Time points and risk factors for RhD immunisations after the implementation of targeted routine antenatal anti-D prophylaxis: a retrospective nation-wide cohort study

Riina Jernman¹; Camilla Isaksson¹; Katri Haimila²; Malla Kuosmanen²; Kaarin Mäkikallio-Anttila³; Suvi Toivonen²; Maija-Riitta Ordén⁴; Kati Sulin²; Kati Tihtonen⁵; Marja Vääräsmäki⁶; Susanna Sainio²

¹ Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
² Finnish Red Cross Blood Service, Helsinki, Finland
³ Obstetrics and Gynecology, Turku University Hospital, Turku, Finland
⁴ Obstetrics and Gynecology, Kuopio University Hospital, Kuopio, Finland
⁵ Obstetrics and Gynecology, Tampere University Hospital, Tampere, Finland
⁶ Obstetrics and Gynecology, Oulu University Hospital, Oulu, Finland

Corresponding author:
Riina Jernman
Department of Obstetrics and Gynecology, Haartmaninkatu 2, P.O. BOX 140, 00029 HUS, Helsinki, Finland

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ABSTRACT

Introduction: Targeted routine antenatal anti-D prophylaxis was introduced to the national prophylaxis program in Finland in late 2013. The aim of this study was to assess the incidence, time points and risk factors for RhD immunisation after the implementation of routine antenatal anti-D prophylaxis, in all women in Finland with antenatal anti-D antibodies detected in 2014-2017.

Material and methods: In a nationwide population-based retrospective cohort study, the incidence, time points and risk factors of anti-D immunisations were analysed. Information on antenatal screening was obtained from the Finnish Red Cross Blood Service database, and obstetric data from hospital records and the Finnish Medical Birth Register.

Results: The study included altogether 228 women (197 with complete data of all pregnancies). After the implementation of routine antenatal anti-D prophylaxis, the prevalence of pregnancies with anti-D antibodies decreased from 1.52% in 2014 to 0.88% in 2017, and the corresponding incidence of new immunisations from 0.33% to 0.10%. Time points for detection of new anti-D antibodies before and after 2014 were the first screening sample at 8-12 weeks of gestation in 52% vs. 19%, the second sample at 24-26 weeks in 20% vs. 50% and at the third screening at 36 weeks in 28% vs. 32%.

Conclusions: The incidence of new anti-D immunisations decreased expectedly after the implementation of routine antenatal anti-D prophylaxis. True failures are rare and they mainly
occur when the prophylaxis is not given appropriately, suggesting a need for constant education of healthcare professionals on the subject.

**Key words**
anti-D, immunisation, routine antenatal anti-D prophylaxis, fetomaternal hemorrhage, prevention, risk factors

**Abbreviations**
FMH: fetomaternal hemorrhage
FRC: Finnish Red Cross
PPH: postpartum hemorrhage
PSE: potentially sensitising event
RAADP: routine antenatal anti-D prophylaxis
RBC: red blood cell

**Key message**
The incidence of antenatal anti-D immunisations has decreased in Finland during 2014-2017 after the implementation of routine antenatal anti-D prophylaxis. True failures of the prophylaxis are rare and they mainly occur when it is not given appropriately.
INTRODUCTION

Primary prevention of hemolytic disease of the fetus and newborn by administering anti-D immunoglobulin to RhD-negative mothers is a major success story in modern obstetrics. However, despite adequate postpartum prophylaxis and in potentially sensitising events (PSE), such as abortions, invasive diagnostic procedures, and bleeding, up to 2% of RhD-negative mothers become immunised.

A further reduction in anti-D immunisations was achieved by the routine administration of anti-D immunoglobulin (RAADP) to all RhD-negative women at the beginning of the third trimester in the mid-1990s. However, despite clinical trials showing that half of the remaining immunisations can be prevented by RAADP, Scandinavian women, including Finns, had to wait for the intervention until the 2010s. By that time large-scale screening methods for determining fetal RHD type from maternal blood had become available, and RAADP in Scandinavia was from the very beginning targeted at women carrying an RhD-positive fetus.

Even the most well-run anti-D-prophylaxis programmes are unable to prevent all immunisations and 0.1-0.3% of RhD-negative mothers develop anti-D antibodies with serious consequences to the affected fetuses. Postpartum failures may be caused by situations where the amount of fetomaternal hemorrhage (FMH) exceeds the neutralisation capacity of the regular anti-D-immunoglobulin dose after complicated delivery. The failure of RAADP in turn may be related to post-term pregnancies or immunisations that occur before 30 weeks of gestation.

The aim of this study was to assess the prevalence and incidence of anti-D immunisations among pregnant women after the introduction of RAADP in Finland in 2014. The time point when the antibody first became detectable and risk factors for immunisation were assessed to better understand the causes of continuing anti-D immunisations.

MATERIAL AND METHODS

A nation-wide cohort study was conducted of all pregnant women with anti-D antibodies detected in the Finnish Red Cross (FRC) Blood Service between 1.1.2014 and 31.12.2017. The number of pregnancies with at least the first antenatal screening test taken was 224 209, 26 793 (12.0%) of which were RhD negative. The total number of births (>22+0 weeks / >500 g neonate) during
these years was 215 048 (13). The annual birth rate decreased 12% from 57 019 in 2014 to 50 155 in 2017.

National screening and prevention program

All antenatal screening tests were performed in the FRC Blood Service laboratory in accordance with the national screening programme (Table 1). RhD-negative women were screened for red blood cell (RBC) antibodies at 8-12 weeks of gestation (1st screening), 24–26 (2nd screening) and 36 weeks (3rd screening).

The national anti-D prophylaxis programme is described in Table 2. Samples for fetal RHD were drawn at 24-26 weeks, and when indicated by the result, anti-D prophylaxis (1250-1500 IU) was given at the maternity clinic at 28-30 weeks. The investigations on fetal RHD screening were described previously9.

Prediction of the nature of anti-D detected for the first time was based on information on anti-D prophylaxis. In uncertain cases, follow-up tests were requested every four weeks to perform repeated titration to obtain a kinetic monitoring of antibody concentration. Distinguishing between low-titre immune anti-D from passive anti-D was not possible at the 3rd screening. As passive anti-D given 6 to 8 weeks earlier rarely exceeds titre of 1 (tested in tubes), only anti-D with a titre of 2 or more were classified as immune15.

Study design

The FRC Blood Service database was searched for the results of antenatal screening including information on anti-D prophylaxis and RBC transfusions. The time point of immunisation was estimated by analysing the results of current and previous pregnancies. If anti-D was detected in the 1st screening, immunisation was assumed to have occurred at the end of the previous pregnancy or during delivery (or after interrupted pregnancies not reported). In cases detected in the 2nd screening, immunisation was assumed to have taken place between 12-24 gestation weeks in the on-going pregnancy, and in cases detected in the 3rd screening, between 24-36 weeks.

Clinical data on pregnancies were collected from hospital medical records, taking into account the estimated time point of immunisation: age, gravidity, parity (multigravidity defined as immunisation at the 4th or later pregnancy), body mass index (BMI, kg/m²) in the...
beginning of pregnancy, twin pregnancy, PSE (interrupted pregnancies, invasive procedures such as chorionic villous/amniotic fluid sampling, trauma, antenatal bleeding), gestation at delivery (post-term described as >41+0 weeks), mode of delivery, postpartum haemorrhage (PPH, >1000 ml), pregnancy-related RBC transfusions, anti-D-prophylaxis given/missed, and intravenous drug abuse. Complicated delivery was defined as Cesarean section, ventouse, manual removal of the placenta. The results were compared with national data on parturients and deliveries in 2014-2017 (n=215 048 women) obtained from the Finnish Institute for Health and Welfare without matching for parity\textsuperscript{13} (Table 3). The data were meticulously analyzed one patient at a time by one researcher (R. J.)

**Statistical analyses**

The data were analysed using SPSS (IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY, IBM Corporation). A p-value <0.05 was considered statistically significant, and 95% confidence interval (CI) was used. The number of observations in the study group and controls was compared using Pearson’s Chi-square, Fisher's Exact test and Student’s $t$-test, depending on the variable. Logistic regression was used to sort out risk factor proportion before and after 2014 and between time-points of immunisation.

**Ethical approval**

This study was approved by the Research Ethics Committee of Helsinki University Hospital (TMK03 §70 16/13/03/03/03/2016, revised on 6 April 2016).

**RESULTS**

**Pregnancy-related anti-D immunisations**

During the study period, there were 274 pregnancies of 228 women with anti-D in Finland, with a decrease from 109 in 2014 to 55 in 2017. In 174 women (76.3%), immunisation occurred in a pregnancy in 1991-2013, and in 54 women (23.7%) in 2014 or later despite the newly introduced
RAADP programme. The corresponding number of new immunisations decreased from 24 in 2014 to 6 in 2017 (Figure 1).

Taking into account the decrease in the annual birth rate, the prevalence of anti-D immunisations in pregnant women decreased from 1.52% (95% CI 1.26-1.84) in 2014 to 0.88% (95% CI 0.68-1.14) in 2017 (Figure 1). The corresponding incidence decreased from 0.33% (95% CI 0.22-0.48) to 0.10% (95% CI 0.05-0.22), respectively. Thus, four years after the introduction of RAADP, the prevalence and incidence had decreased 63% (95% CI 26-101; \( p = 0.0009 \)) and 24% (95% CI 8-40; \( p = 0.0037 \)), respectively. The risk ratio for new immunisation was 0.29 (95% CI 0.10-0.71) with the new programme. The absolute risk difference was 0.20%, corresponding to a number needed to treat (NNT) of 422, but due to fetal RHD screening only 65% of these women needed RAADP (NNT 287). The risk reduction was statistically significant (\( p = 0.0065 \)).

Thirty-one of the 228 (13.6%) women with anti-D detected in the first trimester screening were removed from the time point and risk factor analysis: nine women were presented as primigravidas, and the remaining 22 had not been screened in the FRC Blood Service previously and the time point of immunisation could not be assessed. Thus, complete laboratory history was available from 197 women for the risk analyses. Clinical data on these women are presented in Table 3. Due to the underlying mechanism of maternal immunisation, the mean number of pregnancies was expectedly significantly higher and the proportion of primigravidas and nulliparous women lower in the study group than in the controls. Instead, due to the continuously increasing maternal age and decreasing birth rate in Finland, the mean maternal age and proportion of multiparous (P>4) women immunised after 2014 no longer differed from the general parturient population.

**Time points and potential risk factors for immunisations**

The time points of immunisations are presented in the flow chart (Figure 2) and potential risk factors for each time points in Table 4. In the univariate risk factor analysis, PPH and pregnancy-related RBC transfusions in the previous delivery were statistically significant not only for the immunisations detected in the 1st screening, but also for the 2nd screening, in comparison with the general parturient population (Table 4). Postpartum hemorrhage failed to reach statistical significance for immunisations detected in the 3rd trimester screening, although the p-value of 0.056 suggests that it still might be a weak risk factor. Expectedly, PPH and RBC transfusion were
strongly associated; 25/32 (78%) of the women with reported PPH >1000 ml received transfusion, and in 24 (75%) cases another potential risk factor such as complicated delivery was identified, none of them reaching statistical significance as a sole risk factor. The contribution of these risk factors were not significant in the multivariate logistic regression due to small numbers (statistics not shown). Of the 32 PPH prior to the immunisation, 23 (72%) had occurred before 2014.

For the immunisations detected in the 2nd trimester, twins in the on-going pregnancy reached statistical significance (p=0.039) but can not be regarded as a risk factor as the numbers were small. As information on PSEs is not included in the National Birth Register, their significance could not be calculated.

For anti-D detected in the 1st screening, 40.1% (34/84) of the women had a previous uncomplicated pregnancy and spontaneous delivery. For the cases detected in the 2nd screening in 81.8% (45/55) including eight primigravidae, and in the 3rd screening in 65.6% (38/58) including seven primigravidae, no potential risk factors in the on-going pregnancy were identified. When these 15 primigravidae were discounted from the 197 women there remained 182 with a previous pregnancy and delivery (Table 3). For altogether 40.6% (80/197), no potential risk factor was identified (Table 4).

Failures of RAADP

Of the 54 women immunised after 2014, anti-D was detected in the 1st screening in 10 (18.5%), in the 2nd screening in 27 (50%) and in the 3rd screening in 17 cases (31.5%). Before 2014, the corresponding proportions were 52%, 20% and 28%. None of these were due to false-negative fetal RHD typing.

Among the 27 women with anti-D detected in the 2nd screening and thus unpreventable with RAADP, 24 (88.8%) had no risk factor in the on-going pregnancy, including seven primigravidae; the only possible risk factors were twin pregnancy (n=2), and bleeding in early pregnancy (n=1). Of the 27 immunisations possibly preventable with RAADP (anti-D detected in the 1st or 3rd screening), 10 women (37.0%) did not receive it and in four women it was unclear from the medical records whether it was given. While most of these occurred in 2014 when all health care areas had not yet implemented the RAADP programme, in four cases there was a clear protocol violation. Among 13 women who were immunised despite RAADP, anti-D was detected in the 1st screening in five cases (previous post-term delivery n=1, RBC transfusion
Failure of postpartum prophylaxis and PSEs

A PSE was identified in 29 pregnancies (14.7%); in eight (27.6%) prophylaxis had not been given as instructed. In 19 additional cases (65.5%), it was unclear from the medical records whether it was given. In addition, postpartum prophylaxis was missed or delayed (>72 hours) in two cases. In contrast, postpartum prophylaxis was given to 61/228 women (26.8%) despite existing anti-D.

DISCUSSION

In late 2013, targeted RAADP was added into Finland’s national prophylaxis programme, leading to a significant decrease in anti-D immunisations over the following four years. The risk reduction of new immunisations and NNT are comparable to the first large study evaluating the outcome of administering RAADP only to women carrying an RHD-positive fetus\textsuperscript{12}. It is noteworthy that in these targeted programmes, actual NNTs can be safely decreased 40% in Finland with a slightly smaller proportion of RhD-negative women.

The strength of this study is the thorough data collection of all antenatal anti-D cases in one centre (FRC) during a four-year period. Furthermore, a detailed one-by-one analysis revealed different policies and attitudes regarding anti-D prophylaxis, mainly obscurities concerning its administration.

Limitations were the lack of matched controls, and after 2014 the numbers of new immunisations and possible risk factors were too low for power calculations for true RAADP failures. Distinguishing weak immunisations from traces of anti-D prophylaxis is impossible, hence the number of anti-D immunisations in the 3\textsuperscript{rd} screening is likely an underestimation, but the true number cannot be confirmed until the next pregnancy, an endpoint very few studies reach\textsuperscript{6,15}.

Anti-D detected in the 1\textsuperscript{st} trimester indicates that the immunisation happened at the end of the previous pregnancy or delivery. In the original studies on RAADP, up to half of the cases immunised despite postpartum prophylaxis were assumed to be due to silent FMH in the
In our data, the effect of RAADP on immunisations occurring in the last month of pregnancy was clearly demonstrated by the decline in new cases. The shift of time points for immunisations after implementing RAADP was, however, unexpected. Earlier studies report that up to 50% of prophylaxis failures followed deliveries with larger-than-supposed FMH \(^ {11,16} \). In our study, before 2014, anti-D was detected in the 1\(^{st}\) screening in over half (52%) of the cases but in less than fifth (19%) after RAADP became available. Simultaneously, anti-D detected in the 2\(^{nd}\) screening (and not preventable with RAADP), increased from 20% to 50%. Surprisingly, no significant change was seen in the corresponding figures for the 3\(^{rd}\) screening (28% vs. 32%), cases RAADP could affect.

To verify these residual immunisations, we searched for possible risk factors related to increased FMH, adherence to guidelines, or PSEs unrelated to pregnancy. In almost half of the 84 women with anti-D detected in the 1\(^{st}\) trimester, no risk factor was identified in the previous pregnancy or delivery. In comparison with the general parturient population, only PPH and associated RBC transfusions were observed significantly more often (\(P < 0.001\)), but in contrast to the study by Koelewijn et al., transfusion as a sole risk factor did not reach statistical significance in our material \(^ {11} \). Most PPH cases occurred before 2014, pointing at a less standardised PPH management protocol at that time. We speculate that PPH requiring RBC transfusions represent markers of a complicated delivery involving larger FMH rather than the cause itself, and in these rare situations every effort to quantify FMH should be considered \(^ {17,18} \).

Besides PPH and RBC transfusions, no other risk factors (CS, assisted vaginal deliveries, postmaturity, obesity, younger maternal age) were confirmed in our material \(^ {11,12} \). Causes of these discrepancies include a selection bias in our cohort, with most immunisations occurring before the implementation of RAADP and changes in confounding factors such as increasing maternal age, decreasing birth rate, or management protocols. Another possible explanation is the unavailability of a contemporary control group in the National Birth Register, preventing us from comparing antenatal PSEs. Furthermore, not adjusting for nulliparity, the smaller proportion of primigravidas (related to the immunisation mechanism itself) resulted in fewer non-spontaneous deliveries than in the general parturient population (15% vs. 26%). Therefore, it probably did not emerge as an independent risk factor for immunisation, compared to Koelewijn’s study including only women with previous delivery (para-1) with corresponding proportions as high as 48% vs. 29% \(^ {11} \).
In our study, after the introduction of RAADP, half of the immunisations were detected in the 2nd screening. In almost 90% of these, no risk factor in the on-going pregnancy was identified. Instead, an analysis of women who would have benefited from RAADP revealed that over a third (37%) had not received it. Most of these failures occurred in 2014 when some health care areas had yet to implement the new programme. By late 2017, the coverage had reached 98%. Importantly, none of the failures were due to false-negative fetal RHD typing. With only 13 anti-D immunisations occurring despite RAADP, the number was too low to assess the causes.

In 29/197 pregnancies (16%) with a PSE, prophylaxis had not been given in eight and at least not recorded for additional 19. Several pitfalls are associated with anti-D prophylaxis in PSEs: minimal bleeding or trauma may be dismissed or the prophylaxis not repeated in recurring events. Not all reasons could be verified but they included human error or underestimation of risk, or as reported earlier, failure to follow guidelines. This also indicates how complicated the biology of immunisation is for obstetricians and midwives.

For 41% of our cases, none of the previously reported risk factors associated with delivery or on-going pregnancy were observed, in line with the 43% reported by Koelewijn et al. Clearly, individual immune response plays a considerable role in antibody formation. Despite the stability of anti-D, in some women sensitisation may have had happened in the previous pregnancy or delivery, with the antibody remaining undetectable until the 2nd or 3rd screening when boosted by the RhD-positive fetus. This is supported by our finding that PPH and associated RBC transfusions remained statistically significant also for antibodies detected for the first time in the 2nd trimester. Also, risk factors are difficult to recognise from medical records and continuing collection of data on new cases is needed.

Prevention strategies in Europe differ especially for indications to quantitate FMH in PSEs or at delivery, and with regard to the evaluation of national prevention programmes. Intriguing proposals for reducing remaining anti-D immunisations by administering an additional anti-D immunoglobulin dose after non-spontaneous delivery or prolonged third stage, or repeating RAADP at term to ensure detectable anti-D at delivery have been made. While reasons for prophylaxis failures are unclear, changes in current practices should be considered carefully. Extra doses in non-spontaneous deliveries, obesity or postmaturity would quickly double the use of anti-D immunoglobulin. Appropriate use of anti-D immunoglobulin is not only a question of evidence-based medicine and economics but also of ethics and the availability of a human blood product.
CONCLUSION

The incidence of new RhD immunisations decreased expectedly after RAADP was introduced to the Finnish guidelines, and true failures of prophylaxis are rare. Importantly, immunisations occur when the prophylaxis is not given appropriately. There is a continued need to audit the anti-D pathway and provide education to maternity care professionals.

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Author contributions

RJ and SS were responsible for the study design and the body of the manuscript, CI and RJ for data collection, and MK, SS and RJ for the raw data analysis. Other authors critically revised the manuscript.

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Legends

TABLE 1. Antenatal antibody screening protocol in Finland.

TABLE 2. Anti-D-prophylaxis program for RhD negative mothers in Finland.

TABLE 3. Baseline characteristics of pregnant women with anti-D in Finland during 2014-2017 (n=197). Information from pregnancies where the immunisation was assumed to have occurred.

TABLE 4. Potential risk factors for developing antenatal anti-D, detected in the 1st, 2nd and 3rd screening (n=197) in Finland during 2014-2017 in comparison to the general parturient population.

FIGURE 1. The number of all cases and new cases (=after the introduction of routine antenatal anti-D prophylaxis) with anti-D during pregnancy in Finland in 2014-2017; annual birth rate included.

FIGURE 2. Flow chart of the study population; pregnant women with anti-D detected in the 1st, 2nd or 3rd screening in Finland during 2014-2017.

FRC BS=Finnish Red Cross Blood Service; gw=gestational weeks; G=gravidity; P=parity
TABLE 1.
Antenatal antibody screening protocol in Finland

<table>
<thead>
<tr>
<th>Time point</th>
<th>ABO and RhD groups and RBC antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation week 8-12</td>
<td>All pregnant women</td>
</tr>
<tr>
<td>Gestation week 24-26</td>
<td>RhD negative pregnant women (Including fetal RHD status assessment from maternal sample)</td>
</tr>
<tr>
<td>Gestation week 36</td>
<td>RhD negative pregnant women</td>
</tr>
<tr>
<td></td>
<td>RhD positive pregnant women with a history of blood transfusion or newborn’s jaundice requiring treatment</td>
</tr>
<tr>
<td>Post partum</td>
<td>Newborns to RhD negative mothers (ABO and RhD) if not assessed antenatally</td>
</tr>
</tbody>
</table>

a If any antibodies are detected at any time, antibody follow-up is instructed by the Finnish Red Cross Blood Service.

b If indicated by the result, routine antenatal anti-D prophylaxis is given at 28-30 weeks.
<table>
<thead>
<tr>
<th>Prophylaxis type</th>
<th>Time point</th>
<th>To whom given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postpartum prophylaxis: 250-300 µg anti-D-immunoglobulin</td>
<td>Within 72 h from delivery</td>
<td>RhD negative mothers with a RhD positive newborn or unclear RhD status of the newborn</td>
</tr>
<tr>
<td>Risk-based prophylaxis(^a) during pregnancy: 250-300 µg anti-D-immunoglobulin</td>
<td>Spontaneous abortions after 8 weeks, All terminations of pregnancy, Extrauterine pregnancies, Chorionic villous sampling, Amniocentesis, Abdominal trauma, Antenatal hemorrhage, External version, Intrauterine death</td>
<td>RhD negative mothers with a RHD positive fetus or if the fetal RhD status is unknown</td>
</tr>
<tr>
<td>RAADP: 250-300 µg anti-D-immunoglobulin</td>
<td>At 28-30 weeks of gestation</td>
<td>RhD negative mothers with a RHD positive fetus or if the fetal RhD status is unknown</td>
</tr>
</tbody>
</table>

\(^a\) Risk-based anti-D-prophylaxis should be repeated in two weeks if the immunising event recurs.
TABLE 3. Baseline characteristics of pregnant women with anti-D in Finland during 2014-2017 (n=197). Information from pregnancies where the immunisation was assumed to have occurred.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Study population (n=197)</th>
<th>General parturient population (n=215 048)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>27.3 (5.1)</td>
<td>30.7 (5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gravidity, mean (range/SD)</td>
<td>2.6 (1-12 / 1.6)</td>
<td>1.6 (1-26 / 1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G1</td>
<td>36</td>
<td>63 633</td>
<td>29.6</td>
</tr>
<tr>
<td>G4 or more (multigravidity)</td>
<td>30</td>
<td>44 448</td>
<td>20.7</td>
</tr>
<tr>
<td>Parity, mean (range/SD)</td>
<td>1.2 (0-11 / 1.4)</td>
<td>1.1 (0-18 / 1.4)</td>
<td>NS</td>
</tr>
<tr>
<td>P0</td>
<td>50</td>
<td>88 684</td>
<td>41.3</td>
</tr>
<tr>
<td>P4 or more (multiparity)</td>
<td>8</td>
<td>10 697</td>
<td>5.0</td>
</tr>
<tr>
<td>BMI median (SD)</td>
<td>24.5 (5.4)</td>
<td>24.7 (5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI &gt;30 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
<td>28 543</td>
<td>13.3</td>
</tr>
<tr>
<td>Twin pregnancy &lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2931</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Previous pregnancy and delivery (n= 182)**

<table>
<thead>
<tr>
<th></th>
<th>Study population (n=197)</th>
<th>General parturient population (n=215 048)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cesarean section</td>
<td>23</td>
<td>35 158</td>
<td>16.3</td>
</tr>
<tr>
<td>Assisted vaginal delivery</td>
<td>5</td>
<td>19 751</td>
<td>9.2</td>
</tr>
<tr>
<td>Pregnancy-related transfusion</td>
<td>25</td>
<td>4619</td>
<td>2.1</td>
</tr>
<tr>
<td>Postpartum bleeding &gt; 1000 ml</td>
<td>32</td>
<td>2249 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.7</td>
</tr>
<tr>
<td>Postmaturity &gt; 41 weeks</td>
<td>18</td>
<td>49 173</td>
<td>22.9</td>
</tr>
<tr>
<td>Delivery of twins</td>
<td>2</td>
<td>2931</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> BMI=body mass index (kg/m²), information available of 101 (51.2 %) women

<sup>b</sup> proportions calculated from the 113 with anti-D detected in the 2<sup>nd</sup> or 3<sup>rd</sup> antibody screening test

<sup>c</sup> information available for 33 686 women in 2017 (excluding Helsinki University Hospital)
TABLE 4. Potential risk factors for developing antenatal anti-D, detected in the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} screening (n=197) in Finland during 2014-2017 in comparison to the general parturient population.

<table>
<thead>
<tr>
<th>Potential risk factor</th>
<th>1\textsuperscript{st} screening n=84 (%)</th>
<th>2\textsuperscript{nd} screening n=55 (%)</th>
<th>3\textsuperscript{rd} screening N=58 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous complicated delivery</td>
<td>8</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>- Cesarean section</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>- Assisted vaginal delivery</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>- Delivery of twins</td>
<td>14 (16.7) $P=0.001$</td>
<td>10 (18.2) $P=0.003$</td>
<td>8 (16.0) $P=0.056$</td>
</tr>
<tr>
<td>- PPH &gt; 1000 ml</td>
<td>12 (12.0) $P&lt;0.001$</td>
<td>5 (9.1) $P=0.007$</td>
<td>8 (16.0) $P&lt;0.001$</td>
</tr>
<tr>
<td>Previous delivery &gt; 41 weeks</td>
<td>8</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Complicated</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>PSE in previous pregnancy</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Twins in on-going pregnancy</td>
<td>1 (1.2)</td>
<td>3 (5.5) $P=0.039$</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>PSE in on-going pregnancy</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Missed prophylaxis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Postpartum</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>RAADP</td>
<td>4</td>
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<td>3</td>
</tr>
<tr>
<td>In PSEs</td>
<td>50</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td>Any risk factor</td>
<td>38</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>In previous pregnancy</td>
<td>12</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>In on-going pregnancy</td>
<td>34</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>No risk factor</td>
<td>34</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>
FIGURE 1.

The number of all cases and new cases (=after the introduction of routine antenatal anti-D prophylaxis) with anti-D during pregnancy in Finland in 2014-2017; annual birth rate included.
Flow chart of the study population; pregnant women with anti-D detected in the 1\textsuperscript{st}, 2\textsuperscript{nd} or 3\textsuperscript{rd} screening in Finland during 2014-2017.

FRC BS= Finnish Red Cross Blood Service; gw= gestational weeks; G= gravidity; P= parity

228 women with anti-D in antenatal screening
- 84 G1
- 144 G2-8

115 anti-D detected at 1\textsuperscript{st} screening at gw 8-12
- 31 with no previous samples (9 G1P0) available in FRC CS, removed from analyses

→ 84 (42.6\%) immunised in previous pregnancy after gw 36 or delivery

55 (27.9 \%) anti-D detected at 2\textsuperscript{nd} screening at gw 24-26

→ immunised in ongoing pregnancy before gw 24

58 (29.4 \%) anti-D detected at 3\textsuperscript{rd} screening at gw 36

→ immunised in ongoing pregnancy at gw 24-36 or more