Issues in the Serial Titration of Clinically Significant Antibodies in an Antenatal Patient

Kylie Rusford, Sanjeev Chunilal.
Monash Medical Centre, Clayton, Victoria, Australia.

BACKGROUND
Titrations of antibodies in the antenatal patient have an important role in clinical decision-making in the alloimmunised pregnancy. A case is described where changing obstetric practices over time have led to problems in clearly communicating the significance of titration results.

METHOD
A 39 year old female patient (Gravida 5 Para 3) was referred for management of her current pregnancy. This O Rh(D) Negative (rr) patient had a long history of alloimmunisation to C which was first detected in 2000. She had been monitored during two subsequent pregnancies by serial titrations. However the use of Rh(D) immunoglobulin prophylaxis at 28 and 34 weeks added complexity to the monitoring and reporting of her titration results.

RESULTS
The patient was referred to Monash Medical Centre because of a history of Rhesus alloimmunisation leading to the birth in 2000 of a neonate with Rhesus Haemolytic Disease of the Newborn who required phototherapy. The anti-C was measured on 08-Jan-2011 by another Pathology Laboratory and gave a titre of 128 (cells used not reported). On presentation at Monash Medical Centre her anti-C was titred against pooled cells containing a homozygous dose of the target antigen, as is standard practice. We used our routine column agglutination method and obtained an anti-C titre of 8. (1). R_1, R_2, (Cde/Cde) cells were selected for testing, as is recommended (2). This was the same phenotype of cells that were used during the pregnancy in 2000.

When this rr (Cde/cde) patient was administered prophylactic Rh(D) Immunoglobulin on 13-May-2011, R_1, R_2 cells could no longer be used for titration of the anti-C. Therefore heterozygous r'r (Cde/cde) were selected. A change in titre was noted with the change in testing cell (See Table 1). The Standard Operating Procedure in use at the time reported the titre but not the test cells, so the information reported to the clinical team was confusing.

A further complication to the management of the patient was a lack of reproducibility for the parallel testing on the sample from 31-May-2011. The r'r titre on the original test was 16, but gave a result of 2 when tested in parallel with the sample from 14-June-2011. The discrepant results were noted and reported to the clinical team and repeat testing was recommended. However the follow up sample on 5-July-2011 was not labelled adequately and a further repeat was not received until another 2 weeks later.

The underlying cause of the problem with the parallel testing was not clear.

The results for the R_1, R_2, and R_1R_2 cells gave results within one dilution of the original results, which suggests that sample integrity and pipetting accuracy were not the issue. It was hypothesised that the r'r panel cells used as test cells on 31-May-2011 were the cause of the problem, and now the cells used for testing are sourced from suitable donors from ARCBS.

The patient gave birth on 31-July-2011 to an O Rh(D) Positive baby. The baby had a negative DAT and was C Negative.

The Total Bilirubin on Day 0 was 71 μmol/L (Conjugated Bilirubin 17 μmol/L) The Haemoglobin on Day 0 was 228 g/L (RR 135 – 230 g/L). Occasional nucleated red blood cells (3/100 White cells), mild polychromasia and occasional spherocytes were noted on the blood film.

The Total Bilirubin on Day 1 was unchanged at 72 μmol/L (Conjugated Bilirubin 11 μmol/L). The baby was discharged on 02-August-2011.

<table>
<thead>
<tr>
<th>Sample Collection Date</th>
<th>R_1, R_2 Anti-C</th>
<th>R_1, R_2 Anti-D</th>
<th>r'r Anti-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>03-May-2011</td>
<td>8</td>
<td>Not Present</td>
<td>Not Performed</td>
</tr>
<tr>
<td>31-May-2011</td>
<td>8</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>14-June-2011</td>
<td>16</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>13-July-1011</td>
<td>8</td>
<td>1</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 1: Titre Results and Testing Cells used During the Current Pregnancy.

DISCUSSION
This patient demonstrates some of the laboratory issues encountered in the serial titration of clinically significant antibodies.

On presentation it was noted that there was a marked inter laboratory difference in the titration of this antibody. It is important that such patients are managed by one Pathology Service.

The selection and sourcing of suitable cells is also an issue. The NICE method (3) suggests using pooled homozgyous cells where possible, whereas others feel that heterozygous target cells are more clinically relevant, as the affected foetus cannot be homozgyous. Difficulties can be encountered sourcing suitable cells, and our experience with this patient suggests that sub-optimal cells can give discrepant results.

Finally the cells used should be of the same phenotype during the entire pregnancy, and the anticipated administration of prophylactic Rh(D) immunoglobulin must be factored in when selecting the test cells.

CONCLUSION
The use of prophylactic Rh(D) Immunoglobulin adds complications to the monitoring of antibodies during alloimmunised pregnancies. Our Standard Operating Procedures have been updated to ensure that the appropriate cells are selected in the first trimester so that serial titrations can be performed on the same phenotype of cells throughout the entire pregnancy. Also the reporting of results to the clinician has been updated to include the phenotype of the testing cells to remove any ambiguity of results.

REFERENCES