In conclusion, we suggest that TB should be excluded in any febrile patient with hypercalcemia especially in countries where tuberculosis is endemic.

References

Anti-Rh17 (Anti-Hr0): a rare Diagnostic and Management Problem

N. Salamat, F. A. Bhatti, A. Hussain, Ziaullah
Armed Forces Institute of Transfusion, Rawalpindi.

Introduction
Of all blood group systems, anti Rh antibodies are the commonest cause of haemolytic disease of the newborn (HDN) and its diagnosis and management plan is well established. Also immunization with ‘D’ antigen can be effectively prevented by prophylactic use of anti-D Immunoglobulin. Therefore its prevalence has declined but now less common alloantibodies are frequently encountered posing different management problems. Anti Rh17 is also a rare alloantibody produced after immune stimulus, by individuals who lack C/c and E/e antigens of Rh blood group on their red cells. This rare blood group is designated as D- and was first described by Race and Sanger in 1950. We report diagnosis and management of a pregnant lady with anaemia, mitral valve disease and anti Rh17 alloantibody who required blood for operation and her baby suffered from hydrops foetalis.

Case Report
A 35 years old lady, (gravida 4, term 2, premature 0, abortion 0, live birth 1) diagnosed case of moderately severe mitral valve stenosis was admitted in Armed Forces Institute of Cardiology (AFIC) with amenorrhoea of 7 months duration, shortness of breath NYHA III/IV and palpitations for 5 months. Her first pregnancy ended in intrapartum death in 1998 at full term. Next year a full term healthy boy was delivered by Caesarean-section and patient was transfused one unit of whole blood in post partum period. The child did not have a history of neonatal jaundice or anaemia. In 2001 her next pregnancy ended with intrauterine death at 19th week of gestation and dead hydropic fetus was delivered by Caesarean-section. Fourth pregnancy started in Oct 2002 and she became progressively dyspnoeic, had palpitations and was hospitalized. Her physical examination revealed tachycardia with regular rhythm, BP of 110/70 mmHg, respiratory rate 24/min and pitting edema of both feet. Grade II diastolic murmur was audible on precordium. Abdominal fundal height was 38 weeks while gestational age by dates was 34 weeks. Her haemoglobin level was 4.3 g/dl, with red cells showing microcytosis and hypochromia. Echocardiography showed moderately severe mitral stenosis and ejection fraction of 65%. Rest all of the investigations were within normal limits. Ultrasound of abdomen revealed single alive foetus with ascites and pleural effusion, suggesting hydrops foetalis, whereas placenta was large and fundal and she had developed polyhydramnios. She was prescribed 1/V injection of Iron sucrose (Venofer) 20 mg/day for 6 days and also received oral iron/folic acid 200 mg, Spironolactone 40 mg, and Digoxin 0.25 mg every day. Clotted blood sample of the patient was sent to our centre for grouping and cross matching. The serological test results following this request are summarized in Table 1. Interpretation of these results was that patient is having IgG type, alloantibody, directed against some high frequency (public) red cell antigen. To determine the specificity of antibody and to find compatible blood, rare red cells panels and antisera were
needed which were not available with us therefore the patient’s blood samples were sent to International Blood Group Reference Laboratories (IBGRL), Bristol, UK. Meanwhile search for compatible blood among first-degree relatives was made and yielded no success.

Erythropoietin at a dose of 40 µg/kg/day was started in addition to other antianaemics and haemoglobin was successfully raised to 10.5 g/dl. One autologous unit of 300 ml was collected preoperatively. Because of scar tenderness and distressing dyspnoea due to hydramnios caesarean section was performed, three days after autologous donation on 25 May 2003, delivering dead hydropic foetus. Tubal ligation was not performed. Autologous unit was transfused in postoperative period and the patient made uneventful recovery.

Results from IBGRL were received 10 days after her operation, which are shown in Table 2. The rare alloantibody in proposita was antiRh17, which was further

### Table 1. Initial serological tests.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test</th>
<th>Reagent</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>ABO Grouping</td>
<td>(Monoclonal Bio Tech Labs Ltd, UK)</td>
<td>Tube, IS</td>
<td>BEE</td>
</tr>
<tr>
<td>2.</td>
<td>Rh ’D’ Grouping</td>
<td>Monoclonal IgM + IgG Blended, Biotech Labs Ltd, UK</td>
<td>Tube, IS</td>
<td>D Positive</td>
</tr>
<tr>
<td>3.</td>
<td>Cross match with random donors</td>
<td>Lorne UK, LISS, AHG</td>
<td>Tube LISS IAT</td>
<td>Incompatible with all donors</td>
</tr>
<tr>
<td>4.</td>
<td>Antibody screening with 3 Cell panel</td>
<td>Maxi screen cells Lorne, UK</td>
<td>LISS-Additive IAT in Tube</td>
<td>1+ at 37°C with all cells and 4+ agglutination with all three cells with IAT</td>
</tr>
<tr>
<td>5.</td>
<td>Auto control</td>
<td>-</td>
<td>RT, 37°C, IAT</td>
<td>Negative</td>
</tr>
<tr>
<td>6.</td>
<td>Direct antiglobulin test</td>
<td>AHG, Lorne UK</td>
<td>Tube</td>
<td>Negative</td>
</tr>
<tr>
<td>7.</td>
<td>Antibody identification, 11 cell panel</td>
<td>Lorne, UK</td>
<td>RT, 37°C, LISS Additive</td>
<td>1+ at 37°C with all cells and 4+ agglutination with all cells in both IAT’s</td>
</tr>
<tr>
<td>8.</td>
<td>Kell phenotype</td>
<td>DiaMed-ID antigen</td>
<td>Micro column</td>
<td>K-, k+</td>
</tr>
</tbody>
</table>

### Table 2. Confirmatory serologic tests.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test</th>
<th>Reagent</th>
<th>Result</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Reaction with Rh Null cells</td>
<td>-</td>
<td>-</td>
<td>No agglutination</td>
</tr>
<tr>
<td>2.</td>
<td>Rh phenotype of red cells</td>
<td>DIA Med ID II</td>
<td>No agglutination with anti C, c, E, e</td>
<td>D-- phenotype</td>
</tr>
<tr>
<td>3.</td>
<td>Chemiluminescence test (CLT)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>a.</td>
<td>Prediction of transfusion outcome: Red Cell</td>
<td>Opsonic index without AB serum</td>
<td>Opsonic index With AB serum</td>
<td>R$_1^w$R$_1$ 17.6 29.4</td>
</tr>
<tr>
<td>b.</td>
<td>Prediction of HDFN Red cell</td>
<td>-</td>
<td>-</td>
<td>CLT %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R$_1^w$R$_1$ 176</td>
<td>Rh Null 2</td>
</tr>
</tbody>
</table>

HDFN: Haemolytic disease of fetus and newborn
confirmed by phenotyping patient’s red cells at our center. There were no C/c and E/e antigens on her cells (Table 3, Figure). The opsonic index in chemiluminescence test show significant transfusion and haemolytic disease causing potential of the antibody. Later Rh phenotyping of patient’s family was done, whose results are shown in Table 3 and Figure 1. Both parent's RBC phenotype was B, D+,C+,e+ and their most likely genotypes were DCE/D-- . The proposita and her sister were D-/D--. None of the family members was Rh Null. The patient and her husband were counselled in detail. Patient and her sister having regular follow-up at our institution.

**Discussion**

The proposita developed an unusual alloantibody, anti Rh 17, as a result of a transfusion in her second post partum period. This antibody has reactivity against Rh17 (Hro) antigen of Rh blood group system and is formed by individuals who lack all Rh antigens (C/c and E/e antigen therefore have no Hro antigen) except D¹. Genetically Rh antigens are coded by 2 genes on chromosome 1. RHD coding for D antigen and RHCE is responsible for C/c and E/e antigens.² Molecular basis for D-- phenotype is not clear but recombination of RHD and RHCE genes have been documented.³ This antibody is formed by immune stimulus⁴ like transfusion or pregnancy and usually show single specificity but sometimes separate specificities like anti 'e' may be seen.⁵ Our patient escaped immunization due to fetomaternal haemorrhage during her first two pregnancies hence she was able to have an alive and normal baby after the second pregnancy. Immune causes of hydrops foetalis were not excluded after birth of first hydroyopic baby (third pregnancy) although it required a simple test like antibody screening with standard reagents. Anti Rh ‘D’ is the most common alloantibody causing morbidity and mortality due to HDN⁶ followed by anti c, Kell, C, E, e, Duffy and Kidd⁷. These must be excluded especially with a history of hydroyopic baby, irrespective of the Rh blood group. Determining specificity of antibody is important for future obstetrical management. Therefore all efforts should be made to achieve this.

Initial red cell serological tests and their results are shown in Table 1. These indicated that either patient has clinically significant, pan reactive, IgG alloantibody, against some public antigen or an autoantibody, which is capable of crossing the placenta and causing foetal complications. Negative direct antiglobulin and auto control test excluded the possibility of auto antibodies and also these are rarely associated with hydrops. Other alloantibodies likely to yield similar results with the red cell identification panel used were anti Tja, anti U, and anti 'k' (chelano) antibody. Anti chelano was excluded indirectly by phenotyping patient's cells for 'k' antigen, on the basis that if the cells were 'k' antigen positive then antibody could not be 'anti k'. In the absence of appropriate reagents we could not exclude other suspected antibodies and did not consider Rh system antibodies, as the red cell panel in use was adequate to exclude common anti Rh antibodies. Diagnosis of specificity of antibodies against high frequency, public antigens is important for future counseling, obstetric outcome, neonatal management and arranging transfusion support. Help from a reference center abroad (International Blood Group Reference Laboratories (IBGRL), Bristol UK) was sought and patient's serum samples were sent to them. In the absence of inventory or database for rare donors in any local blood bank it was almost impossible to find compatible blood. We resorted to time old method of checking first-degree relatives of patient for compatible phenotype. None of those brought to us had compatible blood although later family studies did reveal that one sister (fifteen years old) had exactly the same phenotype as proposita (Table 3). There is no national frozen blood bank of rare donors either which could have provided rare phenotype red cells as is usually possible in many developed countries.

Phenotyping of red cells was done at our center as the cells were haemolyzed during transportation, which revealed absence of C/c and E/e antigens on patient's red cells (Table 2). IBGRL, Bristol UK communicated to us that this phenotype is most prevalent in Japanese population and the frozen blood banks there can provide such blood on request. Also we were provided important contacts in Japan. The results of Chemiluminescence test (CLT) done at IBGRL yielded expected results because the past or present history of hydrops is the most sensitive indicator of clinical significance of any alloantibody (Table 2).

Elective Caesarian section had to be done without delay to avoid complications of the dead baby and also worsening polyhydramnios was causing tenderness in previous surgical scar and respiratory distress to the patient.
This precluded liaison with Japanese blood banks, which we were contemplating. Meanwhile small volume autologous donation was collected as patient's haemoglobin had risen adequately within one week with antianaemics and erythropoietin. Autologous donation in the form of predeposit, preoperative haemodilution or cell salvage are very important means of providing blood for cases with rare alloantibodies and limited resources.\textsuperscript{10} It can prove to be a safe option even with co-morbid conditions provided haemoglobin is adequate and blood is collected carefully. This can be done as small volume frequent donations. Tubal ligation was not performed at the time of caesarian section and we are unaware of any contraceptive counselling of the couple, therefore we assume that they may be at risk for future foetal complications.

People have tried to lower the alloantibody levels in post conception period with plasma exchange\textsuperscript{11} without much success therefore such efforts may not help our patient in future. Very few similar cases due to anti Rh17 antibodies have been reported.\textsuperscript{12-15} Usually this antibody causes moderate to severe haemolytic disease of the newborn. The foetal management includes intrauterine transfusions with frozen rare donor blood or maternal blood if diagnosed early during pregnancy.\textsuperscript{13} In other cases exchange transfusions with maternal blood (frozen previously or freshly donated) has been used with successful outcome.\textsuperscript{16} Some authors have reported good neonatal outcome even with repeated incompatible Rh D negative exchange transfusions.\textsuperscript{17,18} Use of intravenous immunoglobulins to inhibit haemolysis before and following the exchange transfusion has been reported to be successful.\textsuperscript{15,19}

The extensive family studies of proposita were undertaken (Table 3 and Figure) with an aim of finding more compatible donors for future as well as for sending blood of those affected to New York blood center, USA, for identification of mutation in the family which may be a new one, not previously reported. Also these individuals can help us to prepare rare red cell reagent panels that are expensive if imported and has short shelf life. These individuals can also become part of rare donors programme in future. Our center is now better prepared to arrange blood for the patient or her foetus in case of intrauterine transfusion, in future. The options are to get blood donation from her sister, autologous donation and/or frozen blood requested from abroad.

We gained useful insight that excluding antibodies in Rh blood group systems before considering any other specificity is the most useful practical approach in case of anti IgG alloantibodies in pregnant ladies. In absence of rare red cell panels, phenotyping for Rh blood group system using basic sera or micro column gel system could help to exclude a significant group of alloantibodies, although this is a deviation from traditional teaching and can not be applied to recently transfused patients. Antibody screening of all pregnant ladies, irrespective of Rh group should be carried out as per standard guidelines for serological screening tests in pregnancy.\textsuperscript{20} This is particularly indicated after birth of a hydropic baby. Maintaining rare red cells reagents may not be possible for every transfusion center, however liaison with international reference centers can have fruitful results.

Acknowledgements

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References