The role of antenatal immunoprophylaxis in the prevention of maternal-foetal anti-Rh(D) alloimmunisation

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Introduction

The first description of haemolytic disease of the newborn (HDN) can be traced back to 1609 and was made by a French midwife, Louise Bourgeois, who, from 1600, worked at the royal court of King Henry IV and Queen Marie de Medicis1-4. In the treatise that Bourgeois wrote in 1609 she described the birth of two twins: the first had hydrops and died immediately, while the second, initially in a better condition, rapidly became jaundiced and, after having developed neurological symptoms (kernicterus), died 3 days after being born.

Hydrops foetalis and kernicterus were correctly interpreted as two aspects of the same pathology only in 19325, when Diamond described foetal erythroblastosis secondary to severe haemolysis, although the cause was still unknown. A few years later, in 1938, Ruth Darrow correctly identified the (antibody-related) pathogenesis of HDN6, although erroneously attributing foetal haemoglobin the role of the culprit antigen, which was suggested to have induced a maternal antibody response after crossing the placenta. The true pathogenesis of the disease was definitively clarified in 1940 with the discovery of the Rhesus (Rh) blood group system by Landsteiner and Wiener7 and with the subsequent identification, in 1941, by Levine8, of the Rh(D) antigen. This antigen was, in fact, identified, in D-negative mothers, as being the cause of the immunisation occurring following transplacental passage of foetal D-positive red blood cells. The subsequent passage of maternal anti-D immunoglobulin G (IgG) across the placenta into the foetal circulation was recognised as the final event able to cause the spectrum of clinical events that characterise HDN.

It did not take long before the risk of immunisation could be quantified1,3: i) 16% in the case of a Rh(D)-negative mother and a Rh(D)-positive, ABO-compatible foetus; ii) 2% in the case of a Rh(D)-negative mother and a Rh(D)-positive, ABO-incompatible foetus (about 20% of the cases); iii) overall risk of immunisation: 13.2%.

Before 1945, about 50% of all foetuses with HDN died of kernicterus or hydrops foetalis. Subsequently, thanks to the progress in treatment, in industrialised countries the mortality decreased to 2-3%; this mortality rate was then very considerably further reduced (100-fold) with the introduction of anti-D immunoprophylaxis to prevent maternal-foetal anti-Rh(D) alloimmunisation9.

At the beginning of the 1960s, Stern demonstrated experimentally that the administration of anti-D IgG could prevent sensitisation to the Rh(D) antigen10; in the same period, other studies clarified the mechanism of Rh iso-immunisation in pregnancy and introduced the clinical practice of passive immunisation with anti-D IgG to protect Rh(D)-negative women from sensitisation against Rh(D)-positive red blood cells11-14. The successes obtained in studies of Rh(D)-negative male volunteers formed the experimental basis for clinical trials in pregnant Rh(D)-negative women15; these trials demonstrated that post-partum immunoprophylaxis decreased the incidence of post-pregnancy anti-Rh(D) immunisation from 12-13% to 1-2%.15,16.

Subsequently, in 1977, it was shown that 1.8% of Rh(D)-negative women, despite post-natal prophylaxis, continued to develop anti-D antibodies
Antenatal anti-D immunoprophylaxis

because of small transplacental haemorrhages during pregnancy\textsuperscript{17,18}.

One year later, a Canadian study by Bowman et al.\textsuperscript{19} showed, in 1,357 Rh(D)-negative primigravida, that the incidence of Rh(D) alloimmunisation could be reduced to 0.1% by prophylaxis with antenatal anti-D IgG, in addition to post-partum prophylaxis.\textsuperscript{19} There is currently sufficient evidence demonstrating that antenatal anti-D prophylaxis also reduces the risk of Rh(D) immunisation in the next pregnancy to below the level of 0.4%.

Forty years after Zipursky and Israels first proposed the use of anti-D IgG to reduce the incidence of Rh alloimmunisation in pregnancy\textsuperscript{14}, immunoprophylaxis has drastically reduced the cases of Rh-induced HDN; nevertheless, this pathology continues to be relevant in 0.4 of 1,000 births (0.04%)\textsuperscript{20}, for various reasons\textsuperscript{21}: i) the possible occurrence of anti-D immunisation during the pregnancy (which occurs in about 1% of Rh(D)-negative women carrying a Rh(D)-positive foetus\textsuperscript{22}); ii) the lack of efficacy of immunoprophylaxis because of the administration of an insufficient dose of anti-D IgG that is not congruent with the volume of the foetal-maternal haemorrhage; iii) immunoprophylaxis not administered; iv) possible errors in typing the pregnant or puerperal woman or the neonate; v) possible errors in transfusion therapy in women of child-bearing age.

Antenatal prophylaxis

Rationale

Systematic anti-D prophylaxis was proposed in the 1970s with the aim of reducing the percentage of Rh(D)-negative women who could be sensitised during pregnancy, despite the use of post-partum prophylaxis. This percentage ranged from 0.9-2.2% in a series of studies carried out between 1977 and 1989\textsuperscript{17,19,23-28}, and from 0.8-1.5% in subsequent studies carried out between 1995 and 1999\textsuperscript{20,32}; this latter modest reduction is probably due to intercurrent changes in obstetric care and the wider use of anti-D prophylaxis following sensitising events during pregnancy\textsuperscript{33}.

The D antigen has been demonstrated to be present on foetal red blood cells from the 7\textsuperscript{th} week of pregnancy onwards\textsuperscript{34}. There is a direct, proportional relationship between the volume of Rh(D)-positive red blood cells to which the Rh(D)-negative subject is exposed and the incidence of anti-Rh immunisation, although it has been estimated that 0.1 mL is already sufficient to induce the formation of antibodies\textsuperscript{14,33}.

During the first trimester of a normal pregnancy, foetal red blood cells can be found in the maternal circulation of about 3% of women, albeit in amounts less than 0.1 mL\textsuperscript{35}. As the pregnancy proceeds, the frequency and volume of foetal red blood cells in the maternal circulation increase, such that during the second and third trimesters these cells can be detected in 12% and 45% of women, respectively. At the time of delivering an ABO-compatible neonate, foetal red blood cells can be found in the maternal circulation in as many as 50% of cases\textsuperscript{32}. However, the main sensitising event for Rh(D)-negative women, in whom prevention is based on post-natal prophylaxis, occurs at the end of the pregnancy, with detachment of the placenta during delivery\textsuperscript{33}.

Other sensitising events, in the form of occult or silent transplacental haemorrhages, are probably the principal cause of the approximately 1% residual rate of immunisation. These events provide the rationale for the strategy, adopted in many countries, of systemic antenatal anti-D prophylaxis for all non-immunised Rh(D)-negative pregnant women, as a complementary measure to post-partum prophylaxis\textsuperscript{36}.

Numerous studies have demonstrated that the antenatal administration of anti-D IgG does not have adverse effects on the foetus, even though small amounts of anti-D can cross the placenta and, by binding to foetal D-positive red blood cells, can cause weak positivity at birth in the direct Coombs' test\textsuperscript{16,23,37-40}.

Antenatal anti-D prophylaxis is generally recommended from the 28\textsuperscript{th} week of pregnancy onwards, because transplacental haemorrhages large enough to cause sensitisation do not occur until the third trimester and, therefore, anti-D antibodies usually develop after the 28\textsuperscript{th} week of gestation\textsuperscript{17,41,42}; studies showing that 92% of Rh(D)-negative women who develop anti-D do so after the 28\textsuperscript{th} week of gestation lend support to this recommendation\textsuperscript{16,37}.

Anti-D prophylaxis has a strong immunosuppressive effect, such that subsequent exposure to the D antigen does not cause a secondary immune response, but rather a primary response, as if the immune system has never encountered the D antigen\textsuperscript{33}. Numerous mechanisms of action have been proposed to explain the efficacy of this strategy, including: (i) accelerated clearance of D-positive cells, (ii) epitope masking, (iii) inhibition due to antibodies.
against FcγRIIB (the specific activating receptor for the Fc fragment of IgG) or anti-idiotype44; (iv) inhibition of immature dendritic cells, and (v) inhibition of B-cell clones specific for the D antigen43; this last, recently proposed, mechanism was suggested by the finding of an increase in the levels of transforming growth factor-β and prostaglandin E₂, found in a cohort of pregnant women who were given antenatal prophylaxis. However, the accelerated destruction of D-positive red blood cells remains, according to some authors, the main mechanism of action43.

**Dose**

A dose of 20 μg (100 UI) of anti-D IgG protects against 1 mL of D-positive red blood cells or 2 mL of whole blood10-21. However, the World Health Organisation (WHO) and the British Medical Research Council consider that 25 μg (125 UI) are needed to protect against the same volume of D-positive red blood cells46; according to Bowman and Pollock, this is equivalent to having a concentration of anti-D IgG in the maternal circulation of 2.4 ng/mL26.

There are essentially two approaches to antenatal prophylaxis: i) a single injection of 300 μg (1,500 UI) in the 28th week of pregnancy19,28,47, or ii) two injections of 100-125 μg (500-625 UI), one in the 28th week, the other in the 34th week24,32,40.

In pharmacokinetic studies, the half-life of the IgG was estimated to be, on average, 17-22 days (range: 11-29)49-51; a recently published pharmacokinetic study50 reported that both protocols are effective and able to ensure, 12 weeks after administration, the minimum residual concentration of 25 μg (2.4 ng/mL) of anti-D IgG indicated by the WHO as a protective level. This is probably an overcautious estimate because it is extremely unlikely that the volume of a transplacental haemorrhage in the antenatal period exceeds 1 mL of foetal red blood, which is equivalent to the volume of foetal-maternal haemorrhage occurring at the time of delivery in 98% of women46,50.

A recent systematic review of the literature on the clinical efficacy of anti-D prophylaxis during pregnancy33 found 11 studies that compared the outcome of Rh(D)-negative women (and their babies)10,23-32 not immunised to D antigen who underwent antenatal prophylaxis in the 28th week of pregnancy or subsequently, with the outcome of Rh(D)-negative controls (and their babies) who did not receive prophylaxis. Table I reports the characteristics of the studies analysed in the review, which showed that the percentage of sensitised women decreased from 1.9-2.2% to 0-0.2% with the use of antenatal prophylaxis; the data also showed that the main clinical benefit that a Rh(D)-negative woman can have from antenatal prophylaxis is the possibility of avoiding HDN in subsequent pregnancies with Rh(D)-positive foetuses.

A meta-analysis, carried out on two of the 11 studies included in the review, demonstrated a notable reduction in relative risk of sensitisation in women treated with the antenatal prophylaxis; indeed, the odds ratio (OR) was 0.37 (95% CI: 0.2-0.65), while the absolute reduction in risk of sensitisation in mothers at risk (carrying a Rh(D)-positive foetus) was 0.6%; the number of women who had to be treated to avoid one case of sensitisation (i.e. the NNT, number needed to treat) was 278.

A meta-analysis by the Cochrane Collaboration in 200042 also included only two studies31,29, for a total of more than 4,500 women treated with immunoprophylaxis in the 28th and 34th weeks of pregnancy; the doses of anti-D IgG used were 100 μg (500 UI) x 2 in the French trial27, and 50 μg (250 UI) x 2 in the British trial29. Three outcomes were evaluated and for each of these the reduction in the relative risk was statistically significant. As far as concerns positivity for the Kleihauer test, which detects foetal red blood cells in the maternal circulation, the OR was 0.6 (95% CI: 0.41-0.88) during pregnancy and 0.6 (95% CI: 0.46-0.79) also after the delivery of a Rh(D)-positive baby. The OR for the incidence of anti-D alloimmunisation was 0.42 (95% CI: 0.15-1.17) both during pregnancy and after the birth of a Rh(D)-positive baby; the OR became 0.41 (95% CI: 0.16-1.04) within 12 months of the birth, while the OR for women at a first pregnancy, within 12 months after the delivery, was 0.11 (95% CI: 0.01-2.04). The reduction in the relative risk of neonatal jaundice was considerably greater, with an OR of 0.26 (95% CI: 0.03-2.3).

According to the Cochrane reviewers, as a result of antenatal prophylaxis [100 μg (500 UI) of anti-D IgG in the 28th to 34th weeks], the risk of alloimmunisation of Rh(D)-negative pregnant women drops from about 1% to 0.2% and the probability of immunisation in subsequent pregnancies is also reduced; the NNT to avoid one case of sensitisation is 213. 

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International recommendations

Various international recommendations (table II) suggest antenatal anti-D prophylaxis (i.m. or i.v.) in unsensitised Rh(D)-negative women as a complement to post-partum prophylaxis.

1) The Society of Obstetricians and Gynaecologists of Canada (SOGC) suggests 300 µg in the 28th week (grade of recommendation: A) or two doses of 100-120 µg in the 28th and 34th weeks of pregnancy56.

2) The Italian Society of Transfusion Medicine and Immunohaematology (SIMITI), together with the Italian Society of Obstetricians and Gynaecology (SIGO) recommends 250-300 µg in the 28th week57.

3) The American Society of Clinical Pathologists (ASCP) suggests 300 µg in the 28th-30th week16.

4) The American College of Obstetrician and Gynaecologists (ACOG) recommends prophylaxis58, around the 28th week, but does not specify the dose (grade of recommendation: A).

5) The U.S. Preventive Services Task Force (USPSTF) (USA) recommends 300 µg in the 24th-28th week59.

6) The National Institute for Clinical Excellence

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**Table I – Summary of the results of the clinical efficacy of antenatal anti-D prophylaxis (adapted from: Jones ML et al.33).**

<table>
<thead>
<tr>
<th>Study [Study design]</th>
<th>Dosage</th>
<th>Patient selection</th>
<th>Anti-D prophylaxis group r/n</th>
<th>Control group r/n</th>
<th>Anti-D prophylaxis group % sensitised or sensitised (95% CI)</th>
<th>Control group % sensitised or sensitised (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowman et al. (1978) [NRCT]8</td>
<td>2 x 1500 IU (28 and 34 weeks) Primigravidae</td>
<td>1/1357 0.1 (0.1 to 0.2)</td>
<td>45/2768 1.6 (1.6 to 2.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bowman and Pollock (1978) [NRCT]5</td>
<td>1 x 1500 IU (28 weeks) Unselected</td>
<td>11/1805 0.6 (0.3 to 1.0)</td>
<td>62/3533 1.8 (1.3 to 2.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tovey et al. (1983) [NRCT]12</td>
<td>2 x 500 IU (28 and 34 weeks) Primigravidae</td>
<td>5/1238 0.4 (0.1 to 0.8)</td>
<td>30/2000 1.5 (1.0 to 2.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hermann et al. (1984) [NRCT]13</td>
<td>1 x 1250 IU (34 weeks) Primigravid primiparae</td>
<td>4/236 1.7 (0.0 to 3.3)</td>
<td>5/286 1.7 (0.2 to 3.3)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td> </td>
<td>Multigravid primiparae</td>
<td>1/332 0.3 (0.3 to 0.9)</td>
<td>3/359 1.9 (0.2 to 2.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td> </td>
<td>Unselected primiparae</td>
<td>5/568 0.9 (0.1 to 1.6)</td>
<td>126/45 1.9 (0.8 to 2.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bowman and Pollock (1987) [NRCT]8</td>
<td>1 x 1500 IU (28 weeks) Unselected</td>
<td>30/295 0.3 (0.2 to 0.4)</td>
<td>62/3533 1.8 (1.3 to 2.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huchet et al. (1987) [Quasi RCT]27</td>
<td>2 x 500 IU (28 and 34 weeks) Primiparae</td>
<td>0/461 0.0 (0.0 to 0.0)</td>
<td>4/454 0.9 (0.0 to 1.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td> </td>
<td>Multigravidae</td>
<td>4/138 0.7 (0.7 to 2.1)</td>
<td>3/359 2.2 (0.3 to 4.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td> </td>
<td>Unselected</td>
<td>1/599 0.2 (0.2 to 0.5)</td>
<td>7/590 1.2 (0.3 to 2.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trolle (1989) [NRCT]28</td>
<td>1 x 1500 IU (28 weeks) Unselected</td>
<td>0/291 0.0 (0.0 to 0.0)</td>
<td>6/322 1.9 (0.4 to 3.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee and Rawlinson (1995) [RCT]9</td>
<td>2 x 250 IU (28 and 34 weeks) Primigravidae</td>
<td>5/513 1.0 (0.1 to 1.8)</td>
<td>9/695 1.5 (0.5 to 2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayne et al. (1997) [Before and after study]9</td>
<td>2 x 500 IU (28 and 34 weeks) Primiparae</td>
<td>4/425 0.3 (0.0 to 0.6)</td>
<td>16/426 1.1 (0.6 to 1.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parsons et al. (1998) [NRCT]31</td>
<td>Not stated* (28 weeks) Not stated</td>
<td>72/9684 0.7 (0.6 to 0.9)</td>
<td>No data 0.8 (not given)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mackenzie et al. (1999) [Controlled before and after study]9</td>
<td>2 x 500 IU (28 and 34 weeks) Primiparae</td>
<td>12/3320 0.4 (0.2 to 0.6)</td>
<td>26/3146 0.8 (0.5 to 1.1)</td>
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</tr>
</tbody>
</table>

NRCT = non randomised controlled trial.; RCT = randomised controlled trial; n = number of deliveries of RhD-positive babies to RhD-negative women; r = number of sensitised RhD-negative women in the trial group. * Most probably the standard Canadian dose of 1500 IU.
Table II – Recommended antenatal anti-D prophylaxis in the main international recommendations

<table>
<thead>
<tr>
<th>Country/Organisation</th>
<th>Recommended dose</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada (SOGC)</td>
<td>300 (or 100-120)</td>
<td>28 (or 28 and 34)</td>
</tr>
<tr>
<td>Italy (SIMTI-SIGO)</td>
<td>250-300</td>
<td>28</td>
</tr>
<tr>
<td>USA (ASCP)</td>
<td>300</td>
<td>28-30</td>
</tr>
<tr>
<td>USA (ACOG)</td>
<td>Not specified</td>
<td>28</td>
</tr>
<tr>
<td>USA (USPSTF)</td>
<td>300</td>
<td>24-28</td>
</tr>
<tr>
<td>UK (NICE)</td>
<td>100</td>
<td>28-28</td>
</tr>
<tr>
<td>UK (BCSH)</td>
<td>100</td>
<td>28 and 34</td>
</tr>
<tr>
<td>Australia (NHMRC)</td>
<td>125</td>
<td>28 and 34</td>
</tr>
<tr>
<td>Australia (RANZCOG)</td>
<td>125</td>
<td>28 and 34</td>
</tr>
<tr>
<td>France (CNGOF)</td>
<td>300</td>
<td>28</td>
</tr>
<tr>
<td>The Netherlands (CHI)</td>
<td>200</td>
<td>30</td>
</tr>
<tr>
<td>Spain (SETS-SEOG)</td>
<td>300</td>
<td>28</td>
</tr>
</tbody>
</table>

SOGC: Society of Obstetricians and Gynaecologists of Canada
SIMTI: Società Italiana di Medicina Trasfusionale e Immunohaematologia
[Society of Transfusion Medicine and Immunohaematology]
SIGO: Società Italiana di Ginecologia e Obstetricia
[Italian Society of Obstetrics and Gynecology]
ASCP: American Society of Clinical Pathologists
ACOG: The American College of Obstetricians and Gynecologists
USPSTF: U.S. Preventive Services Task Force
NICE: National Institute of Clinical Excellence
BCSH: British Committee for Standards in Haematology
NHMRC: National Health and Medical Research Council
RANZCOG: Royal Australian and New Zealand College of Obstetricians and Gynaecologists
CNGOF: Collège National des Gynécologues et Obstétriciens Français
[French College of Obstetricians and Gynaecologists]
CHI: Council of Health Insurances
SETS: Sociedad Española de Transfusión Sanguínea [Spanish Society of Blood Transfusion]
SEOG: Sociedad Española de Obstetricia y Ginecología
[Spanish Society of Obstetrics and Gynecology]

Council (NHMRC) (Australia) suggests 125 µg (625 UI) in the 28th and 34th weeks.
9) The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) confirms the above recommendation.
10) The French recommendations are for a dose of 300 µg in the 28th (± 1) week of pregnancy.
11) The Dutch authorities recommend a single dose of 200 µg in the 30th week.
12) The Spanish Society of Blood Transfusion (SETS) and the Spanish Society of Obstetrics and Gynaecology indicate a single dose of 300 µg in the 28th week.

Prophylaxis should not be given to women with a "weak" D phenotype, in that such women are actually D-positive; however, the use of anti-D IgG in these women does not have significant side effects.

Conclusions
HDN has become a rare disorder since the introduction of anti-D prophylaxis. The foetal Rh(D) antigen is already well developed after 30-40 days of gestation and the risk of antenatal foeto-maternal haemorrhage, already possible from the 6th week, increases exponentially as a pregnancy progresses.

Although the risk of foetal blood entering the maternal circulation is high, the average volume of is very small, being 0.07 mL in the first trimester, 0.08 mL in the second and 0.13 mL in the third.

A Rh(D)-negative woman not immunised and who does not receive prophylaxis has, in every pregnancy, a 16% risk of becoming immunised to a Rh(D)-positive foetus.
However, the global success rate of post-natal Rh immunoprophylaxis has now reached 98.4 - 99.1%

Given that anti-D prophylaxis is effective for about 12 weeks, as indicated by pharmacokinetic studies on anti-D IgG, and given the relatively low risk of significant foeto-maternal haemorrhage before the 28th week of gestation, antenatal prophylaxis is carried out from the 28th week onwards.

The currently available evidence demonstrates that antenatal anti-D prophylaxis, besides being a cost-effective strategy, is able to further reduce the incidence of sensitisation to D antigen down to about 0.2%.

A variety of prophylactic regimens are used in different countries, although 300 µg of anti-D IgG...
in the 28th week is the dose most commonly indicated in international recommendations\textsuperscript{16,20,52,58}. It is not, however, possible to completely eliminate the risk of sensitisation because, despite increased adherence to antenatal immunoprophylaxis\textsuperscript{65}, and although most foeto-maternal haemorrhages able to cause immunisation occur in the last trimester of pregnancy, sensitisation does occur before the 28th week in a small percentage of women; alternatively, there may be cases in which intramuscular administration of the anti-D IgG is insufficient to provide passive prophylaxis.

Furthermore, the risk of intrauterine foetal death of Rh(D)-positive foetuses of Rh(D)-negative mothers appears to be increased despite antenatal immunoprophylaxis, as indicated by a recent, retrospective observational study carried out in Israel on more than 140,000 deliveries occurring between 1988 and 2003\textsuperscript{66}; although the subject undoubtedly needs further investigation, this retrospective study seems to suggest a possible pathogenic scenario not limited to maternal-foetal serological alloimmunisation but also involving the maternal-foetal interface (i.e. the placenta), which could undergo immune-mediated damage leading to intrauterine foetal death.

The IgG subclasses with anti-D activity are IgG1 and IgG3; the anti-D IgG are produced by industrial fractionation from pools of donor plasma containing a high titre of anti-D antibodies; chromatographic procedures, which are well suited to processing small quantities of plasma and guarantee an excellent yield, are used for the fractionation\textsuperscript{67}.

Antenatal prophylaxis with anti-D IgG does not have side effects on the foetus and can be considered complementary to post-partum prophylaxis, but the decision on whether to undertake a programme of prophylaxis during pregnancy can be influenced by the commercial availability of anti-D IgG. The raw material is a limited resource because the hyperimmune plasma comes exclusively from the USA, the only country that still carries out active immunisation of donors which, until about 10 years ago, was also performed in Switzerland and Slovenia (Italian National Blood Centre, \textit{unpublished data}).

An economic analysis carried out in the United
Kingdom\textsuperscript{16,64} demonstrated that the cost per year of life gained from using antenatal anti-D prophylaxis for women in their first pregnancy was low compared to that of other interventions normally offered by the health care service in that country; the same prophylaxis, given to all pregnant women, although costing more, maintained a relatively low cost per year of life gained, thus confirming that antenatal anti-D prophylaxis is a decidedly cost-effective strategy.

Methods of foetal genotyping on maternal plasma\textsuperscript{70-74} are able to detect foetal DNA sequences in the maternal plasma; used on a large scale, these methods could enable elimination of unnecessary antenatal prophylaxis with anti-D IgG in Rh(D)-negative mothers of Rh(D)-negative foetuses, who represent about 40\% of all cases, thus sparing this blood derivative and reserving its appropriate use exclusively to those women at risk of maternal-foetal alloimmunisation (Figure 1). In addition to this benefit, a further advantage of foetal genotyping would be that of knowing whether, in a woman already immunised in an early stage of a pregnancy, the foetus of the current pregnancy is Rh(D)-negative or positive, providing important information for the management of the pregnancy.

The possible use of monoclonal or recombinant anti-D antibodies in clinical practice remains very uncertain\textsuperscript{75}. These antibodies could, in the future, be an alternative to the prophylactic use of plasma-derived polyclonal anti-D IgG\textsuperscript{76-78}; unfortunately, however, none of the products developed by industry so far has been demonstrated to be effective in the numerous phase I clinical trials that have been carried out\textsuperscript{79,80}. During the last 20 years, 19 monoclonal or recombinant antibodies, produced by different cell lines, have been tested in humans\textsuperscript{75}, but the in vivo clinical efficacy and side effects were very heterogeneous. Most of the side effects are probably related to the type of glycosylation to which the IgG molecule is subjected in the various different cell systems used for its production\textsuperscript{75,80}.

The drafting and adoption of national recommendations on the systematic use of antenatal immunoprophylaxis must, however, take into consideration both clinical and organisational implications\textsuperscript{81}, and the potential financial impact of using this blood derivative which, already in the 3 years from 2002 to 2005, led to a 56\% increase in costs, for a 15\% rise in sales (Italian National Blood Centre, \textit{unpublished data}), caused presumably also by the increase in the sale price of the product.

**Key words:** antenatal immunoprophylaxis, maternal-foetal alloimmunisation, haemolytic disease of the newborn, Rh(D).

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