Intravenous immunoglobulin to treat neonatal alloimmune haemolytic disease

Luigi Corvaglia, Elena Legnani, Silvia Galletti, Santo Arcuri, Arianna Aceti & Giacomo Faldella

Neonatology and Neonatal Intensive Care Unit, S. Orsola-Malpighi Hospital, University of Bologna, Italy

Objective: To compare the efficacy of intravenous immunoglobulin (IVIg) and exchange transfusion (EXT) on rhesus haemolytic disease of the newborn (Rh-HDN) and evaluate treatment-related side effects. Methods: Retrospective chart review of two cohorts of newborns with Rh-HDN, treated with (Group 2) or without (Group 1) IVIg. Length of phototherapy, number of EXT, IVIg infusions, intrauterine and top-up red blood cells transfusions, need and permanence of umbilical venous catheter, and length of hospital stay, as well as treatment-related adverse events, were evaluated. Results: Charts of 88 newborns were reviewed (34 in Group 1, 54 in Group 2). Infants in Group 2 received a significantly lower number of EXT, had a lower risk of neurological impairment and needed an umbilical venous catheter for shorter, but required longer phototherapy, longer length of hospital stay, and more top-up transfusions. EXT was associated with a high number of adverse events. Two newborns treated with IVIg developed necrotizing enterocolitis (NEC). Conclusions: IVIg appear as an effective alternative to EXT, reducing the risk of neurological impairment and complications related to EXT. However, side effects of IVIg treatment (higher need of top-up transfusions and longer hospital stay) should be taken into account and the risk of NEC should be carefully monitored during treatment.

Keywords: exchange transfusion, intravenous immunoglobulin, newborn, rhesus haemolytic disease

Introduction

During the last decades an important evolution of prenatal care strategies, including rhesus (Rh)-prophylaxis to Rh-negative mothers after their first sensitisation or exposure to the Rh antigen, use of Doppler ultrasound to detect fetal anaemia, and intrauterine transfusion (IUT) [1,2], has led to a drastic decrease in perinatal mortality and morbidity related to Rh haemolytic disease of the newborn (Rh-HDN). Nonetheless, Rh-HDN is still related to serious morbidities in the neonatal period, such as hypoplastic or haemolytic anaemia and kernicterus [3].

The reduction in the frequency of severe neonatal hyperbilirubinemia and kernicterus are considered of primary importance in the management of infants with hyperbilirubinemia [4]: traditionally, phototherapy and exchange transfusion (EXT) have been used for this purpose. However, it is now recognized that EXT is potentially harmful, because it has a mortality ranging from 0.5 to 4.7% per infant and a morbidity of 2.8–23.5% [5].

The use of prophylactic intravenous immunoglobulin (IVIg) as an alternative to EXT for the treatment of Rh-HDN has been recommended by the American Academy of Pediatrics (AAP) in 2004 [4]. A recent Cochrane review provided some evidence to support the use of IVIg in HDN, showing a significant reduction in the need for EXT in infants treated with a combination of phototherapy and IVIg compared to those treated with phototherapy alone. However, the authors concluded that the clinical applicability of the results was limited, due to the small number of studies and infants included [5]. Furthermore, a recent randomised controlled trial has questioned the recommendation made by the AAP about the use of prophylactic IVIg in Rh-HDN [4], showing no reduction in the need for EXT or in the rates of other adverse neonatal outcomes in infants treated with IVIg [6].

In addition, some cases of necrotizing enterocolitis (NEC) have been reported in newborns with HDN treated with IVIg [7,8].

Given these premises, it is now crucial to understand whether IVIg have a real clinical usefulness and safety in infants with Rh-HDN. In 2003, IVIg were introduced as a treatment option for Rh-HDN in our neonatal intensive care unit (NICU): following this policy change, the number of EXT in infants with Rh-HDN seemed to have decreased substantially.

In order to confirm this clinical perception, we reviewed the medical records of all the infants with Rh-HDN admitted to our NICU from 2003 to 2009, and compared them to an historical cohort: the primary aim of the study was to assess whether the introduction of IVIg had reduced the need for EXT in these infants. In addition, we aimed to evaluate differences between the two cohorts in duration of phototherapy, length of hospital stay, need and permanence of umbilical catheter, need for top-up transfusions, and incidence of treatment-related adverse events.

Methods

Medical records of infants admitted to the NICU of S. Orsola-Malpighi Hospital, Bologna, for Rh-HDN over a 10 years period (1999–2009) were reviewed. Inclusion criteria were the presence of Rh isoimmunisation with positive direct antiglobulin test and/or the need for one or more intrauterine transfusion (IUT) during pregnancy.

Patients were divided into two groups: Group 1 included newborns born between 1999 and 2002 (historical cohort), and Group 2 newborns born between 2005 and 2009. Bilirubin levels in infants in the historical cohort had been monitored according to the Polacek diagram [9]. Treatment options for values exceeding the treatment threshold had included phototherapy
and EXT. Since 2003, all the infants admitted to our NICU with Rh-HDN were treated according to a new policy which included the use of IVIg. In addition, since 2005 Polacek diagram was no longer used and bilirubin levels were monitored according to the Bhutani nomogram [10]. In order to minimize confounders, newborns admitted in 2003 and 2004 (after the introduction of IVIg but before the switch to Bhutani nomogram) were excluded from the study.

Newborns in both groups had regular bilirubin checks (every 4–6 h) and received intensive phototherapy since admission. Newborns in Group 2 who satisfied the criteria for IVIg treatment (positive direct antiglobulin test and/or previous IUT) received one infusion of IVIg (0.5 g/kg of Intratect (Biotest Pharma GmbH, Dreieich, Germany)) in the first 4 h of life, followed if required by a second infusion 12 h after and a third infusion 72 h after the first one. Specifically, the second or third IVIg infusion were given if bilirubin values had not decreased after 12 h from the previous infusion. EXT was performed if bilirubin levels exceeded the treatment level on Polacek diagram for infants in Group 1 and on Bhutani nomogram in Group 2.

As part of the routine clinical monitoring, the following data were prospectively collected for each newborn: bilirubin values every 4–6 h, maximum bilirubin value, days of phototherapy, number of EXT, number of IVIg infusions (for infants in Group 2), number of both intrauterine and top-up red blood cells transfusions, need and permanence of umbilical venous catheter, and length of hospital stay. The presence of any adverse event which was considered as related to treatment was also recorded.

In order to evaluate the absolute effect of IVIg treatment on EXT rate and minimise the possible confounding effect related to different treatment cut-off, bilirubin values of both group were plotted also on the Polacek diagram (scenario 1). Furthermore, in order to evaluate the efficacy of the treatment (EXT and IVIg) on the risk of neurological impairment [11], bilirubin levels in both groups were plotted on the Bhutani nomogram and the time spent above the high-risk line was calculated (scenario 2). In the Bhutani nomogram, total bilirubin values are plotted against hours of life. This hour-specific bilirubin defines the risk of subsequent clinically significant hyperbilirubinemia as high-risk (>95th centile), intermediate-risk (40th–95th centile), and low-risk (<40th centile). The application of this predictive nomogram is potentially useful in reducing the incidence and the risks related to neurological damage induced by bilirubin [10].

Statistical analysis

Statistical analysis was performed by SPSS 16.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) for Windows. Differences between the two groups were tested with Fisher’s exact test for categorical variables and Mann-Whitney U test for continuous variables. The level of significance was set at \( p \leq 0.05 \).

Results

Medical charts of 115 newborns admitted for Rh-HDN between 1999 and 2009 were reviewed: 34 (29.6%) infants were admitted between 1999 and 2002 (Group 1), while 54 (47%) were admitted between 2005 and 2009 (Group 2). Twenty-seven infants, admitted in 2003 and 2004, were excluded.

As shown in Table 1, there were no differences in the demographic characteristics between Groups 1 and 2. Furthermore, demographic characteristics of the infants admitted in 2003 and 2004, who were excluded from the analysis, were similar to those of Group 1 and 2 (mean gestational age 35.7 weeks, birth weight 2736 g, male 60%, C-section 82.1%).

Infants in Group 1 were significantly more likely to receive one or more EXT than infants in Group 2 (82.3 vs. 11%, Table II). In the scenario 1 (bilirubin values in Group 2 plotted on the Polacek diagram), 27/54 (50%) infants in Group 2 treated with IVIg would have received an EXT. This figure is obviously higher than the actual rate of EXT in Group 2, but still significantly lower than the rate of EXT in Group 1 (\( p = 0.003 \)).

Fifty-two infants in Group 2 received at least one infusion of IVIg: 36 (69.2%) infants received 1 infusion, 14 (26.9%) received a second infusion, and 4 (7.7%) a third infusion.

Infants in Group 2 needed longer phototherapy, more top-up red blood cells transfusions, and longer hospitalisation. Infants in Group 1 needed an umbilical venous catheter for longer. No difference in the maximum bilirubin value was found between the two groups (Table II).

Bilirubin values of infants in Group 1 were plotted on the Bhutani nomogram and compared with those of Group 2: in this scenario, despite the increased rate of EXT, bilirubin values of infants in Group 1 would have remained above the high-risk line for a longer period (Figure 1).

In Group 2, two infants developed NEC (stage II according to Bell’s criteria): the first patient (gestational age 34 weeks) required only one IVIg infusion and developed NEC 4 days after. The second patient (gestational age 37 weeks) required two IVIg infusions and developed NEC 7 days after the last infusion.

Statistical analysis

Statistical analysis was performed by SPSS 16.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) for Windows. Differences between the two groups were tested with Fisher’s exact test for categorical variables and Mann-Whitney U test for continuous variables. The level of significance was set at \( p \leq 0.05 \).

Results

Medical charts of 115 newborns admitted for Rh-HDN between 1999 and 2009 were reviewed: 34 (29.6%) infants were admitted between 1999 and 2002 (Group 1), while 54 (47%) were admitted between 2005 and 2009 (Group 2). Twenty-seven infants, admitted in 2003 and 2004, were excluded.

As shown in Table 1, there were no differences in the demographic characteristics between Groups 1 and 2. Furthermore, demographic characteristics of the infants admitted in 2003 and 2004, who were excluded from the analysis, were similar to those of Group 1 and 2 (mean gestational age 35.7 weeks, birth weight 2736 g, male 60%, C-section 82.1%).

Infants in Group 1 were significantly more likely to receive one or more EXT than infants in Group 2 (82.3 vs. 11%, Table II). In the scenario 1 (bilirubin values in Group 2 plotted on the Polacek diagram), 27/54 (50%) infants in Group 2 treated with IVIg would have received an EXT. This figure is obviously higher than the actual rate of EXT in Group 2, but still significantly lower than the rate of EXT in Group 1 (\( p = 0.003 \)).

Fifty-two infants in Group 2 received at least one infusion of IVIg: 36 (69.2%) infants received 1 infusion, 14 (26.9%) received a second infusion, and 4 (7.7%) a third infusion.

Infants in Group 2 needed longer phototherapy, more top-up red blood cells transfusions, and longer hospitalisation. Infants in Group 1 needed an umbilical venous catheter for longer. No difference in the maximum bilirubin value was found between the two groups (Table II).

Bilirubin values of infants in Group 1 were plotted on the Bhutani nomogram and compared with those of Group 2: in this scenario, despite the increased rate of EXT, bilirubin values of infants in Group 1 would have remained above the high-risk line for a longer period (Figure 1).

In Group 2, two infants developed NEC (stage II according to Bell’s criteria): the first patient (gestational age 34 weeks) required only one IVIg infusion and developed NEC 4 days after. The second patient (gestational age 37 weeks) required two IVIg infusions and developed NEC 7 days after the last infusion.

Table I. Demographic characteristics of newborns with rhesus haemolytic disease.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 34)</th>
<th>Group 2 (n = 54)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>35.7 (2.1)</td>
<td>35.9 (2.2)</td>
<td>0.62</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2684 (573)</td>
<td>2737 (527)</td>
<td>0.58</td>
</tr>
<tr>
<td>Male</td>
<td>18 (52.9%)</td>
<td>24 (44.4%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>24 (70.5%)</td>
<td>38 (70.3%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values are reported as mean (SD) for continuous variables, and number (percentage) for categorical variables. Group 1: infants admitted between 1999 and 2002, Group 2: infants admitted between 2005 and 2009.

Table II. Treatment and outcomes of rhesus haemolytic disease of the newborn.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 34)</th>
<th>Group 2 (n = 54)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT alone</td>
<td>6 (17.6)</td>
<td>2 (3.7)</td>
<td>0.051</td>
</tr>
<tr>
<td>IVIg</td>
<td>0</td>
<td>52 (96.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>EXT</td>
<td>28 (82.3)</td>
<td>6 (11)</td>
<td>0.0001</td>
</tr>
<tr>
<td>1 EXT</td>
<td>21 (61.8)</td>
<td>4 (7.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>2 EXTs</td>
<td>5 (14.7)</td>
<td>1 (1.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>3 EXTs</td>
<td>2 (5.9)</td>
<td>1 (1.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>4 EXTs</td>
<td>1 (2.9)</td>
<td>0 (0)</td>
<td>0.39</td>
</tr>
<tr>
<td>IUT</td>
<td>12 (35.2)</td>
<td>16 (30)</td>
<td>0.82</td>
</tr>
<tr>
<td>Maximum bilirubin value (mg/dl)</td>
<td>14.5 (7–19.2)</td>
<td>14.4 (5.0–24.3)</td>
<td>0.966</td>
</tr>
</tbody>
</table>

Values are reported as number (percentage) or median (range). Group 1: infants admitted between 1999 and 2002, Group 2: infants admitted between 2005 and 2009.

EXT, exchange transfusion; IUT, intrathecal transfusion; IVIg, intravenous immunoglobulin; LOS, length of hospital stay; PT, phototherapy; RBC, red blood cells; UVC, umbilical venous catheter.
EXT was associated with some adverse events, including mild to severe thrombocytopenia in 18 (43.9%) patients, hypocalcaemia in 8 (21.9%) patients (two of them required IV calcium supplement), hypomagnesaemia in 2 (4.9%) patients. Furthermore, 4 (9.8%) patients developed complications related to the EXT procedure (apnoeas, bradycardia, tachycardia, one case of seizure requiring phenobarbital administration) and 8 (19.5%) developed complications related to the umbilical venous catheter (venous catheter blockage requiring replacement and catheter-related sepsis).

Discussion

Recent improvements in perinatal care strategies have led to a dramatic decrease in the incidence of Rh-HDN. However, the disease is still related to serious neonatal morbidities, including the risk of severe neurological impairment [3]. For this reason, the choice of an effective and safe treatment for HDN is fundamental.

As reported also in previous studies (Table III) [6,12–16], the introduction of IVIg as a treatment option for Rh-HDN has led to a reduction in the rate of EXT. However, some concerns regarding the effectiveness of prophylactic IVIg were raised after the results of the study by Smits-Wintjens et al. were published. In that well-designed randomised controlled trial, the authors have shown that prophylactic IVIg do not reduce the need for EXT or the rate of other adverse neonatal outcomes compared with placebo [6]. The discrepancy with our study might be partially explained by the extremely high rate of IUTs in the study by Smits-Wintjens, both in the IVIg and in the placebo group; actually, approximately two-thirds of the newborns enrolled in that study (compared to only one-third in our study) received at least one IUT. IUTs reduce haemolysis after birth as fetal erythrocytes are replaced by Rh-compatible red blood cells. Thus, we can speculate that the disease in the population studied by Smits-Wintjens was probably less severe than in our population, and this hypothesis is supported by the low rate of EXT the authors reported also in the placebo group. However, the high rate of top-up transfusions in that study suggests that IUTs are effective in reducing early haemolysis but do not prevent late anaemia.

Similarly to the study by Smits-Wintjens [6], infants with ABO incompatibility were not included in our study, as ABO incompatibility is known to cause less severe haemolytic disease than Rh incompatibility.

In order to minimize the differences in our two study groups and to evaluate the actual effect of IVIg treatment on the rate of EXT, all the bilirubin values of Group 2 were also plotted on the Polacek diagram. In this scenario, 50% of the infants treated with IVIg would have received an EXT: this rate is certainly higher than the rate of EXT in the same population monitored by Bhutani nomogram, but is still significantly lower than the rate of EXT before IVIg treatment was introduced, suggesting that the positive effect of IVIg is independent from the guidelines for EXT.

Beyond reducing the need of EXT, IVIg seemed to decrease also the risk of neurological impairment. In order to evaluate this effect, which is related to the permanence time of bilirubin values above the risk line of the Bhutani nomogram [11], all the bilirubin values of infants in Group 1 were also plotted on the Bhutani nomogram and the time above that line was compared with the one in Group 2. In this scenario, infants treated with IVIg had a significantly lower time in the high-risk zone compared with those treated before the introduction of IVIg. We hypothesise that the use of IVIg soon after birth prevents the rapid spike in bilirubin values which probably occurred in infants treated only with phototherapy before EXT.

In our study, newborns treated with EXT required less phototherapy and shorter hospitalisation than those treated with IVIg, and this is likely to be due to the ability of the EXT of removing the antigen-positive blood. In the review by Gottestein et al. infants treated with IVIg needed less phototherapy and had shorter hospitalisation compared with those receiving phototherapy alone [17], but no comparison was made with those treated with EXT. In the study by Huizing et al. no difference in the duration of phototherapy was found between infants treated with IVIg and with EXT [16].

In addition, infants treated with IVIg required a higher number of top-up transfusions than those treated with EXT: this difference could be related to the specific mechanism of action of IVIg, which is thought to be related to the inhibition of the breakdown of the anti-D sensitised neonatal erythrocytes. IVIg compete with sensitised neonatal erythrocytes in occupying the Fc receptor sites on the cells of the reticuloendothelial system, thus preventing early haemolysis [17]. However, as the antibodies causing haemolysis have not been removed, as would be with EXT, there may be late haemolysis and anaemia when the effect of IVIg comes to an end.

As previously reported [18], a high rate of adverse events was documented in infants treated with EXT. It has been recently suggested an
association between IVIg treatment and NEC [8]: in our population, two infants developed stage II NEC, one after a single infusion of IVIg, and the other after a second infusion. An association between IVIg treatment and NEC was taken into account, as no other risk factor for NEC was documented in the two infants.

One possible limitation of the present study is that it is retrospective. However, following the recommendations made by the AAP regarding the use of IVIg, the treatment policy for Rh–HDN in our centre was changed and this led to the perception of a significant decrease in the rate of EXT. For this reason, we believed that there were no prerequisites for a randomised controlled trial and that a retrospective chart review would have been more appropriate.

In conclusion, our data support the use of IVIg as an effective alternative to EXT for the treatment of Rh–HDN. IVIg also appear to reduce the time in the high-risk zone for neurological impairment and the risk of complications related to EXT. However, side effects of IVIg treatment, such as higher need of top-up red cells transfusion and increased length of hospitalisation, should be taken into account and the risk of NEC should be carefully monitored during treatment. Further data are needed in order to provide definite guidelines on the use of IVIg in infants with Rh–HDN.

Declaration of Interest: The authors have no declaration of interest and have received no financial support for this paper.

References