Forty years of anti-D immunoprophylaxis

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As rightly underscored by Velati in his article published in this issue of *Blood Transfusion*[1], the history of haemolytic disease of the newborn due to foetal-maternal incompatibility for the D antigen, universally and succinctly known as Rh HDN, bears witness to one of the most brilliant successes achieved in Medicine. In fact, in a couple of decades (from 1941 to the early 1960s), not only were the aetiology and immunological pathogenesis[2] of this disease discovered, which until then had escaped a precise nosological classification, but a fairly effective therapy was identified[3] and, above all, valid prophylaxis was introduced [4-6].

The first description of a neonatal disease, undoubtedly due to Rh HDN, can be found in the memoirs of a French midwife, Louise Bourgeois, cited by Bowman[7]. In 1609, Bourgeois assisted at a twin birth: the first twin was markedly hydropic and practically dead at delivery, whereas the second developed rapidly worsening jaundice within a few hours and died three days after birth.

In more recent times, the pioneering contributions of Auden[8], Hubbard[9] and von Gierke[10] must be mentioned, as they described neonatal diseases that were certainly cases of Rh HDN. von Gierke also has the merit of having correlated the states of foetal-placental hydrops and kernicterus with erythroblastosis

To complete this brief historical background, it is still worth emphasising that only in 1932 the three most characteristic clinical signs of Rh HDN, that is, hydrops foetalis, severe neonatal jaundice and delayed anaemia of the newborn, were recognised as expressions (with different incidences and severities) of a single pathological process, confirming the hypothesis proposed by Diamond and his colleagues[11]. These researchers believed that the pathogenesis of the disease involved a defect of the erythron. It was a New York pathologist, Ruth Darrow, who first hypothesised the immunological nature of HDN, reporting that the anatomo-pathological findings in the dead babies "seem to be due to an antigen-antibody reaction"[12]. This hypothesis was first alluded[13] then investigated and fully confirmed, as already reported, by Levine and school[2].

Homing in to the subject of this Editorial, anti-D immunoprophylaxis (IP), it must be accepted that world statistics (or even statistics from the western hemisphere) on the incidence of Rh HDN before and after the era of IP are lacking, so definitive overall evaluations on the impact that IP has had on reducing the frequency of this disease cannot be made. There are, however, national and/or regional data that can be used to provide some estimates. In 1952, Mollison and Walker reported that during the 1940s and 50s there were about 1,000 deaths each year in England due to Rh HDN[14]. In 1968 (at the dawn of IP), Woodrow and Donohoe[15] reported that, again in England, from 1.5 to 2 pregnant women in every 200 had anti-D antibodies, with the inherent risk of D-incompatible offspring developing HDN. Sansone[16] reported that in the 1960s there were about 7,000 cases of Rh HDN per year in Italy and approximately 1,500 deaths. While global statistics on the incidence of Rh HDN in the pre-prophylaxis are not available, there is also a lack of exhaustive information on the decrease of this pathology following the introduction of prophylaxis. In the USA, the percentage of D-negative women alloimmunised following a D-positive pregnancy dropped from about 14% in the 1960s to 1-2% in the 1970s and to 0-1% after 1980, when antenatal prophylaxis became routine[17]. In my own experience, I can report that the number of cases of Rh HDN requiring exchange transfusions at the *Galliera* Hospital in Genoa fell from a total of 86 in the decade 1972-1981 to an average of four each year in the following decade (1982-1991), when, obviously, the full beneficial effect of prevention could still not be appreciated, decreasing to only eight cases in the last 13 years, from 1992 to 2004

(*personal, unpublished observations*). In a Review published in 1992 in *Transfusion Medicine*[18], Tovey reported completely equivalent data collected at the Yorkshire Regional Transfusion Centre: the number of neonates affected by Rh HDN fell from 267 in 1970 to 103 in 1975, to 84 in 1980, to 52 in 1985 and to 37 in 1989; furthermore, the numbers of neonatal deaths or stillbirths due to HDN were 66 in 1970, 14 in 1975, 7 in 1980, 2 in 1985 and only 1 in 1989. It can, therefore, be stated that not only the incidence but also the severity of the disease has been changed significantly by the use of IP.

The numbers are eloquent. Without excessive self-congratulation, it can be safely concluded that anti-D IP has drastically reduced (although not completely to zero) the incidence of a very severe, often fatal, neonatal disorder, that had an enormous psychological impact, could leave survivors with extremely severe neurological sequelae and was a heavy social burden.

Nevertheless, there are still some issues under consideration and debate.

A first point that deserves comment concerns the mechanism of action of IP. Despite the fact that more than 40 years have passed since the first studies on the possibility of preventing HDN by using anti-D IgG, the precise mechanisms through which this system of prophylaxis acts have not yet been completely elucidated. From an historical point of view, it is known that the two groups of researchers (British on the one hand and North American on the other), who reached the final success almost at the same time, started from completely different theoretical premises. The British group[4,5], based on Levine's finding[19] that ABO incompatibility between mother and foetus had a clear protective effect with regards to Rh immunisation, hypothesised that the anti-D IgG introduced into the mother were able to lyse the Rh-incompatible foetal red cells, preventing them from coming into contact with the maternal antibody-generating system and, consequently, an immune response was not triggered. In contrast, the American researchers[6] considered that the anti-D IgG acted, in the mother, with a negative feedback mechanism, in the sense that their presence in the circulation enhanced the activity of T suppressor cells, thus inhibiting the production of the relevant antibodies, as was known to occur in some infectious diseases, such as tetanus and diphtheria, in which the passive administration of anti-tetanus and anti-diphtheria toxins prevented a specific immune response. Unexpectedly, it was the precisely some of the research carried out by the British group from Liverpool (well described in a review by Clarke[20]) that gave strongest support to the American hypothesis. In fact, based on the assumption that the use of anti-D IgM antibodies would have greater lytic effect on D red blood cells, because such antibodies are able to act already at the intravascular level, the British researchers conducted experiments with specific IgM imunoglobulins, but found, to their surprise, that these immunoglobulins, unlike the IgG ones, had no protective effect. The new interpretation, to support their premises, was as follows: the IgG do not have a lytic action on foetal D-incompatible red blood cells but specifically block antigenic D epitopes, hiding these from the maternal antibody-producing system, whereas, the intravascular lysis mediated by IgM leaves some antigenic residues that continue to provide an immunogenic stimulus. Probably prevention involves multiple factors and both the hypotheses could, at least in part, be correct.

Another very important issue concerns the use of IP in the antenatal period, that is, during pregnancy. An International Forum devoted to the current situation of anti-D IP, published recently in *Vox Sanguinis*[21], involved specialists from ten countries (Austria, Finland, France, Japan, Italy, the Netherlands, Poland, Spain, the United Kingdom and the USA), offering a sufficiently wide panorama of what happens in the world (or, rather, the western world). In three countries (the Netherlands, the United Kingdom and the USA), from among those that participated in the *Forum*, antenatal prophylaxis is given systematically to all D-negative pregnant women, because there are compulsory national regulations on this matter. Furthermore, it is well known that this is also the usual practice in Australia and Canada (countries not represented at the Forum). In the other countries involved in the investigation, antenatal IP is administered routinely only on occasion of particular events in the D-negative pregnant woman: abortions (whether spontaneous or elective), ectopic pregnancies, amniocentesis, chorionic villus biopsy, cordocentesis, pre-partum haemorrhages, suspected foetal-maternal haemorrhages (FMH), direct trauma to the abdomen of a pregnant woman, intrauterine foetal death, and twin pregnancies. That said, many Austrian, Polish and Spanish hospitals regularly carry out antenatal IP, despite this not being obligatory according to national legislation in the respective countries. From the survey carried out by our Scientific Society (SIMTI) on the state of IP in Italy, described in the paper by Velati[1], four transfusion facilities routinely perform antenatal IP.

The proposed doses of anti-D IgG vary considerably, ranging from 100 to 300 μg (that is, from 500 to 1,500 UI)

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and are administered (usually intramuscularly) in the 28th week of pregnancy, except in the United Kingdom, where two doses (100 µg) are given, the first in week 28 and the second in week 34 of a pregnancy.

Certainly, the obstacles to the systematic introduction of antenatal IP include, in many countries, the cost-benefit ratio as well as periodic difficulties in obtaining anti-D IgG. The number of women with anti-D antibodies, the natural reservoir of immune plasma, is tending (fortunately) to be ever smaller and the source of deliberately immunised volunteer males is not infinite. Although specific monoclonal antibodies would resolve all the supply problems and attempts to use such antibodies, produced for the purpose (BRAD-3 and BRAD-5), were started several years ago[22], definitive results on their efficacy and safety in clinical use are still lacking.

As mentioned earlier, the doses of anti-D Ig G used in IP, whether antenatal or postnatal, vary considerably both in Italy[1] and in the other countries involved in the abovementioned Forum[21]. The impression gained from the responses to the two surveys is that the doses are related, above all, to the availability of the product in the individual facilities (this is particularly the case in Italy, where the supplies of the products are directly linked to stipulated agreements with the manufacturers). This explains why the most commonly used dose in antenatal IP, whether systematic or dictated by situations requiring its use, is 250 µg, which is undoubtedly an excessive amount. It should be appreciated that 100 µg are able to neutralise the immunising effect of 4 mL of (concentrated) D-positive red blood cells. When the amount of FMH exceeds 4 mL of red cells (a very rare event during pregnancy, but not infrequent at birth), additional doses of anti-D IgG must be used (20 µg for every 1 mL of incompatible foetal red blood cells that enter the maternal circulation). It is, therefore, very important to determine the amount of any FMH. The most widely used and universally adopted method to do this is the acid elution test described by Kleihauer-Braun-Betke[23], although other methods, such as rosette formation or cytofluorimetry, are gaining popularity, particularly when the volume of FMH is thought to have exceeded 4 mL.

The last issue to consider is what to do for weak D (formerly D^u) or D-variant subjects (mothers or neonates). Weak D subjects are considered D-positive for all purposes, since they have all the epitopes distinguishing the antigen, albeit in very small amounts. In consequence, weak D mothers do not need any IP; in contrast, if the foetus or neonate is weak D, D-negative mothers must receive prophylaxis. People with D variants, who lack one or more of the components of the D antigen complex, do, in fact, resemble D-negative subjects (and, if mothers, must receive prophylaxis). The relatively recent discoveries by a group of researchers from Ulm University[24] have, however, led to new causes of concern. Using sophisticated molecular biology techniques, these researchers have identified rare weak D subjects (that is, with a low number of antigenic D sites), who also lack some epitopes. In other words, these are people who could correctly be labelled as weak D-variant. Obviously, given their particular characteristics, these subjects cannot be considered as D-positive and, if mothers, should undergo IP.

In conclusion, IP was –and still is –a powerful means of preventing Rh HDN, which, over the years, has undergone such a huge decrease that the cases of intrauterine foetal death have fallen 100-fold and the incidence of maternal alloimmunisation to RhD antigen has decreased more than 50-fold[25].

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