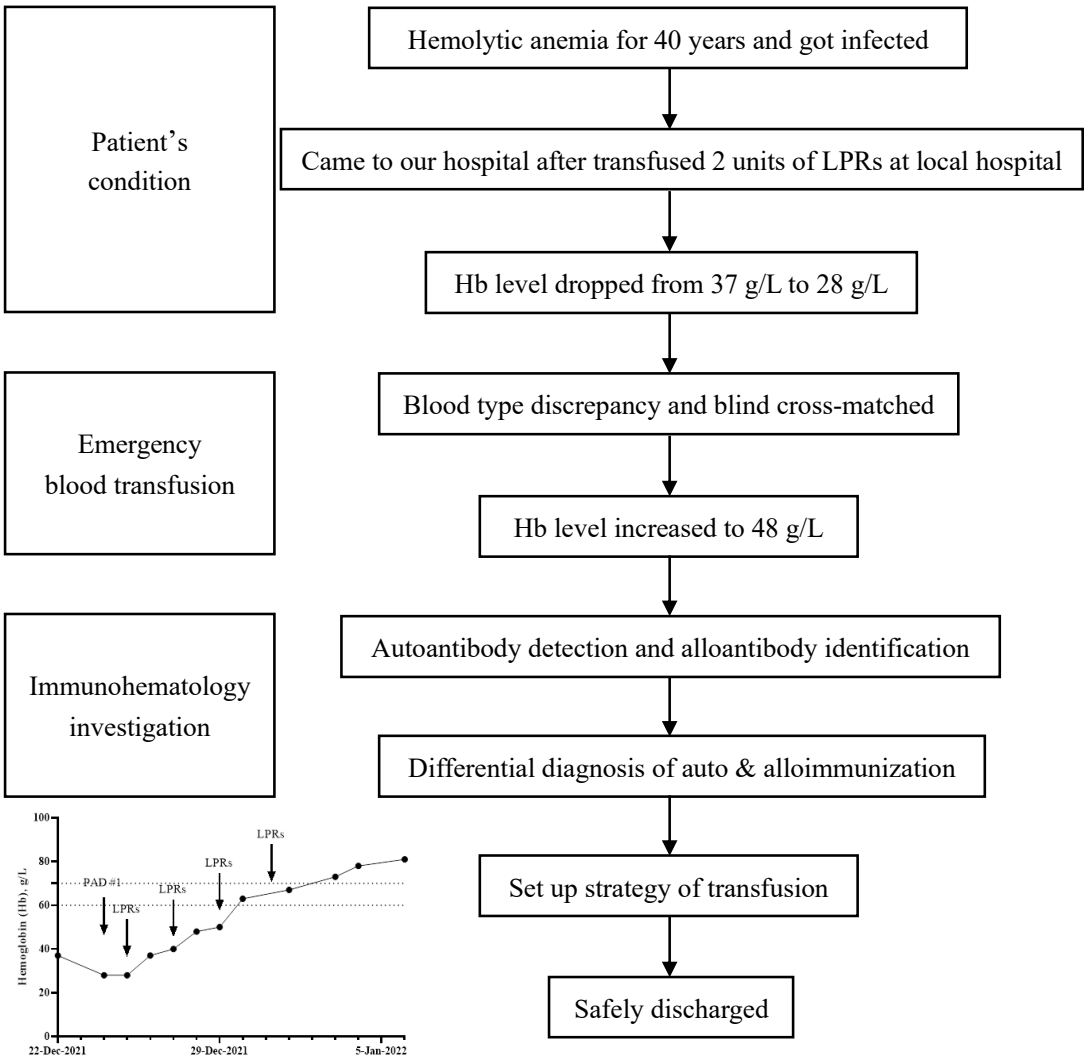

Figure 1. A timeline of the patient's medical history and course of care with the fluctuation of her hemoglobin level. LPRs: leukocyte poor RBCs; Hb: hemoglobin; PAD: post-admission day.

The etiology of her hemolytic anemia was previously uncertain, as her hemoglobin levels continuously ranged from 60 g/L to 70 g/L during outpatient visits without any treatment. She has no history of smoking or illicit substance use. The initial diagnosis for the patient included hemolytic anemia, pulmonary infection, and splenomegaly.

Upon the initial assessment at our hospital, we saw severe hemolysis, as indicated by a hemoglobin level of 28 g/L (Figure 1), and a high reticulocyte count of 14.73%. The total bilirubin level was measured at 169.51 μmol/L, with direct bilirubin accounting for 24.80 μmol/L and indirect bilirubin accounting for 144.71 μmol/L. The direct antiglobulin test showed a significant agglutination at a 3+ level, with positive results for anti-IgG, anti-C3d, and anti-IgG+C3. This indicates that the RBCs have been sensitized. The platelet count, white blood cell count, and C-reactive protein levels were within the normal range.

The patient's RBCs showed strong agglutination (4+) when tested with anti-A and anti-B reagent antibodies during forward typing. In reverse typing, the patient's plasma indicated moderate agglutination (3+) and had two distinct

populations when tested with A1 reagent cells and B reagent cells, respectively (Table 1). Despite obtaining a negative control result, we repeated the blood typing using a different micro-gel reagent card without low ionic strength solution (LISS) at room temperature, which revealed a distinct AB CcDee blood type (Supplementary Figure S1). To address the sensitization of the RBCs, we performed three rounds of warm saline washing at both 37 ℃ and 45 ℃, followed by a 10-min elution process for some of the RBCs. This confirmed the AB CcDee blood type in forward typing. Subsequently, allogeneic adsorption was carried out on a portion of her plasma using reagent O cells to reduce the impact of unexpected antibodies. This process ultimately confirmed the AB blood type in reverse typing (Table 1).

Table 1. Serological results of the patient's blood type under various procedures.

Procedures	Forward Typing			Control	Reverse Typing		
	Anti-A	Anti-B	Anti-D		A_1c	Bc	Oc
LISS	4+	4+	4+	-	$3+^{dp}$	$3+^{dp}$	/
Non-LISS	4+	4+	4+	-	-	-	/
Washed & eluted	4+	4+	/	1+	/	/	/
Oc adsorbed	/	/	/	1+	1+	1+	1+

LISS: the test was incubated at 37 ℃ for 30 min within low ionic strength solution (LISS); Non-LISS: the test was addressed at room temperature without LISS; Washed & eluted: patient's RBCs were washed by 37 ℃ warm saline 3 times and eluted at 45 ℃ for 10 min; Oc adsorbed: patient's plasma was adsorbed by group O panel cells. dp: double population; /: not detected.

Due to the lack of unexpected antibody identification panel cells at that time and considering the patient's critical condition, we opted for blind crossmatch with AB CcDee donor LPRs. Using the patient's self-crossmatch as a control, which yielded a significantly positive result, we administered 2 units of donor LPRs with lower agglutination intensity than the control.

To determine the etiology of the patient's hemolysis, particularly the cause of the fatal anemia post-transfusion, we identified the unexpected antibodies on day 3 post-admission, which possessed the pattern of anti-S and anti-Wr^a antibodies (Figure 2 & Supplementary Figure S2). Significantly, the pattern displayed a clear differentiation with a 3+ agglutination contrast. Therefore, her warm-washed RBCs were subjected to a freeze-thaw process (frozen at −80 ℃ for 30 min and then thawed at 56 ℃ for 10 min). This resulted in an eluate that showed 4+ agglutination reactivity against panel O cells. Subsequently, S and Wr^a antigens were assessed through both serological and genetic analyses. The patient's RBCs were washed and eluted in serological tests at 37 ℃ and 45 ℃. Using the patient's RBCs as the control, testing these two antigens with the corresponding reagent antibodies yielded a weak positive result for the S antigen. Genetic analysis employed a polymerase chain reaction sequence-based typing (PCR-SBT) assay, targeting exon 4 of S (GYPB) gene and exon 16 of Wr^a (DI) gene. There was a cytidine at position 143 of GYPB and a guanine at position 1972 of DI, indicating that her genotype was typical "S" in the MNS system and "Wr^b" in the Diego system (Figure 3). In addition, the weak positive outcome for the S antigen encouraged us to verify the blood units that were first transfused in our hospital. This verification proved that neither the S nor Wr^a antigens were present. Unfortunately, pre-admission samples of the transfused LPRs were unattainable, hindering the confirmation of whether the fatal hemolysis resulted from the local infusion of the S antigen.

Figure 2. The result of the patient's unexpected antibody identification on day 3 post-admission. *: the self-crossmatch contrast, containing the patient's washed and eluted RBCs and her plasma.

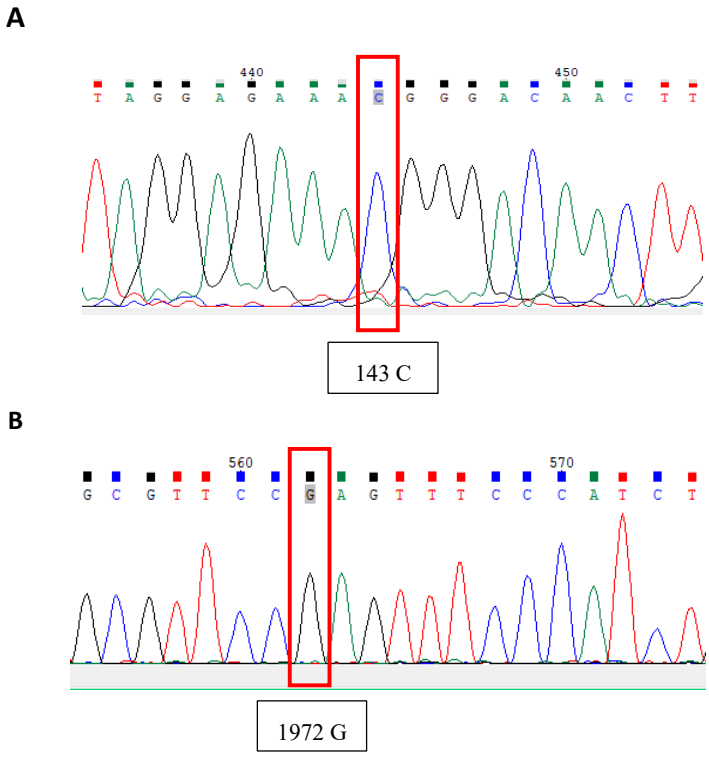

Figure 3. The genetic sequence of the patient's blood type in MNS system and Diego system. (**A**): the sequence of GYPB exon 4 of the patient. (**B**): sequence of DI exon 16 of the patient.

Upon admission, the patient received 2 units of crossmatched AB CcDee LPRs and methylprednisolone, resulting in a significant rise in hemoglobin levels to 37 g/L. Subsequently, an additional 5 units of the same type LPRs, specifically avoiding both S and Wr^a antigens, were transfused to address the patient's hemolytic anemia. Figure 1 illustrates the timeline of hemoglobin level fluctuations. It is worth noting that the assessment of transfusion efficiency was challenged by potential interference from disease progression and therapy.

The autoimmune panel tests showed positive results for antinuclear antibody, anti-mitochondria-M2 antibody, and anti-Ro-52 antibody, indicating an autoimmune reaction from a different perspective. Subsequently, the patient underwent a comprehensive evaluation by the Department of Rheumatology and Immunology. The department proposed a decrease in the dosage of intravenously administered methylprednisolone to 40 mg per day and introduced oral cyclosporine at a dosage of 75 mg twice daily to alleviate the adverse effects of corticosteroids. Financial constraints precluding the use of rituximab led to the consideration of splenectomy as a viable option for significant splenomegaly and potential hypersplenism, until the patient's hemoglobin gradually increased to 81 g/L.

The patient was discharged on day 13 post-admission despite persistent jaundice and without apparent discomfort. Prior to discharge, her hemoglobin levels were maintained within the range of 63 g/L to 81 g/L for a week (Figure 1). On day 30 post-admission, during the third outpatient evaluation, mild symptoms were noted, and her hemoglobin ranged from 59 g/L to 73 g/L. Additionally, the unexpected antibody identification on day 175 post-admission consistently revealed an anti-S and anti-Wr^a antibody pattern (Supplementary Figure S3). It is likely that the patient's autoantibody exhibits a low plasma titer and cannot disrupt the alloantibody pattern.

3. Discussion

The patient's forward typing in this report clearly suggested an AB blood type, whereas the reverse typing showed O type with 3+ agglutination and double population (Table 1). We gave priority to the forward typing procedure, considering the negative control outcome. This required adding RBCs to a saline solution with little plasma. Additionally, the reverse typing was incubated at 37 °C for 30 min as the blood typing card included two out of three unexpected antibody screening tests. We conducted a second blood typing of the patient's blood at room temperature using a different type of typing card that does not contain LISS, a solution that buffers the electric charge of RBCs and promotes better interaction. Hence, we could mitigate the impact of warm antibodies, whether alloantibodies or autoantibodies, on the result of reverse typing. Furthermore, we conducted both forward and reverse typing using a washing elution test and an allogeneic adsorption test, respectively, eventually verifying the blood type as AB CcDee.

As a result, 2 units of donor LPRs with matching blood types were selected for a blind crossmatch because there were no unexpected antibody identification panel cells available.

The unexpected antibody identification addressed in further immunohematology investigation revealed a distinct pattern of anti-S and anti-Wr^a antibodies with a notable 3+ agglutination contrast (Figure 2). Common autoantibodies demonstrated typically pan-reactivity with reagent red cells besides the "mimicking alloantibody" [10]. In this regard, we examined three possible scenarios: (1) The level of autoantibodies may not be high enough to disrupt the pattern of alloantibodies. (2) The autoantibodies may imitate the anti-S and/or anti-Wr^a alloantibodies. (3) The patient may receive LRPs containing S and/or Wr^a antigens. Thus, we initially utilized freeze-thaw release on her washed RBCs to separate the autoantibodies, and the eluate confirmed their presence with strong pan-reactivity. It is reasonable to speculate that both autoantibodies and alloantibodies were present, with the autoantibodies significantly contributing to her anemic condition before the blood transfusion. However, uncertainties remain regarding the production quantity or potential excessive consumption, resulting in a low titer of autoantibodies in the plasma. In addition, we conducted regular serological tests using specific reagent antibodies to examine the presence of S and Wr^a antigens on the patient's RBCs. These tests revealed a slightly positive result for the S antigen. Given the recent LPRs transfusion, we could not definitively ascertain the presence of S or Wr^a antigens on her erythrocytes. Subsequently, DNA sequencing was considered to overcome the limitations and intricacies of serological typing methods and obtain precise results [11–13]. Based on the allelic genes of GYPB and DI genes published by the International Society of Blood Transfusion, we performed sequencing and confirmed the absence of S and Wr^a antigens on her RBCs. Therefore, we considered the possibility of an alloimmunization-mediated HTR in this case due to the infusion of S antigen. The verification of the original LPRs transfused in our hospital revealed the absence of both antigens. Unfortunately, the lack of samples from the LPRs transfused prior to admission hindered confirmation of whether fatal hemolysis resulted from the infusion of S antigen-positive LPRs at local hospital.

AIHA, an autoimmune disorder characterized by autoantibodies against the patient's own RBCs, is susceptible to generating alloantibodies. Due to the possibility of both autoantibodies and alloantibodies intensifying hemolysis by damaging transfused RBCs, blood transfusion should be reserved for essential cases. Moreover, conducting antigen tests on transfused RBCs is crucial to prevent a corresponding alloantibody response. The patient in this atypical scenario, who had long-lasting AIHA and was generating autoantibodies along with simultaneous anti-S and anti-Wr^a alloantibodies, had a fatal HTR following transfusion. It is crucial for medical practitioners to be diligent in diagnosing autoantibodies and alloantibodies in AIHA and to develop a clear plan for blood transfusions.

Supplementary Materials

The following supporting information can be found at: https://doi.org/10.35534/BG20240110007, Figure S1. The results of patient's blood typing by micro-gel reagent cards. A: the typing test was incubated at 37 ℃ for 30 minutes within low ionic strength solution (LISS). B: the unexpected antibody screening tests. C: the typing test was addressed at room temperature without LISS. D: the patient's blood type in Rh system. *: the unexpected antibody screening tests for the patient. Figure S2. The phenotype listing sheet corresponding to the panel used on day 3 post-admission. Figure S3. The result of the patient's unexpected antibody identification during follow-up (day 175 post-admission). A&B: the patterns of the unexpected antibody identification. C: the phenotype listing sheet corresponding to the panel used on day 175 post-admission.

Acknowledgments

We extended our gratitude to the patient in this study.

Author Contributions

Conceptualization, H.G. and Q.Y.; Methodology, H.G. and K.D.; Formal Analysis, H.G.; Investigation, H.G., D.S. and K.D.; Resources, D.L.; Data Curation, S.H.; Writing—Original Draft Preparation, H.G.; Writing—Review & Editing, H.G. and D.L.; Visualization, H.G.; Supervision, D.L.; Project Administration, H.G. and Q.Y.; Funding Acquisition, D.L. All authors made substantial changes in the manuscript and approved the final manuscript.

Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the First Affiliated Hospital of Ningbo University (No. 2023 157RS-01, 23 October 2023).

Informed Consent Statement

Patient consent was waived due to the data or specimens being obtained from previous clinical diagnosis, disease surveillance, or clinical research , with all subjects' privacy and personal identity information protected.

Funding

This research was funded by the grants from the Medical and Health Research Project of Zhejiang Province (No. 2021KY283) and Natural Science Foundation of Ningbo (No. 202003N4228).

Declaration of Competing Interest

The authors declared no conflict of interests.

References

1. Yu Y, Ma C, Sun X, Guan X, Zhang X, Saldanha J, et al. Frequencies of red blood cell major blood group antigens and phenotypes in the Chinese Han population from Mainland China. *Int. J. Immunogenet.* **2016**, *43*, 226–235.
2. Pitan C, Syed A, Murphy W, Akinlabi O, Finan A. Anti-S antibodies: An unusual cause of haemolytic disease of the fetus and newborn (HDFN). *BMJ Case Rep.* **2013**, *2013*, bcr2012006547.
3. Mayne KM, Bowell PJ, Green SJ, Entwistle CC. The significance of anti-S sensitization in pregnancy. *Clin. Lab. Haematol*. **1990**, *12*, 105–107.
4. Yang MH, Chen JW, Wei ST, Hou SM, Chen YJ. The efficacy of ethnic specific blood groups genotyping for routine donor investigation and rare donor identification in Taiwan. *Vox Sang.* **2022**, *117*, 99–108.
5. Greendyke RM, Banzhaf JC. Ocurrence of anti-Wr a in blood donors and in selected patient groups, with a note on the incidence of the Wr a antigen. *Transfusion* **1977**, *17*, 621–624.
6. Schonewille H, van Zijl AM, Wijermans PW. The importance of antibodies against low-incidence RBC antigens in complete and abbreviated cross-matching. *Transfusion* **2003**, *43*, 939–944.
7. Espinosa A, Garvik LJ, Trung Nguyen N, Jacobsen B. A fatal case of acute hemolytic transfusion reaction caused by anti-Wr(a): Case report and review of the literature. *Immunohematology* **2021**, *37*, 20–24.
8. Wallis JP, Hedley GP, Charlton D, Parker PC, Scott Y, Chapman CE. The incidence of anti-Wra and Wra antigen in blood donors and hospital patients. *Transfus. Med.* **1996**, *6*, 361–364.
9. Arriaga F, Llopis F, de la Rubia J, Carpio N, Moscardó J, Marty ML. Incidence of Wra antigen and anti-Wra in a Spanish population. *Transfusion* **2005**, *45*, 1324–1326.
10. Puig N, Carbonell F, Soler MA, Perez MT, Arriaga F, Marty ML. Mimicking anti-S simulating a delayed transfusion reaction. *Vox Sang.* **1987**, *53*, 173–174.
11. Lane WJ, Westhoff CM, Gleadall NS, Aguad M, Smeland-Wagman R, Vege S, et al. Automated typing of red blood cell and platelet antigens: A whole-genome sequencing study. *Lancet Haematol.* **2018**, *5*, e241–e251.
12. Raos M, Lukic M, Pulanic D, Vodanovic M, Cepulic, BG. The role of serological and molecular testing in the diagnostics and transfusion treatment of autoimmune haemolytic anaemia. *Blood Transfus.* **2022**, *20*, 319–328.
13. Al-Riyami AZ, Burnouf T, Wood EM, Devine DV, Oreh A, Apelseth TO, et al. International Society of Blood Transfusion survey of experiences of blood banks and transfusion services during the COVID-19 pandemic. *Vox Sang.* **2022**, *117*, 822–830.