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How clinically important are non-D Rh antibodies?

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ABSTRACT

Introduction: The advent of RhD immunoglobulin prophylaxis to prevent maternal Rh-D alloimmunisation has reduced the incidence of this condition and its associated poor outcomes. Consequently, non-D Rh antibodies now account for a greater proportion of alloimmunised pregnancies. These antibodies have been the subject of comparatively little research. This study investigated the incidence and clinical outcome of pregnancies affected by non-D Rh alloimmunisation at an Australian tertiary maternity service. **Material and Methods:** This was a retrospective study of all pregnancies with non-D Rh antibodies (namely anti-C, -E, -c, -e, -C^w, as well as the compound antibodies anti-CD, -cE and -ce) managed at the Royal Women's Hospital, Victoria, Australia, from 2009 to 2013 inclusive. Information collected included maternal demographics, details of the antibodies, course of the pregnancy, and neonatal outcomes. **Results:** During the study period, 115 non-D Rh alloimmunised pregnancies were identified in 102 mothers. Forty-nine pregnancies reached the critical titer (>16) from non-D Rh alone and 11 fetuses received intrauterine red blood cell transfusion. Labour was induced or cesarean section performed in 38 cases. Forty-three neonates were admitted to the special care nursery and 59 received phototherapy. Nine received treatment for anemia and ten neonates received intravenous immunoglobulin. **Conclusions:** Non-D Rh alloimmunisation is a relatively uncommon complication of pregnancy, occurring in only 0.33% of pregnancies in the study period. It can lead to significant fetal/neonatal morbidity (and may lead to mortality). The most severe outcomes (including perinatal deaths) were mostly associated with the compound antibodies anti-CD and anti-cE, or a non-D Rh antibody in conjunction with anti-D.

Keywords:

Rh, alloimmunisation, non-D, hemolytic disease of the fetus/newborn, blood group antibody, pregnancy

Abbreviations:

HDFN – hemolytic disease of the fetus or newborn

IUT - intrauterine red blood cell transfusion

FDIU – Fetal death in utero

Key Message

Non-D Rh alloimmunisation is a relatively uncommon complication of pregnancy however, non-D Rh antibodies cause mild to moderate hemolytic disease of the fetus or newborn with rare exceptions. Non-D Rh alloimmunisation therefore must be identified and, if present, acted upon by all involved in maternity care provision.

INTRODUCTION

Red blood cell alloimmunisation remains an important cause of adverse perinatal outcome, with antibodies to antigens in the Rh system occurring most commonly. However, the advent of RhD immunoglobulin prophylaxis to prevent maternal RhD sensitisation has reduced the incidence of this specific form of alloimmunisation and its associated poor outcomes (including neonatal anemia, hyperbilirubinemia and perinatal death). The routine use of RhD immunoglobulin post-partum reduces the risk of RhD alloimmunisation from 16% to 2%, and routine antenatal prophylaxis further reduces the rate to 0.3%.(1, 2)

In low-income countries without an anti-D prophylaxis program, stillbirth still occurs in 14% of affected pregnancies, and 50% of the surviving infants either die in the neonatal period or develop cerebral injury.(3) In high-income countries, however, not only is the number of cases significantly reduced, but even of the anemic infants, 94% have normal neurological outcome after intrauterine red blood cell transfusion (IUT).(4)

As a result of RhD immunoglobulin prophylaxis, non-D Rh antibodies – for which prophylaxis is not clinically available – will now account for a greater proportion of alloimmunised pregnancies. Kolewijin et al. found that red blood cell transfusion is the most important independent risk factor for non-D Rh alloimmunisation, followed by parity, major surgery and hematological disease.(5) These antibodies, however, have been the subject of comparatively little research, mostly in the form of isolated case studies that have an inherent bias towards severe outcomes. Notwithstanding these limited data, guidelines continue to recommend close surveillance for women alloimmunised to any Rh antigen.(6)

The present study aimed to investigate the incidence and clinical outcome of pregnancies affected by non-D Rh alloimmunisation at the Royal Women's Hospital, Victoria, Australia, in order to determine the impact of non-D Rh antibodies in a tertiary maternity service. Non-Rh antibodies may also be responsible for adverse perinatal outcomes, of course, however they were only included in this study when they occurred in conjunction with a Rh antibody, to ensure that the focus on this under-researched area was maintained.

MATERIAL AND METHODS:

This was a retrospective cohort study of all pregnancies with non-D Rh antibodies (namely anti-C, -E, -c, -e, -C^w, as well as the compound antibodies anti-CD, -cE and -ce) managed at the Royal Women's Hospital (RWH), Parkville, Victoria, Australia, in the five-year period between January 2009 and December 2013.

A search of the RWH pathology database was performed to identify all women who had non -D Rh antibodies detected in the previous five years. Women were excluded if they did not have a non-D Rh antibody during the course of a pregnancy (e.g. antibodies found in work up for gynecological surgery). Non-Rh antibodies were only included when they occurred in conjunction with a Rh antibody. Once the women were identified, data were extracted from electronic and paper medical records of both the mother and the neonate. The information collected is shown in Supporting Information Table S1. Severe adverse outcomes other than perinatal losses were defined as the need for an intrauterine transfusion, or neonatal red blood cell exchange transfusion or intravenous immunoglobulin administration.

The maternal antibody titer was determined using the indirect antiglobulin test. The critical level (1:16) is the level above which there is thought to be significant risk of fetal hydrops or anemia. If the titer is above 1:16 for Rh antibodies, consideration is given to determining the fetal Rh genotype/phenotype with invasive testing (such as amniocentesis), and fetal monitoring commenced (middle cerebral artery Doppler ultrasound).(7) Prior to invasive testing, patients are counselled regarding the potential risk of enhanced maternal antibody production arising from the procedure, and it is only recommended when the risk of this is deemed to be outweighed by the benefit of confirmation of the fetal antigen status.

Women were excluded from the final data set if they delivered elsewhere, had a fetal death in utero (FDIU), miscarriage or termination. Available details regarding these events were recorded and reported separately.

Ethical Approval

During the process of data collection the data for the mother and baby were collated together and given a study ID which was de-identified. As a retrospective, anonymised, chart-based audit project, this study posed no risk to patients, and met the criteria established for quality assurance activities outlined in the NHMRC guideline *Ethical Considerations in Quality Assurance and Evaluation Activities*.(8) Correspondence confirming this was received from the institutional Human Research Ethics Committee.

RESULTS

Between January 2009 and December 2013, 146 non-D Rh alloimmunised pregnancies were identified in 130 mothers. Among these 146 pregnancies, eight miscarriages, three terminations and six FDIU were excluded from further analysis. The overall pregnancy loss rate in this cohort was thus 11.6%, although only three of these losses (2% of the total) were confirmed pathologically to be the result of alloimmunisation. There were also 14 pregnancies that were excluded as the delivery occurred elsewhere, precluding procurement of outcome data. There were two sets of twins, resulting in 115 pregnancies (in 102 mothers) and 117 neonates with complete data sets available for analysis. Figure 1 presents a flow diagram of the study population.

In the 5 years of the study, there were 34,450 deliveries at the Royal Women's Hospital. Therefore, 0.33% of all deliveries (115/34,450) occurred in pregnancies affected by alloimmunisation with non-D Rh antibodies. The incidence of the different antibody subtypes is shown in Figure 2.

Thirteen pregnancies with anti-C also had anti-D present, not in a compound antibody. Fifteen pregnancies also had a non-Rh antibody present (anti-Fya, anti-Fyb, anti-Jka, anti-Jkb, anti-K, anti-Leb, anti-Lea, anti-P1 or anti-S). In six of these pregnancies, the titer of the Rh antibody was higher than that of the non-Rh antibody. One pregnancy had two different antibodies from two non-D Rh antibodies (anti-E and anti-C^w). When more than one antibody was identified, titration involved separate titers except in the case of the compound antibodies, anti-ce, anti-cE and anti-CD.

Table 1 summarizes the course and outcome of affected pregnancies by type of antibody. Of the pregnancies in which the critical titer was reached (49.6%), amniocentesis was only performed in one case. This pregnancy however was also alloimmunised for anti-K (the father was heterozygous for Kell). Eleven pregnancies (9.6%) required IUT, including one failed attempt where the outcome was early delivery. Of these 11 IUTs, only two cases received an IUT for a non-D Rh antibody alone (anti-E in both cases). In the remaining 9 cases anti-D was present along with another non-D Rh antibody. Four were caused by the compound antibody anti-CD. Four had significant titers of anti-D in addition to the non-D Rh antibody, and one was in conjunction with anti- Fya, the titer of which was again considerably higher than the Rh antibody.

Thirty-eight of the pregnancies (33%) in the study period had either induction of labor or cesarean section prompted by alloimmunisation. Of the 29 preterm deliveries (25.2%), one was extremely preterm (less than 28 weeks' gestation), three were very preterm (28 to <32 weeks) and the remaining 25 were moderate to late preterm (32 to <37 weeks). Excluding pregnancies that had both a non-D Rh antibody and another independent antibody present, the compound antibodies anti-CD and anti-ce resulted in the largest proportion of affected pregnancies leading to preterm delivery - 6 out of 9 of the former, and the single case of the latter.

The outcomes of the 117 neonates are detailed in Table 2. Just over half of all pregnancies affected by non-D Rh alloimmunisation resulted in neonates who required phototherapy. Of the

neonates affected by non-D Rh antibodies alone, the compound antibodies had the highest proportion of neonates requiring phototherapy: the single case with anti-ce and 7 out of 9 neonates affected by anti-CD.

Only 7.7% (9/117) of infants required treatments for anemia. Three were treated with ferrous sulphate (one anti-C, one anti-c and one anti-E), while the other six received an exchange red blood cell transfusion (four anti-C with anti-D independently, one anti-c and one anti-CD). The ferrous sulphate dose used was 6 mg/kg oral daily until six months of age. No infant was recorded as having required a top-up transfusion.

Table 3 summarizes the distribution and highest level of antibody titers for those pregnancies in which a severe adverse outcome other than perinatal loss occurred.

Six FDIUs occurred in the overall study cohort; one was determined at autopsy to have been caused by alloimmunisation. Two had no autopsy performed, two were unrelated to alloimmunisation and in one the cause was unknown. Two miscarriages were suggested to have been a result of alloimmunisation, both to the same mother. The other six miscarriages had no cause identified. The one FDIU and two miscarriages attributed to alloimmunisation were caused by anti-CD, the highest titers of which were 1:16384, 1:32768 and 1:2048.

DISCUSSION

The incidence of non-D Rh alloimmunisation in this study was 0.33% which is in keeping with a study by Chandrasekar et al. in Northern Ireland, who found an incidence of 0.28% (99/34,913).(9) It is, however, considerably higher than the 0.16% (128/78,145) Gotvall and Filbey found in Sweden.(10) This difference may be explained by the genetic variance in populations examined. The distribution of Rh antigens in different populations can vary considerably and there is the potential that the transfusion practices in different countries may play a role in the number of women who become sensitized to non-D Rh antibodies. Currently, in Australia, an extended Rh type is only performed if the patient has known antibodies or if the patient is to undergo a long-term transfusion regimen.(11)

Despite the difference in overall incidence, the proportion of each antibody was largely in keeping with current literature (9, 10, 12, 13) with anti-E being the most common antibody after

anti-D, accounted for 42.6% of non-D Rh alloimmunisation. One noticeable difference however was that these other studies (12-15) all identified the next most common antibody to be anti-c, rather than anti-C as we found.

Across all antibody types, the critical titer was reached in 42.6% of pregnancies from a non-D Rh antibody alone. No severe adverse outcomes (need for IUT, red blood cell exchange transfusion or use of intravenous immunoglobulin) were noted in pregnancies that did not exceed the critical titer, suggesting that this threshold remains clinically appropriate (cf. Table 3).

Although anti-E was by far the most common antibody found, it did not produce the highest titers. In our study, just less than one third (14/49) of pregnancies affected by anti-E reached the critical titer and only 34% (16/47) of neonates had a positive direct antiglobulin test. Anti-E is most commonly associated with mild to moderate hemolytic disease of the fetus or newborn (HDFN).(15) There are, however, case reports and small studies which have reported cases of clinically significant HDFN caused by anti-E.(14, 16, 17) Our results indicated generally less severe outcomes, with 40.4% (19/47) requiring phototherapy and 23.4% (11/47) requiring admission to Special Care Nursery/ Neonatal Intensive Care Unit. However, we did find that of the 49 pregnancies affected by anti-E, three went on to require an IUT (although one was as a result of a much higher anti-Fya titer).

Of the 15 cases of anti-c, eight reached the critical titer, although none of these required an IUT. Two studies have reported that anti-c causes only mild to moderate HDFN in most cases. (20, 21) Our study had similar findings, with two of 15 cases receiving treatment for anemia, and both receiving intravenous immunoglobulin (500mg/kg IV, then repeated 12 hours later). There have also however been studies (22, 23) and a case report (24) suggesting anti-c can cause more severe HDFN.

Aside from an isolated case study (18), anti-e is not usually associated with severe HDFN.(19) In our study only one of three cases reached the critical titer, needing Special Care Nursery/ Neonatal Intensive Care Unit admission and treatment for hyperbilirubinemia.

Three pregnancies were affected by anti-C^w during the study period. One neonate received phototherapy, although this pregnancy was affected by both anti-C^w and anti-S. No other adverse

outcomes were recorded. Anti-C^w is thought to cause only mild disease (6) however there have been rare case reports of anti-C^w causing hydrops fetalis or severe fetal anemia (25, 26).

There are few case reports regarding anti-C alloimmunisation alone.(27) It has more often been reported that anti-C can be additive to the hemolytic effects of anti-D (28) and is more often associated with severe outcomes in compound antibodies or in pregnancies affected by multiple antibodies.(29) This was the case in our study: of the pregnancies affected by anti-C alone, 20% reached the critical titer compared to 84.6% when anti-C occurred with anti-D. As outlined in Tables 1 and 2, rates of cesarean section, induction of labor, direct antiglobulin positivity, and treatments for hyperbilirubinemia and anemia were all more common when anti-D accompanied anti-C. This is in keeping with Spong et al's finding that the presence of anti-D was the most significant factor in determining the clinical outcome of a pregnancy affected by multiple antibodies.(30)

Three compound antibodies were identified during the study period: anti-ce (also known as anti-f), anti-cE and anti-CD. Both anti-ce and anti-cE had mild effects on the course of the pregnancy and neonatal outcome.

Nine pregnancies were identified to have anti-CD alone. Of these nine cases, four required IUTs, which was 36.4% of all IUTs performed in non-D Rh alloimmunised pregnancies. It also accounted for 30% of all cases requiring the use of intravenous immunoglobulin and one sixth of the cases requiring an red blood cell exchange transfusion. During the study period there were two first trimester miscarriages and one FDIU attributed to alloimmunisation. All three occurred as a result of anti-CD. There are few papers that discuss the outcomes and complications associated with compound antibodies directly and therefore little data with which to draw comparisons. At the time of this study our laboratory did not routinely test for anti-G, as a result we were unable to determine if our anti-CD samples were, in fact, anti-G or anti-C+G, and have therefore described them only as anti-CD (as originally reported).

The main strength of this study lies in the fact that there have been, to our knowledge, no other studies into non-D Rh alloimmunisation in the Australian population. However, the Royal Women's Hospital's role as a large, specialist, public hospital that receives referrals from across the state of Victoria may skew the incidence of non-D alloimmunisation, as other hospitals in Victoria will refer women alloimmunised with high risk antibodies. As a proportion of our

deliveries, these antibodies are likely to be overrepresented given the tertiary nature of this hospital.

Unfortunately, there were insufficient data on the miscarriages, terminations and FDIU to include them in the final data sample, as many of these patients received care from other institutions. We could not obtain accurate and complete data sets for these cases and therefore they were removed from subsequent analysis, as we were concerned that their inclusion in the absence of complete data regarding the impact of alloimmunisation on these losses would skew the results. We do acknowledge, however, that this is a significant limitation, and merits further investigation in future studies.

We were also limited by the rarity of non-D Rh alloimmunisation leading to small numbers in our study. This highlights an area for a future broader study with multiple hospitals across various states leading to a larger patient population.

CONCLUSION

This study has shown that although non-D Rh alloimmunisation is still a relatively uncommon complication of pregnancy, it can lead to significant adverse fetal/neonatal outcomes, including miscarriage, FDIU, or consideration of termination of pregnancy on account of fetal morbidity. It therefore must be identified and, if present, acted upon by all involved in maternity care provision. The most severe outcomes (including perinatal deaths) were mostly associated with the compound antibodies anti-CD and anti-cE or a non-D Rh antibody in conjunction with anti-D.

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References:

1. de Haas M, Finning K, Massey E, Roberts DJ. Anti-D prophylaxis: past, present and future. Transfus Med. 2014;24(1):1-7.

2. Qureshi H, Massey E, Kirwan D, Davies T, Robson S, White J, et al. BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn. Transfus Med.. 2014;24(1):8-20.

3. Zipursky A, Paul VK. The global burden of Rh disease. Arch Dis Child Fetal Neonatal Ed. 2011;96(2):F84-5..
4. Moise KJ, Jr., Argoti PS. Management and prevention of red cell alloimmunization in pregnancy: a systematic review. Obstet Gynecol. 2012;120(5):1132-9.
5. Koelewijn JM, Vrijkotte TG, de Haas M, van der Schoot CE, Bonsel GJ. Risk factors for the presence of non-rhesus D red blood cell antibodies in pregnancy. BJOG. 2009;116(5):655-64.
6. Daniels G, Poole J, de Silva M, Callaghan T, MacLennan S, Smith N. The clinical significance of blood group antibodies. Transfus Med. 2002;12(5):287-95.
7. Cacciatore A, Rapiti S, Carrara S, et al. Obstetric management in Rh alloimmunizated pregnancy. J Prenatal Med. 2009;3(2):25-7.
8. National Health and Medical Research Council. Ethical Considerations in Quality Assurance and Evaluation Activities. Canberra, ACT, Australia 2014.
9. Chandrasekar A, Morris KG, Tubman TR, Tharma S, McClelland WM. The clinical outcome of non-RhD antibody affected pregnancies in Northern Ireland. Ulster Med J. 2001;70(2):89-94.
10. Gottvall T, Filbey D. Alloimmunization in pregnancy during the years 1992-2005 in the central west region of Sweden. Acta Obstet Gynecol Scand. 2008;87(8):843-8.
11. National Pathology Accreditation Advisory Council. Requirements for Transfusion Laboratory Practice. In: Health AGDo, editor. 2nd ed. Canberra: Commonwealth of Australia; 2013.
12. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. Transfusion. 2008;48(5):941-52.
13. Zwingerman R, Jain V, Hannon J, Zwingerman N, Clarke G. Alloimmune Red Blood Cell Antibodies: Prevalence and Pathogenicity in a Canadian Prenatal Population. J Obstet Gynaecol Can. 2015;37(9):784-90.
14. Moran P, Robson SC, Reid MM. Anti-E in pregnancy. BJOG. 2000;107(11):1436-8.
15. Joy SD, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-E alloimmunization. Obstet Gynecol. 2005;105(1):24-8.
16. Pepperell RJ, Barrie JU, Fliegner JR. Significance of red-cell irregular antibodies in the obstetric patient. Med J Aust. 1977;2(14):453-6.
17. Chao AS, Chao A, Ho SY, Chang YL, Lien R. Anti-e alloimmunization: a rare cause of severe fetal hemolytic disease resulting in pregnancy loss. Case Rep Med. 2009;2009:471623.
18. McAdams RM, Dotzler SA, Winter LW, Kerecman JD. Severe hemolytic disease of the newborn from anti-e. J Perinatol. 2008;28(3):230-2.
19. Eder AF. Update on HDFN: new information on long-standing controversies. Immunohematology. 2006;22(4):188-95.
20. Hackney DN, Knudtson EJ, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-c isoimmunization. Obstet Gynecol. 2004;103(1):24-30.
21. Thakral B, Agrawal SK, Dhawan HK, Saluja K, Dutta S, Marwaha N. First report from India of haemolytic disease of newborn by anti-c and anti-E in Rh (D) positive mothers. Hematology. 2007;12(5):377-80.
22. Rath ME, Smits-Wintjens VE, Lindenburg IT, et al. Postnatal outcome in neonates with severe Rhesus c compared to rhesus D hemolytic disease. Transfusion. 2013;53(7):1580-5.

23. Slootweg YM, Koelewijn JM, van Kamp IL, van der Bom JG, Oepkes D, de Haas M. Third trimester screening for alloimmunisation in Rhc-negative pregnant women: evaluation of the Dutch national screening programme. BJOG. 2016;123(6):955-63.
24. Sheeladevi CS, Suchitha S, Manjunath GV, Murthy S. Hemolytic Disease of the Newborn Due to Anti-c Isoimmunization: A Case Report. Indian J Hematol Blood Transfus. 2013;29(3):155-7.
25. Kollamparambil TG, Jani BR, Aldouri M, Soe A, Ducker DA. Anti-C(w) alloimmunization presenting as hydrops fetalis. Acta Paediatr. 2005;94(4):499-501.
26. Byers BD, Gordon MC, Higby K. Severe hemolytic disease of the newborn due to anti-Cw. Obstet Gynecol. 2005;106(5 Pt 2):1180-2.
27. Allgood C, Bolisetty S. Severe conjugated hyperbilirubinaemia and neonatal haemolysis. Int J Clin Pract. 2006;60(11):1513-4.
28. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Sem Fetal Neonatal Med. 2008;13(4):207-14.
29. Sharma D, Dannapuneni N, Murki S, Pratap T. Combined Anti e and Anti C Rh Isoimmunisation and Severe Hyperbilirubinemia. Indian J Pediatr. 2015;82(6):570.
30. Spong CY, Porter AE, Queenan JT. Management of isoimmunization in the presence of multiple maternal antibodies. Am J Obstet Gynecol. 2001;185(2):481-4.

Supporting Information legend

TABLE S1: Information collected

Legends

TABLE 1: Course of the pregnancy and delivery

Antibody	**Critical titer reached (> 16)**	**IUT performed (MoM >1.5)**	**IOL or CS prompted by alloimmunisation**	**Preterm delivery (<37 weeks)**
Anti-E (n=46)	14 (30.4%)	2 (4.35%)	12 (26.1%)	7 (15.2%)
Anti-C (n=10)	2 (20%)	0	2 (20%)	2 (20%)
Anti-c (n=15)	8 (53.3%)	0	5 (33.3%)	0
Anti-e (n=3)	1 (33.3%)	0	0	0
Anti-CD (n=9)	8 (88.9%)	4 (44.4%)	6 (66.7%)	6 (66.7%)
Anti-cE (n=11)	10 (90.9%)	0	2 (18.2%)	3 (27.3%)
Anti-ce (n=1)	0	0	0	1 (100%)
Anti-E and Anti-Fya (n=2)	1 (50%)*	1 (50%)	1 (50%)	1 (50%)
Anti-C and Anti-D (n=13)	11 (84.6%)ɾ	4 (30.8%)	9 (69.2%)	7 (53.8%)
Anti-CD and Anti-Kell (n=1)	1 (100%)‡	0`	1 (100%)	1 (100%)
Anti-cE and Anti-Jka (n=1)	1 (100%)§	0	0	0
Anti-C^w and Anti-E (n=1)	0	0	0	0
Anti-C^w and Anti-Fya (n=1)	0	0	0	0
Anti-C^w and Anti-S (n=1)	0	0	0	1 (100%)
Total (n=115)	57 (49.6%)	11 (9.6%)	38 (33%)	29 (25.2%)

*critical titer reached in Anti-Fya

ᴛ critical titer reached in Anti-D alone in 6 cases, critical titer reached in both Anti-D and Anti-C in 4 cases and critical titer reached in Anti-C alone in 1 case

‡ critical titer reached in Anti-CD and Anti-K

§ critical titer reached in Anti-Jka

IUT = intrauterine transfusion, MOM = multiples of median, IOL = induction of labour, CS = cesarean section

Combinations of antibodies were included when an antibody other than a non-D Rh antibody was deemed to be of clinical importance.

TABLE 2: Neonatal outcomes and requirements for specific treatments

Antibody	SCN/NICU Admission	Positive Direct Antiglobulin Test	Phototherapy	Ferrous Sulphate	Red cell exchange transfusion	IVIg
Anti-E (n=47*)	11 (23.4%)	16 (34%)	19 (40.4%)	1 (2.1%)	0	0
Anti-C (n=11*)	1 (9.1%)	4 (36.4%)	3 (27.3%)	1 (9.1%)	0	0
Anti-c (n=15)	9 (60%)	13 (86.7%)	9 (60%)	1 (6.7%)	1 (6.7%)	2 (13.3%)
Anti-e (n=3)	1 (33.3%)	2 (66.7%)	1 (33.3%)		0	0
Anti-CD (n=9)	6 (66.7%)	3 (33.3%)	7 (77.8%)	0	1 (11.1%)	3 (33.3%)
Anti-cE (n=11)	3 (27.3%)	2 (18.2%)	4 (36.4%)	0	0	0
Anti-ce (n=1)	1 (100%)	0	1 (100%)	0	0	0
Anti-E and Anti-Fya (n=2)	1 (50%)	1 (50%)	2 (100%)	0	0	0
Anti-C and Anti-D (n=13)	9 (69.2%)	8 (61.5%)	10 (76.9%)	0	4 (30.8%)	4 (30.8%)
Anti-CD and Anti-Kell (n=1)	1 (100%)	1 (100%)	1 (100%)	0	0	1 (100%)
Anti-cE and Anti-Jka (n=1)	0	1 (100%)	1 (100%)	0	0	0
Anti-C^w and Anti-E (n=1)	0	0	0	0	0	0
Anti-C^w and Anti-Fya (n=1)	0	0	0	0	0	0
Anti-C^w and Anti-S (n=1)	0	0	1 (100%)	0	0	0
Total (n=117)	43 (36.8%)	51 (43.6%)	59 (50.4%)	3 (2.6%)	6 (5.1%)	10 (8.5%)

* = including twins

SCN = special care nursery, NICU = neonatal intensive care unit, IVIg = intravenous immunoglobulin

Combinations of antibodies were included when an antibody other than a non-D Rh antibody was deemed to be of clinical importance.

Non-D Rh Antibody	Non-D Rh Antibody Highest Titer	Other Antibody	Other Antibody Highest Titer	TREATMENT MODALITY		
				IUT	RCEx	IVIg
Anti-c	128	Anti-Fya	8	No	Yes	No
Anti-c	256	Anti-Fya	16	No	No	Yes
Anti-c	256	-	-	No	No	Yes
Anti-C	Below detectable	Anti-D	2048	Yes	No	No
Anti-C	1	Anti-D	1024	No	Yes	Yes
Anti-C	1	Anti-D	2048	Yes	No	No
Anti-C	2	Anti-D	512	No	Yes	Yes
Anti-C	4	Anti-D	1024	Yes	No	Yes
Anti-C	8	Anti-D	16384	Yes	No	No
Anti-C	32	Anti-D	256	No	Yes	No
Anti-C	64	Anti-D	128	Yes	No	No
Anti-C	1024	Anti-D	512	No	Yes	Yes
Anti-E	2	Anti-Fya	512	Yes	No	No
Anti-E	512	-	-	Yes	No	No
Anti-E	512	-	-	Yes (failed)	No	No
Anti-CD	64	Anti-K	4096	No	No	Yes
Anti-CD	256	-	-	No	Yes	Yes
Anti-CD	512	Anti-Fya	16	Yes	No	No
Anti-CD	8192	Anti-D	1024	Yes	No	Yes
Anti-CD	16384	-	-	Yes	No	No
Anti-CD	32768	Anti-S	1	Yes	No	Yes

TABLE 3: Severe outcomes*

IUT = intrauterine red blood cell transfusion, RCEx = Red cell exchange transfusion, IVIg = intravenous immunoglobulin

* Severe outcomes, excluding fetal death in utero, termination and miscarriage, were defined as the **need** for IUT, RCEx or IVIg.

FIGURE 1: Inclusion and exclusion criteria

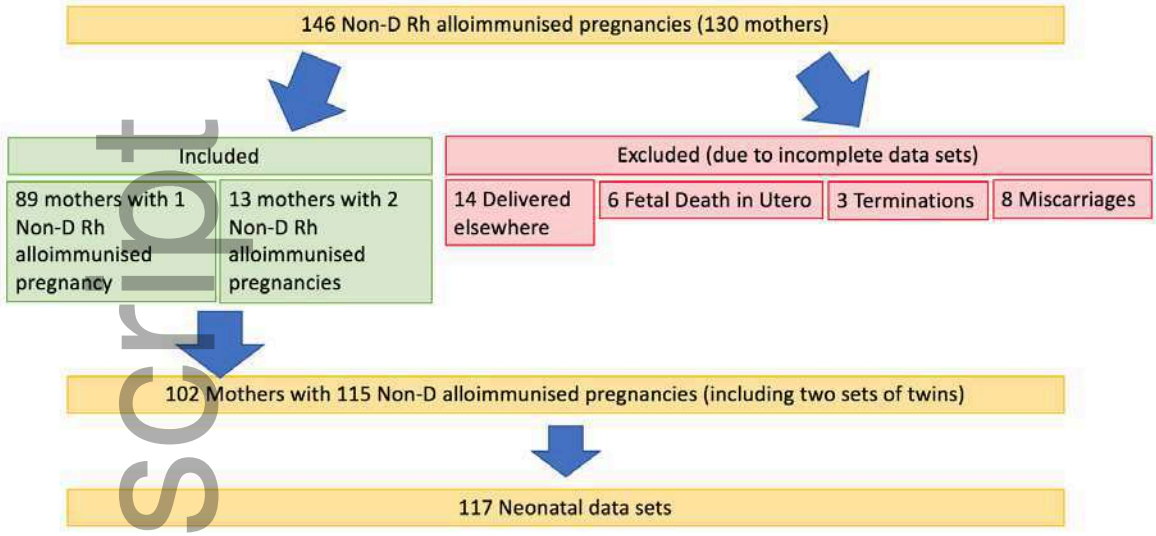
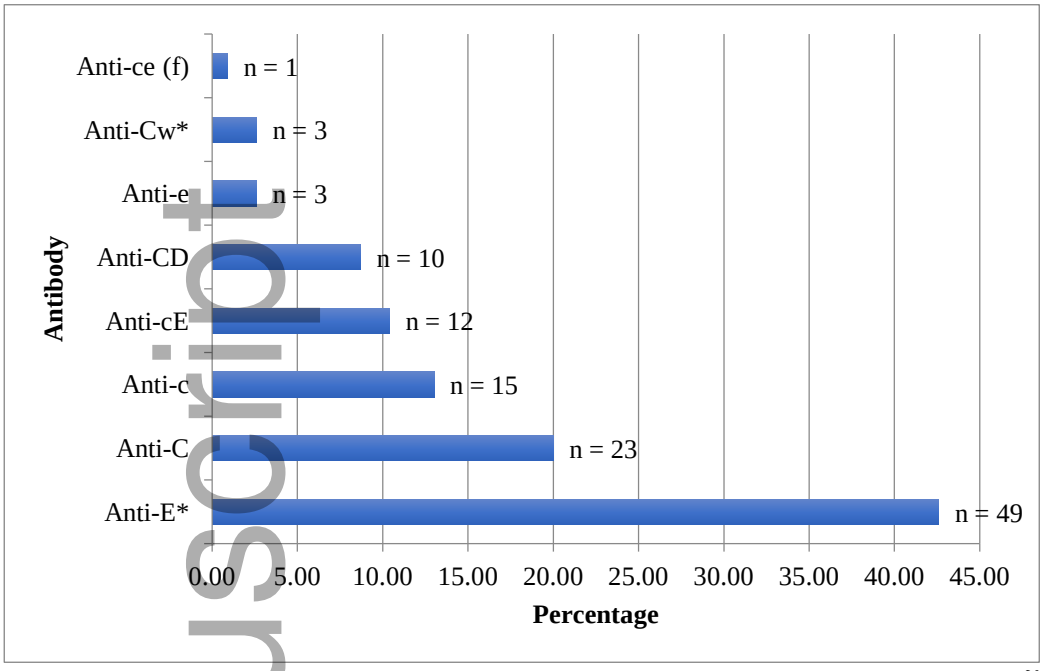


FIGURE 2: Rh antibody incidence



*One pregnancy had independent antibodies from two non-D Rh antibodies (Anti-E and Anti-C^{w}).