

Postpartum anti-D: can we safely reduce the dose?

Bradley M Augustson, Elizabeth A Fong, Dianne E Grey, Janine I Davies and Wendy N Erber

Rhesus (Rh) D immunoglobulin (anti-D) is a human blood product provided by a small group of immunised volunteer donors. It has been used since the 1960s in women who are Rh D-negative to prevent Rh D immunisation after giving birth to a baby who is Rh D-positive. Prevention of Rh D immunisation has been a major medical achievement, as Rh D immunisation was a significant cause of perinatal morbidity and mortality in subsequent pregnancies of affected women. In the United Kingdom, for example, the infant mortality from Rh haemolytic disease of the newborn decreased from 46 per 100 000 before 1969 to 1.6 per 100 000 in 1990 as a result of anti-D.¹

There are differences internationally in the approach to postpartum anti-D prophylaxis, not only in the dose of anti-D used, but also in whether testing is performed to quantitate the volume of fetomaternal haemorrhage (FMH). In Australia, the UK and the United States, postpartum anti-D doses of 600 IU (120 mg), 500 IU (100 mg) and 1500 IU (300 mg), respectively, are administered to Rh D-negative women. These doses are sufficient to cover Rh D-positive fetal red cell bleeds of 6, 5 and 15 mL, respectively. The FMH volume is then quantitated and additional anti-D is given if necessary.^{2,3} In other countries (such as Germany and other European countries) a large dose of anti-D (1500 IU) is given, but FMH volume is not quantitated.

In 1999, the National Health and Medical Research Council (NHMRC) issued guidelines on the use of routine antenatal anti-D prophylaxis.⁴ A phased implementation did not commence until 2002 because of a shortage of domestic supplies of anti-D.^{2,3,5} As a monoclonal anti-D (enabling production of unlimited quantities) has not yet been developed, maintaining a pool of voluntary immunised donors remains critical to the ongoing supply.⁶ After an increase in donor recruitment by the Australian Red Cross Blood Service (ARCBS), Australia has now become self-sufficient with respect to anti-D supply, obviating the need to import anti-D.⁵ One strategy for improving the efficient use of anti-D is to assess whether the amount that is administered routinely postpartum can be reduced. In this article, we report a large

ABSTRACT

Objective: To assess the potential for dose-reduction of prophylactic anti-D postpartum.

Design: Retrospective audit of fetomaternal haemorrhage (FMH) quantitation by flow cytometry.

Participants and setting: 5148 consecutive Rhesus D-negative women aged 15–45 years who had FMH estimation by flow cytometry at a central laboratory in Western Australia in the 65 months between 1 August 1999 and 31 January 2005.

Main outcome measures: Quantitation of FMH volume for adequate prophylactic anti-D administration in a timely fashion.

Results: 90.4% (4651/5148) of the women had an FMH volume of 1.0 mL or less of Rh D-positive red cells, and 98.5% (5072/5148) had a volume of less than 2.5 mL. Only 0.4% of cases had an FMH volume of 6.0 mL or greater (range, 6.0–92.4 mL).

Conclusions: This large retrospective audit shows that a currently available dose of 250 IU (50 mg) of anti-D would have been sufficient for 98.5% of the 5148 Rh D-negative women. On the basis of this evidence, a reduction in the recommended routine postpartum dose of anti-D from 625 IU to 250 IU when flow cytometric quantitation for FMH is available should be considered. Adopting such a strategy would ensure the ongoing provision of a valuable human blood product currently in limited supply.

MJA 2006; 184: 611–613

retrospective audit of our FMH data, obtained by flow cytometry, to provide support for a dose-reduction strategy.

METHODS

We analysed FMH data from 5148 consecutive women within the Western Australian public health system who were Rh D-negative and had delivered an Rh D-positive infant in the state of Western Australia in the 65 months from 1 August 1999 to 31 January 2005. Clinical information such as the mode of delivery were not available. All data were from routine diagnostic tests of consecutive samples from Rh D-negative women of mixed ethnic groups and parity. As this study was a retrospective audit of routine clinical practice, formal ethical approval was not sought.

Within 2 hours of infant delivery, maternal venous blood was collected into EDTA-anticoagulant and transported at 4°C by air or road to the central testing facility in the capital city, Perth, for FMH volume quantitation. All women were given a standard postpartum dose of 625 IU (125 mg) anti-D before the result of the FMH volume quantitation was known, in accordance with Australian guidelines at the time.³

FMH volume quantitation

FMH volume was estimated with fluorochrome-conjugated anti-D in accordance with the manufacturer's instructions (Fluoro-D, CSL, Melbourne, Vic.) and analysed on a Coulter Epics XL flow cytometer (Beckman Coulter, Calif, USA). In brief, the method entailed incubating 2.5 mL of maternal blood with fluorescein isothiocyanate-conjugated anti-D for 15 minutes at room temperature. Controls, consisting of 0, 0.2%, 0.4% and 4% Rh D-positive red cells in Rh D-negative blood, were analysed in parallel with the maternal samples. Red cells were gated by means of forward-scatter and side-scatter, and 100 000 red cell events were analysed. The fluorescing Rh D-positive red cells with bound fluorescein isothiocyanate-conjugated anti-D were determined as a percentage of the total number of red cells analysed. The volume of Rh D-positive red cells in the maternal circulation was then calculated according to the guidelines of the Australian and New Zealand Society of Blood Transfusion, as shown in Box 1.⁷

Reporting

The volume of FMH was reported to the referring physician and available within 72

1 Calculation of volume of fetomaternal haemorrhage (FMH)

$$\text{FMH (mL)} = 1800 \times \frac{1.22 (\text{number positive events} - \text{number events in negative control})}{100\,000}$$

This formula is based on an average maternal blood volume of 1800 mL and assumes that fetal cells are 22% larger than maternal cells.

hours of delivery of the infant. It was advised that mothers with an FMH volume of 6.0 mL or more should receive further calculated postpartum doses of anti-D, in accordance with the guidelines of the NHMRC.³

RESULTS

Blood samples were received from 24 laboratories during the 65 months — five in metropolitan Perth and 19 in regional areas of Western Australia (an area encompassing 2.5 million km²). Samples were received from 5148 women (aged 15–45 years; mean, 28 years), comprising 2471 from metropolitan and 2677 from regional areas.

FMH analysis with fluorescent-labelled anti-D and flow cytometry provided clear differentiation of cells binding anti-D (Rh D-positive red cells) from Rh D-negative cells. The negative (0) and positive (0.2%, 0.4% and 4%) controls gave the expected results and these were reproducible in all analyses.

The flow cytometry FMH results for all 5148 samples analysed are shown in Box 2. The vast majority (98.5%; 5072) had an FMH volume of less than 2.5 mL; 90.4% (4651) had a volume of less than 1.0 mL. Only 1.1% (51) had volumes between 2.5 to less than 6.0 mL, and 0.4% (25) had a volume of 6.0 mL or greater (range, 6.0–92.4 mL).

DISCUSSION

This audit of 5148 FMH volume quantitations provides strong evidence that the amount of prophylactic anti-D currently routinely administered to Rh D-negative women postpartum in most countries, including Australia, is excessive. We thus present evidence on which to base a change in the recommendations for this practice. As highlighted by the Cochrane reviewers, such evidence is currently lacking in the international literature.⁸ Our article also presents a model system capable of managing the accurate assessment of FMH volume over a sizeable geographical area. Our accumulation of data over such a large geographical area and dispersed population as Western

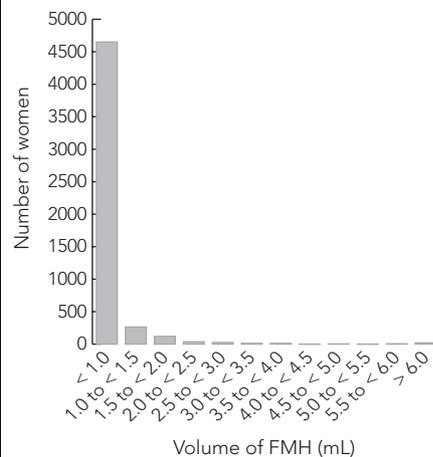
Australia suggests that the implementation of this form of FMH volume quantitation is achievable in other health care systems.

In 1999 in Australia, the ARCBS supplied an average of 15×10^6 IU of anti-D per month for processing by the manufacturer, CSL Bioplasma (Melbourne, Vic). Introduction of antenatal prophylaxis at 28 and 34 weeks' gestation for Rh D-negative primigravidae in late 2002 required the importation of an anti-D product from Canada for postpartum prophylaxis because there was insufficient domestic product. The implementation of antenatal prophylaxis to all Rh D-negative women has required the ARCBS to increase collection of anti-D to 30×10^6 IU per month. A further 15×10^6 IU anti-D per month would have been required to eliminate the need for importation of postpartum prophylactic anti-D.⁵ Following increased production of anti-D by the ARCBS, Australia has become self-sufficient in 2006.

A 250 IU anti-D vial for first trimester sensitising events³ was manufactured for use in 2001 to replace the 625 IU dose. This came 6 years after calls for a reduced dose because of critical shortages of anti-D.⁹ Our retrospective audit shows that this 250 IU (50 mg) dose could also be routinely used for 98.5% of Rh D-negative women postpartum. According to ARCBS data, reducing the postpartum anti-D dose to 250 IU would save an estimated 9×10^6 IU per month.⁵

Changing the postpartum anti-D dosing regimen for Rh D-negative women depends on a practical technique for FMH volume quantitation which has the requisite precision and reproducibility. Specifically, the technique must be capable of identifying the small percentage of women who have an FMH volume greater than that covered by the routine postpartum anti-D dose. These patients, who will require further anti-D, must be identified and treated within 72 hours of infant delivery. Two approaches may be adopted to satisfy these requirements.

Firstly, FMH screening and quantitation could be performed at a centralised laboratory by flow cytometry, as we did in this study. Flow cytometry, in particular with

2 Volume of fetomaternal haemorrhage in the 5148 consecutive women assessed

monoclonal anti-D, is well recognised as being the most accurate and reproducible method for FMH volume quantitation.¹⁰⁻¹² We also found this approach to be extremely practical over a large geographical area.¹³

The second approach is the Kleihauer test followed by flow cytometric quantitation. Despite its general lack of reproducibility for FMH volume quantitation, a well-performed Kleihauer test is accepted by many as an appropriate screening technique for detection of FMH.¹⁴ Therefore, FMH screening using the Kleihauer test followed by accurate quantitation using flow cytometry if fetal red cells are detected could be considered. Based on our data, we estimate that this approach would necessitate flow cytometric FMH volume quantitation in less than 10% of cases.

The major limitation of our data is that they came from a retrospective audit of FMH volume quantitation, so the results must be interpreted with caution. Our report only confirms the low volume of most FMH. Any changes to guidelines with respect to lowering of the anti-D doses would require further prospective studies to assess the efficacy and safety of such changes.

In conclusion, this large study supports a reduction in the dose of anti-D to 250 IU (50 mg) for routine postpartum prophylaxis to minimise unnecessary wastage of this valuable resource. This reduced dose would have major implications in terms of sufficiency and would help alleviate the difficulties associated with maintaining a voluntary Rh D immunised-donor program.

ACKNOWLEDGEMENTS

Sincere thanks to Dr Gary Hoffman for his critical review of the manuscript and to Professor Ian Jacobs for statistical and epidemiological input.

COMPETING INTERESTS

None identified.

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(Received 6 Oct 2005, accepted 3 Apr 2006) □