Delayed hemolytic transfusion reaction due to anti-Jkα

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INTRODUCTION

Immune mediated hemolytic transfusion reactions (HTRs) occur as a consequence of immune conflict between red blood cells (RBCs) membrane antigens and specific alloantibodies in recipient’s plasma1.

Destruction pathway of immune sensitized RBCs, e.g. the mechanism of hemolysis is determined by biological characteristics of the antibody: class, subclass, complement activation ability, specificity, thermal range etc., then by antibody concentration as well as the stage of the complement activation on RBCs membrane. Above mentioned properties of antibodies determine whether RBCs destruction occur within circulation (intravascular hemolysis) or is mediated by macrophages of the mononuclear phagocite system (MPS) predominantly of liver and spleen (extravascular hemolysis)2.

Antibodies that are capable to activate the complement pathway up to final lytic complex (C5b-C9) usually cause the RBCs rupture with release of free hemoglobin into the plasma, usually followed by dramatic symptoms and clinical manifestation of the acute hemolytic transfusion reaction (AHTR)3,4.

It is most likely for IgG antibodies to cause extravascular hemolysis due to their less ability for complete complement activation. Class IgM is most commonly capable to activate the complement only up to C3 stage and therefore those antibodies are likely to be the cause of extravascular hemolysis with or without clinical manifestation of delayed hemolytic transfusion reaction (DHTR). Specific receptors (Fc and C3b) of the macrophages of MPS bind RBCs coated with IgG antibodies and C3b component of the complement followed with RBCs hepatic and splenic sequestration with consecutive extravascular hemolysis5,6.

Background: Kidd antibodies are very heterogeneous and difficult to detect. They have been frequently implicated in delayed hemolytic transfusion reactions (DHRs). Case report: A 64 year old female (6 pregnancies, 2 deliveries, 4 abortions) with none red cell (RBC) transfusions in the history was admitted to hospital due to pneumonia and severe anemia. On admittance hemoglobin (Hb) level was 63g/L and hematocrit (Ht) 0.21L/L. The blood sample of the patient was sent to laboratory for serologic testing since RBC transfusions were required. Patient appeared to be O Rh(D)+ with negative both direct antiglobulin (DAT) and routine antibody screen (ID-DiaCell I+II+III-P). Three units of packed RBCs with negative crossmatch (tube method) were prepared. Patient received two units on Day 2 and one more on Day 3 without any discomfort. Hematological values after the third unit were: Hb 116g/L and Ht 0.37L/L. On Day 6 she started to feel week, tired, with nausea and mild jaundice. Her Hb and Ht had dropped to 99g/L and 0.33L/L respectively, with tendency of dropping further (Day 7: Hb 83g/L, Ht 0.26L/L). Total serum bilirubin was 58.9 umol/L (normal range 20.5 umol/L) and direct fraction was 14.9 umol/L (normal range 7 umol/L). DTHR was suspected. Antibody identification performed by ID-DiaMed Gel Technique (GT) showed the presence of anti-Jkα with dosage phenomenon.

All three previously transfused units were typed Jkα and the patient’s RBCs were Jk(a-b+). She received two units of Jkα negative packed RBCs and was well enough to be discharged on Day 14.

Conclusion: It is important to monitor clinical effect of transfusion regularly and to provide good team work between specialists of transfusion medicine and related medical staff. The policy of transfusion practice is to keep pretransfusion sample for three weeks and to perform cross-match tests on the samples no older then 24h and 48h respectively.
Antigens of the Kidd system are detected only on RBCs and are carried by an integral membrane glycoprotein, which transports urea through the RBC’s membrane. Urea transporter is expressed on RBCs and on endothelial cells of vasa recta in the kidney.

The Kidd antibodies are very heterogeneous, clinically significant immune antibodies which are produced as a consequence of immunisation by transfusion, pregnancy, rarely by transplantation or as a result of autoimmune process. Despite the fact that Kidd antigens are poor immunogens, Kidd antibodies have been frequently implicated in DHTRs but extremely rare are the cause of AHTRs. Following immunization, Kidd antibodies fall rapidly to undetectable levels in the plasma, therefore they are often difficult to detect.

The Kidd antibodies are mainly of the class IgG, subclasses IgG1 and IgG3, capable to bind complement up to C3 stage. They might be the cause of the hemolytic disease of the fetus and newborn (HDN) but rarely severe ones. Those antibodies may manifest a dosage effect reacting only with red cells with double doze of the antigen (homozygous cells).

The Kidd antibodies react better on antiglobulin testing with polyspecific anti-IgG + anti-C3 as well as with monospecific anti-C3 since they are usually detected indirectly through the complement that they bind to RBCs, therefore the reaction with monospecific anti-IgG usually lack. These antibodies usually give stronger hemagglutination with enzyme treated test cells.

Polyethilene glycol (PEG) antiglobulin technique has been reported to be more sensitive compared to indirect antiglobulin test (IAT) and enzyme technique, in detecting antibodies of the Kidd system.

CASE REPORT AND SEROLOGICAL STUDIES

Mrs. S. J. Aged 64, body weight 53kg, with 6 pregnancies, 2 deliveries, 4 abortions and none RBC transfusions in the history, was admitted to the hospital due to bronchopneumonia and severe anemia.

On admittance hemoglobin (Hb) level was 63g/L and hematocrit (Ht) 0,21L/L.

Diagnostic esophagogastroduodenoscopy was performed and showed duodenitis erosiva without gastrointestinal bleeding. On Day 1 routine serologic testing was performed and showed that the patient was group O RhD positive with negative polyspecific direct antiglobulin test (DAT) in the standard tube test. Routine antibody screen was also negative with enzyme treated test cells (ID-DiaCell I+II+III, lot N0 4519.65.01/02) using natural gel cards.

On Day 2 the patient got two units of packed RBCs with negative antiglobulin crossmatch in the standard tube test with RBCs suspended in low ionic strength saline solution (LISS, NBTI).

National Blood Transfusion Institute, Belgrade). Following the transfusion, plasma Hb concentration was 97g/L and Ht increased to 0,33L/L. On Day 3 another packed RBCs unit was administrated with negative antiglobulin tube crossmatch which was carried out on the fresh sample. The patient received all three units without any discomfort and the expected elevation in Hb and Ht levels was achieved (116g/L and 0.37L/L, respectively).

The patient’s condition worsened on Day 6. She started to feel weak, tired, she complained of nousea, experienced fever to 38,6°C and she collapsed. Her Hb and Ht dropped to 99g/L and 0,33L/L, respectively and continued to decline over the next 24h.

On Day 7 hematological values were: Hb 83g/L and 0,26L/L (Figure 1). All the symptoms persisted and mild jaundice appeared on the same day with elevation of total serum bilirubin to 58,9umol/L (normal range 20,5umol/L), direct bilirubin to 14,9umol/L (normal range 7umol/L) and lactate dehydrogenase to 613U/L (normal range 160-320U/L) Since initial decrease in Hb and Ht aroused suspicion of gastrointestinal bleeding, control esophagogastroduodenoscopy and test for microhemorrhages in feces were performed. The absence of bleeding supported the idea that RBC’s hemolysis was responsible for non effective transfusions. On the same day, upon the request of the laboratory for transfusion testing, the new sample was sent for serologic evaluation.

The patient RBCs showed the positive (2+ reaction) DAT in the standard tube test with polyspecific (anti-IgG + anti-C3, goat, NBTI, Belgrade) antihumane globulin reagent (AHG).

The red cells were also 2+ positive in the DAT with polyspecific AHG when tested by the more sensitive ID-DiaMed Gel Technique (GT). Using the ID-DiaMed Monospecific Coombs gel cards the DAT was positive (2+) only with monoclonal anti-C3d but negative with anti-IgG (rabbit).

Antibody screening was carried out by using the gel technique. The test was performed with papain treated test cells (ID-DiaCell I+II+III-P lot N0 4519.65.01/02) using natural gel cards and with non treated test cells (ID-DiaCell I+II+III lot N0 4518.65.01/02) on LISS/Coombs gel cards.

Figure 1.

THE CHANGES IN PATIENT’S HEMOGLOBIN CONCENTRATION DURING HOSPITALISATION. TRANSFUSION ARE INDICATED WITH BLACK STARS, WHITE CIRCLE INDICATES THE CLINICAL MANIFESTATION OF DHTR
With enzyme treated homozygous Jk^a/Jk^a cells, a 3+ reaction was obtained, but with heterozygous Jk^a/Jk^b cells the weaker (2+) agglutination was obtained. Non treated cells gave also positive but weaker reaction (2+ with homozygous cells and 1+ with heterozygous cells). The dosage phenomenon was also clearly demonstrated in the antibody identification test which was performed by using the in-NBTI made erythrocyte panel of ten cells (SERP lot N^0 92/2000 and lot N^0 93/2000). The antibody identified in serum was anti-Jk^a.

Patient was tested against the panel test cells, which were washed and resuspended to 0.8% in LISS (NBTI, Belgrade) by using ID-DiaMed LISS/Coombs gel cards. The identification panels contained four homozygous cells (Jka/Jka) and five heterozygous cells (Jk^a/Jk^b). The stronger agglutination was obtained (2+) with all Jka/Jka cells. With Jka/Jkb cells the reaction was clearly weaker, with some 1+ and with the other ++.

Since the policy of transfusion laboratories for pretransfusion testing is to keep the crossmatched samples for three weeks, the patient’s initial sample was available for serological phenotyping of the Kidd blood group antigens. The RBCs appeared to be Jk^a/Jk^b.

The compatibility testing was then performed on the serum taken on Day 7 and RBCs of recently transfused blood units by the IAT tube crossmatch as well as by the GT.

Since one unit showed clearly stronger reaction compared to other two units, we have performed phenotyping of the RBCs of the transfused units for Jk^a antigen. The unit which gave stronger reaction was determined to be homozygous (Jk^a/Jk^a) and the other two shown to be heterozygous (Jk^a/Jk^b).

Finally, two units of antigen (Jk^a) negative packed RBCs, compatible by antiglobulin crossmatch (tube and GT) were released. Following the transfusion her Hb and Ht rose to 97g/L and 0.31L/L, respectively.

On Day 14 the patient recovered sufficiently to be discharged with negative DAT, Hb 101g/L, Ht 0.32L/L and normal range of LDH and bilirubin.

DISCUSSION

Kidd antibodies are known to be associated with DTHRs, which generally occur due to secondary immune responses. In our case the patient was obviously primarily immunized to Jk^a antigen many years ago through one of the six pregnancies.

Kidd antibodies are well known for their tendency to fall rapidly to very low levels in the Plasma. Our case is in accordance with previous reports since they haven’t been detected in the patient’s plasma in serologic testing even with sensitive serologic techniques, performed prior transfusion.

Our case clearly demonstrates the secondary immune response which had been provoked by the repeated exposure to the Jk^a antigen by transfused Jk^a+ RBCs. The secondary, anamnestic response involves the recognition of the antigen by the persisting B lymphocytes memory pool, followed by proliferation, differentiation and maturation into plasma cells which produce specific alloantibodies in sufficient quantity capable to cause clinical manifestation of DHTR. In our case the whole process of anamnestic reaction was completed within 96h. A rapid increase in antibody concentration was achieved. Hemolysis of allogeneic Jk^a+ RBCs persisted in the next 72h, with the clinical symptoms and laboratory findings which confirmed clinical DHTR.

Clinical manifestation of DHTR suggested rapid and strong anamnestic immune response as well as efficient destruction and elimination of the "sensitized" RBCs by macrophages of the MPS, which was confirmed with negative DAT on Day 9.

The dosage phenomenon in this alloantibody's reaction was shown through the IAT crossmatch by using both the tube and the gel techniques as well as by antibody screening test using papain treated test cells on natural gel card and with non treated cells on LISS/Coombs Dia Med gel cards.

Antibody identification showed most clearly the dosage phenomenon since it yielded very weak agglutination with some heterozygous cells.

In our case the most sensitive technique used in detecting Jk^a antibody was GT with enzyme treated red cells. On the other hand, the Dia Med – ID Microtyping Gel System has been shown to be superior to the conventional tube method.

In detection of complement-binding antibodies of the Kidd system it is very important to use the reagent which contains an anti-complement activity.

It is also necessary to emphasize the need of using red cell diluents which don’t inhibit complement binding of fresh samples. In all serologic procedures we have performed, the in-NBTI manufactured LISS has been used, because it meets all above mentioned requirements.

This case clearly highlights the need of better cooperation between all related medical staff in the course of recognition of adverse effects of hemotherapy, where active role of the specialist of transfusion medicine is a must.

REZIME

KASNA HEMOLIZNA TRANSFUZIONA REAKCIJA UZROKOVANA ANTI-Jk^a ANITETLOM

Uvod: Kidd antitela su vrlo heterogena i teško se otkrivaju. Često su odgovorna za pojavu kasne transfuzijske hemolize reakcije (KTHR). Prikaz slučaja: Osoba četverog pola, starost 64 godine (trudnoća 2, porodjaja 2, prekida trudnoće 4), sa neaktivnom transfuziološkom anamnezom, hospitalizovana zbog pneumonije i teške anemije. Prije prijema, vrednost hemoglobina (Hb) 63g/L i hematokrit (Ht) 0,21L/L. Zbog potrebe za transfuzijom eritrocita, uzork krvi je poslat u laboratoriju za serološko testiranje i pripremu krvi za transfuziju. Krvna grupa BORh(D) + (poz). Direktan antihumanglobulin-iski test (DAT) i rutinski skrining antitela (ID-DiaCell 1+11-III-P) negativni. Pripremljene su tri jedinice deplazmatiziranih eritrocita sa negativnom interreakcijom (klasična metoda i epruveti). Bolesnica je primila dve jedinice eritrocita drugog tipa, a treću trećeg dana, bez ispoljavanja nepovoljnih efekata. Vrednosti hematoloških
parametara nakon primenjene tri jedinice iznosile su: Hb 116g/L i Ht 0,37/L. Šestog dana dolazi do pojave subjektivno lošeg stanja sa osećajem malakalosti, mučninom i blagom žuticom. Vrednosti Hb i Ht se snažavaju na 99g/L odnosno 0,33/L sa tendencijom daljeg pada (sedmog dana Hb 83g/L, Ht 0,26/L). Vrednosti ukupnog i direktnog bilirubina iznosile su 58,9umol/L (normalna vrednost 20,5umol/L i 14,9umol/L (normalna vrednost 7umol/L). Suspetovana je KTHR.

ID-DiaMed gel tehnikom identifikovano je antitelo Jk^a specifičnosti sa ispoljenim fenomenom doze. Tipizacijom je utvrđeno da su sve tri transfundovane jedinice eritrocita Jk^a+, a fenotip eritrocita bolesnice Jk(a-b+). Bolesnica je primila dve jedinice tipiziranih Jk^a negativnih deplazmatsanih eritrocita i u dobro opštem stanju otpuštena iz četrnaestog dana. Zaključak: Pažljivo praćenje kliničkih efekata transfuzije i dobra saradnja sa specijalistima transfuzione medicine je od izузетne važnosti u blagovremenu prepoznavanju transfuzionih reakcija.

U transfuziološkoj praksi, pretransfuzioni uzorak se čuva tri nedelje, a interreakcija se izvodi sa uzorcima koji ništa ne zna od 48 časa.

Ključne reči: transfuzija eritrocita, Kidd krvno grupni sistem, komplement, odložena hemoliza

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