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Chapter

Post-Transfusion Haemolytic Reactions

Jolanta Korsak and Anna Piotrowska

Abstract

Haemolytic post-transfusion reaction is caused by accelerated destruction of erythrocytes by immunological incompatibility between the donor and the recipient. It also occurs for non-immunological reasons: thermal, osmotic or mechanical damage and bacterial infection. Haemolysis can be endogenous (usually acute) and exogenous with macrophages in the reticuloendothelial system of spleen or liver (delayed). The pathophysiology: antibody binding erythrocyte antigens, antibody-coated erythrocytes interaction with monocytes/macrophages activating phagocytosis or antibody-dependent cytotoxicity and the production of inflammatory mediators. Antibodies destroying transfused blood cells are called clinically relevant antibodies that are active in vitro at 37°C. An interesting mechanism is the "bystander immune cytolysis".

Keywords: haemolytic reaction, haemolysis, transfusion reaction, HTR

1. Introduction

Haemolytic transfusion reaction (HTR) is the result of accelerated destruction of red blood cells. The most common cause is immunological incompatibility between a donor and a blood recipient. It is mainly haemolysis that is responsible for the destruction of transfused donor blood cells by antibodies present in the recipient, but in rare cases, destruction may be caused in recipient blood cells by donor antibodies present in transfused plasma or platelet concentrate [1]. Haemolysis may also occur due to non-immunological reasons, such as thermal, osmotic or mechanical damage to the transfused blood; bacterial infection or extremely rare and blood transfusion from a donor with congenital haemolytic anaemia due to deficiency of glucose-6-phosphate dehydrogenase [2].

2. The incidence of haemolytic transfusion reactions

Currently, the incidence of haemolytic transfusion reactions is difficult to estimate. Most data come from retrospective studies that do not include reactions not reported by clinicians. In contrast, prospective studies also contain errors due to reaction symptoms often remaining unrecognised or masked by associated diseases, for example, bleeding or liver disease [1]. The frequency of reporting haemolytic transfusion reactions may also depend on other factors, such as patient population, transfusion response reporting system and medical staff education. Historical research results indicate that the frequency of haemolytic transfusion reactions falls

between 1:10,000 and 1:50,000 transfused blood components [3, 4]. In contrast, the incidence for patients receiving a transfusion is estimated to be higher (about 1:500–1:800 patients) because most recipients receive more than one blood unit. It is worth noting that the estimation of the frequency of haemolytic reactions depends on the number of transfusions in a given centre. Thus, in large clinical centres, where severely ill patients are treated, more of these events are recorded [4]. A report issued by the Quebec Haemovigilance System covering 5 years of observation described 47 ABO incompatibility reactions, 55 cases of acute haemolytic transfusion reaction and 91 cases of delayed transfusion reaction in reference to 7059 all reported transfusion reactions. It was estimated that the frequency of reactions resulting from the ABO incompatibility was 1:27,318, acute haemolytic transfusion reactions 1:14,901 and delayed haemolytic transfusion reactions 1:9313 per unit of transfused red blood cell concentrate [5].

The most common reaction among the acute (approximately 30%) was haemolysis resulting from ABO incompatibility [5]. In the annual report Serious Hazards of Transfusion (SHOT), published in England, in 2017, 42 haemolytic transfusion reactions were reported in reference to 3230 of all reactions observed following transfusion of blood components, of which 13 cases of acute haemolytic transfusion reaction and 29 cases of delayed haemolytic reaction (including 6 cases of hyperhemolysis) were reported. The number of reported cases of delayed haemolytic transfusion reaction was higher than in 2016, but comparable with previous years [6]. Factors that can affect the increase in the number of delayed haemolytic reactions include correctness in carrying out serological tests, longer survival of patients after transfusions and an increase in the number of transfused blood components. Since most patients receive more than one unit of red blood cell concentrate, the estimated incidence of delayed haemolytic transfusion reactions is from 1:854 to 1:524 per patient who has been transfused and is higher than per transfused unit [7]. In the population, delayed haemolytic transfusion reactions occur with a frequency of 1.69/100,000 per year [7].

3. Mechanisms of haemolytic transfusion reactions

Red blood cells undergo haemolysis in the intravascular mechanism, in blood or extravascular vessels, that is, organs involving cells of the reticuloendothelial system, primarily spleen and/or liver. Clinically significant differences between the above mechanisms of red blood cells destruction are based on the time of onset of haemolysis and the destruction rate of red blood cells. Intravascular haemolysis is characterised by the destruction of red blood cells at a rate of about 200 ml of transfused cells within 1 h of transfusion. It is manifested by a rapid decrease in haemoglobin, haemoglobinemia and haemoglobinuria and can potentially be life threatening [2]. In contrast, extravascular haemolysis is less dramatic, with a rate of destruction of red blood cells of approximately 0.25 ml/h/1 kg of recipient's body weight. For example, for 70 kg recipient, about 18 ml of transfused red blood cells are destroyed per hour. However, it is worth noting that despite the low intensity of haemolysis, the survival time of red blood cells after transfusion is significantly reduced [2]. In general, intravascular haemolysis is called as an early acute haemolytic transfusion reaction. It can occur during transfusion or up to 24 h after transfusion of red blood cells. In comparison extravascular haemolysis is called delayed haemolytic transfusion reaction and usually occurs 24 h or days after the end of the transfusion. The quoted breakdown of reactions is somewhat artificial, because the symptoms associated with haemolytic reactions sometimes overlap [1].

The occurrence and severity of individual clinical symptoms can vary widely and are often non-specific [1, 8].

Red blood cell transfusion can also stimulate the production of alloantibodies without the occurrence of haemolysis. This phenomenon is called delayed serologic transfusion reaction (DSTR) and should be differentiated from delayed haemolytic transfusion reaction [9].

3.1 Intravascular haemolysis

Most often intravascular haemolysis is the result of the destruction of red blood cells by the complement system, stimulated by the presence of alloantibodies or autoantibodies. Among alloantibodies, such haemolysis is induced by anti-A and anti-B, rarely anti-Jka, anti-Jkb, anti-Vel, anti-P, anti-Lea and very unique antibodies with other specificities [10, 11]. In all these cases, haemolysis takes place via the classical pathway of complement activation. Its occurrence and severity, in addition to the class of antibodies, is also affected by the number of antigenic determinants with which the antibodies react. The reaction is most severe in the case of antigens A and B, because their number is estimated at about 5×10^5 per cell [12, 13]. In contrast, the presence of antigens from the Rh, Kell, Kidd and Duffy systems on the surface of red blood cells is determined in the range of 10^3–10^4 per cell [12]. **Table 1** shows the number of antigenic determinants on the cell surface for selected red blood cell antigens.

Antibodies combined with antigens by triggering the complement cascade lead to cell lysis. This mechanism is called the classic pathway for complement activation and is shown in **Figure 1**.

The starting point is the antigen-antibody complex present on the surface of the cell membrane [14, 15]. Antibodies of the IgM and IgG class (outside the IgG4 subclass) bind the C1q protein in the initial stage of activation. The condition for complement activation is the binding of the C1q molecule by two Fc fragments of adjacent IgG antibodies or by one IgM molecule. It should be noted here that the IgM class is more efficient in starting the process of complement activation than the IgG class [2, 15]. The C1qrs complex is created and activates the C_2 and C_4 components and their distribution into C2a and C2b as well as C4a and C4b. The C4b2a

Blood group system	Antigens	The number of antigenic determinants on the surface of the cell membrane
ABO	A_1	8.5×10^5
	A_2	2.5×10^5
	B	7.5×10^5
Rh	D	1–2×10^5
	C	7–8×10^4
	e	1.8–2.4×10^4
Kell	K	6.1×10^3
Duffy	Fy^a	1.7×10^4
Kidd	Jk^a	1.4×10^5
MNSs	S	1.2×10^4

Table 1.
Number of antigenic determinants on the cell surface of the red blood cell (according to [12, 13]).
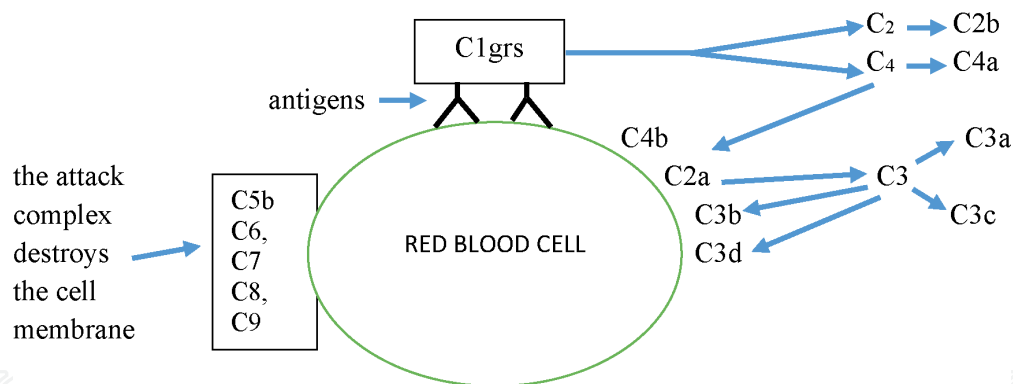
Figure 1.
The classic pathway for complement.

complex has proteolytic properties and is called C3 convertase. Convertase breaks down molecules of C3 into C3a, C3b, C3c and C3d. The C3b and C3d components bind with the red blood cell membrane and in many cases the complement cascade process ends. In other cases, the C3b component activates C5 and C5a and C5b are formed. C5b binds to C6, then to C7. This creates a complex of three C5b-6-7 particles, which is partially incorporated into the cell membrane and further binds C8. The C5b-8 complexes create holes in the cell membrane that increase when exposed to the C9 component. The C5B-C9 complex called membrane attack complex (MAC) creates pores in the cell membrane of a red blood cell that are 1/700 of its size. Haemoglobin escapes from the cells into the plasma, and the effects of haemolysis are visible macroscopically in the plasma of the blood sample [15].

The alternative path of complement activation and the lectin path of complement activation do not play a role in the destruction of red blood cells. Although the mechanism of the lectin route may be the reason for the in vivo ineffectiveness of the use of monoclonal and recombinant antibodies, which are thus eliminated from the body before they fulfil their function, for example, anti-D Ig for prevention purposes in RhD maternal-foetal conflict [16].

However, the complement system does not work specifically. The safety of body cells is enabled by factors that regulate complement activity present in plasma and on cells of various tissues, including red blood cells. Membrane inhibitor of reactive lysis (MIRL) (CD59) and decay accelerating factor (DAF) (CD55) are essential to protect red blood cells from haemolysis. The expression of these membrane inhibitors is associated with Cromer group system and CD59. On blood cells with the Cromer mull phenotype, known as Inab, DAF inhibitor expression is absent [17, 18]. DAF regulates C3a-converting activity. MIRL inhibits membrane attack complex [15, 17]. Lack of these particles may increase the susceptibility of red blood cells to intravascular haemolysis due to complement activation [19].

3.2 Extravascular haemolysis

In a situation in which, despite activation of the complement system, through antigen-antibody reaction, there is no intravascular haemolysis, red blood cells with detectable C3b component remain in the circulation. This kind of mechanism of red blood cell destruction occurs for IgG antibodies with complement system [13]. They may interact with CR1 and CR3 receptors on macrophages and consequently undergo phagocytosis. Most of the cells coated by the complement C3b component are destroyed by liver macrophages, that is, by Kupffer cells, while the cells coated with antibody molecules are mainly destroyed by spleen macrophages. They have surface receptors that recognise antibody classes and subclasses, and complement components,

of which the Fc R1 receptor is specific for red cells coated with antibodies [1]. Blood cells connected to this receptor are destroyed in the process of antibody-dependent cytotoxicity. Red blood cells can be absorbed and completely "digested" inside the macrophage. They can also be partially absorbed and then the integrity of the cell membrane is disturbed by the loss of proteins and lipids, which changes its osmotic properties. Such a blood cell, after being released from the macrophage, circulates in the blood as a spherocyte, whose survival is short. The macrophage cytotoxins are another mechanism that plays a role in the destruction of red blood cells. As a consequence of antibody-dependent cell-mediated cytotoxicity (ADCC) haemoglobinemia and haemoglobinuria may occur similarly to intravascular haemolysis, although the antibodies that caused it do not bind complement components.

4. Mediators of inflammatory reactions in haemolytic transfusion reactions

Receptors for complement activation products C3a and C5a are found on many cells: monocytes, macrophages, neutrophils, platelets, endothelium and smooth muscle. Their release causes an increase in the concentration of oxygen radicals, leukotrienes, nitric oxide and cytokines. The increase in cytokine release may also be due to the interaction of Fcγ R1 receptors with IgG molecules associated with red blood cells. Udani et al. [20] showed in vitro that in the case of ABO incompatibility, monocytes are directly involved in the formation of acute haemolytic transfusion reaction [15]. Incompatible red blood cells reduce CD14 expression and increase CD44 expression on monocytes in whole blood. After 24 incubations with incompatible red blood cells, monocytes show a significant increase in CD44 levels. The results of these studies indicate a critical role of monocyte activation in the development of intravascular haemolytic transfusion reaction [15].

In ABO incompatibility, in which anti-A, anti-B and anti-AB antibodies activate complement leading to intravascular haemolysis, a large amount of tumour necrosis factor-α (TNF) and interleukins CXCL8 (IL-8) and CCL2 are released into the plasma (MCP-1) [19–21]. TNF-α is released first, its elevated concentration is already detected within first 2 h. It carries a pro-inflammatory potential that is responsible for fever, leukocyte activation, stimulation of procoagulant activity, increased antibody production and vascular wall permeability [22]. TNF-α also stimulates endothelial cells to synthesise adhesion molecules and chemotactic cytokines [22]. CXCL8 and CCL2 produced in the blood during ABO incompatibility will appear later than TNF-α in very high concentrations. CXCL8 primarily activates neutrophils, which leads to the accumulation of leukocytes in the lung vessels of small diameter and damage to the endothelium of blood vessels and their higher permeability [1, 12]. CCL2 is mainly a chemotactic and activating factor for monocytes [1, 12].

In incompatibility, in which non-complement IgG antibodies cause extravascular haemolysis, cytokines belonging to two categories differing in response rates are produced: (1) synthesised at a concentration higher than 1 μg/ml within 24 h and (2) synthesised at a concentration of about 100 pg/ml. Low concentration cytokines include IL-1β, IL-6 and TNF-α. CXCL8 concentration is similar to that in intravascular haemolysis, whereas TNF-α is synthesised at low concentration, estimated at <100 pg/ml [1, 2]. IL-1ra (receptor antagonist) is produced in extravascular haemolysis, which is an IL-1 receptor antagonist. Its presence to some extent affects some clinical differences between extravascular and intravascular haemolysis [23]. IL-1β concentration and IL-6 produced by monocytes in response to red blood cells coated with IgG antibodies increase progressively within 24 h to a concentration of 100 pg/ml. Since IL-1β and IL-6 affect proliferation and differentiation

of β-lymphocytes, the synthesis of these two cytokines enhances the synthesis of allo- and autoantibodies, which are often involved in the formation of delayed haemolytic transfusion reaction [1, 24, 25].

5. Complications of haemolytic transfusion reactions

5.1 Disseminated intravascular coagulation associated with haemolytic transfusion reaction

The key pathogenetic phenomenon in DIC is excessive thrombin generation in the tissue factor (TF)-dependent pathway and activated factor VII (FVIIa-activated factor VII) [26]. In the pathogenesis of DIC, interactions between the blood coagulation system and mediators of the inflammatory response are also of great importance [27]. Proinflammatory cytokines affect blood coagulation and fibrinolysis, for example, TNF-α and IL-1 increase TF expression and inhibit thrombomodulin (TM) expression on vascular endothelial cells [28]. On the one hand, these processes lead to the production of a large amount of thrombin that converts fibrinogen to fibrin. Fibrin creates blood clots in the light of small vessels trapping the platelets. If the activation of coagulation is not timely inhibited, the resulting clots will block the blood supply to vital organs, which will be manifested in their failure. On the other hand, the formation of a large amount of blood clots will "consume" blood coagulation factors and platelets, which will manifest as a haemorrhagic diathesis. In addition, their degradation products (fibrinogen/fibrin degradation products (FDP)) resulting from the breakdown of fibrinogen and fibrin exhibit anticoagulant properties, inhibit platelet function, act as cytotoxic vascular endothelium and increase capillary permeability, further disrupting haemostasis mechanisms [26].

Clinically, this is manifested by unexpected bleeding and/or a decrease in blood pressure. The course is acute, dynamic, with thrombocytopenia, increased concentration of fibrin degradation products, prolonged prothrombin time (PT), extended partial thromboplastin time after activation (activated partial thromboplastin time (APTT)) and hypofibrinogenaemia. **Table 2** presents the point algorithm for the diagnosis of acute disseminated intravascular coagulation.

However, this complication is rare and predominantly accompanies intravascular haemolysis, but in recipients who have received non-compliant blood in the ABO system, it occurs even in 25% of cases [1].

5.2 Hypotension and shock

Hypotension occurs in about 1 in 10 cases of intravascular haemolytic transfusion reaction, but is also sometimes observed in extravascular haemolysis. Complement activation appears to be the most important determining factor in these cases. During the haemolytic reaction, C3a, C4a, C5a and C5a-des-arg anaphylatoxins are released. Furthermore, consumption of a C1-esterase inhibitor contributes to the activation of the kinin pathway associated with the release of bradykinin [32]. In addition, tumour necrosis factor (TNF) and interleukin-1 (IL-1), released by phagocytes during haemolytic transfusion reaction may also contribute to hypotension and shock [32].

5.3 Impaired renal function

Impaired renal function is observed in both intravascular and extravascular haemolytic transfusion reactions, although definitely more frequently in the case of

Test	Result	Score
Platelet count ($\times 10^9$/l)	>100	0
	>50, ale ≤ 100	1
	≤50	2
Concentration of fibrinogen/fibrin degradation markers (FDP; D-dimery)	Normal	1
	Moderate	2
	Growth significant increase	3
Prothrombin time extended	o < 3 s	0
	o ≥ 3 s, ale < 6 s	1
	o ≥ 6 s	2
Fibrinogen concentration (g/l)	>1.0	0
	≤1.0	1
DIC acute diagnosis	≥5	

Table 2.
Point algorithm for the diagnosis of acute disseminated coagulation Intravascular [29–31].

intravascular. The severity of this abnormality varies greatly—from asymptomatic increase in urea (BUN) and serum creatinine up to complete anuria. Concomitant hypotension and intravascular coagulation syndrome may increase renal impairment. Blood clots that form in the renal arterioles cause cortical kidney attacks. Haemoglobin released from red blood cells also reacts nephrotoxically with nitric oxide (NO), damaging the epithelial cells of the renal tubules and the stroma that remains after their breakdown [33, 34].

Intravascular haemolysis modulates blood pressure and local blood flow through changes in the metabolism of the physiological vasodilator—nitric oxide (NO). NO can bind to thiol groups and haemoglobin haeme [35]. The connection of NO with haeme Fe^{2+} impairs oxygen transport through Hb. The presence of O_2 leads to oxidation of NO to NO_3 and oxidation of Fe^{2+} to Fe^{3+} and the formation of methaemoglobin. The interaction between Hb and NO is regulated by the allosteric transition of haemoglobin R (oxyHb) to the T form (deoxyHb). In oxyHb, cysteine is exposed at position 93 of the haemoglobin amino acid chain (Cys 93β). It is known that a significant proportion of NO does not immediately bind to $HbFe^{2+}$ heme, instead it binds to cysteine, resulting in the formation of the S-nitrosothiol derivative Hb (SNO-Hb). This process is reversible, so SNO-Hb releases NO, which is transported to endothelial receptors, where it participates in the regulation of vascular wall tone and blood flow. In the case of haemolysis of red blood cells, the free haemoglobin released from them reacts with NO much faster and more strongly than Hb inside cells [35]. The effect of intravascular haemolysis described above may be very similar to the side effect caused by transfusion of first-generation stromal haemoglobin solutions. This has been tested for its use as a substitute for red blood cells. It had vasoconstrictive and, as a result, hypertensive effect. This effect is largely attributed to the binding nitric oxide by free haemoglobin (NO) [36].

6. Clinical symptoms of transfusion haemolytic reactions

Intravascular haemolysis is accompanied by haemoglobinaemia and usually also haemoglobinuria, whereas extravascular haemolysis can only be

Initial symptoms of haemolytic transfusion reactions	Complications
Intravascular haemolysis Nausea or vomiting Ache Dyspnoea Decrease in RR and/or tachycardia Dark urine	Kidney failure Disseminated intravascular coagulation (DIC)
Extravascular haemolysis Fever and/or chills Nausea or vomiting Ache Dyspnoea	Kidney failure disseminated intravascular coagulation (DIC)

Table 3.
Initial symptoms of haemolytic transfusion reactions.

accompanied by anaemia. In both cases, the patient's serum bilirubin increases, but it depends on the degree of haemolysis as well as liver function [1]. Elevated LDH is always observed with intravascular haemolysis, not always with extravascular haemolysis. Reduced haptoglobin levels usually occur in both types of haemolysis. Drop in blood pressure is much more common in patients with intravascular than extravascular haemolysis. Renal failure and DIC are also more commonly associated with intravascular haemolysis. Some patients may experience organ failure such as the pancreas, heart and even multiple organ failure that threatens the patient's life.

In unconscious patients and patients under general anaesthesia, it may be difficult to recognise a haemolytic transfusion reaction, as some symptoms may go unnoticed (e.g. pain and nausea). Pain, which is described as a symptom of haemolytic reactions, is located at the puncture site, back, chest, groin and head. The occurrence of pain in the haemolytic transfusion reaction is not clear. It is probably the result of direct stimulation of nociceptive nerves in perivascular tissue by bradykinin, which, in turn, is released during sudden activation of complement [37]. Clinical manifestations are shown in **Table 3**.

7. Causes of haemolytic transfusion reactions

7.1 Haemolytic transfusion reactions caused by alloantibodies

The most common cause of haemolytic transfusion reactions is the immunological destruction of red blood cells resulting from the reaction of antibodies in the recipient's blood and the antigens present on the transfused donor's blood cells to which these antibodies are made.

Antibodies capable of destroying transfused blood cells are called clinically relevant antibodies, and the transfusion reaction in the event of immunological incompatibility depends on: (1) specificity of antibodies; (2) thermal amplitude of the antibodies; (3) IgG classes and IgG subclasses; (4) number, density and spatial configuration of antigenic sites on red blood cells; (5) the ability of antibodies to activate the complement system; (6) plasma concentrations of antibodies and (7) volumes of transfused red blood cells. A very important feature of all antibodies responsible for causing a haemolytic transfusion reaction is its in vitro activity at 37°C.

Antibodies detected at a lower temperature are not considered clinically relevant, for example, anti-A1, anti-M and anti-P1, whose optimal reaction is usually at low temperature, but if detected at 37°C, they can cause destruction of red blood cells with the appropriate antigen. They then become clinically significant.

Features of antibodies (specificity, class and heat amplitude) and antigens (density of antigenic sites and their distribution) against which the antibodies directed are interconnected. In different people, antibodies with a particular specificity most often occur in the same class of immunoglobulins and have a similar heat amplitude, for example, anti-A, anti-B and anti-AB from the ABO system often belong to both IgM and IgG classes, they bind complement and have an extended thermal amplitude of up to 37°C.

A and B antigens are highly immunogenic. Anti-A, anti-B and anti-AB antibodies are involved in causing an early intravascular transfusion reaction, and transfusion of incompatible blood in the ABO system poses a threat to the recipient's life, especially when group A red blood cells are transfused to a patient with group O. Sixty-one (61%) of all haemolytic transfusion-related fatal reactions are associated with the ABO incompatibility [38, 39]. A contrasting example is the Lua antigen and anti-Lua antibodies. They are usually IgM molecules, are rarely active at 37°C and usually do not bind complement. Lua antigens have uneven distribution on red blood cells and are weakly immunogenic. No cases of acute haemolytic reaction caused by anti-Lua antibodies have been reported, delayed transfusion haemolytic reaction is rare and occurs only in mild form.

Not all detectable alloantibodies that react with red blood cells can cause a haemolytic reaction. The specificity of the antibodies potentially responsible for intravascular and extravascular haemolysis is shown in **Table 4**.

Similar reactions to anti-A and anti-B come from anti-PP_1P^k, anti-P1 and anti-Vel. Other antibodies cause intravascular haemolysis, but sometimes they may be accompanied by intravascular haemolysis. Such reactions were observed in the following blood group systems: Rh, MNSs, Lutheran, Kell, Duffy, Diego and Lewis. The mechanism of appearance of intravascular symptoms has not been fully explained, because although some of the antibodies bind complement components,

Blood group system	Intravascular haemolysis	Extravascular haemolysis
ABO, H	Anti-A, -B, -AB, -H in the Bombay phenotype	
Rh		All
Kell	Anti-K	All
Kidd	Anti-Jk^a	Anti-Jk^a, -Jk^b, -Jk^3
Duffy		Anti-Fy^a, -Fy^b
MNS		Anti-M, -S, -s, -U
Lutheran		Anti-Lu^b
Lewis	Anti-Le^a	
Cartwright		Anti-Yt^a
Vel	Anti-Vel	
Colton		Anti-Co^a
Dombrock		Anti-Do^a, -Do^b

Table 4.
Specificity of selected antibodies associated with haemolytic transfusion reactions.

their reactions end with C3 components. Only in the case of rare haemolytic reactions due to anti-Lea it was shown that the coated cells are destroyed by the spleen macrophages very slowly and in the event of transfusion of large volumes of red blood cells, they become inefficient. Then intravascular haemolysis coincides with visible haemoglobinuria [40, 41]. Interesting clinical point of view are antibodies from the Kidd system. They activate the complement system to the stage of binding of the C3b component, causing extravascular haemolysis. However, the symptoms in some recipients, or the occurrence of a reaction already during a blood transfusion and haemoglobinuria, indicate that the destruction of blood cells also takes place inside the vessel. In the laboratory setting, anti-Jka antibodies are called "insidious" antibodies because they are often difficult to detect due to their low concentration, and yet they can cause a severe haemolytic complication [41].

Patients with antibodies found to be clinically insignificant may theoretically be given a blood transfusion from a donor with the antigen to which they are directed. In clinical practice, however, such antibodies can sometimes destroy donor blood cells. Therefore, if possible, blood without this antigen should be selected [41].

Another cause for haemolytic transfusion reaction may be a secondary immune response in patients who have developed alloantibodies during previous transfusions of blood components or pregnancy. This is called delayed haemolytic transfusion reaction (DHTR) in which current blood transfusion stimulates memory lymphocytes and stimulates the production of alloantibodies directed at incompatible antigen found on transfused blood cells [21, 42]. In approximately 50% of cases, alloantibodies produced after transfusion or pregnancy cease to be detected after a few months, and this period of time depends on the specificity of the antibodies and the individual characteristics of the immune system. Schonewille et al. found that, using current laboratory methods, 25% of red blood cell antibodies become indeterminate on average after about 10 months from production [43]. Therefore, pre-transfusion tests may not always detect the presence of antibodies. Antibodies stimulated for synthesis may cause symptoms of haemolysis after 3–10 days, usually very mild and their presence can be detected after 10–21 days. **Table 5** presents features of delayed haemolytic transfusion reaction and the time of their occurrence.

Antibodies that cause a delayed haemolytic transfusion reaction are IgG molecules that are binding or non-binding for complementary components. Their specificity is most often directed to the antigens of the Rh, Kidd, Duffy, MNS and Kell systems [14]. In approximately 11% of cases, more than one antibody specificity is

Time (days)	Occurrence	Explanation
0	Negative pre-transfusion test	Antibody titres below detection threshold
1	Red blood cell transfusion	
3–10	Clinical symptoms of haemolysis	Acceleration of transfused blood cells destruction
10–21	Post-transfusion testing of blood samples: DAT and screen of antibodies positive	Increase in antibody titre; donated blood cells coated with antibodies
>21	DAT can be negative	Destruction of donor blood cells in reticuloendothelial system and/or liver
>21–300	DAT may be positive, eluate testing may show presence of alloantibodies or panagglutination	Alloantibodies not specifically associated with autologous red blood cells or produced warm antibodies

DAT—direct antiglobulin test.

Table 5.
Features of late hemolytic transfusion reaction and time of their occurrence [21].

detected. In rare cases, the result of transfusion alloimmunity in DHTR may be the production of autoantibodies (warm IgG autoantibodies or cold autoagglutinins). This phenomenon occurs in patients with sickle cell disease [44–46].

7.2 Haemolytic transfusion reactions due to passive transmission of alloantibodies in blood components or in blood products

Transfusion of plasma, platelet or granulocyte concentrate from donors incompatible in the ABO system with the recipient may lead to acute haemolytic transfusion reaction and even death. The severity of the reaction depends on the titre of anti-A and/or anti-B antibodies in the transfused plasma or in the blood component containing the plasma, and on its volume [47–49]. Tests on the ABO system titre in group O apheresis concentrates of platelets show that 26% of samples have an anti-A or anti-A, B antibody titre of 64 or higher. This concentration may be responsible for causing a haemolytic reaction [50]. In turn, the results of studies by Coolig et al. [51] carried out in pooled platelet concentrates of whole blood groups showed that 60% of them had anti-A titres of at least 64 [51]. Repeated transfusions of ABO incompatible platelet concentrate may lead to accumulation of anti-A antibodies in the recipient's plasma, which may result in severe haemolytic reactions [52]. Unfortunately, despite many studies, it has not been possible to determine the critical titre of anti-A and/or anti-B antibodies that would be safe in the event of transfusion of ABO incompatible platelet concentrates, and in many countries, proprietary haemolysis prevention programs have been developed for recipients of incompatible platelets [48–50, 53].

Haemolytic transfusion reactions due to passively transferred anti-A and/or anti-B antibodies have also been observed in patients after intravenous immunoglobulin administration [54]. Spath et al. [55] analysed reports available in the literature describing cases of haemolysis in patients treated with intravenous immunoglobulins [55]. They showed that the haemolytic reaction is induced by IgG anti-A/B antibodies present in immunoglobulin products. The reaction generally occurs in high-dose IVIG recipients [55].

7.3 Haemolytic reaction associated with the "bystander immune cytolysis"

The haemolytic transfusion reactions may have a different immunological origin than the reactions of antibodies in the recipient's blood and the antigen present on the donor's blood cells. This additional mechanism occurs when recipient's red blood cells are destroyed by a reaction called "bystander immune cytolysis". It is defined as the immunological destruction of red blood cells by antibodies whose specificity corresponds to antigens found on other cells/blood cells (e.g. HLA antigens found on leukocytes and plasma proteins), while red blood cells are only close to this immunological "confusion" [56]. They are destroyed by the complement system, although they did not participate directly in the antigen-antibody reaction. One of the reasons for this haemolytic reaction is the binding of the C567 complement complex, activated in an immune reaction, to the membrane of red blood cells not participating in the reaction but located in the vicinity [56]. Blood cells are destroyed as a result of the activation of the binding of the remaining components of C8 and C9 complement and the formation of the MAC complex on the blood cells [56]. The mechanism of "bystander" haemolysis is similar to the destruction of blood cells in patients with paroxysmal nocturnal haemoglobinuria [57, 58]. A characteristic feature of the cell membrane of these blood cells is the lack or weak expression of the CD55 (DAF) and CD 59 (MIRL) proteins, which are complement inhibitors. This makes the subject more susceptible to haemolysis. It was found that

when red blood cells became the "bystander" of leukocyte reactions and antibodies directed to them, they underwent haemolysis. The reaction of anti-HLA antibodies with leucocytes caused complement activation, which resulted in haemolysis of the patient's red blood cells sensitive to the complement [59]. It is noteworthy that in patients with a haemolytic reaction associated with the immune cytolysis of the "bystander" not only transfused red blood cells but also autologous blood cells of the patient were destroyed.

8. Differentiation

Early haemolytic transfusion reactions should be differentiated with septic shock due to bacterial contamination of the blood component, as well as anaphylaxis and bleeding. In addition, immune haemolysis of nocturnal paroxysmal haemoglobinuria or autoimmune anaemia should also be considered. The cause of an early haemolytic reaction may also be congenital haemolytic anaemia, for example, glucose-6-phosphate dehydrogenase deficiency or microangiopathic haemolytic

- Alloantibodies responsible for haemolysis
- Delayed serological transfusion reaction
- Autoimmune haemolytic anaemia
- Cold agglutinins disease
- Non-immune haemolysis
  - use of inappropriate fluids
  - improper storage of blood components
  - defects in the blood-heating devices
  - needle diameter too small, haematocrit of transfused red blood cells too high
  - an inappropriate method of freezing/thawing red blood cells
  - using the wrong infusion pumps
  - bacterial infection of blood components
  - mechanical damage to blood cells, artificial valves
- Congenital haemolytic anaemia
- Haemoglobinopathies
- Drug-induced haemolysis of red blood cells
- Microangiopathic haemolytic anaemia
  - thrombocytopenic thrombosis (TTP)
  - haemolytic uremic syndrome
  - HELLP syndrome
- Bleeding
- Nocturnal paroxysmal haemoglobinuria
- Polyagglutination
- Infections
  - *Clostridium perfringens*
  - Malaria
  - Babesiosis

Table 6.
Differential diagnosis of haemolytic transfusion reactions [1].

anaemia (TTP, HUS and HELLP). In differential diagnosis, attention should also be paid to non-immune reasons related to improper blood storage, transfusion of red blood cells through a small needle diameter, etc.

Differential diagnosis of delayed haemolytic transfusion reactions includes latent sources of infection, autoimmune haemolytic anaemia, cold agglutinin disease, nocturnal paroxysmal haemoglobinuria, bleeding, mechanical destruction of red blood cells, for example, artificial heart valves and TTP.

It should be noted that an increase in body temperature and white blood cell count, typical for DHTR, can be interpreted as a sign of infection. In some patient groups, it may be difficult to recognise a delayed haemolytic transfusion reaction. Patients with liver failure are a special problem. Haemoglobinemia is not diagnosed in the serum of these patients due to jaundice, often direct antiglobulin reaction (DTA) is positive and elevated bilirubin and LDH are found.

Another group are patients with absorbing haematomas. They may be similar to delayed haemolytic reactions. Elevated unbound bilirubin, LDH and decreased haptoglobin are observed. The presence of fibrinogen degradation products from an absorbing haematoma can be interpreted as a DIC symptom. DHTR can be identified in these patients by the presence of antigen on the transfused red blood cells to which the antibodies may be directed. **Table 6** presents the differential diagnosis of haemolytic transfusion reactions.

9. Diagnosis of transfusion haemolytic reactions

If a haemolytic transfusion reaction is suspected, medical personnel should immediately stop transfusing a blood component. The blood unit should be checked at the patient's bedside, whether it was properly administered. Often, the clinical manifestations of haemolytic reactions are not clear, and the cause of the complication should be differentiated with bacterial infection. Therefore, prior to conducting laboratory tests of donor blood, bacteriological examination of the component remaining after the transfusion cessation should be conducted.

9.1 Tests carried out in case of suspected early hemolytic transfusion reaction

Laboratory tests—mainly serological—are crucial for the diagnosis of an early haemolytic reaction. The type of laboratory tests performed for early transfusion haemolytic reactions is shown in **Table 7**.

The basic serological examination consists of direct antiglobulin testing (DAT); determination of blood group and RhD in donor and recipient; repetition of the serological compliance test. A test should be performed for the presence of antibodies in the recipient before and after the transfusion. Positive DAT indicates haemolysis of red blood cells of immunisation origin. A negative DAT result does not exclude haemolysis, it may mean that the transfused blood cells have been destroyed by alloantibodies or the method used is not very sensitive. Alvarez et al. [60] compared the sensitivity of DAT performed by technique using monospecific IgG antiglobulin, flow cytometry and antibody elution. The study showed that DAT could only indicate 10% of antibody coated cells [61]. Performing DAT in the red blood cell eluate, its sensitivity was 1%. Flow cytometry proved to be a similarly sensitive method.

The re-determination of the ABO and RhD blood group of the recipient before and after the transfusion and in the donors' blood will exclude errors in the identification of the recipient or blood sample (wrong blood in tube (WBIT)). Test results carried out by Biomedical Excellence for Safer Transfusion Working Party of The International Society for Blood Transfusion in 10 countries with 62 institutions,

Blood product	Confirm ABO, Rh
	Confirm other antigen types, if indicated
Blood bank laboratory	Direct antiglobulin test (DAT)
	Confirm ABO, RH, antibody screen and identification
	Post-transfusion haemolysis check
Clinical laboratory	Complete blood count, platelet count
	Urinalysis for haemoglobin
	Serum bilirubin, creatinine, urine quantitation, coagulation profile
	DIC evaluation

Table 7.
Type of laboratory tests and the location of their performance in the case of early transfusion reaction.

Laboratory indicators	Frequency (%)
Low haptoglobin 92	92
Positive DAT	89[*]
Increased bilirubin concentration medium/slow	80
The presence of haemoglobin in plasma and/or urine	88 vs. 52[**]

[*]*Negative DAT mainly associated with HTR in ABO incompatibility.*
[**]*IHTR vs. DHTR.*

Table 8.
Changes in laboratory indicators in haemolytic transfusion reactions [56].

which examined a total of 690,000 blood samples, showed that the frequency of WBIT is 1 in 165. In two countries, Sweden and Finland, which have implemented national identification systems, this frequency was 1 for 1986 samples [61].

All-antibody screening for recipients is generally performed using routine testing on standard blood cells. A panel of standard cells should contain clinically important antigens in a homozygous form to detect the presence of weak antibodies. The test should be performed on serum/plasma samples taken before and after transfusion. If positive results indicate alloantibodies are present, they should be identified. Detection of a specific antigen on the donor's blood cells is the confirmation that the detected alloantibodies were responsible for the haemolytic transfusion reaction. If negative results are obtained, additional tests should be performed, for example, PTA PEG, polybrene test and PTA NaCl test. If negative results persist, the test should be repeated after a week and after 2 weeks, as in some patients, the antibodies may have been consumed to destroy transfused incompatible red blood cells.

Laboratory tests that help to differentiate haemolysis include determination of free haemoglobin in the blood and urine, haptoglobin and lactate dehydrogenase (LDH) and bilirubin. While interpreting the obtained test results, it should be kept in mind that haemolysis or shortening the survival time of red blood cells can be caused by non-immunological factors, for example, adding hypotonic fluids to red blood cells, inefficient heating or freezing devices, etc. [62]. **Table 8** presents changes in laboratory indicators in transfusion haemolytic reactions [56].

9.2 Tests carried out in case of suspected delayed haemolytic transfusion reaction

Laboratory tests show anaemia, increased LDH and bilirubin, decreased haptoglobin and higher white blood cell counts in post-transfusion haemolytic reactions. Bilirubin concentration depends on the severity of haemolysis and liver function.

Serological tests show positive DAT and the presence of all red blood cell antibodies that were not detected prior to transfusion. This means that after transfusion of red blood cells, the production of alloantibodies directed to the antigen found on the transfused blood cells occurs.

Positive DAT with anti-IgG reagents or with anti-IgG and anti-C3 reagents is generally seen as two red blood cell populations. It has been observed that in some patients, the coating of blood cells includes not only transfused, but also autologous red blood cells. Positive DAT with anti-IgG and anti-C3d reagents may persist for several months [9].

Alloantibody testing should be performed in the intermediate antiglobulin test (IAT) and enzyme test. In both methods, in addition to the reference blood cells, the patient's autologous blood cells should be included. Depending on the specificity, alloantibodies responsible for the delayed transfusion reaction activate in characteristic tests, for example, antibodies from the Rh system react in an enzymatic test, often also in anti-globulin testing. In contrast, anti-K, anti-Fya antibodies react in an anti-globulin test. Positive reactions with allogeneic blood cells are accompanied by positive auto control of the patient's red blood cells. Ness et al. [9] showed that the formation of warm autoantibodies after the onset of DHTR is relatively common. Approximately one-third of patients who were examined 25 days after the onset of the reaction presented a positive DAT due to autoantibodies with broad specificity [9]. The incidence of autoantibodies after DHTR may be even higher because autoantibodies may mimic the specificity of alloantibodies.

Usually, plasma alloantibodies are detectable at 4–7 days after the transfusion and reach maximum activity between 10 and 15 days after the transfusion. When examining recipient red blood cells using a diagnostic reagent with a specificity corresponding to alloantibodies detected in the patient, mixed agglutination is observed, which indicates the presence of two blood cell populations in the patient's circulation. One of them, which does not react with diagnostic antibodies, is the recipient's autologous blood cells, the other population is antigenically incompatible transfused donor cells, not yet removed from the recipient's circulation.

10. Treatment of transfusion haemolytic reactions

Treatment of early haemolytic transfusion reactions depends mainly on the patient's condition, which must be closely monitored. It is most important to observe the clinical symptoms of the recipient and stop the blood transfusion at the right moment. Particular attention should be paid to the patient's circulation. In the event of a marked decrease in blood pressure, make-up fluids should be transfused and pressure amines should be administered. However, it is important to avoid overloading the circulation with fluids, especially in patients with heart or kidney failure. Catheterisation of the pulmonary artery helps to monitor the situation.

In some cases, an exchange transfusion should be considered, bearing in mind that the haemolysis intensity depends mainly on the volume of incompatible blood transfused. For exchange transfusion, red blood cells without an antigen should be used against which the patient has developed alloantibodies. The decision to carry it out must be balanced and the course carefully monitored. It should be emphasised that in patients with an early reaction due to ABO incompatibility, exchange transfusion may reduce the risk of serious complications or death. For patients with ongoing haemorrhage choosing a blood for transfusion may be difficult. However, it should be remembered that these difficulties must not cause risk of haemorrhage. Often the way out of this situation is transfusion of O RhD negative red blood cells.

Therapeutic intervention	Indication	Typical dose
Hydration	Prevent renal impairment Maintain urine output >100 ml/h	Normal saline and/or 5% dextrose 200 ml/m^2/h
Alkalinisation of urine	Prevent renal impairment Maintain urine pH > 7.5	NaH$_2$CO$_3$ 40–70 mEq
Diuresis	Prevent renal impairment	Mannitol 20% 100 ml/m^2 Furosemide 40–80 mg
Vasodilation	Increase renal blood flow	Dopamine 1–5 µg/kg/min
Anticoagulant	Treat DIC	Heparin 5–10 u/kg/h
Red cells exchange transfusion	Decrease load of incompatible red cells	Exchange of one estimated red cells mass
Plasma or platelet transfusion	Treat haemorrhagic complications of DIC	Platelet—1 unit platelet/10 kg or 1 unit apheresis platelet FFP—10 ml/kg
Intravenous immunoglobulin (not standard therapy)	Prevent extravascular haemolysis	0.4 mg/kg

DIC—disseminated intravascular coagulation; FFP—fresh frozen plasma.

Table 9.
Therapeutic options in haemolytic transfusion reactions [1].

The prevention of renal failure is aided by an early prevention of hypotension. A fluid balance should be maintained, the use of dehydrating agents (mannitol and furosemide) is helpful, but their oliguria should be closely monitored. Low doses of dopamine (1–5 µg/kg/min) may be used to maintain renal circulation, but this may not be effective.

Treatment and prevention of DIC during haemolytic transfusion reaction is controversial. Heparin is recommended because it additionally acts as an inhibitor of the complement activity and limits haemolysis. However, there is a danger of bleeding. Another method of treating early haemolytic transfusion reaction is to use a high dose of 0.4/kg intravenous immunoglobulin per 24 h after blood transfusion.

Delayed haemolytic transfusion reactions are well tolerated by most patients. Additional fluid and diuretic therapy are usually not necessary. Depending on the severity of the anaemia, transfusion of blood components should be avoided until the antibodies responsible for the reaction have been identified and the appropriate selection of blood cells has been made. Attempts have been made to use high doses of intravenous immunoglobulins to prevent haemolytic reactions in patients who have been immunised for winter and for whom compatible red blood cells have not been selected [63]. The main procedure for subsequent transfusions is to select red cells that do not contain the antigen for which all antibodies have been detected. **Table 9** summarises the treatment options used in haemolytic transfusion reactions.

11. Prevention of haemolytic transfusion reactions

Data on the incidence of haemolytic transfusion reactions vary from country to country and change over time. There are several causes. One of them was the use of improved techniques for detecting clinically relevant alloantibodies, which reduce the number of haemolytic transfusion reactions observed in blood recipients. In addition, the widespread introduction of automation and computerisation to

pre-transfusion studies, which significantly limits the possibility of errors in serology laboratories and blood banks.

The above improvements, however, did not significantly affect the elimination of mistakes made in hospitals leading to transfusion of inappropriate blood to the patient. These include, among others, errors in collecting blood samples from patients and blood transfusions to a wrong patient. These errors are the most common cause of ABO incompatible transfusions, threatening the patient's life.

The introduction of haemovigilance transfusiological surveillance systems has enabled the analysis of all fatal and severe transfusion reactions. It allows to identify malfunctioning procedures leading to transfusion reactions. It enforces the introduction of procedures eliminating further errors.

12. Summary

Preventing haemolytic transfusion reactions by focusing on advances in serology and transfusion medicine has significantly reduced their incidence. Progress in understanding reaction pathophysiology has helped clinically assess patients and treat them effectively. It is possible that technological progress enabling modification of red blood cells and the use of red blood cell substitutes will significantly change transfusion practice in the future and eliminate the occurrence of haemolytic transfusion reactions. But until then, HTRs will remain the most important adverse post-transfusion reaction.

Author details

Jolanta Korsak* and Anna Piotrowska
Military Institute of Medicine, Poland

*Address all correspondence to: jkorsak@wim.mil.pl

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