

RhD immunisation in pregnancy

Postnatal prophylaxis with anti-RhD immunoglobulin has reduced the immunisation rate for RhD-negative women with an RhD-positive foetus by 80 %. Haemolytic disease of the foetus and newborn has become a rare cause of death. However, some foetuses do still develop severe anaemia as a result of RhD immunisation and require transfusion. RhD typing of the foetus via blood samples from RhD-negative mothers and antenatal prophylaxis for women with an RhD-positive foetus will enable RhD immunisation to be reduced further.

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Hydrops foetalis and severe icterus of the newborn were first described by a French midwife in a set of twins in 1609 (1). A number of authors subsequently reported similar conditions: icterus gravis neonatorum, erythroblastosis foetalis and anaemia neonatorum. Besides icterus and general oedema, the infants showed enlargement of the spleen and liver as well as a very high number of erythroblasts in peripheral blood. This gave rise to the term «erythroblastosis foetalis» (2).

In 1932, Diamond and colleagues demonstrated commonalities and intermediate forms between the aforementioned disorders and concluded that they represented the same pathophysiological condition (2). A metabolic disturbance of the bone marrow with abnormal erythroblast function was proposed as a contributory factor. In 1934, however, Hawksley & Lightwood suggested that the condition could be due to haemolysis, but the cause of the haemolysis was unclear (3).

In 1938, Ruth Darrow claimed that the haemolysis was the result of an antigen-antibody reaction and that the antibodies stem from the mother and pass into the foetal circulation via the placenta (4). This was confirmed in 1939 when Levine & Stetson published a case of hydrops foetalis where the mother had experienced a severe reaction to a blood transfusion from the father and had formed antibodies that also reacted with the child's erythrocytes (5).

Shortly afterwards, in 1940, Landsteiner & Wiener discovered an antibody after immunising a rabbit with erythrocytes from a rhesus monkey (6). The antibody reacted with erythrocytes from the same blood donors as Levine & Stetson's antibody (7), suggesting that both antibodies were reac-

ting with the same antigen. The antigen was named «rhesus». Although the antibodies were later shown to react with unrelated erythrocyte antigens, the pathogenesis behind haemolytic disease of the foetus and newborn (HDFN) had been identified (8, 9). Today, the term RhD is used to refer to the human alloantigen, and LW (Landsteiner-Wiener) to the xenoantigen against which the rabbit antibody is directed.

Development of prophylaxis

In 1957, Levine showed that ABO incompatibility between mother and child (mother: O, child: A, B or AB) reduced the risk of RhD immunisation in pregnancy (10). In 1958, Betke & Kleihauer published their method for detecting foetal erythrocytes in maternal blood (11). In 1960, Ronald Finn discovered that foetal erythrocytes were present more frequently in the blood of mothers with ABO-compatible than ABO-incompatible offspring (12). He proposed therefore that immunisation of the mother could be prevented by administering an antibody that reacted with foetal erythrocytes. This led to several clinical studies in which anti-RhD immunoglobulin was given within 72 hours postpartum as prophylaxis if the newborn was RhD-positive, because most immunisations are the result of intrapartum fetomaternal haemorrhage.

The results indicated a protection rate of 80–85 % against RhD immunisation (13, 14). The protection rate is limited by immunisations that occur as a result of antenatal fetomaternal haemorrhage, especially in the third trimester (15, 16). Tiblad and colleagues suggested that half of these immunisations could potentially be prevented by antenatal prophylaxis in the final trimester (17). Postnatal prophylaxis was introduced in most industrialised nations around 1970. Together with improved therapeutic options for affected children, this led in the UK to a 98.5 % reduction in perinatal mortality due to RhD immunisation (15). Nevertheless, postnatal prophylaxis will not prevent RhD immunisation that occurs as a result of fetomaternal haemorrhage in pregnancy, mainly

in the final trimester. In the event of major haemorrhage, a higher dose of RhD prophylaxis is needed to provide adequate protection.

Prophylaxis in Norway

In 1945, the head of the Serodiagnostic Department of the Norwegian Institute of Public Health, Otto Hartmann, travelled to the USA to learn RhD typing. Upon his return, he began to RhD type blood samples that were sent to the Institute for statutory syphilis testing (18). This made it possible to identify the women most at risk of HDFN so that they could be given special monitoring. It is unclear whether serum from RhD-negative women was also screened from the outset for anti-RhD antibodies (anti-RhD). However, such screening was underway in the department in 1969 when routine prophylaxis was adopted by maternity wards, overseen by the Norwegian Institute of Public Health (19). Prophylaxis was probably introduced at about the same time in the rest of the country, but data on this have not been published.

In 1987, Kornstad published the incidence of anti-RhD in the maternity wards overseen by the Institute (19). If his findings are extrapolated to the country as a whole, this corresponds to an estimated 300–350 RhD immunisations annually in pregnant woman before 1970. After the introduction of prophylaxis, this number fell rapidly and was probably 50–60 per year at the end of the study period in 1985. Prophylaxis had thus reduced the incidence of anti-RhD by about 85 %, as in the UK.

Status in Norway

In 2013, the Oslo Blood Bank reported new cases of RhD immunisation in 11 pregnant women (internal statistics). In 2013, they held 11 097 (internal statistics) of the country's 53 710 blood samples (20.7 %) for initial screening in pregnancy (20). If new cases of anti-RhD in pregnant women occur equally across the country, one can assume an incidence of 50–60 new cases in pregnant women in Norway in 2013.

This is roughly the number of new cases per year that Kornstad's numbers would suggest (19). The actual figure may nevertheless be somewhat higher due to female immigrants who have probably not received adequate postnatal RhD-prophylaxis in their native countries. We have previously argued that the prophylaxis system does not always function optimally (21), but there is hardly systemic failure.

We obtained anonymised information on morbidity and mortality due to HDFN from the Medical Birth Registry of Norway and the Cause of Death Registry respectively at the Norwegian Institute of Public Health. Data from the two registries are presented separately and are not linked. The data were analysed by authors CAA and HEH. The number of deaths due to HDFN in Norway decreased from 80 in the five-year period 1965–69 to one in the four-year period 2010–13; i.e. > 95 % reduction (Fig. 1). Some of this reduction was probably achieved through advanced monitoring and treatment during pregnancy. The National Center for Fetal Medicine at St. Olavs Hospital gave transfusions to 133 fetuses in the period 1988–2014, and 70 % were performed because of anti-RhD immunisation. In the period 2010–2014, 22 foetuses received 105 transfusions at St. Olavs Hospital, while Haukeland University Hospital performed 15 transfusions in seven foetuses.

Data on morbidity are generally not of sufficient quality to allow analysis. However, in the period 1998–2013, eight infants were reported to have been born with hydrops foetalis as a result of blood group immunisation, with most cases probably due to anti-RhD (Medical Birth Registry of Norway) (Fig. 2). There are no statistics available on the number of abortions or miscarriages due to RhD immunisation, or on how many women choose not to have further children because they have been immunised. There are also unfortunately no statistics on the number of children who sustained lasting injuries. Nevertheless, it is clear that RhD immunisation is still an issue for maternal health in Norway.

New possibilities

Postnatal RhD prophylaxis prevents most RhD immunisations, which are due to intra-partum fetomaternal haemorrhage. This prophylaxis will not, however, prevent immunisations caused by fetomaternal haemorrhage in pregnancy, or major haemorrhage. Antenatal RhD prophylaxis should thus be able to reduce the immunisation rate further.

In 1989, Birgitta Trolle published a Danish intervention study in which RhD-negative pregnant women were given both antenatal and postnatal anti-RhD prophylaxis (22). While there were six immunisations in the control group (n = 291) that received only postnatal prophylaxis, there were none in the intervention group (n = 322). Other, larger studies have confirmed that a combination of antenatal and postnatal prophylaxis is more effective than postnatal prophylaxis alone, but the degree of risk reduction varies with the study design (23–25).

A significant problem with extending antenatal prophylaxis to all RhD-negative women, as practised in some countries including the USA, is that about 38 % of the women will be carrying an RhD-negative foetus and so not require prophylaxis (26). Giving prophylaxis to these women seems unethical. It is unnecessary treatment, and anti-RhD immunoglobulin must still be made by immunising RhD-negative men,

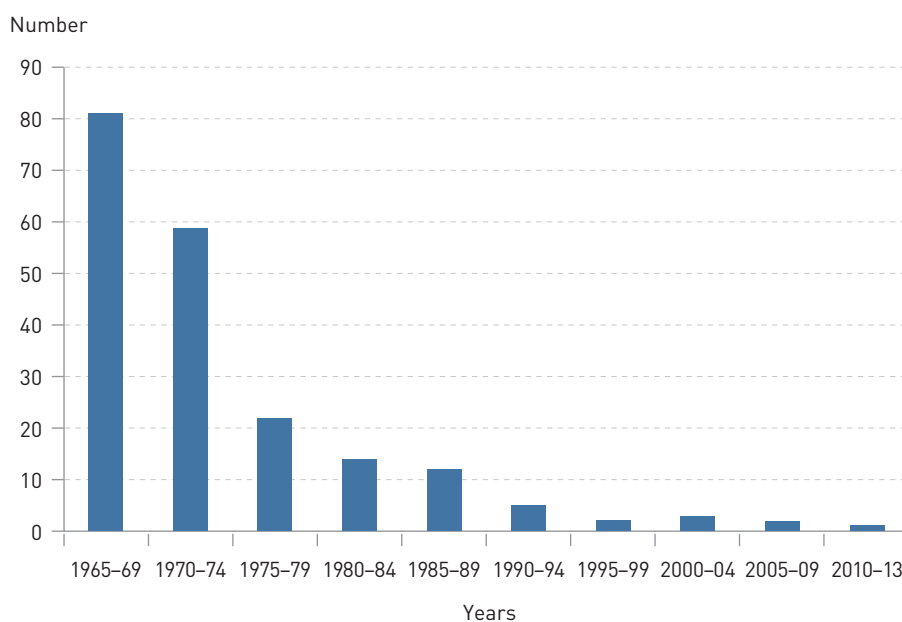


Figure 1 Deaths due to haemolytic disease of the foetus and newborn (HDFN) over five-year periods, 1969–2013 (both anti-RhD and other immunisations) (1969–85: ICD-8: 774 and 775; 1986–95: ICD-9: 773, 774 and 696; 1996–2013: ICD-10 P55–58). Source: Norwegian Institute of Public Health (Cause of Death Registry)

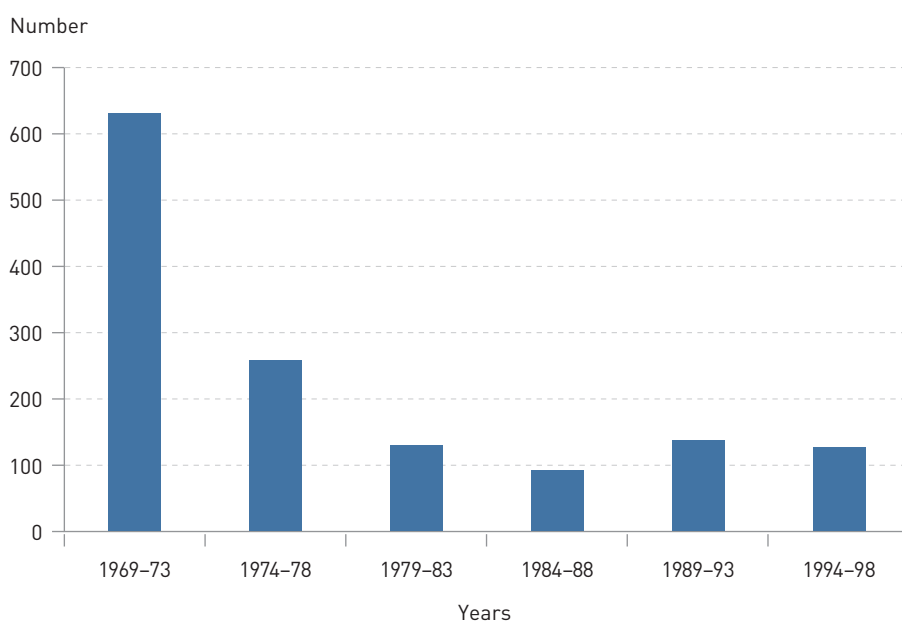


Figure 2 Newborns with haemolytic disease (anti-RhD and other immunisations) (ICD-8: 7740–7759). Due to the transition to ICD-10 with its different subdivisions in 1998, we have not included figures beyond this year. Source: Norwegian Institute of Public Health (Medical Birth Registry of Norway)

who are paid to undergo the procedure and then donate plasma for use in manufacturing these drugs. Globally, there is a shortage of such immunoglobulin.

In the last few years, methodology has been developed for genomic RhD typing of the foetus using cell-free DNA from maternal blood at gestational weeks 10–12 (27). At such an early stage in the pregnancy, the amount of cell-free foetal DNA in maternal blood is quite small. This can lead to false-negative results (2.4%); that is, an RhD-positive foetus could be typed as RhD-negative because of the low quantity of foetal DNA (27). The mother would not then receive the necessary antenatal prophylaxis. The method, which was introduced in Denmark in 2010 and in the Netherlands in 2011, does however have specificity and sensitivity of over 98% when typing is performed in gestational weeks 25–27 (28, 29). A similar method has recently been established at the Norwegian National Advisory Unit on Blood Group Serology at Oslo Blood Bank. In Norway, the *Guidelines in Obstetrics* from 2014 recommend RhD typing of the foetus from a maternal blood sample in week 25 (30). This is beyond the abortion time-limit, such that the result can only be used as grounds for RhD prophylaxis and not as grounds for late abortion. There are therefore no ethical concerns over the typing.

Even if the analysis is performed in gestational weeks 25–27, there may still be a few false negative results (0.087%) (28). However, postpartum prophylaxis will be given if RhD typing reveals the newborn to be RhD-positive. It is therefore important to keep the umbilical cord blood samples of newborns during the introductory period as these will hold the answer key. The guidelines recommend giving prophylaxis with anti-RhD immunoglobulin in week 29. The Norwegian Directorate of Health has approved the new method, and the regional health authorities, through the National System for the Introduction of New Health Technologies (methods) within the Specialist Health Service, have decided that it is to be adopted (31).

The typing method established at the Norwegian National Advisory Unit on Blood Group Serology, Oslo University Hospital, Ullevål, is based on amplification of exons 7 and 10 of the *RHD* gene and has sensitivity of 100% and specificity of 98.9% (unpublished data).

When immune anti-RhD is detected, the woman will be monitored with respect to HDFN and given appropriate treatment, in accordance with current practice. It is

also important to keep in mind that HDFN may occur as a result of other blood group immunisations.

The prophylaxis programme must be maintained for the foreseeable future as the risk of immunisation will always be present in an RhD-negative woman carrying an RhD-positive foetus. With this strategy, we expect to see a further significant reduction in RhD immunisation in pregnancy.

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