Analysis of Titre Score as a valid alternative to Continuous Flow Analysis for the prediction of the nature of Anti-D in Pregnancy

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Introduction and Background

Anti-D is known to cause severe Haemolytic Disease of the Fetus and Newborn (HDFN). With the introduction of anti-D prophylaxis in the 1960's, the number of deaths dramatically reduced from 46 to 1.6 in 100,000, with a further reduction following the introduction of antenatal prophylaxis at 28 weeks gestation. BSH (2016) antenatal guidelines state that all clinically significant red cell antibodies should be quantified (anti-D, anti-c) or titrated to guide clinical management of the pregnancy.

Passive and immune anti-D cannot be differentiated. SHOT (2012) reported six cases where the anti-D was assumed to be passive, when in fact it was immune. The latest BSH guidelines now recommend that all anti-D detected in pregnancy should be quantified by Continuous Flow Analysis (CFA) or by a method that has been extensively validated against CFA and that gives a result that can be reported in IU/ml.

Some blood transfusion automated systems have the ability to perform titrations. Whilst the end-point titre is semi-quantitative, it does not represent the clinical picture and correlates poorly with the severity of HDFN. However the adoption of a titre score provides a quantitative result, by taking into account the strength of the reaction and the avidity of the antibody, which is thought to be better correlated with risk of HDFN.

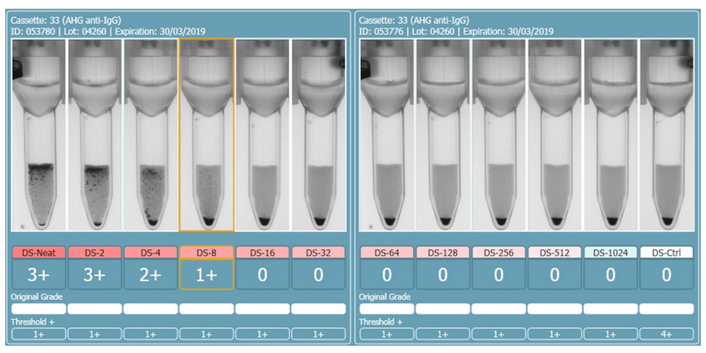
Aim

The aim of the first phase of this study was to assess if titration, converted into a titre score, determined by the automated BioVue® column agglutination technology (CAT) is a comparable alternative to CFA to determine the nature of detected anti-D.

Method

The ORTHO VISION® automated platform was used to make doubling dilutions of the patient's plasma. The dilutions were tested against pooled OR1r 0.8% NHSBT cells in CellStab in anti-IgG cassettes. The reaction grades were read and graded automatically. Each positive reaction grade was converted into a score value, and the sum of the scores gave the Titre Score (TS).

Reaction strength	4	3	2	1	0.5
Score	12	10	8	5	3



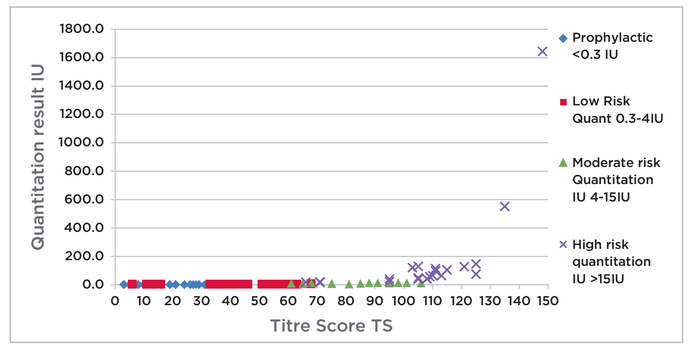
Anti-D was classified as passive based on a CFA result <0.2IU/ml, patient clinical history, evidence of anti-D prophylaxis and D status of the fetus (if available). If there was no evidence to suggest a passive nature, then the antibody was classed as immune.

The quantitation results by CFA were compared directly to the TS. The sensitivity, specificity and positive/ negative predictive values were determined at various TS cut-off points, and used to determine the most appropriate score to differentiate between passive and immune anti-D in conjunction with patient history.

Results

196 patient samples were tested across five UK hospital laboratories. Of the anti-D detected in these samples, 128 were classified as passive and 68 as immune.

Figure 1: TS vs CFA quantitation based upon HDFN Risk categories.



Statistical analysis was performed to determine the optimal TS cut-off to use in conjunction with the BSH criteria, to decide if CFA and clinical referral would be required. Potential TS cut-off values were selected based on passive and the immune low risk boundary, as below.

Figure 2: Passive and Low risk CFA vs. TS comparison.

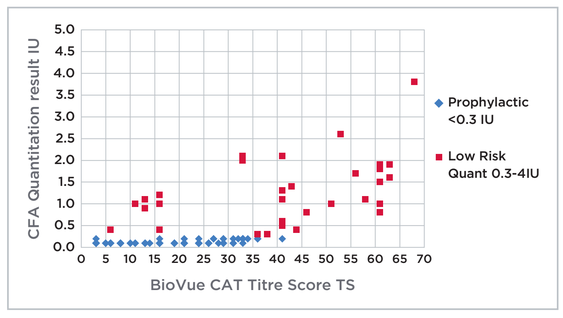


Table 2: Diagnostic testing of potential TS cut off values.

TS	30	31	32	33	34	35
TP	59	59	59	57	57	57
TN	106	110	111	117	118	118
FP	22	18	17	11	10	10
FN	9	9	9	11	11	11
Sensitivity	86.76%	86.76%	86.76%	83.82%	83.82%	83.82%
Specificity	82.81%	85.94%	86.72%	91.41%	92.19%	92.19%
PPV	72.84%	76.62%	77.63%	83.82%	85.07%	85.07%
NPV	92.17%	92.44%	92.50%	91.41%	91.47%	91.47%

Using a TS cut-off of 35, 173(TP+TN)/196 (88%) patient samples were correctly assigned as passive or immune anti-D when compared to CFA. The TS cut-off of 35

- correctly categorised 118(TN)/128 (92%) patient samples as prophylactic anti-D, signifying a NPV of **91.47%** (95% CI of **86.17%-94.86%**) and negative likelihood ratio of 0.18.
- correctly categorised 57/68 (83%) patient samples as immune anti-D, signifying a PPV of **85.07%** (95% CI of **75.70%-91.25%**) and a positive likelihood ratio of 10.73.

Conclusions

The data shows good correlation between TS and CFA quantitation to determine the nature of anti-D.

A TS cut-off of 35 can be used to predict the nature of detected anti-D; a TS of <35 indicates potentially passive in nature, a TS of ≥35 indicates potentially immune anti-D.

The data for TS ≥35 does not yet have enough power to replace CFA completely. As such we would currently still recommend all samples with a TS of ≥35 to be referred to NHSBT for CFA confirmation and risk stratification.

As a procedure validated against CFA, with results that can be traced back to quantification value expressed in IU/mL, this test, when used as a screen, can be performed at local hospital sites. The reduced cost and improved turn-around time, with associated clinical impact improvement, represents a real alternative to a referral to NHSBT for CFA.

Future Plans

To continue gathering TS data on samples known to contain immune anti-D, in association with clinical outcomes, with the aim of recommending TS as a whole-scale replacement for CFA.

- Begin looking at other specificities to determine whether TS would be a suitable replacement.

References

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