Following targeted routine antenatal anti-D prophylaxis, almost half of the pregnant women had undetectable anti-D prophylaxis at delivery

Kirsten Sørensen1 | Helena Eriksson Stjern2 © | Bente Anita Grande Karlsen2 | Geir Tomter1 | Inger Ystad2 | Ida Herud1 | Mette Silihagen Bævre1 | Abid Hussain Llohn2 | Çiğdem Akalın Akkök1 ©

Abstract

Introduction: In September 2016, a nationwide targeted routine antenatal anti-D prophylaxis program was implemented in Norway. The prophylaxis (anti-D immunoglobulin) aims to cover the whole third trimester and is administered in gestational week 28 to RhD-negative women who carry RhD-positive fetuses. However, in many women, antibody screening at delivery does not detect anti-D immunoglobulin. The goal of this study was to investigate the presumable role of dose and timing of antenatal anti-D immunoglobulin administration in non-detectable prophylaxis at the time of delivery.

Material and methods: In this retrospective observational study, RhD-negative pregnant women who gave birth at Oslo University Hospital and Akershus University Hospital between January 2017 and December 2019 were analyzed. Women who received antenatal anti-D immunoglobulin (1500 IU at Oslo University Hospital and 1250 IU at Akershus University Hospital) when fetal RHD genotyping at gestational week 24 predicted an RhD-positive fetus were included if an antibody screen at delivery was available. Data from the blood bank, maternity information systems, and electronic patient records were used.

Results: Analysis of the 984 RhD-negative women at the two hospitals revealed that 45.4% had non-detectable anti-D at delivery. A significant difference between the two hospitals was observed: 40.5% at Oslo University Hospital (n = 509) and 50.7% at Akershus University Hospital (n = 475) (p = 0.001). The proportion with non-detectable anti-D increased to 56.0 and 75.3%, respectively (p = 0.008) in the group of women who gave birth 12 weeks after routine antenatal anti-D prophylaxis. Significantly fewer women had detectable anti-D at delivery when the lower anti-D

Abbreviations: AUH, Akershus University Hospital; FMH, fetomaternal hemorrhage; GW, gestational week; OUH, Oslo University Hospital; RAADP, routine antenatal anti-D prophylaxis.
A nationwide targeted routine antenatal anti-D prophylaxis (RAADP) program was implemented in Norway in September 2016, with fetal RHD genotyping at gestational week (GW) 24 together with antibody screening and administration of anti-D prophylaxis at GW 28. Targeted RAADP is administered in addition to the postnatal anti-D prophylaxis given within 72 h when the newborn is typed as RhD positive. The antenatal dose of anti-D immunoglobulin (Ig) recommended in Norwegian national guidelines for obstetrics is 1500 IU. The aim of the RAADP program is to reduce pregnancy-related RhD immunizations and anti-D-associated hemolytic disease of fetus and newborn. The Norwegian Directorate of Health, which approved the implementation, advised systematic monitoring of the program to assess whether these goals are achieved. The Norwegian National Advisory Unit on Immunohematology at Department of Immunology and Transfusion Medicine, Oslo University Hospital (OUH), Ullevaal, therefore developed and established a local quality registry with parameters necessary for the assessment. Other transfusion services, Akershus University Hospital (AUH) being the first, also established local registries with the same parameters.

The goal of RAADP is to cover the whole third trimester until delivery and protect pregnant women from RhD immunization due to silent fetomaternal hemorrhage (FMH). There is no international consensus regarding anti-D Ig dose and timing. The recommended dose of prophylaxis varies, with either a single dose of 1500 IU anti-D Ig at GWs 28–30 or two doses of 500–625 IU at GW 28 and GW 34 being used in different countries. The proportions of non-detectable anti-D at delivery after RAADP at GW 28 are reported to vary from 20.5 to 78%. The aim of this study was to assess the proportions of non-detectable anti-D in the Norwegian setting when targeted RAADP was given at about GW 28. Furthermore, the presumable impact of dose and timing of prophylaxis administration on non-detectable anti-D Ig at delivery is investigated and discussed to seek potential optimization of the RAADP program.

Key message
Of women who received routine antenatal anti-D prophylaxis at about gestational week 28, 45% had a negative antibody screen at delivery. This may leave women at risk of anti-D immunization.
2.3 Targeted routine antenatal anti-D prophylaxis

All pregnant women are ABO/RhD typed and antibody screened at the first antenatal visit. For those who are RhD-negative, fetal RHD genotyping and antibody screening are performed at GW 24. Anti-D prophylaxis at GW 28 is recommended to non-D-immunized women when the fetus is predicted to be positive and when the result is inconclusive. Two different anti-D Ig products are available in Norway: 1500 IU Rhophylac (CSL Behring GmbH, Germany) and 1250 IU Rhesonativ (Octapharma, Switzerland). Due to a misunderstanding the latter product was in use at AUH during the whole study period and until April 2020, whereas the recommended dose of 1500 IU anti-D Ig was used from the start at OUH.

2.4 Fetal RHD genotyping

Fetal RHD genotyping is performed regionally at four laboratories, and 18 local laboratories perform ABO/Rhd typing and antibody screening in samples from pregnant women. The Norwegian National Advisory Unit on Immunohematology at Department of Immunology and Transfusion Medicine, OUH, performs fetal RHD genotyping for the whole health region of South-Eastern Norway. AUH and the other laboratories in the region forward samples for fetal RHD genotyping at OUH.

2.5 Antibody screening and identification of anti-D

Antibody screening is routinely performed at the first prenatal visit and at GW 24, and antibody screening at delivery is requisitioned.
when there is risk of bleeding. Additionally, women admitted to the observation ward at AUH are routinely antibody screened. The indirect agglutination test at 37 °C on fully automated platforms is used. At OUH, antibody screening is performed with three in-house screening cells using LISS/Coombs ID-Cards (BioRad) with the IH-1000 (BioRad) or Ortho LISS/Coombs cassettes (Ortho Clinical Diagnostic, Mannedorf, Switzerland) with Vision Max (Ortho Clinical Diagnostic). At AUH, commercial screen cells, Surgiscreen, using BioVue cassettes with IgG on Vision Max is the standard. Antibody identification is performed with a panel of group O cells of varying phenotypes. OUH uses an in-house panel of 14 cells and LISS/Coombs ID-Card (Bio-Rad), and AUH uses commercial cell panels from Ortho, Panel C untreated, with BioVue polyspecific cassettes on Vision Max.

2.6 Statistical analyses

Univariate and multivariate analyses were performed to assess the potential factors (anti-D Ig dose, interval between RAADP and antibody screen at delivery, and maternal age at delivery) that may be associated with detectable anti-D at the time of delivery. Women were categorized into two time interval groups—short (1–72 days) and long (73–100 days)—and further into two age groups—younger (18.4–31.9 years) and older (32.0–50.5 years)—using the median values of the study group as the cut-off points. Results are reported as unadjusted odds ratios (ORs) and adjusted ORs (aORs) and 95% confidence intervals (CIs). An independent sample Student’s t-test was used to compare maternal age at delivery, length of pregnancy, and GW for administration of RAADP between the women who gave birth at OUH and AUH.

The chi-squared test was used to test for associations between categorical variables and detectable anti-D at the time of delivery at OUH and AUH.

The level of statistical significance was set at a p value of <0.05. Statistical analyses were performed in Excel and IBM SPSS Statistics.

2.7 Ethical approval

The quality registries were approved by the local data protection officers for research at OUH (ref. 21/10417) on September 4, 2016, and at AUH (ref. 16–152) on October 12, 2016. Data protection officers for research judged that informed consent was not required as they considered the study to be a quality assurance study without any intervention and the women were managed in precisely the same way as in routine management.

3 RESULTS

During the study period, we registered 4350 pregnancies with a fetal RHD genotyping performed at about GW 24. The number of incompatible pregnancies (n = 2659) corresponded to approximately 60% of the total number, that is, as expected when the frequency of RhD negatives in the population is about 15%, as it is in Norway. In total, 1675 deliveries were excluded, mainly because antibody screening had not been performed at delivery (Figure 1). We investigated 984 deliveries to assess the proportion of RhD-negative women with non-detectable anti-D at delivery when targeted RAADP was

### TABLE 1 Variables in the local quality registries

| Variable                                                                 | Total (n = 984) | OUH (n = 509) | AUH (n = 475) |
|---------------------------------------------------------------------------|----------------|--------------|--------------|
| Name/unique ID and social security number of the pregnant woman           |                |              |              |
| Estimated date of delivery                                                |                |              |              |
| Date and gestational week of first antibody screen                        |                |              |              |
| Result of first antibody screen                                           |                |              |              |
| Received anti-D prophylaxis due to a sensitizing event (date)? Yes/no     |                |              |              |
| Date of antibody screen and fetal RHD genotyping at GW 24                |                |              |              |
| Result of antibody screen at GW 24                                        |                |              |              |
| Result of fetal RHD genotyping at GW 24                                   |                |              |              |
| Date RAADP given                                                          |                |              |              |
| Date of delivery                                                          |                |              |              |
| Social security number of the child                                       |                |              |              |
| RhD type of the newborn                                                   |                |              |              |
| Date of postnatal anti-D prophylaxis                                      |                |              |              |
| If performed, result of antibody screen at delivery                       |                |              |              |

Abbreviations: GW, gestational week; RAADP, routine antenatal anti-D prophylaxis.

### TABLE 2 Characteristics of women included in the study

|                          | Total (n = 984) | OUH (n = 509) | AUH (n = 475) |
|--------------------------|----------------|--------------|--------------|
| Maternal age at delivery (years) | 32.3 (4.8) | 33.3 (4.4) | 31.3 (4.9) |
| Length of pregnancy (weeks)            | 39.2 (2.0) | 39.1 (2.1) | 39.4 (1.8) |
| Administration of RAADP (GW)           | 29.2 (1.8) | 28.9 (1.4) | 29.7 (2.0) |

Abbreviations: AUH, Akershus University Hospital; GW, gestational week; OUH, Oslo University Hospital; RAADP, routine antenatal anti-D prophylaxis; SD, standard deviation.
given at about GW 28. Negative antibody screening at delivery was found in 45.4% of the women (Table 3), with a significant difference between the two hospitals: 40.5 and 50.7% at OUH and AUH, respectively (p = 0.001) (Table 3). Administration of RAADP ranged from GW 25 to 39 in the whole study population (Table 2, Figure 2). Analyzing only women who received anti-D prophylaxis at GW 28 (n = 368), as recommended by the Norwegian national guidelines for obstetrics, showed that the proportion of negative antibody screens at delivery was 52.7%, and a significant difference at the two hospitals was also observed: 45.8% (OUH) and 70.2% (AUH) (p < 0.001) (Table 3).

The mean maternal age at delivery was lower at AUH (p < 0.001), and the mean length of pregnancy was shorter at OUH (p = 0.032) (Table 2). Significantly more women at AUH (67.2%) than at OUH (32.8%) had available antibody screen results 0–7 days prior to delivery (p < 0.001) (Table 3). Almost 90% of the screens were performed on the day of or the day before delivery (data not shown).

3.1 | Timing of RAADP administration

Figure 2 shows the timing of RAADP. The majority (70.7%) received the prophylaxis at GW 28–29: 82.3% at OUH and 58.3% at AUH. At both hospitals, <4% of the RAADP was given earlier than GW 28, whereas 25.4% received the prophylaxis at GW 30–39. Shipping samples from AUH and waiting to receive fetal RHD results probably delays the administration of anti-D prophylaxis. Pregnant women at AUH received anti-D prophylaxis later than those at OUH: GW 29.7 vs. 28.9, respectively (p < 0.001) (Table 2).

3.2 | Relation between detectable anti-D at delivery and time interval from RAADP to antibody screen at delivery

We investigated how the interval between the administration of RAADP and delivery influenced the detectability of anti-D Ig at delivery (Figure 3a and b). The percentage of women with a negative antibody screen was <20% when the interval was <9 weeks. However, when analyzing the two hospitals separately, we observed a marked increase in non-detectable anti-Ds at 11 and 8 weeks following RAADP at OUH and AUH, respectively, illustrating a faster increase in the proportion of negative antibody screens at delivery at AUH than at OUH. With an interval of 12 weeks between RAADP and delivery, the percentages of negative antibody screen was 65.1% for the whole group, while it was 56.0% and 75.3% at OUH and AUH (p = 0.008), respectively (Figure 3b and Table 3).
3.3 | Potential factors associated with detectable anti-D at delivery

Univariate analyses indicated that higher anti-D dose (60.0 vs. 49.3%), shorter time interval (79.0 vs. 31.1%), and older age (58.2 vs. 51.0%) were significantly associated with detectable anti-D at delivery ($p < 0.03$ for all comparisons based on the chi-squared test; Table 4). In adjusted multivariate analyses (Table 4), anti-D dose (aOR 2.2; 95% CI 1.6–2.9; $p < 0.001$) and time interval (aOR 9.4; 95% CI 6.9–12.8; $p < 0.001$) remained significantly associated with detectable anti-D at delivery. However, maternal age was no longer a statistically significant factor. The model accounted for 31% of the variance within the dataset (Nagelkerke $R^2 = 0.314$).

4 | DISCUSSION

Guidelines and management strategies to prevent anti-D immunization in pregnancy vary widely worldwide. Studies have demonstrated a decrease in the number of pregnancy-related RhD immunizations after implementation of RAADP. A meta-analysis comparing several regimens found strong evidence for the effectiveness of RAADP in general: 500 IU at GW 28 and GW34, or 1500 IU at GW 28–30, or 1250 IU at GW 28 and GW 34. Administration of 1250 IU at GW 28 and GW 34 had the highest probability (83%) of effectiveness, and the single dose of 1500 IU had the least effectiveness (76%). A one-dose regimen has the advantage over two doses because it eliminates the risk of the second dose being skipped because of compliance issues.

We investigated 984 deliveries at two hospitals and showed that 45.4% of the women had a negative antibody screen at delivery. The antenatal prophylaxis dose administered at the two hospitals differed (1500 IU at OUH and 1250 IU at AUH). We showed a significant difference in the proportion of negative antibody screens at delivery between OUH and AUH: 40.5 and 50.7%, respectively. Our findings support the reports from Davies et al. and White et al, which showed that 78 and 44% of women ($n = 157$ and $n = 125$) who received 1500 IU anti-D Ig at GW 28 had no detectable anti-D at delivery. However, Wikman et al. found that only 20.5% ($n = 4280$) of women who received 1250 IU anti-D Ig at GW 28 had negative antibody screens at delivery.

The lack of detectable anti-D Ig at delivery and its implications for the prevention of RhD immunization during the last weeks of pregnancy is a common concern and indicates that the prophylaxis does not last long enough to cover the whole third trimester, especially the critical last couple of weeks when the risk of FMH is highest. It does not necessarily mean that the RhD-negative pregnant woman is unprotected and prone to RhD alloimmunization, but it does predict clearance due to the half-life of anti-D Ig and it may imply a silent FMH consuming anti-D Ig. The current
FIGURE 3 (A and B) The time interval between the administration of routine antenatal anti-D prophylaxis (RAADP) and negative antibody screen at delivery. (A) The whole study group. (B) The study group separated into deliveries at Oslo University Hospital (OUH) and Akershus University hospital (AUH)
study had no follow-up after delivery, and the number of cases would have been insufficient to demonstrate a clinical impact on the RhD-immunization rate. Nevertheless, with a recently introduced national routine, it is important to search for possible explanations for and the consequences of a lack of detectable anti-D at delivery in almost half of the pregnant women.

One exclusion criterion was an inconclusive fetal RHD genotype because of a maternal RHD variant since D variants may express RhD epitopes that anti-D Ig binds to, leaving less anti-D Ig in the circulation. We also excluded women with other alloantibodies, as these women may be more prone to make further antibodies (i.e., anti-D). We used the last antibody screen available (0–7 days before delivery) because earlier positive screens that may have become negative within the final weeks of pregnancy could have biased the results.

The lower maternal age at AUH (Table 2) may be because the hospital serves a younger population. Both hospitals have similar indications for requesting type and screen at delivery; however, more antibody screens were performed at AUH than at OUH. This seems paradoxical as OUH is the largest hospital in the country, with national responsibilities, including more complicated cases, so we expected the need for screening to be higher. Similarly, this may be the reason for the shorter length of pregnancy at OUH, as labor is often induced before the due date in complicated cases.

When investigating the impact of the time interval between RAADP and delivery on the detectability of anti-D at delivery, we observed that the number of non-detectable anti-Ds increased with the interval (Figure 3a and b) as previously shown.

According to the manufacturers, the half-life of both 1250 IU Rheonativ and 1500 IU Rhophylac anti-D Ig is 3–4 weeks, but there are individual variations. A half-life of 3–4 weeks should give a residual amount of anti-D in the plasma of approximately 80–190 IU after 12 weeks. Antibody screening using gel technology, as at OUH, can detect approximately 60 IU of anti-D Ig in circulation, the amount required to prevent RhD immunization from a 0.5 mL bleed. Using a lower anti-D Ig dose affected the duration of the prophylaxis. OUH used 1500 IU, and AUH used 1250 IU during the whole study period. Following RAADP at GW 28, 45.8% (OUH) and 70.2% (AUH) negative antibody screens at delivery implied the impact of the anti-D Ig dose. It may also indicate that 1250 IU is insufficient to cover the whole last trimester. Guidelines recommend 1500 IU at GW 28–30 if using a one-dose regimen. However, an anti-D Ig dose of 1500 IU given at GW 28 may not be optimal either, because more than 50% of those who received 1500 IU had non-detectable anti-D after 12 weeks. The much later RAADP administration at AUH, with 37.7 vs 13.9% at OUH after GW 29, did not outweigh the lower anti-D Ig dose.

In the multivariable analysis (Table 4), anti-D dose, together with the time interval, remained significantly associated with detectable anti-D at delivery, but the impact of maternal age was not significant. The time interval between RAADP and delivery was the most important predictor of detectable anti-D at delivery. Women in the short-interval group were 9.4 times more likely to have detectable anti-D at delivery than those in the long-interval group, indicating that a longer time interval may be associated with poorer coverage in the last weeks of pregnancy. However, the model only explains 31% of the variance, indicating that other unmeasured factors such as maternal body mass index (BMI), twin pregnancy, individual variations in half-life of anti-D Ig, and/or undetected larger silent FMH may play an important role.

### Table 4

| Variable                           | Unadjusted OR (95% CI) | p-value | Unadjusted OR (95% CI) | p-value |
|------------------------------------|------------------------|---------|------------------------|---------|
| Anti-D dose*                        |                        |         |                        |         |
| 1500 IU (303 of 509)               | 1.5 (1.2–1.9)          | 0.001   | 2.2 (1.6–2.9)          | <0.001  |
| 1250 IU (234 of 475)               | 1.0                    |         | 1.0                    |         |
| Time interval*                     |                        |         |                        |         |
| Short (382 of 485)                 | 8.2 (6.2–11.0)         | <0.001  | 1.0                    | <0.001  |
| Long (155 of 499)                  | 1.0                    |         | 1.0                    |         |
| Age group*                         |                        |         |                        |         |
| Younger (253 of 496)               | 1.0                    | 0.024   | 1.1 (0.82–1.48)        | 0.527   |
| Older (284 of 488)                 | 1.3 (1.04–1.72)        |         | 0.59                  |         |

Notes: Short interval: 1–72 days (n = 485), long interval: 73–100 days (n = 499). Younger age: 18.4–31.9 years (n = 496), older age: 32.0–50.5 years (n = 488).

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; Ig, immunoglobulin; OR, odds ratio.

*Figures in parentheses indicate the number of women with detectable anti-D Ig at delivery out of the total number of women in the respective categorical groups.
Despite information on BMI, twin pregnancy, and mode of delivery being missing in our study, the difference being highly significant does not imply a demographic difference as the sole probable explanation.

Failures of antenatal and/or postnatal anti-D prophylaxis may be due to the following:

- omission of ante- and/or postnatal anti-D Ig,
- delayed administration or omission of anti-D Ig after sensitizing events,
- lower anti-D Ig dose than recommended due to administration errors,
- incorrect storage of anti-D Ig rendering it ineffective
- (significantly) delayed administration of ante- and/or postnatal anti-D Ig
- immunization that may occur during the pregnancy before administration of antenatal anti-D Ig at GW 28
- immunization that may occur during the pregnancy despite administration of antenatal anti-D Ig due to a large FMH or repeated FMH after GW 28, and
- inadequate postnatal anti-D Ig dose due to increased FMH, cesarean section, high BMI (controversial), postmaturity, or twin pregnancy (or triplets, quadruplets, etc.).

Our results demonstrated that prophylaxis given 2–4 weeks later than GW 28 increased the proportion of women with detectable anti-D Ig at delivery. As an interval of 8–10 weeks between administration of prophylaxis and delivery demonstrated detectable anti-D in about 80% of the women who received 1500 IU, rescheduling must be weighed against exposing the pregnant women to alloimmunization at the beginning of the last trimester. Nevertheless, the risk of FMH is highest in the last weeks of pregnancy. As mentioned, a two-dose regimen is an alternative and has been demonstrated to increase the proportion of women with detectable anti-D at delivery. However, evidence is insufficient to show superior efficacy. Any change in routine, such as postponing administration of the RAADP or introducing a two-dose regimen, must be discussed with maternal caretakers and can only be appropriate when combined with a scheduled routine control to avoid extra antenatal visits and costs. Antibody screening should also be performed closer to the administration of RAADP so as not to leave alloimmunization unrevealed during the second trimester.

Finally, compliance with postnatal prophylaxis is crucial, since the risk of FMH is highest during labor. We have not investigated failures of postnatal prophylaxis systematically, but there have been incidents (n = 9) where no documentation was found regarding the administration of prophylaxis or where prophylaxis was given later than 72 h. If such failures are revealed, we inform the woman and perform an antibody screen.

5 | CONCLUSION

Our study showed that anti-D was not detectable in 45.4% of the deliveries, and detectability depended on the dose as well as when the anti-D prophylaxis was given. Administration of RAADP closer to delivery could be considered to increase the proportion of women with detectable anti-D. Compliance with the recommended dose of 1500 IU is essential, as using 1250 IU increased the proportion of women with a negative screen at delivery. We do not know which women with a negative antibody screen at delivery are at risk of immunization, so further studies are necessary to address the issue. Despite a lack of detectable anti-D Ig at delivery, studies have shown that antenatal prophylaxis at GW 28 has halved the anti-D immunization rate. A nationwide study including follow-up after delivery with a sufficient number of cases will illuminate the outcomes of the Norwegian RAADP program and the incidence of de novo anti-Ds.

ACKNOWLEDGMENT

The authors gratefully acknowledge Dr. S.A. Mousavi, Department of Immunology and Transfusion Medicine, Akershus University Hospital, for multiple regression analyses.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

KS, HES, AHL, and ÇAA were responsible for the study design; KS, HES, BAGK, GT, IY, IH, and MSB for data collection; and KS and HES for the data analysis. All authors critically revised and approved the submitted version of the manuscript.

ORCID

Kirsten Sørensen https://orcid.org/0000-0002-3508-4764
Helena Eriksson Stjern https://orcid.org/0000-0002-1543-9994
Çiğdem Akalin Akkök https://orcid.org/0000-0001-7615-2906

REFERENCES

1. Sørensen K, Baevre MS, Tomter G, et al. The Norwegian experience with nationwide implementation of fetal RHD genotyping and targeted routine antenatal anti-D prophylaxis. Transfus Med. 2021;31:314-321.
2. Norwegian Society of Gynecology and Obstetrics (Norsk gynekologisk forening). Veileder i fødsels hjelp- Alloimmunisering mot erytrocytt-antigener (The Norwegian national guidelines for obstetrics - RBC alloimmunization) 2020 [cited 2021 09.06]. Available from: https://www.legeforeningen.no/foreningsled/fagmed/norsk-gynekologisk-forening/veileder/veileder-i-fodselshjelp-2020/alloimmuniserings-mot-erytrocytt-antigener/.
3. Arentz-Hansen B, Brurberg KG, Kramme MK, et al. NIPH systematic reviews. Determination of fetal rhesus D status from maternal plasma of rhesus negative women. Oslo, Norway: Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH); 2014.
4. Qureshi H, Massey E, Kirwan D, 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.
5. Fung KFK, Eason E. 133-prevention of rh alloimmunization. J Obstet Gynaecol Can. 2018;40:e1-e10.
6. Sperling JD, Dahlke JD, Sutton D, Gonzalez JM, Chauhan SP. Prevention of RhD alloimmunization: a comparison of four National Guidelines. Am J Perinatol. 2018;35(2):110-119.
7. Davies J, Chant R, Simpson S, Powell R. Routine antenatal anti-D prophylaxis - is the protection adequate? *Transfus Med*. 2011;21:421-426.

8. White SW, Cheng JC, Penova-Veselinovic B, et al. Single dose vs two-dose antenatal anti-D prophylaxis: A randomised controlled trial. *Med J Aust*. 2019;211:261-265.

9. Wikman A, Mörterberg A, Jalkesten E, et al. Altered strategy of prophylactic anti-D administration in pregnancy to cover term and post-term - a pilot study. *Vox Sang*. 2021;116:1005-1011.

10. Norwegian Institute of Public Health. Medical Birth Registry of Norway. [cited 2021 10.08]. Available from: http://statistikkbank.fhi.no/mfr/.

11. Koelewijn JM, de Haas M, Vrijkotte TG, van der Schoot CE, Bonsel GJ. Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. *BJOG*. 2009;116:1307-1314.

12. Jernman R, Isaksson C, Haimila K, et al. Time points and risk factors for RhD immunisations after the implementation of targeted routine antenatal anti-D prophylaxis: A retrospective nation-wide cohort study. *Acta Obstet Gynecol Scand*. 2021;100:1868-1875.

13. Turner RM, Lloyd-Jones M, Anumba DO, et al. Routine antenatal anti-D prophylaxis in women who are rh(D) negative: Meta-analyses adjusted for differences in study design and quality. *PLoS One*. 2012;7:e30711.

14. MacKenzie IZ, Findlay J, Thompson K, Roseman F. Compliance with routine antenatal rhesus D prophylaxis and the impact on sensitisations: observations over 14 years. *BJOG*. 2006;113:839-843.

15. Sebring ES, Polesky HF. Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects. *Transfusion*. 1990;30:344-357.

16. Bowman JM, Pollock JM. Antenatal prophylaxis of rh isoimmunization: 28-weeks'-gestation service program. *Can Med Assoc J*. 1978;118:627-630.

17. Daniels G. Variants of RhD - current testing and clinical consequences. *Br J Haematol*. 2013;161:461-470.

18. Lubenko A, Contreras M, Habash J. Should anti-rh immunoglobulin be given D variant women? *Br J Haematol*. 1989;72:429-433.

19. Gehrie EA, Tormey CA. The influence of clinical and biological factors on transfusion-associated non-ABO antigen alloimmunization: responders, hyper-responders, and non-responders. *Transfus Med Hemother*. 2014;41:420-429.

20. Crowther CA, Middleton P, McBain RD. Anti-D administration in pregnancy for preventing rhesus alloimmunisation. *Cochrane Database Syst Rev*. 2013;2:CD000020.

21. Practice Bulletin No. 181: Prevention of rh D alloimmunization. *Obstet Gynecol*. 2017;130:e57-e70.

22. Xie X, Fu Q, Bao Z, Zhang Y, Zhou D. Clinical value of different anti-D immunoglobulin strategies for preventing rh hemolytic disease of the fetus and newborn: a network meta-analysis. *PLoS One*. 2020;15:e0230073.

**How to cite this article:** Sørensen KP, Stjern HE, Karlsten BA, et al. Following targeted routine antenatal anti-D prophylaxis, almost half of the pregnant women had undetectable anti-D prophylaxis at delivery. *Acta Obstet Gynecol Scand*. 2022;101:431-440. doi: 10.1111/aogs.14328