

ABO INCOMPATIBLE KIDNEY TRANSPLANTATION: AN APPROACH TO STANDARDISE ANTIBODY TITRATION METHODS

Barry Doyle¹, John Quigley², James Kelleher³

¹Irish Blood Transfusion Service, Dublin 8, Ireland, ²The National Maternity Hospital, Dublin 2, Ireland, ³Beaumont Hospital, Dublin 9, Ireland.



Introduction

Antibody mediated acute rejection can be an adverse complication of ABO incompatible (ABOi) kidney transplantation (Diagram 1). Efficient antibody reduction methods and immunosuppression have now made ABOi transplantation possible. Long term outcome data shows that ABOi transplantation is comparable to ABO compatible transplantation with regards to patient survival, graft survival and incidence of acute rejection. (Gloor *et al*, 2007). The availability of ABO compatible kidneys can be a limiting factor for some patients especially blood group B patients.

Antibody Reduction Methods

Current antibody reduction methods involve the removal of blood from the patient, separation of plasma, then removal of plasma or antibodies from the plasma. Plasmapheresis involves the removal of all antibodies and requires FFP or albumin replacement. More recently introduced immunoadsorption based techniques which use antigen bound columns, allow more selective removal of antibody, but can be costly.

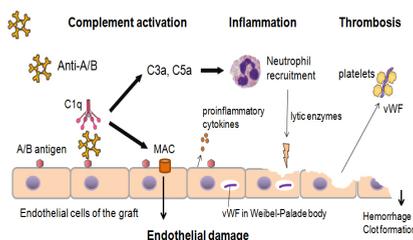
Importance of Accurate Antibody Titration

Antibody titration results are important indicators of eligibility for ABOi transplantation, rejection risk and antibody reduction guidance (pre and post operatively). Underestimation of antibody titre could result in transplantation while the patient is still at risk. Overestimation could lead to unnecessary, invasive and costly antibody reduction procedures or elimination from ABOi programmes.

UK Neqas Pilot Study

Recent UK Neqas pilot study results have shown wide ranges in antibody titration results between centres (gel column methods range: 16-512, tube method range: 16-1024, Rowley M, BBTS 2012). Standardisation of methods is necessary.

Diagram 1 Mechanism of Hyperacute Rejection



Methods

An observational study was carried out in seven blood transfusion laboratories internationally including; UK (4), Irish (1), Spanish (1) and Swiss (1). Each laboratory was surveyed regarding their anti-A/B titration method. The data collected was analysed for variability in protocols, reagents used and control measures in place.

Results

Isotype Reported (IgG/IgM) - 6/7 laboratories reported both IgG and room temperature IgM (RT). One laboratory reported IgG only.

Agglutination Method - 5/8 laboratories used column methods with 3 using traditional tube methods.

Red Cells Used - All the laboratories used the routine A/B reverse grouping cells that were available. There were no specialised cells in use.

Controls Used - Most centres titred the current sample in parallel with the previous sample where possible as a control measure. However, there is a critical lack of specialised anti-A/B controls or reference standards in use.

Use of Card Readers - There were no automated card readers in use in any of the laboratories.

End Point Reading - Results varied from 'last positive result' or '+w' to 1+ for column methods. Tube methods were consistent with +5 used by every laboratory.

Titration Method - All laboratories used a manual dilution method and no automated diluters were in use.

Variables Identified

- Lack of standardised protocols in general.
- Lack of specialised reagent red cells.
- End point reading.
- Agglutination method used.
- Lack of specialised controls.
- Lack of reference standards.

Ways to Standardise Anti-A/B Titration Methods

- Establish specialised A/B reagent cells, assessed by flow cytometry for antigen density and pooled (minimum four) for further antigen consistency.
- Use automated card readers to ascertain consistent end point reading.
- Establish anti-A/B reference standards and controls to be used in conjunction with patient samples. These could be used to monitor reagent cells, methods and technical variability.
- Use automated sample diluters instead of manual serial dilution to help eliminate laboratory technical error and user inexperience.
- Standardise protocols between centres and use one standard operating procedure like with anti-D/c quantification in the UK and Ireland.

Conclusions

Antibody titration methods still remain to be subjective. Whilst other methods such as flow cytometry hold much promise for the future, there is much room for improvement in the current titration methods, especially when optimal patient care could be compromised when the decisions pertaining to transplant are influenced by a substandard technique.

References

- Gloor JM and Stegall MD: ABO incompatible kidney transplantation. *Current Opinion in Nephrology and Hypertension*, 2007 Vol 16 No 6: 529-534
- Rowley M: Anti-A and Anti-B Titrations Pilot EQA Scheme, BBTS Presentation 2012.
- Diagram 1 taken from: <http://www.intechopen.com/books/understanding-the-complexities-of-kidney-transplantation/abo-incompatible-kidney-transplantation>

Acknowledgements

Special thanks to all the laboratories that took part in this survey.