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

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Introduction

Discrepancies in ABO and Rh typing due to the presence of mixed field reactions may be encountered in the transfusion medicine laboratory^{1,2}. In order to resolve the discrepancy and to provide compatible blood for transfusion, there is a requirement to obtain relevant historical information from the patient and to perform additional laboratory investigations. Often the cause of the mixed field reactions is easily ascertained on history, when the patient is found to have had recent transfusion or stem cell transplantation from a non-group identical donor. Certain ABO subgroups and pathologic or physiologic conditions may also lead to mixed field reactions on ABO typing; however, they should rarely affect Rh typing^{1,2}. Similarly, a change in RhD phenotype has been described in a patient with leukaemia, but this did not affect ABO typing³.

One of the potential causes of mixed field reactions on ABO and Rh typing is the presence within an individual of a chimeric state or mosaicism^{4,5}. A chimera is present when two or more distinct cell populations containing genetic material from more than one zygote exist within an individual. Although descriptions highlighting the concept of chimerism as a cause of blood group typing discrepancies have existed for decades, chimerism is uncommon and presents challenges when mixed-field agglutination is encountered on forward or reverse ABO typing during routine pre-transfusion testing^{6,7}.

There are several forms of chimerism which have varied manifestations^{1,4,5}. Congenital chimerism refers to the situation where there is either embryo fusion or dizygotic twin-twin blood vessel anastomoses between two dichorionic placentas which results in the exchange of haematopoietic cells. Partial haematopoietic chimerism also exists and is the result of transfusion or stem cell transplantation; in these situations evidence of chimerism is confined to one body compartment (haematopoietic cells).

Both serologic and molecular methodologies may be useful in determining the cause of mixed field reactions.

In addition, a highly sensitive flow cytometric method may assist in characterizing ABO subgroups through the detection of low levels of A and B antigen on red blood cells (RBCs) or on low fractions of RBCs in a sample⁸. Here we describe the use of blood group genotyping and flow cytometry to investigate a patient with mixed field reactivity in ABO, RhD, and RhE typing.

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 Novaclone, and Immucor-Gamma Gammaclone; 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

Table I - Summary of serologic and molecular typing.

ABO group	
Forward group	
Anti-A	1-2+mf
Anti-B	0
Anti-AB	1-2+mf
Reverse group	
A1 cells	0
A2 cells	0
B cells	3+
O cells	0
Molecular typing <i>ABO</i>*A2.01, <i>ABO</i>*0.01.01 present	
RhD	1+mf
RhE	1+mf

mf=mixed field, macroscopic

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^{9,10}.

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.*¹¹. 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 RBCs⁸. 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 A₂ 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^{1,3}.

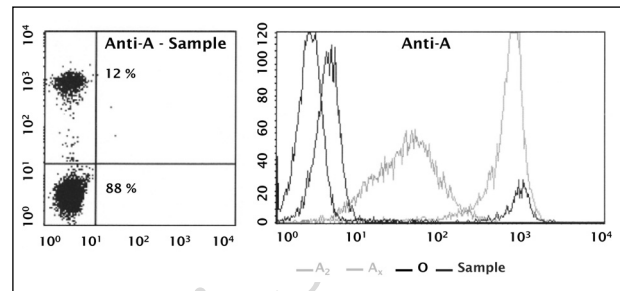


Figure 1 - The sample was tested with a primary antibody, anti-A (clone ES-15) with a PE conjugated rat anti-mouse Ig as the secondary antibody. In the histogram the x axis represents PE-derived fluorescence on a logarithmic scale and the y axis counts on a linear scale. Included in the histogram are 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.

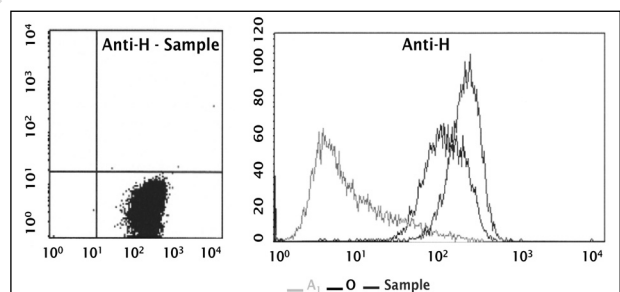


Figure 2 - The sample was tested with a FITC conjugated anti-H (clone BRIC231).

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.

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

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*O.01.01* 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.

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Keywords: mixed field reactions, chimerism, ABO.

The Authors declare no conflicts of interest.

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