ANTI-D TITRE SCORE AS AN ALTERNATIVE TO CONTINUOUS FLOW ANALYSIS

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The introduction of 2016 BSH guidelines in blood grouping and red cell antibody testing in pregnancy created a new challenge for transfusion laboratories in the management of antenatal women with Anti-D detected in their plasma. A collaborative study was created to look into providing an alternative method to assist in determining the nature of Anti-D and reduce the burden on the hospital transfusion laboratory.

Awkward Anti-D

Advances in antenatal transfusion medicine including the use of prophylactic Anti-D immunoglobulin have seen a decline in the incidence of Immune Anti-D and the associated clinical features of Haemolytic Disease of the Foetus and Newborn (HDFN). However, despite all the efforts of antenatal prophylactic anti-D programmes, allo Anti-D sensitisation can still occur and must be prevented where possible. One of the most complicated issues surrounding detection of allo Anti-D is the differentiation of the nature between immune and passive.

Detecting and identifying Anti-D is a relatively easy practise but the determining of the nature, strength and associated clinical risk is the tricky part. Laboratories cannot assume Anti-D is prophylactic, this must be confirmed. In the UK, the leading process used to assist in the differentiation and clinical risk of Anti-D is by performing quantification using Continuous Flow Analysis (CFA). In most instances this requires the referral of the patient's specimen to a specialist NHSBT reference centre. CFA is also the technique referenced by the Royal College of Obstetrics and Gynaecology (RCOG) guidance with HDFN risk categories based upon CFA quantitation levels.

In the transfusion laboratory, distinguishing between immune and passive Anti-D can require somewhat detective skills, including determining if a mother has received any prophylactic Anti-D, and if she has, when and what dose? Other questions include, if she has had any sensitising events or has she been transfused elsewhere, all ultimately trying to establish if there is any potential that immune Anti-D may have developed. Good communication between the transfusion laboratory and the antenatal clinical team is vital to gaining this important information.

New Recommendations, New challenges

Misinterpretation of the nature of Anti-D can be clinically detrimental, ultimately resulting in HDFN, as highlighted in the 2012 SHOT report. As a result of such findings, the BSH Guidelines for blood grouping and red cell antibody testing in pregnancy 2016 recommended that all Anti-D detected should be referred for CFA, as a preventative measure. However, adhering to these recommendations can result in a substantial increase in CFA referral rates which incurs increased time, cost, and reporting implications for laboratories. As such, there has been a mixed response from laboratories in observance of the recommendation. Some are referring all Anti-D detected, while others use internally validated procedures or algorithms to decide when to send for CFA in order to prevent excessive referrals workload/costings.

The 2016 BSH guidelines also stated that an alternative option could be used 'by a technique that has been validated using large numbers of samples of known concentration'. At present there were no known other methodologies currently validated to this extent.

The Automated Titre

The Ortho Vision system provides a platform to perform automated serial dilutions which can be used for antibody titration. At the 2016 Ortho User group, a group discussion reflecting on the 2016 guidelines resulted in a collaborative team of Transfusion Managers, Biomedical Scientists and Ortho Technical Specialists joining together with the aim of using automated titres, specifically using a Titre Score (TS) to compare against that of the gold standard method of CFA. This was in the hope of easing the potential burdens of complete CFA referral by assessing a potential screening method for those that are suspected to be passive.

Twelve Ortho Vision automated systems were used within the study, with a total of 196 Anti-D titres being performed. The prophylactic category results were very promising and it became quickly apparent that TS could be a useful screening method. Together with transfusion history, Anti-D injection date / dose and the date of the last negative antibody screen and D status of the fetus, a TS cut off value to determine CFA referral could be possible.

Outliers results found within the prophylactic category were mainly due to patients receiving multiple prophylactic injections, a manifestation often also seen in CFA. Reporting of such cases would include appropriate follow up and guidance.

Future application

Once a potential TS cut-off point was established, some of the research sites have been in discussion with their fetal medicine departments and the use of TS prior to referral for CFA has received positive feedback from the clinical teams. In light of this, the hospital transfusion laboratories of the research are eager to validate the TS process within their trusts, some of which are already close to implementation.

Hopefully, this methodology will allow laboratories an alternative to complete CFA referral of all anti-D detected. All immune or suspected immune Anti-D samples should always be referred for CFA quantitation until further validation of the TS has been assessed against clinical outcomes.