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Mixed field reactions in ABO and Rh typing chimerism likely resulting from twin haematopoiesis

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Case Report

A 70-year-old female was found to have mixed field reactions in both ABO forward typing and RhD and RhE typing prior to an operative procedure. She had no history of transfusion or transplantation, but had seven previous pregnancies. The patient underwent surgery without a requirement for blood transfusion prior to full resolution of the ABO and Rh discrepancy.

ABO testing was performed on patient EDTA anticoagulated blood samples using both tube testing and gel column agglutination (MTS, Ortho Clinical Diagnostics, Pompano Beach, Florida, USA). Repeat testing was performed on a second sample. Patient RBCs were evaluated for reactivity with anti-A, anti-B and anti-A,B from four manufacturers (Ortho BioClone Murine Monoclonal Blend, Ortho Clinical Diagnostics, Raritan, New Jersey, USA; Novaclone, Dominion Biologicals Limited, Dartmouth, Nova Scotia, Canada; Gamma-Clone, Immucor-Gamma, Norcross, Georgia, USA; SeraClone, Bio-Rad Medical Diagnostics, GmbH, Dreieich, Germany). For RhD and RhE typing, monoclonal blend antisera were used by tube test (anti-D reagents Ortho BioClone, Dominion Biological Limited Novacline, and Immucor-Gamma Gammanclone; anti-E reagents Ortho BioClone and Bio-Rad Seraclone).

Serologic and genotyping results are summarized in Table I. Tube testing and the MTS gel method demonstrated macroscopic mixed field reactions on forward grouping using anti-A and anti-A,B while anti-B reagents were non-reactive. Reaction strength on immediate spin ranged from 1 to 2+, depending on the antisera used. On reverse grouping, patient plasma samples reacted 3+ with blood...
Mixed field reactions due to chimerism

Group B RBCs by immediate spin, all other cells (A₁, A₂ and O) tested were nonreactive. Macroscopic mixed-field reactivity was observed with anti-D and with anti-E reagents on immediate spin. Lewis or other blood group phenotypes were not performed.

Since a chimera was suspected on serologic testing, the patient was further questioned and revealed that she had a twin brother, who sadly died a few months after birth.

Samples were evaluated further by genotyping and flow cytometry. Genotyping analysis performed on peripheral blood by PCR-ASP and PCR-RFLP methods routinely used in the laboratory demonstrated an apparent ABO*A₂.01/O.01.01 genotype.

Genomic DNA was used for analysis of 21 short tandem repeat (STR) loci dispersed over 17 chromosomes to establish the presence of more than two alleles. This was performed with the established and validated method used for clinic routine analysis at the Transplantation Immunology Laboratory, University and Regional Laboratories, Region Skåne, Lund, Sweden. In six of the 21 STR loci tested there was clear evidence of presence of more than two alleles confirming that the chimerism was present and detectable at the DNA level.

Genomic DNA was used for real-time PCR analysis for presence of a marked specific for the Y chromosome, the SRY (Sex Determining Region Y) gene, since the patient’s twin was a male. This was performed with the established and validated method for prediction of fetal RHD status from maternal plasma at the Nordic Reference Laboratory for Genomic Blood Group Typing, University and Regional Laboratories, Region Skåne, Lund Sweden based on a publication by Finning et al. This assay includes a marker for the SRY gene. The presence of male DNA in this female patient was shown by a positive signal for the SRY gene.

Flow cytometry performed by the laboratory with monoclonal anti-A revealed two distinct populations of RBCs. The majority (88%) were very weakly reactive, while the remaining 12% were strongly positive at the same expression level as the A₂ control RBCs (Figure 1). All RBCs were non-reactive with anti-B. Anti-H testing was strongly positive, showing that all RBCs in the patient express the H antigen at the high level observed in group O and A₁ RBCs (Figure 2). In order to explain why the majority of the patient’s "group O RBCs" appear to express low levels of A antigen, FUT2 genotyping was performed to establish that she is a secretor (data not shown), whose A glycolipids in plasma can get adsorbed onto the group O RBCs, which will therefore be stained weakly by anti-A.  

Table 1 - Summary of serologic and molecular typing.

<table>
<thead>
<tr>
<th>ABO group</th>
<th>Forward group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>1-2+mf</td>
</tr>
<tr>
<td>Anti-B</td>
<td>0</td>
</tr>
<tr>
<td>Anti-AB</td>
<td>1-2+mf</td>
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</tbody>
</table>

<table>
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<tr>
<th>Reverse group</th>
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</thead>
<tbody>
<tr>
<td>A1 cells</td>
</tr>
<tr>
<td>A2 cells</td>
</tr>
<tr>
<td>B cells</td>
</tr>
<tr>
<td>O cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Molecular typing ABO<em>A₂.01, ABO</em>O.01.01 present</th>
</tr>
</thead>
<tbody>
<tr>
<td>RhD</td>
</tr>
<tr>
<td>RhE</td>
</tr>
</tbody>
</table>

mf=mixed field, macroscopic

Discussion

This case is an example of an ABO and Rh discrepancy that was encountered during routine serological pre-transfusion testing in a patient who was not previously known to be a chimera. Although an uncommon occurrence, this situation can arise as some chimeric individuals are previously undiagnosed and asymptomatic. It is particularly poignant in this case that

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Figure 1 - The sample was tested with a primary antibody, anti-A (clone ES-15) with a PE conjugated rat-anti-mouse Ig as the secondary antibody. In the histogram the x axis represents PE-derived fluorescence on a logarithmic scale and the y axis counts on a linear scale. Included in the histogram are relevant control RBCs of different phenotypes. For dot plots the x and y axes represent FL1- and PE-derived fluorescence, respectively, on logarithmic scales. Two populations of cells can clearly be distinguished when tested with anti-A and the percentage of each population is given in the dot plot.

Figure 2 - The sample was tested with a FITC conjugated anti-H (clone BRIC231). In the histogram the x axis represents FITC-derived fluorescence on a logarithmic scale and the y axis counts on a linear scale. Included in the histogram are relevant control RBCs of different phenotypes. For dot plots the x and y axes represent FITC- and FL2-derived fluorescence, respectively, on logarithmic scales. One population is seen indicating that all RBCs express H-antigen at a normal level.
biological evidence remains of a twin who died in infancy so many years ago.

Mixed field reactions were observed in ABO, RhD and RhE typing using both tube testing and the MTS gel technique. Flow cytometric analysis also revealed a dual population of RBCs (a group A minority and a group O majority weakly stained with A glycolipids as expected in a secretor). Genotyping revealed the presence of both the ABO*A2.01 and ABO*O1.01 alleles. It is probable that sufficient DNA from the minority population was present to be amplified by the genotyping assays, and it is likely that the patient's majority genotype is ABO*O1.01/O1.01, and that the minor population of cells, most likely acquired in utero from her twin, is ABO*A2.01/O1.01 or ABO*A2.01/A2.01. STR analysis confirmed the presence of more than 2 alleles, and the presence of male DNA was shown by detection of the SRY gene in real time PCR. Since the patient was not the recipient of a recent blood transfusion and had not undergone stem cell or organ transplantation, additional historical information was sought which revealed that she was the product of a twin pregnancy and was the sole survivor.

As it has been previously described, this case illustrates that it is possible for an immunologically immature twin to receive cells in utero, including haematopoietic stem cells, from the other twin through placental blood vessel anastomoses, with subsequent production of a natural permanent genetic chimera within haematopoietic cells resulting in mixed field typing for ABO and Rh. In addition, the finding of mixed-field agglutination for other blood group antigens such as D and E in this patient is a finding well compatible with chimerism and makes an A subgroup as the underlying explanation for the serological findings very unlikely.

In conclusion, this case identified a case of a permanent genetic chimera resulting from twin-twin exchange of genetic material in utero. Suspicion for the presence of a chimera was initially prompted by the findings of an ABO and Rh discrepancy on a routine pre-transfusion sample. Additional questioning uncovered the existence of a deceased twin. Further characterization of the discrepancy was achieved in a reference laboratory, including a demonstration of the use of flow cytometry to delineate and quantify the presence of two distinct cell populations.

Acknowledgements

We would like to acknowledge Mariska Maguire, Canadian Blood Services Crossmatch Laboratory; Asa Hellberg, Lund University for performing the ABO genotyping and real time PCR, and Jill Story, Lund University for helpful comments on the manuscript.

Keywords: mixed field reactions, chimerism, ABO.

The Authors declare no conflicts of interest.

References