A RE-AUDIT ON ANTI-D POST-PARTUM AND THE USE OF KLEIHAUER TESTING

Final Report

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ABSTRACT

Kleihauer testing is recommended following the birth of a baby and following any potential sensitising event after 20 weeks' gestation. This is to detect large fetomaternal bleeds which may require additional doses of RhD Immunoglobulin to prevent immune anti-D antibody formation.

This audit aimed to determine, in RhD negative women following the birth of an RhD positive or unknown baby, the level of Kleihauer testing being completed and the level of RhD Immunoglobulin use. These results were compared with the 2009 audit of RhD Immunoglobulin use. DHB policies were checked for a current policy regarding Kleihauer testing and whether flow cytometry for fetal red cells is performed and under what circumstances.

10% of the 40,405 women who gave birth over the course of a year in the nine participating DHBs were RhD negative. Two-thirds of those had a baby that was RhD positive or RhD unknown.

Kleihauer testing was performed in 76% of the RhD negative women with RhD positive or RhD unknown babies. Although not perfect, this result was a significant improvement on the previous audit. It is estimated that one or two women were underdosed with RhD Immunoglobulin as a result of a lack of Kleihauer testing and were potentially sensitised.

96% of women received RhD immunoglobulin perinatally. That leaves approximately 87 RhD negative women with RhD positive or unknown babies unprotected from sensitisation at birth. In this audit, this lack of RhD Immunoglobulin administration represents a significantly higher risk for the formation of anti-D antibodies than that due to lack of Kleihauer testing.

A small proportion of women did not have blood groups identifiable through the audit's methodology but an expected number did receive RhD Immunoglobulin, suggesting these women were appropriately cared for.

All audited DHBs now have policies in place requiring Kleihauer testing for RhD negative women giving birth to RhD positive or RhD unknown babies. Kleihauer testing remains the principal test used across all DHBs with only two offering HbF flow cytometry but only where the Kleihauer test indicated a bleed greater than 2 or 2.4 mL.

BACKGROUND

Kleihauer testing is recommended[1,2] following a potential sensitising event after 20 weeks' gestation to detect large fetomaternal bleeds which may require additional doses of RhD Immunoglobulin to prevent immune anti-D antibody formation.

In a 2009 audit[3] on the use of RhD immunoglobulin in the eight large District Health Boards (DHBs) in New Zealand, it was found that less than half of the women (44%) were Kleihauer tested following an antenatal indication after 20 weeks or at birth.

Two distinct groups of DHBs were apparent – those that perform Kleihauer testing at births and antenatal sensitising events (87% tested) and those that don't (2% tested).

It was found that there were no policies for Kleihauer testing within Counties-Manukau, MidCentral or Waitematā DHBs and that the Auckland DHB had several policies in place discussing Kleihauer testing but there was not one specific Kleihauer testing policy.

The recommendations from the audit included the following that:

- the importance of testing for fetomaternal haemorrhage is reiterated
- this importance is promulgated in DHB policies throughout New Zealand.
- laboratories should anticipate a large increase in Kleihauer testing
- laboratories consider other technologies such as gel agglutination micro columns as a screening test

AIM

To ascertain, in RhD negative women following the birth of an RhD positive or unknown baby,

1. the level of Kleihauer testing being completed,
2. the level of RhD Immunoglobulin use and dosing

and to compare this to the results obtained during the 2009 Clinical Audit of RhD Immunoglobulin in New Zealand (NZBS).

METHOD

Data were collected by the six New Zealand Blood Service (NZBS) Transfusion Nurse Specialists (TNS) based in Auckland, Hamilton, Palmerston North, Wellington, Christchurch and Dunedin, and Clinical Nurse Specialists in Transfusion Medicine employed by, Northland, Waitematā and Counties-Manukau District Health Boards. The involvement of these nine DHBs covered 74.9% of births in the country.

Data was collected as follows:

- The Ministry of Health provided a list of mothers who had given birth within the participating DHBs during the audit timeframe (1st July 2018 – 30th June 2019). The data was from the Ministry of Health's National Maternity Collection, a subset of the National Minimum Dataset, covering publicly funded hospitals and birthing centres (approximately 95% of deliveries)
- Blood groups of the mothers were obtained from NZBS records as well as those of the community laboratories serving the DHBs.
- A list of the NHIs and dates of the RhD negative mothers was sent back to the Ministry who then provided an unlinked list of babies from that list.
- The blood groups of the babies were obtained in the same way as the mothers and a list of RhD positive or unknown babies' NHIs was sent back to the Ministry.
- The Ministry provided an unlinked list of mothers' NHIs from the list of RhD positive/unknown babies.

- Laboratories providing Kleihauer testing provided a list of patients tested during the audit period.
- The list of RhD negative mothers with RhD positive or RhD unknown babies was used to search for RhD Immunoglobulin administration
- Combining these lists, results were obtained of Kleihauer tests and RhD immunoglobulin administration.
- The DHB policies were perused for evidence of:
    - a current policy regarding Kleihauer testing
    - whether flow cytometry for fetal red cells is performed and under what circumstances

The lead Transfusion Medicine Specialist (TMS) was responsible for obtaining the data from the Ministry of Health. Each TNS/CNS was responsible for obtaining the laboratory data from the local DHB.

Where the Kleihauer test or administration of RhD Immunoglobulin was within 10 days of the identified event date in the Ministry of Health's data, then that was attributed to the birth of the baby.

SECURITY

The data was collated in a secure PostgreSQL database with restricted access, located on the NZBS internal network. Only the lead TMS overseeing the audit has access to the database. The only identifying data used was the NHI number. On completion of the final audit report, the NHI numbers will be removed from the database and the database archived.

ETHICS

The audit proposal was reviewed by the TNS/CNS group and NZBS's Clinical Advisory Group following which ethics approval was sought from the National Health and Disability Ethics Committee. The proposal was sent to each DHB's Hospital Transfusion Committee with an invitation to participate in the audit and all agreed to participate. As this was an observational study, no consent was sought or required from the audited women. As the audit was retrospective and took place beyond the timeframe within which any clinical action could be taken on finding a woman who should have had a Kleihauer test but didn't, no clinical interventions were undertaken or necessary.

REPORTING

Reporting was done in draft to the TNS/CNS group, NZBS Clinical Advisory Group, and then to the Hospital Transfusion Committees of the participating DHBs, with an invitation for comment. Following this, the report will be finalised and issued to all DHBs via their NZBS Demand Management contacts.

RESULTS

The first list from the Ministry yielded 41,036 entries. Two entries were excluded. One was a male patient and one was a mother whose pregnancy miscarried at 12 weeks.

In the audit period, there were 58,704 pregnancies giving birth (Ministry of Health) and 42,132 live and stillbirths in the DHBs taking part in the audit (Stats NZ) (table1). The audit thus covered approximately 70% of the country's births and 93% of the participating DHBs' births. Note that the number of births exceeds the number of mothers because of women giving birth twice in a year (beginning and end of the year) as well as multiple pregnancy births.

The proportion of RhD negative women varied from 6-14%, depending on the DHB, reflecting the ancestral ethnicities of the mothers. It is known that mothers of Maori, Pasifika and East Asian descent have higher rates of RhD positivity. 0.6% of women had no blood group on record.

DHB	Recorded births	RhD positive	RhD negative	RhD Unknown	% RhD negative/unknown	Total mothers
Auckland	5,424	5,916	584	12	9%	6,512
Canterbury	6,417	4,710	666	60	13%	5,436
Capital and Coast	3,195	2,911	386	11	12%	3,308
Counties Manukau	2,157	6,895	357	88	6%	7,340
MidCentral	1,419	1,446	183	5	12%	1,634
Northland	5,472	1,764	171	5	9%	1,940
Southern	7,605	2,644	417	2	14%	3,063
Waikato	8,247	4,004	456	18	11%	4,478
Waitematā	2,196	6,105	553	36	9%	6,694
Total	42,132	36,395	3,773	237	10%	40,405

Table 1: *Mothers giving birth by DHB and mothers' RhD types*

From the list of RhD negative mothers, we obtained the list of babies born (table 2). A small proportion of babies (3.8%) had no group available, due to no sample being sent (134 babies), technical problems with the result (6), or stillbirths (5).

DHB	RhD negative	RhD positive	Unknown	% RhD positive/unknown	Total
Auckland	184	393	21	69%	598
Canterbury	234	400	39	65%	673
Capital and Coast	129	254	9	67%	392
Counties Manukau	102	215	26	70%	343
MidCentral	65	131	8	68%	204
Northland	61	111	3	65%	175
Southern	172	237	14	59%	423
Waikato	170	286	6	63%	462
Waitematā	190	347	19	66%	556
Total	1307	2374	145	66%	3826

Table 2: *Babies of RhD negative mothers by DHB and RhD type*

The mothers of the babies who RhD positive or unknown group were provided by the Ministry of Health, giving a final auditable set of data as shown in table 3.

DHB	Mothers	Tested	% Tested	Previous audit % tested of (n)	p	Change
Auckland	402	248	62%	0% (26)	<0.00001	Improved
Canterbury	415	361	87%	93% (44)	0.34	No change
Capital and Coast	258	236	91%	83% (36)	0.13	No change
Counties Manukau	245	195	80%	3% (32)	<0.00001	Improved
MidCentral	124	59	48%	0% (32)	<0.00001	Improved
Northland	111	82	74%	-	-	No comparator
Southern - Otago	134	115	85%	95% (40)	0.17	No change
Southern - Southland	107	47	44%	-	-	No comparator
Waikato	283	254	90%	97% (31)	0.33	No change
Waitematā	366	317	87%	2% (42)	<0.00001	Improved
Overall	2445	1863	76%	50% (283)	<0.00001*	Improved

Table 3: *RhD negative mothers of RhD positive or unknown babies with the proportion having had a Kleihauer test by DHB, compared with the previous audit using a Fisher's exact test*
** statistical comparison excludes Northland and Southland*

While laboratories were only asked to provide the NHIs of women who had had Kleihauer tests, most actually provided the actual result as well. Counties Manukau and Waitematā provided what was asked for. The results (table 4) show that 3 in 1,411 Kleihauer tests were positive. This is statistically similar with the previous audit showing a positive Kleihauer test in 3 out of 357 (0.9%) (Fisher's exact test comparing the two audits: p=0.25).

DHB	negative	positive	% positive
Auckland	265	1	0.38%
Canterbury	377	0	0.00%
Capital and Coast	241	0	0.00%
Counties Manukau	-	-	-
MidCentral	65	0	0.00%
Northland	85	0	0.00%
Southern	113	1	0.88%
Waikato	265	1	0.38%
Waitematā	-	-	-
Total	1411	3	0.21%

Table 4: *Proportion of Kleihauer tests showing greater than 6mL fetal red cells*
notes: 1. no results were available for Waitematā and Counties Manukau DHBs
2. positive means > 6mL fetomaternal haemorrhage detected
3. more Kleihauer tests were done than women tested due to repeat testing

From all three test results, the requirement was for only one more vial of RhD Immunoglobulin. Two of the three women (Waikato and Southern DHBs) (table 4), received the additional RhD Immunoglobulin. The third woman (Auckland DHB), with an estimated fetomaternal haemorrhage of 6.5mL fetal red cells, did not. It is unknown if this last woman subsequently formed an anti-D antibody. 6mL is of note because this is the amount of fetal red cells cleared by a single vial of RhD Immunoglobulin.

Looking at RhD Immunoglobulin issues, a further woman was identified who had had a 57mL bleed requiring 6,357 IU RhD Immunoglobulin to prevent her from becoming sensitised. Her birth was at Waitematā DHB which had not provided results for Kleihauer tests for this audit, only whether women had been tested.

Figure 1 shows the overall breakdown of the audit. This identified a single woman who should have received an additional dose of RhD Immunoglobulin but did not. However, 582 women did not get a Kleihauer test at all. Extrapolating from the results of the women who were tested, we expect that this lack of testing missed two women who missed out on additional RhD Immunoglobulin and were possibly sensitised to RhD as a result.

Lack of blood group testing, the very first step in the process, affecting 237 women, is calculated to include approximately 22 RhD negative women, with 14 of these having given birth to RhD positive babies. Interestingly, 26 of this group of women with unknown blood groups received RhD Immunoglobulin around the time of the birth, suggesting that they may have sourced a blood group elsewhere (e.g. overseas).

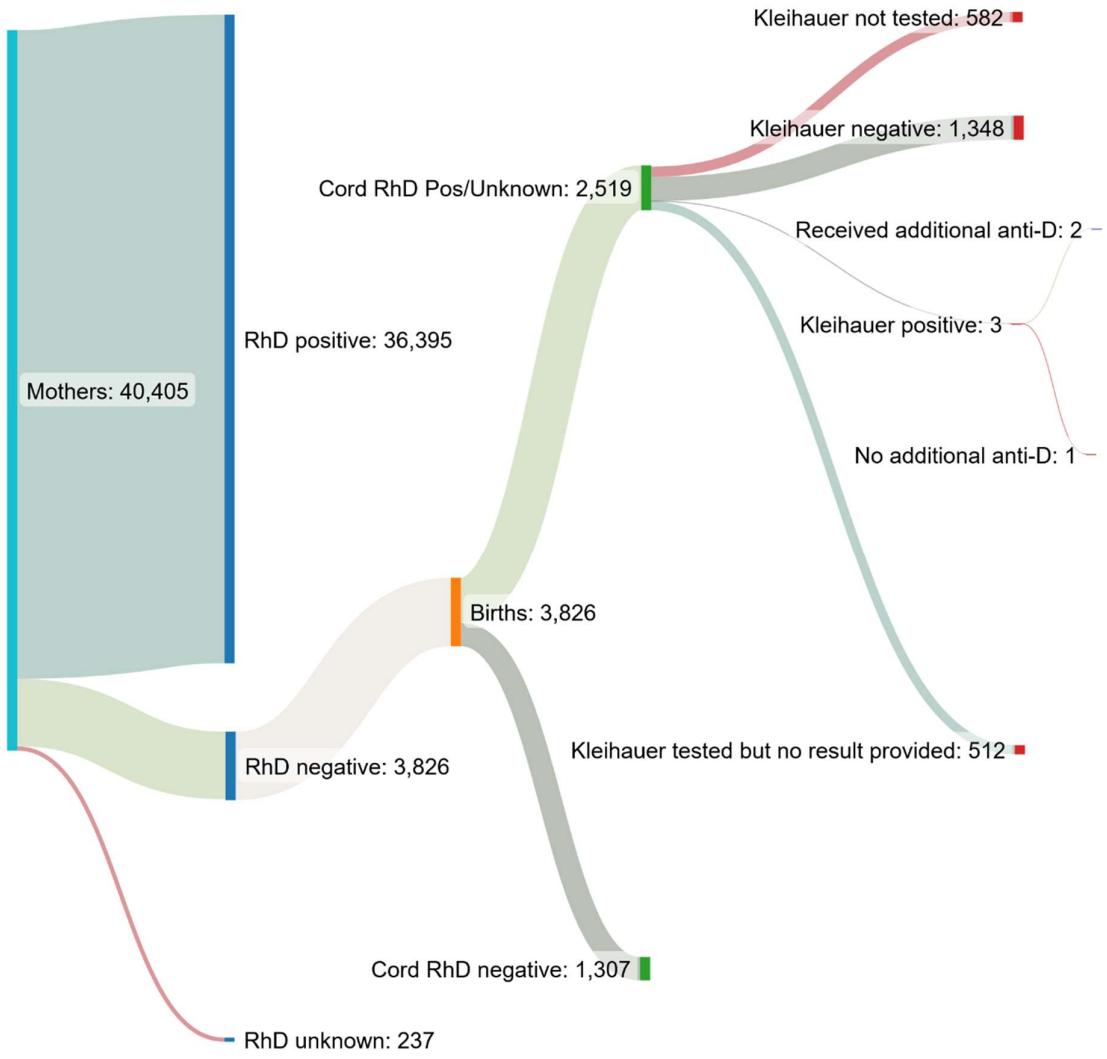

Figure 1: *Sankey chart of the process flow of RhD negative women through to Kleihauer testing*

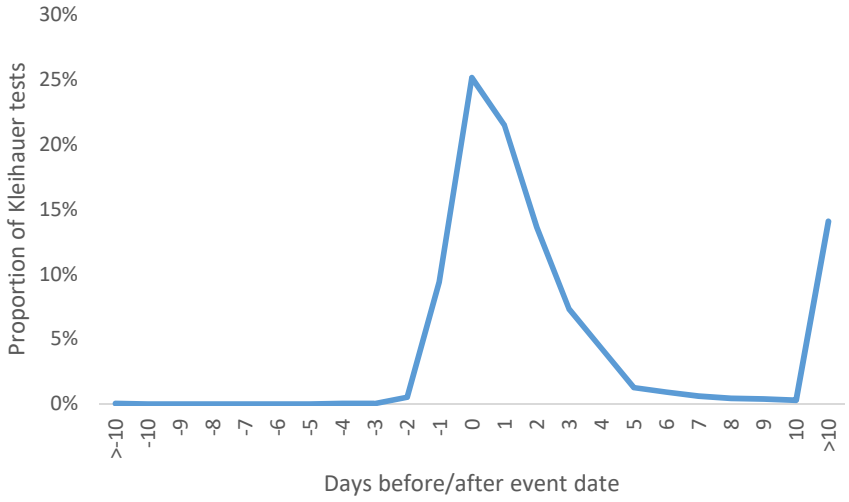

Figure 2: *Timing of Kleihauer testing in relation to the reported event date*

Timing of Kleihauer testing, within the limitations of the data quality, appeared to be reasonably good with 78% of tests within 72 hours of birth (figure 2).

RhD Immunoglobulin was given to almost all RhD negative women with RhD positive or unknown babies (table 5). This compares well with the previous audit that showed 98% of women (267 of 272) (Chi-squared test, p=0.165) had received RhD Immunoglobulin.

DHB	RhD neg mothers with RhD pos/unknown babies	received RhD Immunoglobulin	% received RhD Immunoglobulin
Auckland	388	377	97%
Canterbury	388	363	94%
Capital and Coast	250	244	98%
Counties Manukau	244	243	100%
MidCentral	117	113	97%
Northland	109	106	97%
Southern	232	224	97%
Waikato	271	259	96%
Waitematā	368	351	95%
Overall	2367	2280	96%

Table 5: *RhD negative mothers with RhD positive/unknown babies and whether they received perinatal RhD Immunoglobulin by DHB*

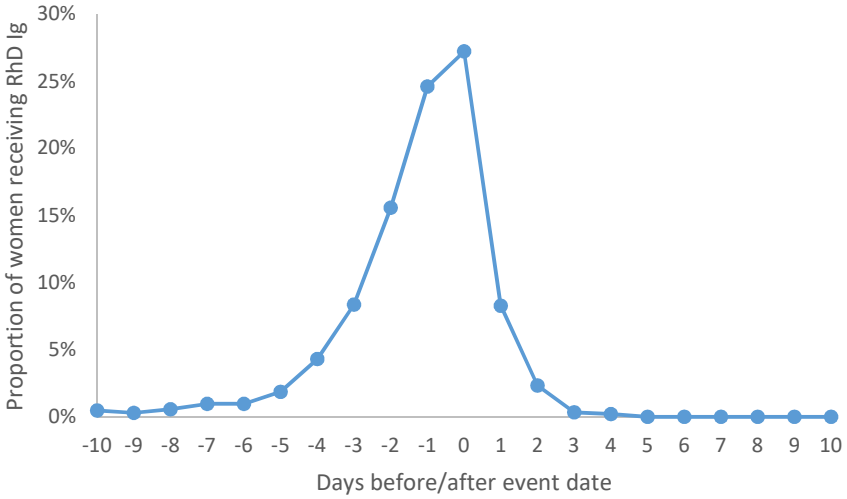

Figure 3: *Timing of RhD immunoglobulin administration in relation to the reported event date*

Where RhD Immunoglobulin was administered, this was consistent with the national recommendations (table 6) of 625 IU.

Three women received Rhophylac, the intravenous preparation. It is assumed that the 875 IU dose was an error, with the woman receiving the incorrect 250 IU dose and then the correct 625 IU dose. Women receiving 1250 IU (two vials of 625 IU) were seen at all nine participating DHBs. The woman receiving 6,375 IU had had a 57mL fetomaternal bleed.

Dose (IU)	Mothers	percentage
625	2209	98.4%
875	1	0.0%
1250	35	1.6%
6375	1	0.0%

Table 6: *Distribution of RhD Immunoglobulin doses*

DHB policies and testing capabilities were checked (table 7).

DHB	Appropriate FMH testing policy	Testing capability
Auckland	Yes	Kleihauer,
Canterbury	Yes	Kleihauer, flow cytometry if FMH > 2mL
Capital and Coast	Yes	Kleihauer
Counties Manukau	Yes	Kleihauer
MidCentral	Yes	Kleihauer
Northland	Yes	Kleihauer
Southern	Yes	Kleihauer
Waikato	Yes	Kleihauer, flow cytometry if FMH > 2.4mL
Waitematā	Yes	Kleihauer

Table 7: *DHB policies and testing capability for fetomaternal haemorrhage (FMH: fetomaternal haemorrhage)*

All DHBs audited now have appropriate policies in place for defining when fetomaternal haemorrhage testing is indicated. Testing at all sites was via the Kleihauer test with Waikato and Canterbury hospital laboratories also offering flow cytometry for bleeds greater than 2 or 2.4 mL. It is worth noting that flow cytometry in these two laboratories detects fetal haemoglobin (HbF) rather than RhD.

Finally, of the 3,773 RhD negative women entering this audit, 68 (1.8%) already had an anti-D antibody.

AUDIT LIMITATIONS

This audit represents a full year view of DHB practice. One limitation is the ability to obtain data on all births. Most but not all are reported on. The data itself is limited by the quality of the data collected. Specifically, this relates to the quality of coding, as seen by the one male patient included. Additionally, the date of birth appears to be coded somewhat variably, either being the actual date of birth or possibly the date of reporting the birth. This means that it is possible, even likely, that some Kleihauer testing and RhD Immunoglobulin administration occurred in the final days of the pregnancy rather than post-partum.

Kleihauer test results are reported in quite different ways across the country with some labs reporting millilitres of fetomaternal haemorrhage and others reporting simply reporting less than 6 mL. Two laboratories didn't provide quantitation, as the data request did not actually require this.

RhD Immunoglobulin is issued as a stock item to some facilities, e.g. birthing centres. Although there are systems in place to prevent this, it is possible that a few women may have received RhD Immunoglobulin without NZBS being informed.

Access to RhD Immunoglobulin is an issue for some rural communities and it is possible that some women may not have received RhD Immunoglobulin for this reason.

There is an implicit assumption in this audit that all women would comply with guidelines. However, it is reasonable to expect that a small number of patients may have refused additional blood tests (a post-partum Kleihauer) or further doses of RhD Immunoglobulin. This is not recorded in the data sets.

Lastly, the data received for the audit was limited to the specific questions proposed. As a result, it is not possible to look for other demographic factors to identify which women were more likely than others to miss out on RhD Immunoglobulin or Kleihauer testing.

DISCUSSION

This audit shows that almost all DHBs audited have improved in their Kleihauer testing when compared with the 2011 audit[3]. There is still room for improvement but the direction is positive. Using the data presented, looking at the number of Kleihauer tests missed and the proportion that had fetomaternal bleed greater than six millilitres, it is likely that only one woman has been exposed to an fetal red cells in excess of the cover provided by the RhD Immunoglobulin given. This is a remarkable improvement on the previous audit.

This improvement may be in part to the policies now in place in all audited DHBs and it is gratifying to see that the feedback from the previous audit has been taken up. It is also good to see that two DHBs are offering flow cytometry for a more precise definition of larger fetomaternal bleeds. While the test detects fetal haemoglobin (HbF) rather than RhD, largely because of the far wider utility of an HbF assay, this limitation is well recognised and can be mitigated.

A larger concern is that while 96% of women received RhD Immunoglobulin around the time of giving birth, that leaves 4% or 87 RhD negative women with RhD positive or unknown babies who did not receive RhD Immunoglobulin. In this audit, this represents a significantly higher risk for the formation of anti-D antibodies than that due to lack of Kleihauer testing.

While there are no immediate health consequences to women who form anti-D, 70% of babies born to sensitised mothers are affected, ranging from minimally affected to needing phototherapy, and intra-uterine transfusion[4]. Failure to provide adequate care can result in tragic outcomes including permanent neurologic damage from kernicterus or even neonatal death.

Prophylaxis was first proposed 45 years ago[5] and has progressed from post-partum only, adding antenatal post-exposure events and now also routine prophylaxis at 28 and 34 weeks[1]. Of these three phases, post-partum prophylaxis has the biggest impact. RhD Immunoglobulin has proved to be a very safe product. Although one might expect the RhD positive fetal red cell to bind RhD Immunoglobulin, in practice, there is no evidence of any harm to the fetus[4], most likely due to dilution in the mother's circulation. Additionally, RhD Immunoglobulin has proved safe in mothers with no viral infection transmissions reported in New Zealand and only very rare allergic reactions noted[2].

Future developments in New Zealand include the implementation of fetal RhD genotyping using a peripheral blood sample from the mother. Initially, this will allow determination of the RhD type of the fetus in high-risk women, those with anti-D antibodies, and potentially reduce the intrusive level of monitoring otherwise required. With increased capacity, this could be rolled out to all RhD negative women to identify those needing RhD Immunoglobulin and Kleihauer testing.

However, two-thirds of RhD negative women, those with RhD positives fetuses will, for the foreseeable future, need anti-D prophylaxis, ideally both post-exposure and routine prophylaxis, together with Kleihauer testing, and it is incumbent on us to provide the highest level of care possible for these women.

RECOMMENDATIONS

1. There have been significant improvements in Kleihauer testing in the sites with previously low rates. However, there is still room for further improvement.
2. Giving birth is the event most associated with sensitisation to RhD. Although only 4% of women appear to have missed out on getting RhD Immunoglobulin, all 4% are at risk of forming anti-D antibodies. Efforts should be made to achieve as high a rate of RhD Immunoglobulin as possible.
3. Although not clear from this audit, it is essential that the timing of Kleihauer testing, reviewing the results of the test and administration of RhD Immunoglobulin is undertaken within the first 72 hours after birth. Robust processes need to be in place to manage this, especially as women are frequently changing hands for their care or being discharged home at this time.
4. Flow cytometry, as used in Waikato and Canterbury, may be a useful addition to Kleihauer testing to confirm the size of large fetomaternal haemorrhages and to distinguish these from hereditary persistent fetal haemoglobin (HPFH) in the mother. Using a threshold of 2-3 mL of fetal red cells limits the number of requests for flow cytometry and focuses efforts on the women most at risk of sensitisation.

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