Acute hemolytic transfusion reaction due to a warm reactive anti-A_1

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BACKGROUND: Anti-A_1 are regularly observed by reverse testing and are generally considered clinically irrelevant. For compatibility testing and the selection of blood, we use the type-and-screen (T&S) strategy, in which ABO confirmation of patients with a definitive blood group is performed by forward grouping only. Because anti-A_1 seem clinically irrelevant, it is our policy to provide group A blood in patients with an anti-A_1.

STUDY DESIGN AND METHODS: This is a case report of a 96-year-old woman who died shortly after transfusion of blood group A red blood cells (RBCs). She was known to have blood group A_2 with an anti-A_1 and the absence of other RBC antibodies. Directly after starting transfusion, acute dyspnea was observed, while other clinical signs for a transfusion reaction were absent. In the laboratory, indications for a severe hemolytic transfusion reaction (HTR) triggered serologic investigations and complement deposition experiments.

RESULTS: Analyses revealed that the anti-A_1 was present as a high-titer IgM class immunoglobulin that induced complement deposition on A_1 RBCs. The anti-A_1 reacted in a wide temperature amplitude up to 37°C with A_1 RBCs, while weak agglutination was observed with A_2 RBCs at room temperature.

CONCLUSION: A pretransfusion detectable anti-A_1 caused a severe HTR that, in view of the rapid onset of clinical symptoms and concomitant deterioration, contributed to the death of the patient. Considering its clinical significance in this case, we encourage an unambiguous procedure for patients with an anti-A_1, especially when T&S is used for donor RBC selection.

In Caucasians, approximately 79% of individuals with blood group A have the subgroup A_1 and 21% are a weak A subgroup with most being A_2. Of persons with blood group AB, approximately 83% are A_1B and 17% are A_2B.[1] A_1 cells possess a mixture of distinctive A and A_1 antigens (total, 0.8×10^6-1.2×10^6 per red blood cell [RBC]), while A_2 cells possess the A antigen only at a lower antigen loading of 0.1×10^6 to 0.4×10^6 per RBC. In subjects with A_2 or A_2B, an anti-A_1 is detectable in 1% to 2% and 22% to 26% of cases, respectively. An anti-A_1 primarily appears as an antibody of immunoglobulin (Ig)M class that is active at temperatures lower than 25°C and is therefore rarely clinically significant.[2] However, we describe in this case report a subject known with an anti-A_1 who was transfused with A_1 RBCs, which resulted in a severe hemolytic transfusion reaction (HTR), shortly after which the patient died.

ABBREVIATIONS: AF = atrial fibrillation; HTR(s) = hemolytic transfusion reaction(s); INR = international normalized ratio; RR = Riva-Rocci; RT = room temperature; T&S = type and screen; VB = veronal buffer; VBG = VB with 0.05% gelatin.

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MATERIALS AND METHODS

Type-and-screen policy

The Dutch transfusion guideline recommends type-and-screen (T&S) for pretransfusion compatibility testing.[3] Herein, the ABO and D blood group of new patients is determined twice by forward and reverse grouping in two samples that have been drawn independently of each other; only then the blood group is definitive. Donor RBC selection for patients with a definitive blood group requires a recent (<72 hr) antibody screening and ABO and D confirmation by forward grouping only using test reagents. By this routine, an anti-A_1 is not detected in the category of patients with a definitive blood group and for whom crossmatching is not necessary, for example, in the absence of RBC alloantibodies.

Policy for patients with an anti-A_1

In case an anti-A_1 is found, the Dutch transfusion guideline advises to test its reactivity at 37°C.[3] If reactivity is possible at this temperature, RBCs of blood group A may be selected after a negative crossmatch in the indirect antiglobulin test (IAT).

Blood products

In the Netherlands, all donor products are supplied by Sanquin Blood Supply, as the national blood bank. Standard RBCs are leukoreduced ($<10^6$ per donor unit) and nonirradiated. One unit (280 mL) contains 100 to 110 mL SAGM, at least 40 g hemoglobin (Hb; hematocrit, 0.50%-0.65%) and citrate plasma (5-10 mL). As part of T&S compatibility testing, all RBCs are subjected to ABO and Rh(D) confirmation upon delivery at the transfusion laboratory in the hospital.

Transfusion reaction protocol

If a transfusion reaction is suspected, transfusion is stopped and the laboratory is informed by the clinical staff. Blood samples are immediately drawn for hematologic tests (Hb and reticulocytes), hemolysis variables (free Hb, potassium, lactate dehydrogenase [LDH], bilirubin, and haptoglobin), other chemistry tests (sodium, creatinine, and urea), and serologic tests (repetition of forward and reverse grouping, repetition of the antibody screening, IAT with transfused RBCs, and the direct antiglobulin test [DAT]). These variables are compared with the most recent pretransfusion sample and a sample drawn 24 hours after the transfusion reaction. The laboratory specialist consults the physician on the outcome; a new transfusion may only be considered after this consult.

Serologic testing

EDTA specimen tubes were drawn and stored at room temperature (RT). The blood group was determined by the column technique (Ortho BioVue system, Ortho Clinical Diagnostics) using ABO-D/reverse (forward + reverse) and ABD-Confirmation (forward only, including D) reagent cassettes at RT. For reverse grouping, a 0.8% suspension of A_1 and B test cells (Affirmagen) was used in the BioVue cassettes. Antibody screening was performed in the IAT at 37°C in a low-ionic strength solution (LISS) using a set of three test cells of blood group O (Ortho Surgiscreen; homozygous for Rh, Fya, Fyb, Jka, Jkb, M, and S antigens) and anti-IgG reagent cassettes. This technique was also used for crossmatching donor RBCs with plasma of the patient. Subgrouping of blood group A was performed with the *Dolichos biflorus* lectin in saline, following the instructions of the manufacturer (Sanquin).

The DAT was performed with RBCs directly taken from the patient sample and diluted to a 3% to 5% cell suspension with a LISS/antiglobulin test differentiation card with anti-IgG/IgA/IgM/C3c/C3d at 37°C (BioRad Diamed). Antibody elution from the RBCs was performed with the acid elution kit (Peli Elustrip, Sanquin Reagents) and heat elution at 56°C according to the procedures described in the AABB Manual.[4]

The reactivity of the anti-A_1 was investigated in a three-stage test in round-bottom glass tubes (macroscopic evaluation of agglutination after each reading stage): at RT, a suspension of test cells was added to plasma (or eluate) of the patient, mixed, and immediately spun down (Stage 1); the mixture was incubated at 37°C for 15 minutes, mixed, and spun down (Stage 2); and the suspension was washed with phosphate-buffered saline (PBS; three times at RT or 30/37°C) after which polyspecific anti-human serum (Pelikloon, Sanquin Reagents) was added (Stage 3) and antiglobulin test control cells (Sanquin) were added if no agglutination was observed.[4] The antibody specificity of the eluate to A_1, A_2, and O test RBCs from adult donors (Sanquin) was performed in the three-stage test using bovine serum albumin (BSA; Sanquin) as a potentiator. The temperature amplitude of the anti-A_1 was investigated in saline by the addition of prewarmed plasma at 30/37°C to prewarmed A_1 test cell suspensions. The IAT for determination of the antibody titer with or without dithiothreitol (DTT)-treated plasma was performed in saline (no prewarmed suspensions were used), in which the plasma was incubated with 10 mmol/L DTT (Sigma-Aldrich) in PBS at 37°C for 30 minutes.

Complement deposition experiments

Fractionation of the serum was performed using a chromatographic separation column (10/300 Superdex200, GE Healthcare) that was equilibrated with two column

volumes of veronal buffer (VB; 3.12 mmol/L barbital + 1.8 mmol/L sodium barbital + 145 mmol/L NaCl, pH = 7.4). A quantity of 250 μL of posttransfusion plasma was filtered through a 0.45-μm filter before fractionation was done in VB. A total of 1.5 column volumes were eluted and fractions of 0.5 mL were collected and stored at –20°C.

An enzyme-linked immunosorbent assay (ELISA) was performed to measure IgG and IgM concentrations in the different fractions. Nunc Maxisorp plates were coated with 2 μg/mL monoclonal mouse anti-human IgG (Clone MH16-1, Sanquin Reagents) or 2 μg/mL monoclonal mouse anti-human IgM (Clone MH15-1, Sanquin Reagents) overnight at RT. Standard curve antibodies and fractions were diluted in high-performance ELISA buffer (Sanquin Reagents) and added for 1 hour at RT after washing of the plate. After incubation, the plates were washed again and 1 μg/mL horseradish peroxidase (HRP)-linked monoclonal mouse anti-human IgG (Clone MH-16-1, Sanquin Reagents) or 1 μg/mL monoclonal mouse anti-human IgM HRP (Clone MH-15-1, Sanquin Reagents) was added to the wells. After an additional incubation step of 1 hour at RT and washing of the plates, substrate solution (0.11 mol/L sodium-acetate buffer pH 5.5 in water, 100 μg/mL TMF, 0.1% H_2O_2 3% solution) was added. Finally, conversion of HRP was stopped by adding 2 mol/L H_2SO_4.

Flow cytometric analysis of complement deposition was performed with A_1, A_2, and O test cells (Sanquin), which were treated with bromelain and added to a 96-well round-bottom plate to a final concentration of 0.083%, diluted in VB with 0.05% gelatin (VBG). Fractions were added in 25% of the final volume and unfractionated serum was included as a positive control, to a final concentration of 5%, the difference in volume made up for with VBG. Twenty-five percent of the final volume consisted of healthy AB recalcified plasma, added as a source of complement proteins. To prevent complement-mediated lysis of the RBCs, anti-C5 monoclonal antibody (eculizumab, 20 μg/ml) was added in VBG, 10 mmol/L $CaCl_2$, and 2 mmol/L $MgCl_2$ (VBG). After 1-hour incubation at 37°C on a shaker, RBCs were washed with PBS supplemented with 0.5% BSA and stained in 75 μL of PBS 0.5% BSA with 1.5 μg/mL anti-C3-FITC (Clone anti-C3-19, Sanquin Research) and 1 μg/mL anti-C4-APC (Clone anti-C4-10, Sanquin Research) for 30 minutes at RT while shaking. After being stained, cells were washed again in PBS 0.5% BSA and fluorescence of RBCs was measured on a flow cytometer (FACSCanto, BD).

CASE REPORT

A 96-year-old female with a history of atrial fibrillation (AF) and recent trauma-related costa fracture was admitted to our hospital for treatment of anemia, which was diagnosed by her general practitioner (Hb, 6.4 [normal, 12.1-16.1] g/dL). There were no signs for critical illness and considering her age and wishes, an investigation into the cause of her anemia was deferred. It was decided to treat her symptomatically with intravenous iron supplementation and 2 units of RBCs at the daycare ward.

According to the record in our laboratory blood bank management system, the patient was A_2 D+ with the presence of an anti-A_1 (no history of transfusions or other detected [allo]antibodies). The anti-A_1 was discovered twice, 3 years earlier upon her first presentation at the hospital by reverse grouping with A_1 test cells; at that moment, the patient was typed A_1 negative and the temperature amplitude of her antibody was not investigated. According to T&S, we performed ABO and D confirmation by forward grouping only and excluded the presence of alloantibodies by an IAT using a three-cell screening panel. Two random RBC units of blood group A D+ were selected after electronic crossmatching (transfusion time, 1.5 hr/unit).

Ten minutes after starting the transfusion, the patient became progressively dyspneic while other vital signs remained normal (temperature, 36.1°C; Riva-Rocci [RR], 140/104 mmHg; pulse rate, 77/min; saturation, 94%). The transfusion was stopped after 50 mL of infusion and the patient was physically examined (after 15 minutes—RR, 177/98 mmHg; pulse rate, 88/min; saturation, 98%). The symptoms were interpreted to be of pulmonary cause since her costa fracture 2 months earlier revealed the presence of pleural fluid on the chest X-ray. A new radiograph revealed an increase of pleural fluid and mild atelectasis, while no signs for a transfusion-related acute lung injury (TRALI) or indications for a transfusion-associated circulatory overload (TACO) were found. According to the physician, the actual findings were not suggestive for a transfusion reaction and the transfusion reaction protocol was not initiated. A new RBC unit was ordered at the laboratory and upon transfusion of the second unit of blood group A D+, similar symptoms were observed. Once again, the transfusion was stopped after 150 mL and it was decided to perform an emergent pleural effusion aspiration. Again, the transfusion reaction protocol was not initiated.

The patient deteriorated rapidly (RR, 174/94 mmHg; pulse rate, 102/min with AF; saturation, 93% with 3 L of oxygen; respiratory rate, 42/min), blood was drawn for analysis, and she was immediately prepared for thoracocentesis. Since she used a vitamin K antagonist for AF, her international normalized ratio (INR) was checked using a point-of-care analyzer. An INR of 2.9 was treated with 1000 IE prothrombin complex concentrate and just before the procedure a venous sample was taken for a control INR.

In the laboratory, the aspect of the plasma pointed to severe hemolysis both in the citrate sample tube (INR) and in the formerly drawn heparin tube (Fig. 1A). By now, all indications were strongly suggestive for a HTR, which

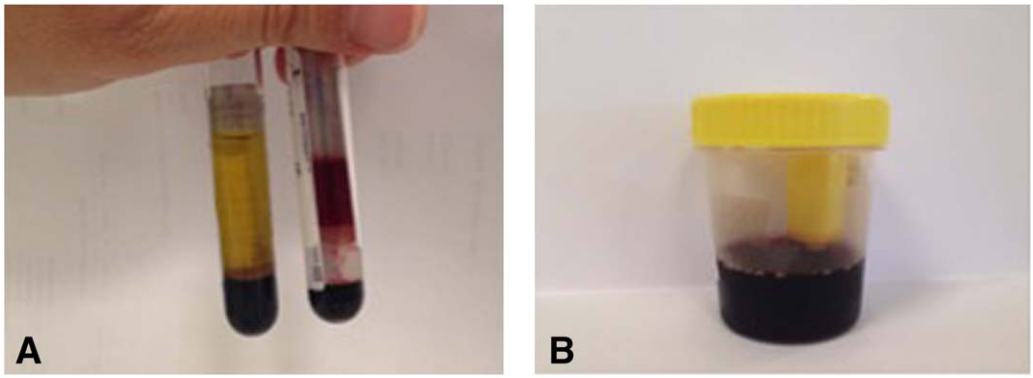

Fig. 1. Heparin plasma before and after transfusion (A) and urine after transfusion (B). [Color figure can be viewed at wileyonlinelibrary.com]

triggered our transfusion reaction protocol. Biochemical and serologic investigations confirmed intravascular hemolysis caused by her existent anti-A_1 (see next section). Upon treatment with prothrombin complex, the INR reduced to 1.6 and the pleural effusion aspiration was conducted at the medium care unit yielding a transudative fluid (no hemothorax). No clinical improvement was evidenced, while an increase of the hemolysis variables was deduced from subsequent blood analyses. Corroborating the findings in the laboratory, the patient was now treated for a HTR with 2 L of 0.9% NaCl under supplemental oxygen (15 L).

After Rh phenotype and K typing, two compatible O D+ RBCs were selected and fully transfused, while none of the earlier transfusion-related symptoms were observed. Nevertheless, the patient deteriorated (RR, 90/50 mmHg; pulse rate, 102/min with AF; respiratory rate, 9/min) and she became anuric since the onset of the HTR. Upon catheterization, a small amount of strongly colored red/brown urine was collected (Fig. 1B). Fourteen hours after the first transfusion, the patient became bradycardic and died.

Biochemical investigation

One day before admission, the general practitioner requested laboratory tests, which revealed a severe microcytic anemia with normal white blood cell and platelet (PLT) counts (Table 1). The hemolysis variables were within the reference range. Retrospective analysis of samples drawn at 10:34 AM and 12:10 PM (after the partly transfused units of A D+) showed that the hemolysis variables were highly indicative for a HTR. While the plasma was negative for the presence of free Hb before transfusion, a concentration of 0.54 mg/dL was deduced from the hemolytic index after transfusion that was macroscopically evidenced. Concomitantly, a rapid increase of total bilirubin and LDH was observed up to 4.47 (<1.00) mg/dL and 1563 (<247) U/L, respectively. These variables progressively increased including the concentrations potassium and creatinine to 5.1 (3.5-5.0) mmol/L and 1.65 (0.55-1.02) mg/dL, respectively, as well as decreasing levels of haptoglobin. Before transfusion, a mild complement C3 deficiency was evidenced, which further reduced to 40 (90-180) mg/L after transfusion.

Serologic testing

After observation of the aspect of the plasma, the transfusion reaction protocol was initiated at the transfusion laboratory and serologic tests were performed. Reverse grouping with A_1 test cells and the IAT with both partly transfused A D+ RBCs revealed strong positive (4+) reactions with the samples drawn before and after transfusion. The IATs with both fully transfused O D+ units were negative. No alloantibodies were detected in both samples. A weak positive (+) DAT with anti-IgG and anti-C3d was found in the posttransfusion sample.

Further serologic testing was performed at the Sanquin reference laboratory for immunohematology (Table 2). First, RBC antibodies to common low-incidence antigens were excluded. *D. biflorus* lectin tests revealed that both transfused units of blood group A D+ were A_1 positive and the patient was A_1 negative. After transfusion, a mixed-field reaction was observed, indicative for the persistence of a small number of transfused A_1 cells in the blood of the patient.

At the reference laboratory, a very weak positive DAT (+/–) with anti-IgG and anti-C3d was observed in the pretransfusion sample as well. Eluates of the pre- and posttransfusion sample that were prepared with acidic reagents were nonreactive with A_1 test cells, whereas eluates prepared by heat (56°C) agglutinated A_1 test cells (Table 2). Both 56°C eluates revealed strong agglutination (4+) at RT, which persisted throughout the second and third reading stages. Only direct agglutination of A_2 test cells was observed, but with weaker reactivity (2+) disappearing after heating to 37°C and in the IAT. No reactivity was observed between the 56°C eluates and group O test

TABLE 1. Biochemical variables measured 1 day before transfusion and after transfusion of the 4 units of RBCs*

Test	Reference	Unit	1 DB	10:34	12:10	14:30	15:49	16:09	20:57	22:57	1:11	2:46
Hb	12.1-16.1	g/dL	6.4	Transfusion 1:	Transfusion 2:	7.4	NA	7.4	Transfusion 3:	ND	Transfusion 4:	NA
Free Hb	NA	mg/dL	0.01	A_1 R0r, 50 mL	A_1 R1r, 150 mL	0.54	0.47	0.51	O R_1R_1,	0.23	O R_1R_1,	0.35
Na	135-145	mmol/L	137	(20%)	(55%)	136	INR sample	135	280 mL (100%)	136	280 mL (100%)	Urine sample
K	3.5-5.0	mmol/L	3.6			4.0		4.2		5.1		
Creatinine	0.55-1.02	mg/dL	0.90			0.92		1.31		1.65		
Urea	7.0-17.9	mg/dL	20.2			21.0		21.0		24.4		
Bilirubin total	<1.00	mg/dL	0.53			2.56		2.87		4.47		
LDH	<247	U/L	152			1386		1327		1563		
Haptoglobin	37-221	mg/dL	150			91		85		38		
C3	90-180	mg/dL	62			ND		ND		40		
C4	10-40	mg/dL	18			ND		ND		15		

* In all samples reticulocytes were not determined.
DB = day before admission; NA = not applicable; ND = not determined.

TABLE 2. Follow-up of the serologic investigations performed at the Sanquin reference laboratory (Amsterdam)*

Before transfusion					After transfusion				
D. biflorus lectin test in glass tubes with RBCs									
Patient		RBC1	RBC2		Patient		RBC1	RBC2	
—		4+	4+		MF		NA	NA	
DAT with patient RBCs, BioRad Diamed (LISS at 37°C), anti-IgG ǀ -A ǀ -M ǀ C3c ǀ -C3d									
Anti-IgG+/– ǀ anti-C3d+/–					Anti-IgG+ ǀ anti-C3d+				
IAT with test RBCs/RBCs and patient 56°C eluate, BSA in glass tubes (20°C ǀ 37°C ǀ anti-IgG/C3d)									
A_1	A_2	O	RBC1	RBC2	A_1	A_2	O	RBC1	RBC2
4+ ǀ 4+ ǀ 4+	2+ ǀ – ǀ +/–	– ǀ – ǀ –	ND	ND	4+ ǀ 4+ ǀ 3+	2+ ǀ +/– ǀ –	– ǀ – ǀ –	4+ ǀ 4+ ǀ 4+	4+ ǀ 4+ ǀ 4+
IAT with prewarmed A_1 test RBCs and prewarmed patient plasma, saline in glass tubes (30 or 37°C ǀ anti-IgG/C3d)									
30°C			37°C		30°C			37°C	
3+ ǀ 3+			– ǀ –		3+ ǀ 2+			– ǀ –	
IAT with A_1 test RBCs and patient plasma, saline in glass tubes (20°C 37°C ǀ anti-IgG/C3d)									
Before DTT			After DTT		Before DTT			After DTT	
128 ǀ 8 ǀ 4			8 ǀ – ǀ –		16 ǀ (2) ǀ (2)			8 ǀ – ǀ (2)	

* Weak reactions are denoted by "+/–" or "()."
MF = mixed field; NA = not applicable; ND = not determined.

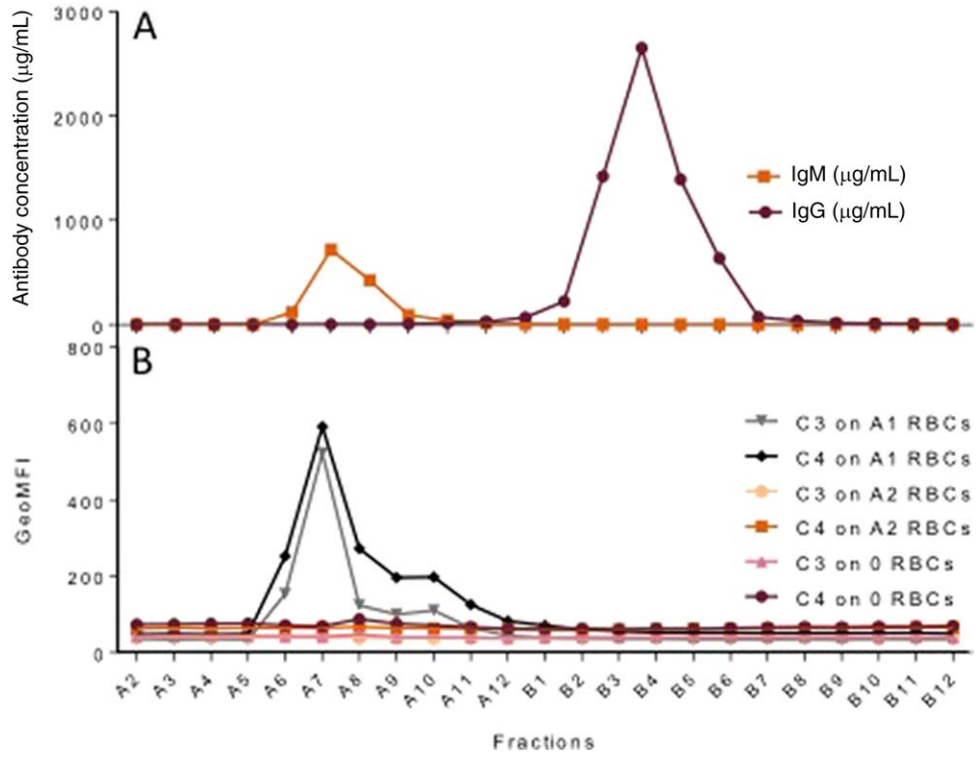

Fig. 2. Presence of separated IgM and IgG antibodies in different fractions of the patient plasma (ELISA, A). C3 and C4 deposition on different RBCs after incubation with plasma fractions (FACS, B). [Color figure can be viewed at wileyonlinelibrary.com]

cells. Next to antibody specificity, the temperature amplitude and immunoglobulin class of the anti-A_1 were investigated. Upon the addition of prewarmed plasma to a prewarmed suspension of A_1 test cells, strong agglutination (3+) was observed up to 30°C in saline (no direct agglutination upon warm addition at 37°C). In the same technique, an agglutination titer of 128 was found at RT in the pretransfusion sample, which reduced to 8 after treatment with DTT. In the antiglobulin stage, agglutination remained absent with the DTT-treated plasma. A similar agglutination trend, yet at significantly lower titers, was observed with the plasma after transfusion, suggestive for antibody scavenging by the transfused A_1 RBCs.

Complement deposition analyses

Fractionation of posttransfusion plasma by size exclusion chromatography followed by ELISA tests revealed the presence of IgM antibodies in fractions A6 to A9 (highest concentration in A7) and IgG antibodies in fractions B1 to B5 (highest concentration in B3, Fig. 2A). FACS analyses revealed C3 and C4 deposition on A_1 cells only that had been incubated with IgM fractions A6, A7, and A8 (Fig. 2B). These fractions did not induce C3 or C4 deposition on A_2 or O cells, confirming that these antibodies are specifically directed to cells with blood group A_1. Fractions containing IgG antibodies did not induce complement deposition on any of the three blood groups.

DISCUSSION

This is a very rare case in which a subject with an anti-A_1 developed a HTR after transfusion of A_1 RBCs and subsequently died. Evidence for a HTR is based on the apparent clinical symptoms that were observed directly after starting the transfusion. This highly causal response corroborates with serologic findings and strongly positive hemolysis variables. Because of the lack of an updated health status, we cannot exclude with certainty the possibility of a concurrent event. Apart from her iron deficiency anemia, there were no clinical suspicions of infection, ischemic disease, or heart failure upon presentation. Neither was she suspected of disseminated intravascular coagulation (no bleeding, normal PLT counts, proper INR response upon prothrombin complex concentrate), nor a TRALI or TACO (X-ray negative, no symptoms after 2 entire units of blood group O and 2 L of saline).[5]

Clinically, the immediate onset of symptoms attributes to the hypothesis that the immunogenic cause for the HTR is based on IgM class antibodies. Analogous to HTRs by major ABO incompatibility, complement binding IgM-anti-A/B features an instantaneous response. These clinical symptoms include back or flank pain, fever, chills, fainting or dizziness, and flushing of the skin, which have not been observed in our patient.[6] With respect to anti-A_1, predominantly delayed HTRs have been observed in patients;[7-12] however, some cases report on the acute

onset of symptoms. These reactions have been observed in immunodeficient patients only and the clinical symptoms are heterogeneous, diverging from characteristic ABO incompatibility[13] to milder symptoms in the absence of pain.[14,15] The clinical symptoms of our patient were limited to severe dyspnea and this atypical symptomatology complicated a swift clinical diagnosis for a HTR.

Indications for a HTR due to anti-A_1 agglutinins were confirmed by serologic results and the exclusion of alloantibodies including antibodies to low-incidence antigens. Direct agglutination was markedly observed after reverse grouping and crossmatches with the transfused units of blood group A were positive, while the IAT with both units of blood group O were negative. The antibody was predominantly present as an IgM as saline agglutination was abolished after DTT treatment, while additional antiglobulin testing did not enhance agglutination titers. The antibody was present in high titers, which dropped by at least three dilution steps (128 to 16 at RT and 8 to 2 at 37°C) after transfusion of approximately 200 mL A_1 RBCs (partly transfused RBCs 1 and 2, Table 1). Direct agglutination of A_1 RBCs was observed in a wide temperature amplitude (up to 30°C upon the addition of prewarmed plasma to a prewarmed cell suspension in saline) and FACS experiments revealed that specifically IgM immunoglobulins induced complement deposition on A_1 cells only.

Considering these in vitro properties, we envision the in vivo clinical relevance of the observed anti-A_1, especially since the temperature of RBCs is usually approximately 10°C when transfusion is started. Apart from its agglutination properties, we observed weak reactivity toward A_2 test cells. This is known to be a weak interaction, which has been observed for anti-A_1 agglutinins in serum.[16] Our investigation of the anti-A_1 specificity was conducted in the 56°C eluates revealing weak direct agglutination of A_2 RBCs at RT only and identical reaction patterns of the pre- and posttransfusion eluate. The same holds for the pre- and posttransfusion DATs (both weakly positive), in which slightly stronger reactivity was observed in the posttransfusion sample likely due to residual transfused A_1 RBCs. The similarity of the pre- and posttransfusion outcomes of these sensitive tests provides—unlike the other serologic experiments performed—no proof that the anti-A_1 caused the HTR. We assume that the anti-A_1 detected in this patient acts as a weak A_2 cold agglutinin and a A_1-specific agglutinin with a broad temperature amplitude. We consider reactivity to A_2 RBCs clinically irrelevant, since negative hemolysis variables were found before transfusion.

This case describes another example of a HTR based on an anti-A_1, yet with different serologic properties and clinical symptoms. In fact, published case reports confirm this diversity and only few contributions comprehensively elucidate the evidence of HTRs by an anti-A_1 in A_2(B) subjects. Serologically, our case is similar to Jakobowicz and colleagues[10] who found IgM-anti-A_1 with increased temperature amplitude in a male with a lymphosarcoma. After transfusion of A_1 RBCs, a mild and delayed HTR was evidenced and an agglutinin titer of 64 was determined in saline at RT that reduced to 4 at 37°C. In other examples, allo-IgG-anti-A_1 antibodies were found to be responsible for HTRs,[8,17] while few contributions report on the combination of IgM- and IgG-anti-A_1 antibodies.[9,11,18] Clinically, the symptoms in our patient mostly resemble the case report by Preece and colleagues,[13] who described dyspnea shortly after starting transfusion of A_1 RBCs in a A_2B female with plasma cell myeloma and recent autologous marrow transplant. However, clinical symptoms were worsened by back pain and chills and despite hemoglobinemia (disappearing after 6 hr), bilirubin and haptoglobin levels remained normal. Identical to our case, a positive DAT for IgG and complement was found, an anti-A_1 was eluted, and her plasma agglutinated A_1 cells at 37°C. Furthermore, an anti-A_1 was discovered by reverse typing with pretransfusion serum, which has been described in only one other case report involving a HTR by an anti-A_1.[11]

To our surprise, the majority of published cases describe the absence of an anti-A_1 in pretransfusion serum,[7-10,17,18] which may be counterintuitive since this agglutinin is frequently observed in nontransfused subjects.[1] Unlike the presence of a pretransfusion anti-A_1, severe immunodeficiency seems associable with HTRs. Although specific tests were not performed, our patient was clinically not suspected for immunodeficiency or a monoclonal element (normal total protein level, normal anti-B titer). A negative transfusion history was established by consulting blood bank managing systems of regional hospitals and considering the absence of a serologic change after the HTR, we find it highly likely that naturally occurring anti-A_1 was responsible for this serious event.

In the past 50 years or more our transfusion policy was based on the clinical irrelevance of anti-A_1 agglutinins. Patients with an anti-A_1 have always been transfused with blood group A and the investigation of the temperature amplitude has not been performed. According to T&S, pretransfusion crossmatching was not conducted in these patients and transfusion reactions have never been encountered. This case, however, illustrates that such a policy may not be safe, especially considering the absence of typical clinical symptoms for the HTR that caused a delay in triggering the transfusion reaction protocol. In the light of this case the question arises what should transfusion policy be using T&S and the detection or discovery of a newly formed anti-A_1. Once discovered, it should also cover the serologic properties to be investigated in an appropriately assigned technique and the blood group selection of donor RBCs. The Dutch guideline states that confirmation of ABO and D blood group in a

patient with a definitive blood group can be performed by forward grouping only.[3] The British guideline states that it is very unlikely that an anti-A_1 is of clinical significance, yet they do recommend an IAT compatible at 37°C if blood group A donor units are selected for transfusion.[19] Other practice guidelines suggest the exclusion of clinical significance by an IAT at 37°C and the selection of A_2 or O RBCs in case the anti-A_1 is reactive at this temperature.[4,20]

CONCLUSION

Hemolytic transfusion reactions based on anti-A_1 remain a relatively rare phenomenon, which is covered by infrequent and heterogeneous case reports in literature. Still, an anti-A_1 is found in ±0.7% of Caucasians. Serologic management for patients with an anti-A_1 is complex, especially in conjunction with T&S. Complicating factors include the natural presence of an anti-A_1, rare complication rates of A_1 transfusions in A_2(B) subjects, full patient transfusion history, and incoherent etiologies concerning immunization. We changed our policy so that patients with an anti-A_1 are excluded from T&S and blood group O is transfused in case the IAT with the RBCs of blood group A is positive at 37°C. We thereby encourage universal unambiguous procedures for patients with an anti-A_1 and their implementation in T&S.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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