An acute haemolytic transfusion reaction due to anti-Jkα

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Introduction
The Kidd system antibodies are characteristically difficult to detect. They show variability in immunoglobulin class, subclass and serological characteristics. They are generally detected by an antiglobulin test, using a polyspecific antiglobulin or complement antiserum. Often, the antibodies are only detected using cells with a double dose (homozygous) expression of Kidd antigens, enzyme-treated cells or by using sensitive immunohaematological techniques.

Case report
A 73-year-old woman, with a history of two pregnancies and no red cell transfusions, was admitted to our hospital. She had severe anaemia, cirrhosis related to hepatitis C virus infection, cryoglobulins, mild ascites infection and mild renal failure. There were no reported incidents of red cell immunisation.

On admission, her haemoglobin concentration was 7.5 g/dL and her haematocrit 23%.

On day 3 of hospitalisation, the first red blood cell (RBC) transfusion was required and performed (day 0). The patient’s group was A1B, Rh+.

The antibody screening was negative, using our standard automated method for pre-transfusion testing (AutoVue System with Ortho BioVue microcolumn, Ortho-Clinical Diagnostics, Inc., Raritan, New Jersey, USA) with polyspecific anti-human globulin (anti-IgG+C3d) and an EDTA plasma sample. She received an ABO/Rh compatible standard packed red cell unit (about 170 mL of packed red cells) with the Type & Screen procedure, as indicated by national legislation.

The post-transfusion level of haemoglobin was 8.4 g/dL.

Additional pre-transfusion tests were performed on day 14, although no transfusion of RBC units was performed.

On day 19 after the first RBC transfusion, two RBC units were requested because the woman’s haemoglobin had decreased to 7.2 g/dL.

The antibody screening tests were still negative, according to our standard automated method. The same day of the request, she received one AB, CCDee standard packed red cell unit, with the Type & Screen procedure.

The transfusion was interrupted 2.5 hours after being started because of a transfusion reaction: chills, lumbar pain and dark red urine.

The pre-transfusion and post-transfusion data indicate that there was no significant change in body temperature (pre-transfusion +37.2 °C - post-transfusion +37.5 °C) and minor modifications of blood pressure (pre-transfusion 130/65 - post-transfusion 140/85) and heart rate (pre-transfusion 78 beats per min - post-transfusion 88 beats per minutes).

Dark red urine was still observed 24 hours after the reaction.

To determine the cause of the post-transfusion haemolysis, immediately after the reaction, the patient’s post-transfusion serum and plasma samples were inspected for evidence of haemolysis and compared with the plasma pre-transfusion sample, using the scale of values proposed by Elliot1.

The post-transfusion samples were grossly haemolysed, with a dark red hue similar to haemolysis of 200 mL of RBC in 3,000 mL of plasma.

The post-transfusion biochemical values (Figure 1) revealed an increase in free plasma haemoglobin from 18.0 mg/dL to 260 mg/dL (reference value <5.5 mg/dL).

The post-transfusion level of haemoglobin was 6.5 g/dL.

It was concluded that the patient suffered from the classic clinical symptoms of an acute haemolytic transfusion reaction (AHTR), confirmed by concomitant biochemical changes.
Material and methods

Since other clinical or therapeutic causes of acute haemolysis, such as clerical error and mechanical haemolysis were excluded, an extensive immunohaematological work-up was carried out to search for the antibody responsible.

This work-up included the following tests.

1) Direct antiglobulin tube test (DAT): two volumes of reagent to one volume of a 3% red cell suspension, centrifugation, macroscopic reading, incubation at 20 °C for 5 minutes of all non-reactive tubes with polyspecific and anti-C3 antisera, centrifugation and macroscopic reading, addition to each negative tube of IgG-coated and C3-coated Coombs control cells (Immucor Inc., Norcross, GA, USA). We used undiluted and diluted (serial two-fold dilutions in saline) polyspecific and monospecific anti-human globulin reagents: anti-IgG+C3d from three manufacturers (Immucor; Diagnostics Scotland, Edinburgh, UK; Ortho-Clinical Diagnostics); anti-IgG from three manufacturers (Immucor; Diagnostics Scotland; Ortho-Clinical Diagnostics); anti-IgA and anti-IgM (Biotest AG, Frankfurt/Main, Germany). The rapid acid elution (ELU-KIT™ II, Immucor) was used for the recovery of antibody bound to red cells.

2) Indirect antiglobulin test (IAT), using plasma and serum and the standard method (Ortho BioVue, Ortho-Clinical Diagnostics) with polyspecific anti-human globulin, 50µL of LISS (Ortho® BLISS, Ortho-Clinical Diagnostics), 10µL of a 3% red cell suspension (from a screening panel - Surgiscreen Ortho-Clinical Diagnostics or red cells from selected blood donors), 40µL of plasma or serum, 15 minutes of incubation at 37 °C, centrifugation for 5 minutes and macroscopic reading; in addition, to demonstrate complement dependence of antibody in stored and aged sera, we also performed the IAT using fresh complement (from a pool of 50 blood donors of AB blood group) added to sera (one volume in three volumes of test sera).

3) Tube IAT (100µL of plasma or serum, 100µL of Gamma PeG™ additive, Immucor), one drop of a 3% red cell suspension, 15 minutes of incubation at 37 °C, four washes with saline, addition of two drops of monoclonal anti-IgG (Immucor), centrifugation for 1 minute and macroscopic reading.

4) Standard tube ficin-IAT using 100µL of plasma or serum, one drop of a 3% red cell suspension, 45 minutes of incubation at 37 °C, four washes with saline, addition of two drops of monoclonal anti-IgG (Immucor), centrifugation for 1 minute and macroscopic reading. The ficin-treated red cells (from the screening panel or from blood donors) were prepared using a commercial

Figure 1 - Free plasmatic haemoglobin levels. The clinical symptoms of an acute haemolytic post-transfusion reaction were confirmed by increases of free plasmatic haemoglobin from 18 mg/dL to 260 mg/dL (reference value <5.5 mg/dL).

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stabilized ficin solution (Ortho-Clinical Diagnostics) as suggested by the manufacturer.

5) Saline 20 °C: 100µL of plasma or serum, one drop of a 3% red cell suspension, 30 minutes of incubation at 20 °C, centrifugation for 1 minute and macroscopic reading.

6) Solid-phase IAT using a commercial panel for identification (Ready-ID® Extend I and II, Immucor), 100µL of LISS additive (Capture® LISS, Immucor), 50µL of plasma or serum, 20 minutes of incubation at 37 °C, six washes with saline, addition of one drop of indicator cells (Capture-R® Indicator Red Cells, Immucor), centrifugation and macroscopic reading.

Instead, for cross-matching (Capture-R® Select, Immucor), we prepared a 0.3-0.5% suspension of well washed red blood cells in saline, 50µL of the red blood cell suspension, centrifuged the strip, carried out six washes with saline, and proceeded with LISS and plasma or serum as described for identification.

7) Erythrocytes Magnetized® technology3 (ScreenLyys, Diagnost, France), which uses IgG-coated plates. To prevent the neutralisation of anti-IgG, 60µL of low-ionic and diluent (NanoLyys and Screen Diluent, Diagnost) solutions were dispensed before the addition of samples and RBC (12 µL plasma, 15µL of a 1% three-cell panel, Hemascreen, Diagnost).

After incubation, without any washing/centrifugation, magnetisation was performed and the plates were placed on a magnetic workstation (FreeslyNano, Diagnost) that allowed the adherence of sensitised-magnetic RBC.

8) Typing of the red cells of the patient and transfused units was performed using the standard agglutination tube methods.

**Results**

During our investigations, at the time of collecting samples, no haemodilution was performed in the patient by infusion of saline or other liquid.

### Table 1 - Results of serological investigations

<table>
<thead>
<tr>
<th>Time</th>
<th>IAT* column (standard screening)</th>
<th>Cross-match</th>
<th>IAT† (tube)</th>
<th>Identification of antibodies in serum</th>
<th>No. of transfused RBC units</th>
<th>AHTR</th>
<th>Erythrocytes Magnetized® Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Negative</td>
<td>No, T&amp;S</td>
<td>Negative</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td>1 (Packed RBC)</td>
<td>No</td>
<td>nd</td>
</tr>
<tr>
<td>Day 14</td>
<td>Negative</td>
<td>No</td>
<td>Negative</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td></td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>Day 19</td>
<td>Pre-transfusion</td>
<td>Negative</td>
<td>No, T&amp;S</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td>Yes</td>
<td>nd</td>
</tr>
<tr>
<td>Day 19</td>
<td>Post-transfusion</td>
<td>Negative</td>
<td>No, T&amp;S</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>Day 20</td>
<td>Post-transfusion</td>
<td>Negative</td>
<td>No</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Day 21</td>
<td>Post-transfusion</td>
<td>Weak score at only with Jk(a+)</td>
<td>No</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>Day 25</td>
<td>Post-transfusion</td>
<td>Negative</td>
<td>No</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td></td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>Day 32</td>
<td>Post-transfusion</td>
<td>Negative</td>
<td>No</td>
<td>Anti-IgG only in SP and EMT(‡)</td>
<td></td>
<td></td>
<td>nd</td>
</tr>
</tbody>
</table>

(* IAT: indirect antiglobulin test; † DAT: direct antiglobulin test; ‡ SP: solid-phase; # EMT: erythrocytes magnetized technology; ($) AHTR: acute haemolytic transfusion reaction. |
The results of our investigation are reported in table I. An anti-Jka was detected only by the solid-phase method and Erythrocytes Magnetized® technology in the sample of day 14 (after the first transfusion) while all other methods gave negative results. No differences were detected between serum and EDTA plasma. Two out of four cells Jk(a+b-) show weak positive results for the presence of anti-Jka by the column agglutination method, using the plasma sample of day 21 (2 days after the reaction).

These two cells had a double dose expression of the target antigen. This result was not confirmed by further investigations on the samples drawn on day 25 and day 32 after the first transfusion.

The tube DAT was negative for all samples tested. Despite this, we performed an elution from pre-transfusion and post-reaction blood samples (day 19) and tested them by tube PEG-IAT and by the solid phase method.

The anti-Jkα was detected in the post-transfusion eluate, using only the solid phase method.

No antibodies were detected in any samples by microcolumn agglutination, using serum with fresh complement added.

The patient's RBC phenotype, determined on pre-transfusion and on all post-transfusion samples, was A, B, CCDee, Jk(a-b+); Fy(a+b+); M+N+S+s+.

Samples from the first RBC unit transfused and the unit involved in the reaction were available and typed as Jk(a+b+) and Jk(a+b-), respectively.

The antibody screening test was negative in both blood donors using the solid-phase method and positive results in cross-matches were detected only in solid-phase, using the pre and post-reaction samples (day 19), while results were negative in solid-phase using selected AB, CCDee, Jk(a-b+) units.

After our investigations, the patient recovered and needed no further transfusions.

Discussion

After the discovery of the first anti-Jkα, many cases involving Kidd system antibodies have been reported and a substantial number have been implicated in AHTR. Kidd antibodies are usually IgG or a combination of IgG and IgM, pure IgM examples are rare.

IgM Kidd antibodies can bind the complement and are consequently detected only by broad spectrum antiglobulin reagents, containing an anti-complement component.

It has been generally considered that the major antibody component of Kidd antibodies must be IgG with complement-fixing ability.

Current guidelines for pre-transfusion testing indicate that antiglobulin reagents with a potent anti-IgG can effectively detect such antibodies, without the need of an anti-complement component.

This has encouraged Blood Transfusion Centres to use techniques which compromise the detection of the complement, on the basis that the antibodies will be detected by the anti-IgG component of the antiglobulin reagent.

We report a case in which the IgG component of an alloantibody-Jkα was demonstrated only in solid-phase and by Erythrocytes Magnetized® technology, but not detected in the patient's plasma or serum, when tested by microcolumn agglutination with broad spectrum antiglobulin or in tubes with anti-IgG antiglobulin reagent.

The antibody was the cause of an AHTR associated with the transfusion of Jk(a+) blood units.

The use of plasma rather than serum was not the cause of false negative results in the initial screening tests, although the column agglutination tests (using gel technology) can compromise the detection of Kidd antibodies.

This problem may sometimes be associated with shear forces.

Because the primary immune response could have already occurred with a previous pregnancy, the first transfusion of random Jk(a+b+) could have caused a secondary immune response, that resulted in the haemolysis of the second Jk(a+b-) transfused unit.

As recently reported by the results of a large retrospective multicentre study, we determined that the anti-Jkα was the cause of a secondary response in a short interval of time after transfusion (14 days after the first transfusion); the level of antibody was such that serological detection was only possible using the most sensitive technique.

This same circumstance was reported by Callahan et al.; the Authors described an anti-Jkb, detected only in solid-phase and not by PEG or LISS techniques, which caused a delayed haemolytic transfusion reaction in a patient affected by sickle cell disease.

Naturally occurring anti-Jkα antibodies, detectable only in solid-phase, were previously reported by Beamsey et al. and the use of manual polybrene7 was the method of choice for the detection of weak anti-Jkα.

During the investigations, 2 days after our patient's reaction, the antibody increased to levels detectable by our standard method and then immediately declined to a point at which antibody was no longer detectable in
This case demonstrates one example of a haemolytic transfusion reaction due to anti-Jkα, which was not detectable with a highly sensitive routine test method (microcolumn agglutination).

Our data document the well-known difficulties in the detection of Kidd antibodies and highlight the importance of using additional sensitive techniques and multiple methods, particularly in cases of haemolytic transfusion reactions.

In this case, significant haemolysis caused by anti-Jkα was detected only in solid-phase and by Erythrocytes Magnetized™ technology and not by column or liquid-tube technologies.

References
2) American Red Cross Immunohematology. Methods and Procedures. 1993 American Red Cross, Rockville.