Hemolytic disease of the fetus and newborn
Zwiers, C.

Citation
Zwiers, C. (2019, March 12). *Hemolytic disease of the fetus and newborn*. Retrieved from https://hdl.handle.net/1887/68703

Version:	Not Applicable (or Unknown)
License:	[Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](https://hdl.handle.net/1887/68703)
Downloaded from:	https://hdl.handle.net/1887/68703

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

Leiden University Repository

The following handle holds various files of this Leiden University dissertation:
http://hdl.handle.net/1887/68703

Author: Zwiers, C.
Title: Hemolytic disease of the fetus and newborn
Issue Date: 2019-03-12

CHAPTER 7

IMMUNOGLOBULIN FOR ALLOIMMUNE
HEMOLYTIC DISEASE IN NEONATES

Carolien Zwiers
Mirjam E.A. Scheffer-Rath
Enrico Lopriore
Masja de Haas
Helen G. Liley

ABSTRACT

Background
Exchange transfusion and phototherapy have traditionally been used to treat jaundice and avoid the associated neurological complications. Because of the risks and burdens of exchange transfusion, intravenous immunoglobulin (IVIg) has been suggested as an alternative therapy for alloimmune hemolytic disease of the newborn (HDN) to reduce the need for exchange transfusion.

Objectives
To assess the effect and complications of IVIg in newborn infants with alloimmune HDN on the need for and number of exchange transfusions.

Search methods
We performed electronic searches of CENTRAL, PubMed, Embase (Ovid), Web of Science, CINAHL (EBSCOhost), Academic Search Premier, and the trial registers ClinicalTrials.gov and controlled-trials.com in May 2017. We also searched reference lists of included and excluded trials and relevant reviews for further relevant studies.

Selection criteria
We considered all randomized and quasi-randomized controlled trials of IVIg in the treatment of alloimmune HDN. Trials must have used predefined criteria for the use of IVIg and exchange transfusion therapy to be included.

Data collection and analysis
We used the standard methods of Cochrane and its Neonatal Review Group. We assessed studies for inclusion and two review authors independently assessed quality and extracted data. We discussed any differences of opinion to reach consensus. We contacted investigators for additional or missing information. We calculated risk ratio (RR), risk difference (RD) and number needed to treat for an additional beneficial outcome (NNTB) for categorical outcomes. We calculated mean difference (MD) for continuous variables. We used GRADE criteria to assess the risk of bias for major outcomes and to summarize the level of evidence.

Main results

Nine studies with 658 infants fulfilled the inclusion criteria. Term and preterm infants with Rh or ABO (or both) incompatibility were included. The use of exchange transfusion decreased significantly in the immunoglobulin treated group (typical RR 0.35, 95% CI 0.25 to 0.49; typical RD -0.22, 95% CI -0.27 to -0.16; NNTB 5). The mean number of exchange transfusions per infant was also significantly lower in the immunoglobulin treated group (MD -0.34, 95% CI -0.50 to -0.17). However, sensitivity analysis by risk of bias showed that in the only two studies in which the treatment was masked by use of a placebo and outcome assessment was blinded, the results differed; there was no difference in the need for exchange transfusions (RR 0.98, 95% CI 0.48 to 1.98) or number of exchange transfusions (MD -0.04, 95% CI -0.18 to 0.10). Two studies assessed long-term outcomes and found no cases of kernicterus, deafness or cerebral palsy.

Authors' conclusions

Although overall results show a significant reduction in the need for exchange transfusion in infants treated with IVIg, the applicability of the results is limited because of low to very low quality of evidence. Furthermore, the two studies at lowest risk of bias show no benefit of IVIg in reducing the need for and number of exchange transfusions. Based on these results, we have insufficient confidence in the effect estimate for benefit of IVIg to make even a weak recommendation for the use of IVIg for the treatment of alloimmune HDN. Further studies are needed before the use of IVIg for the treatment of alloimmune HDN can be recommended, and should include blinding of the intervention by use of a placebo as well as sufficient sample size to assess the potential for serious adverse effects.

PLAIN LANGUAGE SUMMARY

Review question
Is IVIg effective in reducing the need for exchange transfusion in newborns with alloimmune hemolytic disease of the newborn (HDN)?

Background
In alloimmune HDN, maternal antibodies (circulating proteins that are produced by the immune system in response to the presence of a foreign substance) are produced against fetal blood cells. These antibodies are transferred across the placenta and destroy red blood cells, leading to fetal anemia (deficiency of red cells in the unborn baby). Intrauterine (within the womb) blood transfusion is used to treat severe fetal anemia. After birth, the antibodies persist in the infant and cause hyperbilirubinemia (a raised blood level of an orange-yellow pigment (bilirubin, a waste product of a degrading red blood cell)) with the risk of serious brain damage (kernicterus) and anemia. Traditional treatment of hyperbilirubinemia consists of (intensive) phototherapy (light treatment) and exchange transfusion (where the baby's blood is replaced with that of a donor; ET). Because ET is an invasive, high risk procedure, alternative treatments such as intravenous immunoglobulin (IVIg), have been investigated. IVIg is thought to reduce the rate of hemolysis and consequently the need for ETs.

Study characteristics
We searched the medical literature to 19 May 2017 and found nine randomized (clinical studies where people are randomly put into one of two or more treatment groups) or partly (quasi) randomized trials (including 658 participants) that assessed the efficiency of IVIg in infants with alloimmune HDN.

Key results
Analysis of all included studies showed a reduction in the need for and number of ETs in infants treated with IVIg combined with phototherapy compared to infants treated with phototherapy only. However, this was not confirmed in an analysis of the two placebo-controlled studies (where a pretend treatment was given). There was no difference in the need for or number of top-up transfusions.

Quality of evidence

The evidence from the studies was very low quality. However, two studies used a placebo, thereby minimizing bias and allowing blinding of the researchers assessing the response. These studies were consistent with each other and yielded moderate quality evidence (with a relatively small total number of participants involved (172) being the only reason to not regard the level of evidence from them as high) that IVIg was ineffective in preventing ET or top-up transfusions.

Conclusion

Based on all included studies, we could make no conclusions on the benefit of IVIg in preventing ET or top-up transfusion. However, the two placebo-controlled trials provided evidence of moderate quality that IVIg was ineffective in preventing ET or top-up transfusion, and therefore routine use in alloimmune HDN should not be recommended. However, since there was some evidence that IVIg reduced hemolysis (in laboratory studies), future high-quality studies are needed to determine whether IVIg has limited role in some infants with alloimmune HDN.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON

Intravenous immunoglobulin plus phototherapy compared to phototherapy alone for alloimmune hemolytic disease in neonates
Patient or population: neonates with alloimmune hemolytic disease
Settings: -
Intervention: IVIg + phototherapy
Comparison: phototherapy

Outcomes	№ of participants (studies)	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects[a] (95% CI)	
				Risk with phototherapy alone	**Risk difference with IVIg + phototherapy**
Use of ET (≥ 1); all studies	658 (9 RCTs)	⊕⊖⊖⊖ **Very low**[1,2,3]	**RR 0.35** (0.25 to 0.49)	Study population	
				329 per 1000	214 fewer per 1000 (247 fewer to 168 fewer)
Use of ET (≥ 1); placebo-controlled studies	172 (2 RCTs)	⊕⊕⊕⊖ **Moderate**[4]	**RR 0.98** (0.48 to 1.98)	Study population	
				153 per 1000	3 fewer per 1000 (80 fewer to 150 more)
ETs performed per infant; all studies	658 (9 RCTs)	⊕⊖⊖⊖ **Very low**[1,2,3,5]	-	The mean ETs per infant for all studies was 0	MD 0.34 lower (0.5 lower to 0.17 lower)
ETs performed per infant; placebo-controlled studies	172 (2 RCTs)	⊕⊕⊕⊖ **Moderate**[4,5]	-	The mean ETs per infant for placebo-controlled studies was 0	MD 0.04 lower (0.18 lower to 0.1 higher)
Use of top-up transfusion in 1st week of life; all studies	378 (4 RCTs)	⊕⊕⊖⊖ **Low**[6,7]	**RR 1.05** (0.65 to 1.69)	Study population	
				130 per 1000	6 more per 1000 (45 fewer to 89 more)
Use of top-up transfusion after 1st week of life; all studies	507 (7 RCTs)	⊕⊖⊖⊖ **Very low**[1,8,9]	**RR 1.16** (0.97 to 1.38)	Study population	
				219 per 1000	35 more per 1000 (7 fewer to 83 more)
Maximum total serum bilirubin (μmol/L); all studies	451 (6 RCTs)	⊕⊖⊖⊖ **Very low**[10,11,12]	-	The mean maximum serum bilirubin (μmol/L) for all studies was 0	MD 25.39 lower (34.07 lower to 16.7 lower)

[a]**The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **ET:** exchange transfusion; **IVIg:** intravenous immunoglobulin; **MD:** mean difference; **RCT:** randomized controlled trial; **RR:** risk ratio.
GRADE Working Group grades of evidence
High quality**:** we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate quality**:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low quality**:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low quality**:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from estimate of effect.

[1]In three studies, the method of randomization was not stated and there was inadequate concealment of random sequence (selection bias). In seven studies, there was no blinding of personnel (performance bias) and in five studies, no blinding of outcome assessment (detection bias). Among other potential sources of bias were that mean bilirubin levels at study entry were already higher than the threshold for the outcome (ET) in one study, differences between study groups despite randomization (one study), postrandomization withdrawals or cross-over between study groups (two studies) and criteria for ET differing between treatment arms (one study).

[2]Substantial heterogeneity: X^2 = 34.63, df = 8 (P = 0.0003), I^2 = 77%.

[3]Four studies did not clearly specify use of intensive phototherapy (which should be a routine intervention for infants at high risk of ET).

[4]Total number of participants in these two trials was low, increasing the risk of possible bias.

[5]Only a few infants needed a second ET.

[6]In one trial, the methods of randomization and allocation concealment were not stated. In two studies, there was no blinding of personnel (performance bias).

[7]Combined studies were underpowered for use of top-up transfusion in 1st week. A total of 378 infants were enrolled in all four trials, and the overall frequency of top-up transfusion was low (13.8%).

[8]Substantial heterogeneity: X^2 = 15.60, df = 5 (P = 0.008); I^2 = 68%.

[9]Due to small differences between treatment groups, the combined studies were underpowered for use of top-up transfusion after 1st week.

[10]Four studies used no method of blinding the intervention.

[11]Substantial heterogeneity: X^2 = 14.82, df = 5 (P = 0.01); I^2 = 66%.

[12]Peak serum bilirubin in the control group varied 1.86-fold between studies; there was considerably greater variation between studies than between groups within studies.

BACKGROUND

Description of the condition

The use of anti-D immunoglobulin prophylaxis in D-negative women has led to a marked decline in Rh hemolytic disease of the newborn (HDN).[134] Sensitization can occur despite immunoprophylaxis, particularly if it is given too late or in insufficient dose. A proportion of HDN is caused by antibodies to antigens other than D and is, therefore, not preventable with anti-D immunoglobulin. Fetal therapy has significantly improved outcome in Rh sensitized fetuses, but it does not comprehensively prevent need for neonatal treatment (van Kamp 2004). Primary modes of postnatal therapy include phototherapy and exchange transfusion (ET) to reduce risk of mortality and kernicterus. Top- up transfusions are used to treat early and late anemia. In contemporary perinatal centers, 15% to 40% of neonates admitted for Rh or ABO HDN require at least one ET.[174,175]

The safety of ET has been reported for over 50 years. Published mortality rates vary from 0.53% to 4.7% per infant.[178-184] ET-related death is more common in sick or

premature infants than in healthy term infants.[175,178-180] Risks related to ET include adverse cardiorespiratory events; catheter-related complications; those related to the use of blood products; metabolic derangements; and other serious complications such as pulmonary hemorrhage, necrotizing enterocolitis and bowel perforation. In the last two decades, ET-related risks have been reported to be as high as 74%, although the incidence of severe adverse events is approximately 3-10%.[175,181,184,185]. Because improved perinatal care has reduced the need for ET, the complication rate could increase as clinicians become less experienced with the procedure.[175] However, Steiner 2007 reported that over a 21-year period, despite a sharp decline in the number of ETs performed, there was no increase in morbidity and mortality.

Description of the intervention

Intravenous immunoglobulin (IVIg) is an alternative therapy that may be effective in treating alloimmune HDN. In 1987, the first report of successful treatment of late anemia due to E-incompatibility with IVIg was published.[186] Subsequent case reports and case series reported success of IVIg treatment in neonates with both Rh or ABO incompatibility.[187-189] However, Hammerman 1996a found a reduced or no response to IVIg treatment in infants with ABO incompatibility who had early and severe hemolysis. Since the early 1990s, several quasi-randomized or randomized controlled trials on the use of IVIg (including variations on timing of administration and dose) to reduce ET have been published.[173,174,190-207]

The potential benefits of IVIg over ET include that the treatment is less complicated and less labor intensive. In addition, IVIg could allow safe treatment of some infants in less sophisticated neonatal units, or avoid delaying treatment while transferring infants for ET. Comprehensive assessment of IVIg in premature infants, particularly in the treatment of sepsis, has shown that it is safe and well tolerated.[208] It is a well-established therapy for alloimmune thrombocytopenia due to maternal and fetal human platelet antigen incompatibility.[209] The risk of transmission of viral infection is extremely low.[210] Hemolysis and acute renal failure are uncommon complications of IVIg treatment.[211] One study showed an increased incidence of sepsis in premature infants receiving prophylactic IVIg.[212] Since about 2010, several cases of necrotizing enterocolitis in infants with HDN treated with IVIg have been reported.[213-215] Other rare serious adverse effects of IVIg have been described in pediatric and adult cohorts, but not in newborns.[216]

How the intervention might work

IVIg might reduce the rate of hemolysis in alloimmune HDN by nonspecific blockade of Fc-receptors on the macrophages that are thought to mediate the destruction of antibody-coated red cells.[217] Ergaz 1995 demonstrated a decline in carboxyhemoglobin levels in four of five infants treated with IVIg for alloimmune HDN.[218] Hammerman 1996b demonstrated a significant reduction in carboxyhemoglobin levels in 19 of 26 Coombs-positive infants treated with IVIg.[219] Carboxyhemoglobin levels are a sensitive index of hemolysis and hence these studies suggest that immunoglobulin could decrease hemolysis. IVIg is typically formulated in 6% to 12% solutions, so at doses of 0.5 g/kg to 1 g/kg the volume administered is 4 mL/kg to 16 mL/kg. It is possible that this is a sufficient fluid bolus to reduce bilirubin levels modestly through dilution, temporarily slowing their rate of rise and allowing more time for intensive phototherapy to have effect.

Why it is important to do this review

This is an update of a Cochrane Review first published in 2002. Although results of the previous review showed a significant reduction in the need for ET in infants treated with IVIg, the applicability of the results was limited because none of three included studies was at low risk of bias. Nevertheless, American Academy of Pediatrics (AAP) guidelines recommend the administration of 0.5 g/kg to 1 g/kg IVIg in alloimmune HDN if total serum bilirubin (TSB) is rising despite intensive phototherapy or if TSB level is within 34 μmol/L to 51 μmol/L (2 mg/dL to 3 mg/dL) of exchange level.[220] As a result of these guidelines, despite the equivocal conclusions of the previous Cochrane Review, the use of IVIg in alloimmune HDN has become widespread in many countries. However, supplies of IVIg are limited and it does present some hazards. Therefore, use of IVIg should be restricted to treatment of conditions for which it is of confirmed benefit.

OBJECTIVES

To assess the effect and complications of IVIg in newborn infants with alloimmune HDN on the need for and number of exchange transfusions.

CHAPTER 7

METHODS

Criteria for considering studies for this review

Types of studies

All randomized and quasi-randomized controlled trials of IVIg in the treatment of alloimmune HDN.

Types of participants

Neonates with alloimmune HDN due to either Rh (or other red cell antigens) or ABO blood group antibodies with or without any other blood group antibodies.

Types of interventions

IVIg given for treatment of alloimmune HDN versus control (placebo or 'standard care'). Phototherapy, which is widely regarded as a safe and effective standard treatment may have been used in both IVIG and control groups. Early and late IVIg administration were defined (for this review) as IVIg started within (early) or after (late) the first 12 hours of life. Studies must have included predefined criteria for both IVIg and ET therapy.

Types of outcome measures

Primary outcomes

Efficacy:
–   use of ET (proportion of infants receiving one or more ETs);
–   ETs performed per infant.

Secondary outcomes

Efficacy:
–   use of top-up transfusion(s) in first week of life (% of infants);
–   number of top-up transfusions performed in first week of life per infant;
–   use of top-up transfusion(s) after first week of life (% of infants);
–   number of top-up transfusions performed after first week of life per infant;
–   maximum TSB (µmol/L (mg/dL));
–   duration of phototherapy (days);
–   duration of hospitalization (days);
–   incidence of sensorineural hearing loss (any severity);
–   incidence of kernicterus;
–   incidence of cerebral palsy.

Safety:

– neonatal mortality;
– incidence of adverse reactions possibly related to the use of IVIg or ET (statement of adverse events from individual trials only).

Search methods for identification of studies

Electronic searches

We performed electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library), PubMed, Embase (Ovid), Web of Science, CINAHL (EBSCO- host), Emcare and Academic Search Premier. The subject query was applied in all databases taking into account the terminological differences between these databases. The query consisted of the combination of four subjects: immunoglobulins, alloimmune hemolytic jaundice, newborn infants and randomized controlled trials. Various synonyms and related terms for all subjects were used. Two search strategies were used: the first strategy was limited to randomized trials and systematic reviews, the second strategy included only the subjects immunoglobulins and alloimmune hemolytic disease (and synonyms and related terms for those subjects). The search was performed on 19 May 2017. The bibliographic databases yielded 1565 references in total of which titles and abstracts were screened. The complete search strategy is attached in the appendix "Complete Search Strategy." In addition to database searches, we searched the trial registers ClinicalTrials.gov and controlled-trials.com. We applied no language restrictions.

Searching other resources

We searched the reference lists of all included and excluded trials and relevant reviews for further relevant studies.

Data collection and analysis

We used the standard method of Cochrane and its Neonatal Review Group.

Selection of studies

Two review authors independently screened the titles and abstracts of all references for possible inclusion using predefined criteria for inclusion (see below). We obtained a full-text version of the article if a report appeared to meet inclusion criteria for the review, or if it was not clear based on title and abstract. We resolved any disagreements through discussion with other review authors. The inclusion criteria for this review were:

– randomized and quasi-randomized controlled trials;

- study compared IVIg with any definition of "standard care" plus placebo, or with any definition of "standard care" without placebo;
- study included neonates with alloimmune HDN due to either ABO or Rh blood group antibodies with or without any other blood group antibodies;
- study measured ETs (primary outcome) for each study arm or at least one of the secondary outcomes (see below) (or both) for each study arm;
- study used predefined criteria for both IVIg and ET therapy.

Data extraction and management

Two review authors independently extracted data using a data collection form that was pilot tested before use. We resolved any disagreements through discussion and if necessary with the help of a third review author blinded to trial author, institution and journal of publication. One review author contacted authors of studies that did not report all required data or information. One review author entered data into Review Manager 5, and at least one review author checked them.

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias of included studies using the 'Risk of bias' tool as described in the Cochrane Handbook for Systematic Reviews of Interventions.[221] The following items for risk of bias were assessed: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias. Each item was rated as 'low risk of bias', 'unclear risk of bias' or 'high risk of bias.' Any differences of opinion were discussed with a third blinded review author until consensus was reached. For selective reporting, we used the following criteria to rate a study as 'low risk of bias:'

- for studies enrolling neonates with Rh or both Rh and ABO HDN: reporting (in paper or subsequent correspondence) at least one outcome related to each of ET, bilirubin and top-up transfusion, plus adverse effects and hospitalization.
- for studies enrolling only neonates with ABO HDN: reporting (in paper or subsequent correspondence) at least one outcome related to each of ET and bilirubin, plus adverse effects and hospitalization. Top-up transfusion was not considered to be a preferred outcome measure because anemia requiring treatment is an unusual consequence of ABO alloimmune hemolysis;
- study protocols or methods section of papers should not describe an intention to report outcomes that were not subsequently reported in the paper.

Measures of treatment effect

We calculated the risk ratio (RR) and risk difference (RD) for categorical outcomes, such as the incidence of ET. We calculated the mean difference (MD) for continuous variables, such as the maximum bilirubin level. We also calculated the number needed to treat for an additional beneficial outcome (NNTB) to avoid ET, where the assumed control risk was derived from the mean baseline risk from the studies.[222] We presented 95% confidence intervals (CI).

Dealing with missing data

We contacted investigators for missing information about study design, results or both.

Assessment of heterogeneity

We assessed clinical heterogeneity by determining whether clinical characteristics of participants, interventions, outcome measures and timing of outcome measurements were similar for included studies. We assessed statistical heterogeneity using X^2 and I^2 tests. An I^2 statistic of 50% or greater was considered as substantial or considerable heterogeneity according to the Cochrane Handbook for Systematic Reviews of Interventions.[221]

Assessment of reporting biases

We contacted investigators to request missing outcome data when selective reporting bias was suspected based on the criteria described under Assessment of risk of bias in included studies. If the data remained unavailable and the absence was thought to introduce serious bias, the study was excluded.

Data synthesis

We used Review Manager 5 to synthesize the available data (RevMan 2014). Whether we used a fixed-effect model or a random-effects model depended on the level of clinical heterogeneity and the results of the X^2 test and I^2 statistic for heterogeneity.[221] If there was substantial heterogeneity, we used a random-effects model was used and examined the sources of heterogeneity. If there was no substantial statistical heterogeneity, we used a fixed-effect model.

Quality of evidence

We used the GRADE approach, as outlined in the GRADE Handbook,[222] to assess the quality of evidence for the following (clinically relevant) outcomes:

– use of ET (proportion of infants receiving one or more ETs; assessment for all studies and separately for placebo-controlled studies;
– ETs per infant; assessment for all studies and separately for placebo-controlled studies;
– use of top-up transfusion(s) in first week of life (% of infants); all studies;
– use of top-up transfusion(s) after first week of life (% of infants); all studies;
– maximum serum bilirubin; all studies.

Two review authors independently assessed the quality of the evidence for each of the outcomes. We considered evidence from randomized controlled trials as high quality but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates and presence of publication bias. We used the GRADEpro Guideline Development Tool to create a 'Summary of findings' Table to report the quality of the evidence.

The GRADE approach results in an assessment of the quality of a body of evidence in one of four grades.
– High: we are very confident that the true effect lies close to that of the estimate of the effect.
– Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
– Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
– Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Subgroup analysis and investigation of heterogeneity
Subgroup analyses were conducted to determine if effects depend on:
– population:
o Rh incompatibility;
o gestational age at birth (less than 37 weeks and 37 weeks or greater);
– intervention:
o early administration of IVIg: start of IVIg 12 hours or less after birth;

o   late administration of IVIg: start of IVIg more than 12 hours after birth;

o   single versus multiple doses.

As in contemporary care intensive phototherapy is standard care for ABO incompatibility and therefore ETs hardly ever occur in this subgroup nowadays, no subgroup analysis was performed for ABO incompatibility only.

Sensitivity analysis

We conducted a sensitivity analysis based on whether or not the included studies used a placebo and treatment blinding (which had potential to reduce performance bias and detection bias). The two studies that used a placebo were also at low risk of other forms of bias in that they used random sequence generation, allocation concealment, reported complete outcome data for all prespecified outcomes and did not have other apparent risks of bias.

RESULTS

Description of studies

See Characteristics of included studies; Characteristics of excluded studies; and Characteristics of studies awaiting classification tables.

Results of the search

The search conducted up to 19 May 2017 identified 1565 references (see: Appendix 1). After title and abstract screening, the full text of 27 references was screened. After full text screening, nine studies were included in the meta-analysis.[173,174,190-196] Details of the studies are given in the Characteristics of included studies Table. Eleven studies were permanently excluded from this review. Details of these studies are given in the Characteristics of excluded studies Table. We found no additional studies searching reference lists of included and excluded studies and relevant reviews. A flow diagram of the study selection process is presented in Figure 1. No additional studies were included after an additional search for ongoing studies in the trial registers ClinicalTrials.gov and controlled-trials.com.
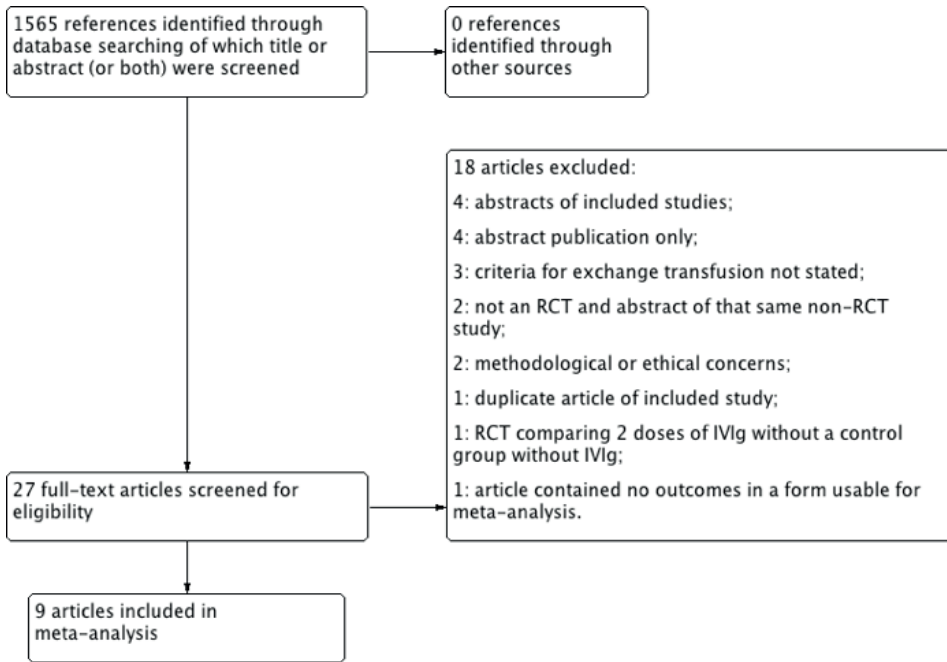
Figure 1. Flow diagram of study selection process. IVIg: intravenous immunoglobulin; RCT: randomized controlled trial.

Included studies

The review included nine randomized controlled trials published between 1992 and 2013.

Participants

The nine studies included 658 participants. Five studies included only infants with Rh incompatibility.[173,174,191,192,195] One study included only infants with ABO incompatibility.[193] Two studies enrolled mostly infants with ABO incompatibility but also some with Rh incompatibility and both ABO and Rh incompatibility.[190,194] Tanyer 2001 included 34 infants with ABO incompatibility, 18 with Rh incompatibility, two with "subgroup" incompatibility and seven with "more than one incompatibilities."[196] Only Nasseri 2006 reported results for each type of incompatibility separately and Alpay 1999 provided this information through correspondence.[190,194] Four studies enrolled only term infants of 37 weeks of gestation or greater.[190,192,194,196] None of the studies only included premature infants of less than 37 weeks of gestation. Rübo 1992 did not describe details of the gestational age at birth of enrolled infants.[195] Santos 2013 and Smits-Wintjens 2011 provided outcomes for term and preterm infants separately.[173,174]

Interventions

Seven of nine studies that met the inclusion criteria examined the effect of a single dose of IVIg in combination with phototherapy.[173,174,190-193,195] One study examined multiple doses,[194] and one study compared groups treated with a single dose or multiple doses with a control group,[196] but was inconsistent in describing which group received a single dose or multiple doses of IVIg and therefore this study was excluded from the (planned) subgroup analysis of single and multiple doses. Two studies used a placebo in addition to phototherapy for the control groups.[173,174] The intensity and topography of phototherapy fits the definition of intensive phototherapy in only three studies.[173,174,192] Tanyer 2001 used an obsolete model with three overhead lights from a single angle and Miqdad 2004 did not use a phototherapy blanket beneath the baby.[193,196] The remainder of included studies did not describe the intensity and topography of phototherapy in sufficient detail to allow a conclusion as to whether it is reasonable to describe it as intensive phototherapy. Five studies started IVIg 12 hours or less after birth,[173,174,191,192,195] and three studies started IVIg more than 12 hours after birth.[190,194,196] Miqdad 2004 started IVIg within 12 hours in nine neonates and more than 12 hours in 47 neonates, but they did not report outcomes for early and late IVIg administration separately.[193]

Since phototherapy was used in both treatment and control groups in all studies and is now considered standard of care in HDN, this review is effectively an analysis of the effectiveness of IVIg plus phototherapy versus phototherapy alone.

Outcomes

All included studies reported ET as the primary outcome. Six studies reported mean (or median) number of ETs per infant[174,194] or supplied enough data to calculate these.[191,192,195,196] The authors of four studies provided unpublished data (standard deviation or mean, or both) for ET.[173,174,190,193] Four studies reported the maximum bilirubin level.[173,174,191,195] Two studies provided unpublished data on maximum bilirubin levels.[190,192] Although all studies commented on the duration of phototherapy in their results, only seven studies reported or subsequently provided the numerical.[173,174,190,192-194,196] These studies all used predefined criteria for commencing phototherapy but not all for ceasing it. Six studies reported or subsequently provided numerical data on the duration of hospitalization.[173,174,190,192-194] Only two studies reported (after correspondence) predefined criteria for hospital discharge.[173,193] Six studies included top-up transfusion as an outcome.[174,190,191,193-195] Three studies provided additional data on top-up transfusions.[173,174,192] Smits-Wintjens 2011 did not report top-up transfusions separately for the first week and after the first week of

life, but subsequently provided this information.[174] Elalfy 2011 had a follow-up period of only one week after discharge.[192] Three studies reported predefined criteria for top-up transfusions,[174,190,194] and one study later provided data through correspondence.[173] All studies reported short-term adverse events. None of the included studies reported data on neurodevelopmental outcomes. Two studies provided additional information on neurodevelopmental outcomes.[173,193]

Excluded studies

We excluded 11 studies. One study only compared groups with a high or a low dose of IVIg,[199] and four studies were only reported in abstract form and our request for additional information was not (sufficiently) answered.[200,202,203,205] Three studies did not report predefined criteria for the primary outcome ET.[198,201,207]. One study did not report any outcome in a usable form for meta-analysis.[206] Two studies were excluded due to methodological or ethical (or both) concerns.[197,204] Details of excluded studies are given in the Characteristics of excluded studies Table.

Additional data

We attempted to contact the authors of all studies (except for the six studies that were identified for the previous review[190,191,195,196,205,206] to request further methodological information and results. We successfully contacted the authors of 11 papers[173,174,190,192,193,195,200,201,204] (including contact for the previous review)) in order to obtain additional data or to assist with the determination to include or exclude the study.

Risk of bias in included studies

For details of risk of bias of included studies, see the Characteristics of included studies Table and Figure 2.

Allocation

Only five studies reported an adequate method of randomization.[173,174,191-193] Miqdad 2004 and Elalfy 2011 provided information on randomization method only through correspondence. One quasi-randomized controlled trial allocated participants by order of admission.[196] This study was rated as high risk of bias for both random sequence generation and allocation concealment. Alpay 1999 did not state what method of randomisation was used either in the paper or in response to a query from the review authors, commenting only that that the group allocation was decided by attending neonatologists who differed from those who were conducting the study, which we

Figure 2. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

construed to mean that the allocation was not random, and that the allocation was at high risk of bias. Nasseri 2006 and Rübo 1992 stated that babies were randomly assigned to treatment groups but did not provide any detail about the method used and the allocation was therefore considered at unclear risk of bias.

Blinding

Only two studies used a placebo in the control group,[173,174] and were therefore rated as low risk for performance bias and detection bias. After correspondence with the authors of two additional studies, the risk of detection bias was rated as low; Miqdad

2004 explained that data were kept and entered to their database by personnel who were not involved in the management of the cases and Elalfy 2011 explained that the person who performed the randomization was different from the one who conducted the study and the one who analyzed the data. None of the other studies described any method of blinding of intervention after allocation and, therefore, they were rated as high risk of bias on both items.

Incomplete outcome data

Reporting of outcome data was at low risk of bias in seven studies.[173,174,190,191,193-196] For six of these studies, there were no missing data. In Rübo 1992, the amount of and reasons for missing data were similar between groups (low risk). One study was at high risk of bias because of a substantial amount of missing data on bilirubin levels.[192]

Selective reporting

Reporting bias was suspected in four studies because important outcomes were either not reported or were not reported in a form that was useable for meta-analysis, or that allowed judgment about local treatment practices (e.g. if the authors only stated that there was no significant difference between groups).[191,192,195,196] The remaining studies were at low risk of bias.

Other potential sources of bias

Elalfy 2011 had non-random cross-over after randomization and another study used an additional criterion for ET in the control group only.[192,193] These two studies were at high risk of bias. Dagoglu 1995 used post-randomization consent and although follow-up was complete for all infants for whom consent was obtained, four infants (two randomized to each arm of the study) were excluded because consent was withheld. Two infants were also excluded post-randomization in one other study because of "protocol violations" but no details were given.[195] The latter two studies were rated at unclear risk of bias because the review authors were unable to assess the impact of these withdrawals on overall outcomes. Three other studies were rated as unclear risk of bias,[190,194] or low risk of bias[174] for a potential risk of bias. For details see 'Risk of bias' tables.

Effects of interventions

See: Summary of findings for the main comparison (page 130).

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Use of exchange transfusion (≥ 1)	9	658	Risk Ratio (M-H, Fixed, 95% CI)	0.35 [0.25, 0.49]
1.1 Studies without a placebo group	7	486	Risk Ratio (M-H, Fixed, 95% CI)	0.26 [0.18, 0.39]
1.2 Placebo-controlled studies	2	172	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.48, 1.98]
2 Exchange transfusions per infant, by study quality	9	658	Mean Difference (IV, Random, 95% CI)	-0.34 [-0.50, -0.17]
2.1 Studies without a placebo group	7	486	Mean Difference (IV, Random, 95% CI)	-0.44 [-0.64, -0.25]
2.2 Placebo-controlled studies	2	172	Mean Difference (IV, Random, 95% CI)	-0.04 [-0.18, 0.10]
3 Use of top-up transfusion in 1st week by study quality	4	378	Risk Ratio (M-H, Fixed, 95% CI)	1.05 [0.65, 1.69]
3.1 Studies without a placebo group	2	206	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.24, 2.12]
3.2 Placebo-controlled studies	2	172	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.70, 2.00]
4 Top-up transfusions in 1st week per infant by study quality	3	262	Mean Difference (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
4.1 Studies without a placebo group	1	90	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.2 Placebo-controlled studies	2	172	Mean Difference (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
5 Use of top-up transfusion after 1st week by study quality	7	507	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [0.97, 1.38]
5.1 Studies without a placebo group	5	335	Risk Ratio (M-H, Fixed, 95% CI)	1.40 [1.08, 1.82]
5.2 Placebo-controlled studies	2	172	Risk Ratio (M-H, Fixed, 95% CI)	1.01 [0.80, 1.27]
6 Top-up transfusions after first week per infant, by study quality	4	316	Mean Difference (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]
6.1 Studies without a placebo group	2	144	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.2 Placebo-controlled studies	2	172	Mean Difference (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]
7 Maximum total serum bilirubin (μmol/L) by study quality	6	451	Mean Difference (IV, Fixed, 95% CI)	-25.39 [-34.07, -16.70]
7.1 Studies without a placebo group	4	279	Mean Difference (IV, Fixed, 95% CI)	-29.05 [-38.32, -19.78]
7.2 Placebo-controlled studies	2	172	Mean Difference (IV, Fixed, 95% CI)	0.93 [-23.94, 25.79]
8 Duration of phototherapy (days) by study quality	7	585	Mean Difference (IV, Random, 95% CI)	-0.98 [-1.31, -0.66]
8.1 Studies without a placebo group	5	413	Mean Difference (IV, Random, 95% CI)	-1.06 [-1.41, -0.72]
8.2 Placebo-controlled studies	2	172	Mean Difference (IV, Random, 95% CI)	-0.50 [-1.24, 0.24]

Comparison 1. Intravenous immunoglobulin plus phototherapy versus phototherapy.

Intravenous immunoglobulin plus phototherapy versus control (phototherapy only)
Primary outcomes
   Exchange transfusion
The results of nine included studies could be entered into the meta-analysis.[173,174,190-196] Most studies found a statistically significant reduction in the use of ET for IVIg treated infants.[190-196] Two studies concluded that the use of (one or more) ETs was not reduced despite using early IVIg in combination with phototherapy.[173,174] The meta-analysis of all nine studies (658 participants) showed that IVIg reduced the need for an ET (typical RR

0.35, 95% CI 0.25 to 0.49; typical RD -0.22, 95% CI -0.27 to -0.16; NNTB 5) (Analysis 1.1). However, overall, we rated this as very low quality evidence, because, although it was derived from randomized trials, there was very serious risk of bias in most trials, and moderate heterogeneity and serious indirectness, related to the fact that some trials did not use intensive phototherapy, which would be considered standard practice.

Review: Immunoglobulin for alloimmune hemolytic disease in neonates

Comparison: 1 Intravenous immunoglobulin plus phototherapy versus phototherapy

Outcome: 1 Use of exchange transfusion ($\geq$ 1)

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Studies without a placebo group					
Alpay 1999	8/58	22/58		20.3 %	0.36 [0.18, 0.75]
Dağ oğ lu 1995	4/22	15/19		14.9 %	0.23 [0.09, 0.58]
Elalfy 2011	2/40	11/50		9.0 %	0.23 [0.05, 0.97]
Miqdad 2004	4/56	16/56		14.8 %	0.25 [0.09, 0.70]
Nasseri 2006	3/17	11/17		10.2 %	0.27 [0.09, 0.81]
Rübo 1992	2/16	11/16		10.2 %	0.18 [0.05, 0.69]
Tanyer 2001	3/40	7/21		8.5 %	0.23 [0.06, 0.78]
Subtotal (95% CI)	**249**	**237**		**87.8 %**	**0.26 [0.18, 0.39]**
Total events: 26 (Treatment), 93 (Control)					
Heterogeneity: Chi² = 1.26, df = 6 (P = 0.97); I² =0.0%					
Test for overall effect: Z = 6.70 (P < 0.00001)					
2 Placebo-controlled studies					
Santos 2013	6/46	7/46		6.5 %	0.86 [0.31, 2.36]
Smits-Wintjens 2011	7/41	6/39		5.7 %	1.11 [0.41, 3.01]
Subtotal (95% CI)	**87**	**85**		**12.2 %**	**0.98 [0.48, 1.98]**
Total events: 13 (Treatment), 13 (Control)					
Heterogeneity: Chi² = 0.13, df = 1 (P = 0.72); I² =0.0%					
Test for overall effect: Z = 0.07 (P = 0.94)					
Total (95% CI)	**336**	**322**		**100.0 %**	**0.35 [0.25, 0.49]**
Total events: 39 (Treatment), 106 (Control)					
Heterogeneity: Chi² = 11.32, df = 8 (P = 0.18); I² =29%					
Test for overall effect: Z = 6.23 (P < 0.00001)					
Test for subgroup differences: Chi² = 10.07, df = 1 (P = 0.00), I² =90%					

```
        0.01   0.1    1    10   100
      Favours treatment   Favours control
```

Analysis 1.1. Comparison 1. Intravenous immunoglobulin plus phototherapy versus phototherapy, Outcome 1 Use of exchange transfusion (≥ 1).

Subgroup analysis of infants with only Rh incompatibility supported a reduction in the use of ET with IVIg treatment (371 participants, typical RR 0.38, 95% CI 0.25 to 0.58; NNTB 5) (Analysis 2.1).[173,174,190-192,194,195]

In only those infants born at 37 weeks of gestation or greater, IVIg reduced the use of ETs (391 participants, typical RR 0.39, 95% CI 0.25 to 0.61; NNTB 6) (Analysis 6.1).[173,174,190,192,194,196] In the subgroup of infants born at less than 37 weeks of gestation, IVIg did not reduce the use of ETs (82 participants, typical RR 0.77, 95% CI 0.31 to 1.91; NNTB 20) (data not shown).[173,174]

Five studies found reductions in the use of ET where IVIg was used 12 hours or less after birth (335 participants, typical RR 0.41, 95% CI 0.26 to 0.66; NNTB 6) (Analysis 3.1).[173,174,191,192,195] Reductions were also found in the three studies which used IVIg more than 12 hours after birth (211 participants, typical RR 0.31, 95% CI 0.18 to 0.53; NNTB 4) (data not shown).[190,194,196] Subgroup analyses of infants receiving a single dose of IVIg and infants receiving multiples doses of IVIg supported a reduction in the use of ET with IVIg treatment, although there was insufficient evidence to support a dose-response effect (single dose of IVIg: 563 participants, typical RR 0.37, 95% CI 0.26 to 0.53; NNTB 6; Analysis 4.1;);[173,174,190-193,195] multiple doses of IVIg: 34 participants, RR 0.27, 95% CI 0.09 to 0.81; NNTB 1; Analysis 5.1).[194]

However, despite these apparently promising results, analysis of the only two placebo-controlled studies at low risk of all forms of bias showed no reduction in the use of ET (172 participants, typical RR 0.98, 95% CI 0.48 to 1.98) (Analysis 1.1.2).[173,174]

Furthermore, when all studies were considered, heterogeneity was moderate for use of ET (X^2 = 11.32, degrees of freedom (df) = 8 (P = 0.18); I^2 = 29%) and was high for ETs per infant (Tau^2 = 0.04; Chi^2 = 36.77, df = 8 (P < 0.0001); I^2 = 78%), whereas the results of both these outcomes for the placebo-controlled trials were highly consistent (I^2 = 0% for both). We rated the quality of evidence from the two placebo-controlled studies as moderate, downgrading it only for imprecision because of the low total number of participants.

Overall, immunoglobulin treatment also led to a reduction in the mean number of ETs per infant (658 participants, MD -0.34, 95% CI -0.50 to -0.17). We assessed the level of evidence from the whole group of studies as very low, again downgrading the evidence from randomized trials because of very serious risk of bias, high heterogeneity, indirectness and imprecision. In contrast, analysis of the two placebo-controlled studies were consistent with each other and when considered alone, yielded moderate quality of evidence that IVIg did not reduce the number of ETs (172 participants, MD -0.04, 95% CI -0.18 to 0.10) (Analysis 1.2.2).

CHAPTER 7

Secondary outcomes

    Top-up transfusions during and after the first week

The results of four studies could be entered in the meta-analysis of the use of top-up transfusions in the first week of life[173,174,190,192] and of seven studies for the use of top-up transfusions after the first week of life.[173,174,190,191,193-195] IVIg did not increase the need for top-up transfusions during the first week (378 participants, typical RR 1.05, 95% CI 0.65 to 1.69) (Analysis 1.3) or in the period after the first week (507 participants, typical RR 1.16, 95% CI 0.97 to 1.38) (Analysis 1.5). IVIg also did not increase the need for top-up transfusions in the first week and after the first week of life in the following subgroups: infants with Rh incompatibility only (first week: typical RR 1.08, 95% CI 0.65 to 1.77 (Analysis 2.3); after first week: typical RR 1.09, 95% CI 0.92 to 1.28 (Analysis 2.5)); infants born 37 weeks or more of gestation (first week: typical RR 0.91, 95% CI 0.48 to 1.74 (Analysis 6.3); after first week: typical RR 1.18, 95% CI 0.81 to 1.71 (Analysis 6.5)); infants born less than 37 weeks of gestation (first week: typical RR 1.39, 95% CI 0.70 to 2.73; after first week: typical RR 1.24, 95% CI 0.93 to 1.67 (data not shown)); infants treated with IVIg 12 hours or less after birth (first week: typical RR 1.18, 95% CI 0.70 to 2.00 (Analysis 3.3); after first week: typical RR 1.04, 95% CI 0.89 to 1.22 (Analysis 3.5)); and in infants treated with a single dose of IVIg (first week: typical RR 1.05, 95% CI 0.65 to 1.69 (Analysis 4.3); after first week: typical RR 1.13, 95% CI 0.95 to 1.33 (Analysis 4.5)). Although the need for top-up transfusions during the first week of life was not increased for the subgroup of infants treated with IVIg more than 12 hours after birth (typical RR 0.71, 95% CI 0.24 to 2.12) (data not shown), the need for top-up transfusions after the first week of life was increased with late IVIg treatment (typical RR 8.00, 95% CI 1.03 to 62.26) (data not shown). However, the CIs were very large and the lower CI limit was nearly one. For infants treated with multiple IVIg doses, the use of top-up transfusions after the first week of life was not increased (typical RR 5.65, 95% CI 0.25 to 126.87) (Analysis 5.3) and not estimable for the first week of life.
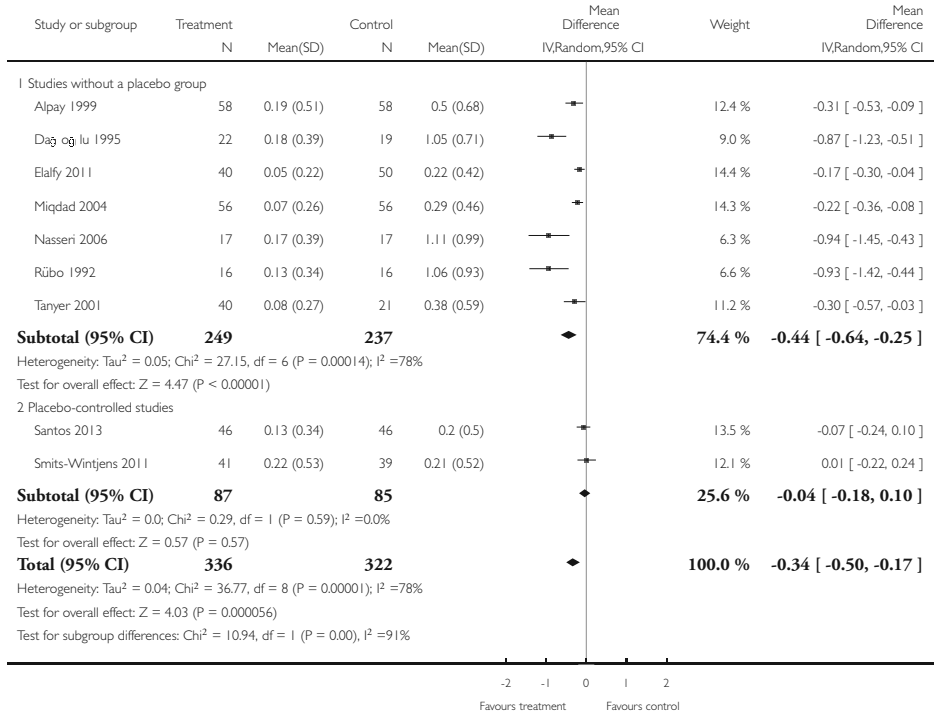
For the subgroup of infants included in placebo-controlled studies only, at low risk of all forms of bias, the need for top-up transfusions in the first week of life and thereafter was also not altered in infants treated with IVIg (first week: 172 participants, typical RR 1.18, 95% CI 0.70 to 2.00 (Analysis 1.3.2); after first week: typical RR 1.01, 95% CI 0.80 to 1.27 (Analysis 1.5.2)).[173,174]

Smits-Wintjens 2011 and Santos 2013 were the only studies included in the analysis of the number of top-up transfusions per infant. In the first week of life and thereafter, the

Review: Immunoglobulin for alloimmune hemolytic disease in neonates

Comparison: 1 Intravenous immunoglobulin plus phototherapy versus phototherapy

Outcome: 2 Exchange transfusions per infant, by study quality

Study or subgroup	Treatment		Control		Mean Difference	Weight	Mean Difference
	N	Mean(SD)	N	Mean(SD)	IV,Random,95% CI		IV,Random,95% CI
1 Studies without a placebo group							
Alpay 1999	58	0.19 (0.51)	58	0.5 (0.68)		12.4 %	-0.31 [-0.53, -0.09]
Dağ oğ lu 1995	22	0.18 (0.39)	19	1.05 (0.71)		9.0 %	-0.87 [-1.23, -0.51]
Elalfy 2011	40	0.05 (0.22)	50	0.22 (0.42)		14.4 %	-0.17 [-0.30, -0.04]
Miqdad 2004	56	0.07 (0.26)	56	0.29 (0.46)		14.3 %	-0.22 [-0.36, -0.08]
Nasseri 2006	17	0.17 (0.39)	17	1.11 (0.99)		6.3 %	-0.94 [-1.45, -0.43]
Rübo 1992	16	0.13 (0.34)	16	1.06 (0.93)		6.6 %	-0.93 [-1.42, -0.44]
Tanyer 2001	40	0.08 (0.27)	21	0.38 (0.59)		11.2 %	-0.30 [-0.57, -0.03]
Subtotal (95% CI)	**249**		**237**			**74.4 %**	**-0.44 [-0.64, -0.25]**
Heterogeneity: Tau² = 0.05; Chi² = 27.15, df = 6 (P = 0.00014); I² =78%							
Test for overall effect: Z = 4.47 (P < 0.00001)							
2 Placebo-controlled studies							
Santos 2013	46	0.13 (0.34)	46	0.2 (0.5)		13.5 %	-0.07 [-0.24, 0.10]
Smits-Wintjens 2011	41	0.22 (0.53)	39	0.21 (0.52)		12.1 %	0.01 [-0.22, 0.24]
Subtotal (95% CI)	**87**		**85**			**25.6 %**	**-0.04 [-0.18, 0.10]**
Heterogeneity: Tau² = 0.0; Chi² = 0.29, df = 1 (P = 0.59); I² =0.0%							
Test for overall effect: Z = 0.57 (P = 0.57)							
Total (95% CI)	**336**		**322**			**100.0 %**	**-0.34 [-0.50, -0.17]**
Heterogeneity: Tau² = 0.04; Chi² = 36.77, df = 8 (P = 0.00001); I² =78%							
Test for overall effect: Z = 4.03 (P = 0.000056)							
Test for subgroup differences: Chi² = 10.94, df = 1 (P = 0.00), I² =91%							

```
        -2    -1    0    1    2
     Favours treatment   Favours control
```

Analysis 1.2. Comparison 1. Intravenous immunoglobulin plus phototherapy versus phototherapy, Outcome 2 Exchange transfusions per infant, by study quality.

number of top- up transfusions was not altered in IVIg treated infants (first week: MD 0.05, 95% CI -0.07 to 0.17 (Analysis 1.4); after first week: MD -0.00, 95% CI -0.12 to 0.12 (Analysis 1.6)).

When all studies reporting these outcomes were considered, there was low to very low quality evidence (downgraded for risk of serious to very serious bias, and serious imprecision) that IVIg did not alter the risk of early or late top-up transfusion. These results were consistent with the findings of the placebo-controlled trials.

Maximum total serum bilirubin

Six studies reported results for maximum serum bilirubin. [173,174,190-192,195] The meta-analysis of all six studies showed that the mean maximum serum bilirubin decreased by 25.39

µmol/L in infants receiving IVIg (MD -25.39 µmol/L, 95% CI - 34.07 to -16.70) (Analysis 1.7). Furthermore, subgroup analyses showed that IVIg decreased maximum bilirubin levels in infants with Rh incompatibility, infants of more than 37 weeks of gestation, infants treated early or late, and infants treated with a single dose of IVIg. However, subgroup analyses of the only two placebo- controlled studies[173,174] and of infants born at less than 37 weeks of gestation[173,174] showed that IVIg did not reduce maximum serum bilirubin (placebo-controlled trials: MD 0.93 µmol/L, 95% CI - 23.94 to 25.79 (Analysis 1.7.2); infants born at less than 37 weeks of gestation: MD -18.91 µmol/L, 95% CI -54.49 to 16.68 (data not shown)). The quality of evidence regarding maximum serum bilirubin was very low, with evidence from six randomized controlled trials downgraded for risk of bias and serious inconsistency; (heterogeneity: X^2 = 14.82, df = 5 (P = 0.01); I^2 = 66%). Of note, the peak serum bilirubin in the control groups varied nearly two-fold between studies, indicating that there were likely to be very different thresholds for ET between the studies.

Duration of phototherapy

Results of seven studies could be included in the meta-analysis of the duration of phototherapy.[173,174,190,192-194,196] Although all studies gave criteria for commencing phototherapy, only five studies described or provided predefined criteria for ceasing phototherapy.[173,174,190,192,196] Analysis of all seven studies showed that duration of phototherapy decreased by 0.98 days with IVIg treatment (MD -0.98 days, 95% CI -1.31 to -0.66) (Analysis 1.8). All subgroup analyses showed a decrease in duration of phototherapy in IVIg-treated infants varying from a mean decrease of 1.12 days in infants treated with a single dose of IVIg (MD -1.12 days, 95% CI -1.30 to -0.94) (Analysis 4.8) to 1.24 days in infants treated with IVIg 12 hours or less after birth (MD -1.24 days, 95% CI -1.44 to -1.03 (Analysis 3.8)). However, as for maximum bilirubin levels, analyses of the two placebo-controlled studies and of infants born less than 37 weeks of gestation showed no reduction in duration of phototherapy (placebo-controlled trials: MD -0.50 days, 95% CI -1.24 to 0.24 (Analysis 1.8.2); infants born less than 37 weeks of gestation: MD -0.91 days, 95% CI -1.96 to 0.14) (data not shown)).

Duration of hospitalization

Results of six studies could be entered in the meta-analysis.[173,174,190,192-194] None of these studies described predefined criteria for hospital discharge and only two studies provided them through correspondence.[173,193] The analysis showed that IVIg treatment shortened duration of hospitalization by 1.34 days (MD -1.34 days, 95% CI -1.60 to -1.09)

(data not shown). All subgroup analyses showed a shorter duration of hospitalization with IVIg treatment (data not shown).

Incidence of adverse reactions

All studies reported or subsequently provided data on adverse reactions, although for most of the trials, we did not know any details of what protocols were used to identify adverse events or how they were defined. Nine studies reported that there were no adverse reactions of IVIg treatment.[173,174,190-196] None of the adverse reactions were necrotizing enterocolitis. In the study by Alpay 1999, two control infants receiving ET developed hypoglycemia and hypocalcemia after ET. In the study by Rübo 1992, one control infant who required ET developed sepsis and one control infant who required ET developed inspissated bile syndrome. However, the authors stated that a causal relationship with ET could not be established in either infant. In the study by Dagoglu 1995, one control infant developed inspissated bile syndrome. Miqdad 2004 described that "no immediate adverse effects related to IVIg were noted, including fever, allergic reactions, volume overload or hemolysis;" however, they also stated that "ten of the babies who had ET, from both groups, had to be treated for blood culture-positive or clinical sepsis." In the study by Smits-Wintjens 2011 one infant from the IVIg group developed a Bacillus cereus sepsis with brain abscesses a few days after ET. Sterility tests on the used IVIg batches and cultures of all donor blood products used for intrauterine transfer (IUT) and ET were sterile. The sepsis may have been related to the umbilical venous catheterization and ET. A case report provided information on this exceptional case.[174]

Long-term outcomes

Only two studies had a relatively long follow-up period of one year[173] and two years.[193]. In both studies, there were no cases of kernicterus, deafness or cerebral palsy. All participants of the Smits-Wintjens 2011 study were included in a subsequent long-term follow-up study and neurodevelopmental outcome in children of at least two years of age was equal in children treated with IVIg and children treated with placebo.[223] The authors stated that their findings may have been limited by a small sample size.

Neonatal mortality

None of the studies reported neonatal mortality data.

> *Incidence of adverse reactions possibly related to the use of intravenous immunoglobulin or exchange transfusion*

None of the studies reported Incidence of adverse reactions possibly related to the use of IVIg or ET.

DISCUSSION

Summary of main results

Data from nine studies with 658 participants provided limited evidence that IVIg treatment in neonates with alloimmune HDN reduced the need for ET. Although this review update showed a significant reduction in the need for ET, most of the included studies were at high risk of bias. IVIg treatment was also associated with a significant reduction in maximum bilirubin level and duration of phototherapy when all included studies were analyzed and for most of the subgroup analyses based on type of alloimmunization, gestational age at birth, and timing and number of doses of IVIg. Duration of hospitalization was significantly reduced when analyzing all studies that reported this outcome and for almost all subgroup analyses, including the analysis of studies at low risk of bias only. Although there was some evidence that IVIg reduced hemolysis and shortened hospital stay, these results should be interpreted with considerable caution because the studies reporting these benefits were not blinded, only two studies used predefined criteria for hospital discharge, and criteria for stopping phototherapy were not reported in most studies. In addition, since the late 1980s, guidelines for phototherapy have recommended using it more promptly for infants at risk of hemolysis.[224] In many hospitals, the quality of phototherapy has also improved over the years. Nevertheless, the quality/intensity of phototherapy can still vary today, especially in low-resource settings and if good quality control is not applied. The incidence of late top-up transfusions is an important outcome, especially in areas where follow-up of infants is difficult or where supply of safe blood for transfusion is limited. However, as thresholds for top-up transfusions in neonates vary widely, this outcome is susceptible to bias, particularly in unblinded studies. Seven of nine studies were included in the analysis of the incidence of top-up transfusion after the first week of life.[173,174,190,191,193-195] However, only five of the seven studies used predefined criteria for top-up transfusions.[173,174,190,193,194] In addition, the predefined criteria varied between studies, thus conclusions were limited. Data on adverse events of IVIg seemed to indicate that it can be used safely. Although we found reports of a higher incidence

of NEC in infants with HDN treated with IVIg in the literature,[213-215] there were no cases of NEC in the current meta-analysis.

Importantly however, subgroup analysis of the only two studies that were placebo-controlled, blinded, at low risk of all forms of bias, including 172 participants, were very consistent with each other and showed that IVIg treatment had no effect on the need for ET or the number of ETs per infant.[173,174] As for ET, analysis of these two studies at low risk of bias demonstrated no difference in maximum bilirubin level and duration of phototherapy.

Overall completeness and applicability of evidence

This review included all (quasi-) randomized controlled trials on the use of IVIg in alloimmune HDN. We identified 27 trials, of which nine trials, comprising 658 infants, fulfilled inclusion criteria for the review. The only two included studies that were placebo-controlled comprising a total of 172 infants, enrolled only infants with Rh HDN and the intervention consisted of a single dose of 0.5 g/kg to 0.75 g/kg IVIg administered within four to six hours after birth.[173,174] Santos 2013 included infants of 32 gestational weeks or greater and Smits-Wintjens 2011 included infants of 35 gestational weeks or greater. Criteria for phototherapy and ET were similar in both studies. Evidence from subgroup analysis of these two studies with 172 participants showed that early administration of IVIg in a single dose of 0.5 g/kg to 0.75 g/kg did not reduce ETs or had other benefits in the treatment of Rh HDN. There was no clear evidence from this review that a higher dose improved efficacy. The only randomized controlled trial comparing the effect of two doses of IVIg in Rh HDN showed that 0.5 g/kg and 1 g/kg had a similar effect on the duration of phototherapy, duration of hospitalization and ET requirements.[199] However, this study was not powered to detect a difference in the need for ET. Only two studies examined long-term neurodevelopmental outcome, which found no cases of kernicterus, deafness or cerebral palsy in a follow-up period of one year[173] and two years.[193] All participants of the Smits-Wintjens 2011 study were included in a subsequent long-term follow-up study and neurodevelopmental outcome in children of at least two years of age was equal in children treated with IVIg and children treated with placebo.[223]

American Academy of Pediatrics guidelines of 2004 recommend the administration of 0.5 g/kg to 1 g/kg IVIg in alloimmune HDN if TSB is rising despite intensive phototherapy or if TSB level is within 34 µmol/L to 51 µmol/L (2 to 3 mg/dL) of exchange level.[220] Based on the results of this review and because IVIg administration is not completely without

risks,[211-213] and supplies of IVIg are limited, we do not recommend routine use of IVIg. However, since there is some evidence that it reduces hemolysis and it appears safe in infants with alloimmune HDN, it might be reasonable to consider using it in special circumstances, such as during transfer of an infant to a location that can perform an ET, where the risk of ET is considered to be much higher than usual, such as in very or extremely low birth weight infants, or in the context of a future research study.

Quality of the evidence

The quality of included studies ranged from fulfilling none of the 'risk of bias' criteria to fulfilling all criteria (see 'Risk of bias' section of Characteristics of included studies Table, and Summary of findings for the main comparison). Only two of nine trials ful-filled all criteria to be rated as high-quality studies. We made the decision to evaluate the quality of evidence using GRADE criteria separately for the seven studies at high risk of bias and the two studies at low risk of bias, because evaluation of the seven studies at high risk of bias as a group also demonstrated other concerns including inconsistency and indirectness. For the outcomes of use and number of ETs in the first week, the quality of evidence from the seven studies at high risk of bias was very low, whereas the evidence from the two studies at low risk of bias was moderate (down- graded only for small number of participants). For the outcome of top-up transfusions after the first week, we evaluated the level of evidence only for Rh HDN and only for the two studies at low risk of bias, because we deemed this outcome to usually be irrelevant for infants with ABO incompatibility (who are at much lower risk of late anemia) and because of incomplete reporting of data in other studies. The evidence was of very low quality. Analysis of the effect of IVIg on the need for ET in infants with ABO incompatibility included only three studies at high risk of bias,[190,193,194] because other studies only enrolled infants with Rh HDN, did not use predefined criteria for top-up transfusion or did not provide sufficient detail to separate Rh- and ABO-affected infants. The quality of evidence for IVIg for ABO incompatibility was very low (GRADE analysis not shown). For several of the secondary outcomes of the review, the RR (or other relevant statistic) was not estimable for included studies (no events in either intervention or control groups), highlighting the extent to which these studies were seriously underpowered. In summary, we considered that the evidence from the two trials at low risk of bias provided a sufficient quality of evidence to guide practice.

It was unclear why placebo-controlled, high-quality trials yielded such different results. Possibilities included that when administration of IVIg was not compared with use of a

placebo administered in similar dose and over similar duration, there were differences in timing of the next bilirubin measurement, meaning that in IVIg-treated infants, there was longer exposure to phototherapy before the decision about ET was made. Another possibility was that there was bias in the decision to perform an ET, influenced by knowledge of group allocation. A third possibility was that rather than a specific immunomodulatory effect, IVIg (and where used, the placebo solution) has a sufficient non-specific dilutional effect to change the rate of rise of bilirubin, altering duration of exposure to phototherapy and decision making about ET.

Potential biases in the review process

We tried to minimize bias by working with two review authors who independently assessed eligibility for inclusion of trials, extracted data and assessed risk of bias. However, we were aware that these parts of the review process were based on personal judgment because reviewing research is influenced by prior beliefs. In addition, one included trial was performed by two of the five review authors. Nevertheless, we attempted to review all studies in a similar way. In addition, we were unable to contact authors of all potentially eligible studies and, therefore, we could not include all available data. While the translator of the Turkish included study was a medical doctor from Turkish parents, he may have missed some details regarding the risk of bias of that study.

Agreements and disagreements with other studies or reviews

The overall findings of this review were consistent with previous systematic reviews. Louis 2014 included 12 studies (813 participants) and concurred with our finding that high-quality studies found no effect of IVIg, whereas low-quality studies found IVIg effective in HDN. Gottstein 2003 included three studies that were also included in our review,[190,191,195] and one study that was excluded from our review.[206] They concluded that with IVIg treatment significantly fewer infants required ET. Duration of hospitalization and phototherapy were also significantly reduced in their review. However, based on our judgment, none of their included studies was of high quality. Two Chinese systematic reviews[225,226] also found a reduction in ET requirements, duration of phototherapy and hospitalization but concluded that well-designed trials with a larger sample size were required for further evaluation of the efficacy and safety of IVIg. Until the date we conducted our search, our review was the most recent, extensive and up-to-date review of all randomized and quasi-randomized trials on the effect of IVIg in alloimmune HDN.

CHAPTER 7

AUTHORS' CONCLUSIONS

Implications for practice

Based on the overall outcomes of the review, there is insufficient evidence to conclude that intravenous immunoglobulin (IVIg) is beneficial in neonates with hemolytic disease of the newborn (HDN). We gave particular weight to the results of the only two studies that provide evidence at sufficient low risk of bias to guide routine clinical practice, and that show no reduction in the use of exchange transfusion (ET), or improvement in any other important outcomes of the review. In addition, IVIg has risks that have been identified in other contexts of treatment. Therefore, we believe routine use of IVIg for HDN should not be recommended.

The effect of IVIg plus phototherapy compared to phototherapy alone on eventual neurodevelopmental outcomes remains unknown, although there was no difference in neurodevelopmental outcome between these groups in a (small) long-term follow-up study.[223] However, since there is some indirect evidence that IVIg reduces hemolysis and because it appears safe in neonates with alloimmune HDN (acknowledging that the combined sample size of all studies is insufficient to assess uncommon, put potentially serious adverse effects), it may have a limited role in special circumstances, such as where ET is impossible, or is considered particularly high risk. Nevertheless, undertaking preparations for ET, including ensuring earliest possible use of intensive phototherapy, and birth at or transfer to a center that can perform ET, would seem to be strongly indicated in high-risk infants, and should not be abandoned in the expectation that IVIg will be efficacious.

Implications for research

Future research into the role of IVIg in the early treatment of alloimmune HDN may be warranted, especially for infants for whom ET carries particularly high risks. Such a trial should examine the safety and efficacy of IVIg by recording both short-term outcomes such as the need for transfusion therapy and the incidence of adverse events and also long-term neurodevelopmental outcomes. Both ETs and (late) top-up transfusions should be recorded because reduction of ETs can increase the number of top- up transfusions.[227] Consideration should also be given to including additional measures to assess the severity of hemolysis such as carboxyhemoglobin or end tidal carbon monoxide. Based on evidence from the two placebo-controlled trials at low risk of all forms of bias, the conclusion of the review authors is that IVIg is of very limited usefulness

in Rh HDN. However, neither of these placebo-controlled studies enrolled infants with severe established jaundice due to ABO incompatibility. In contrast to Rh incompatibility, ABO incompatibility mainly results in hyperbilirubinemia without significant anemia. This is primarily due to the relatively few group A and B antigenic sites on neonatal red blood cells.[228] Furthermore, infants with ABO- mediated hemolysis often present for neonatal care when they already have severe jaundice. Due to these differences between Rh and ABO incompatibility it is conceivable that IVIg has a different effect in anti-A or anti-B-mediated jaundice. Due to the relative rarity of severe jaundice caused by ABO incompatibility in many countries and the fact that this condition almost always resolves with phototherapy alone,[229] exploring the use of IVIg to treat established jaundice would require a multicenter randomized controlled trial. Based on the discordance of results in this review between trials that were conducted with and without careful blinding of the intervention using a placebo, we recommend that any future trials should either use a placebo or a robust alternative method for blinding of treatment and outcome assessment. Future trials should be well designed and give priority to establishing guidelines for the "conventional" management of alloimmune HDN, focusing on the criteria for performing both top-up and ETs and on the role of intensive phototherapy.

ACKNOWLEDGEMENTS

CHAPTER 7

REFERENCES TO STUDIES INCLUDED IN THIS REVIEW

Alpay 1999 [published and unpublished data]
Alpay F, Sarici SÜ, Okutan V, Erdem G, Özcan O, Gökçay E. High-dose intravenous immunoglobulin therapy in neonatal immune haemolytic jaundice. Acta Paediatrica 1999;88(2):216–9.

Dagoglu 1995 [published data only]
Dagoglu T,OvaliF,SamanciN,BengisuE.High-dose intravenous immunoglobulin therapy for rhesus haemolytic disease. Journal of International Medical Research 1995;23 (4):264–71.

Elalfy 2011 [published and unpublished data]
Elalfy MS, Elbarbary NS, Abaza HW. Early intravenous immunoglobulin (two-dose regimen) in the management of severe Rh hemolytic disease of newborn - a prospective randomized controlled trial. European Journal of Pediatrics 2011;170(4):461–7.

Miqdad 2004 [published and unpublished data]
Miqdad AM, Abdelbasit OB, Shaheed MM, Seidahmed MZ, Abomelha AM, Arcala OP. Intravenous immunoglobulin G (IVIG) therapy for significant hyperbilirubinemia in ABO hemolytic disease of the newborn. Journal of Maternal-fetal & Neonatal Medicine 2004;16(3):163–6.

Nasseri 2006 [published data only]
Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. Saudi Medical Journal 2006;27(12):1827–30.

Rübo 1992 [published data only]
Rübo J, Albrecht K, Lasch P, Lauftkötter E, Leititis J, Marsan D, et al. High-dose intravenous immune globulin therapy for hyperbilirubinemia caused by Rh hemolytic disease. Journal of Pediatrics 1992;121(1):93–7.

Santos 2013 [published and unpublished data]
Santos MC, Sá C, Gomes SC Jr, Camacho LA, Moreira ME. The efficacy of the use of intravenous human immunoglobulin in Brazilian newborns with rhesus hemolytic disease: a randomized double-blind trial. Transfusion 2013;53(4):777–82.

Smits-Wintjens 2011 *[published and unpublished data]*
Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, et al. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011;127(4):680–6.

Tanyer 2001 *[published data only]*
Tanyer G, iklar Z, Dallar Y, Yildirmak Y, Tira Ü. Multiple dose IVIG treatment in neonatal immune hemolytic jaundice. Journal of Tropical Pediatrics 2001;47(1):50–3.

REFERENCES TO STUDIES EXCLUDED FROM THIS REVIEW

Atici 1996 *[published data only]*
Atici A, Satar M, Gö ebakan M. Intravenous immunoglobulin therapy for neonatal hyperbilirubinemia due to ABO or Rh incompatibility [ABO veya Rh Uyu mazli inin neden oldu u neonatal hiperbilirübinemide intravenöz immüglobülin tedavisi].

Çocuk Sa li i ve Hastaliklari Dergisi 1996;39:623–30.

Garcia 2004 *[published and unpublished data]*
Garcia MG, Cordero G, Mucino P, Salinas V, Fernandez LA, Christensen RD. Intravenous immunoglobulin (IVIg) administration as a treatment for Rh hemolytic jaundice in Mexico City. Pediatric Research 2004;55:65.

Girish 2008 *[published data only]*
Girish G, Chawla D, Agarwal R, Paul VK, Deorari AK. Efficacy of two dose regimen of intravenous immunoglobulin in Rh hemolytic disease of newborn - a randomized controlled trial. Indian Pediatrics 2008;45(8): 653–9.

Hematyar 2011 *[published and unpublished data]*
Hematyar M, Zareian M. The effects of intravenous immunoglobulin(IVIG) in hemolytic jaundice of the newborn due to ABO and Rh isoimmunization. Acta Paediatrica 2011;100(Suppl 463):13–83.

CHAPTER 7

Huang 2006 *[published data only]*
Huang WM, Chen HW, Li N, Yang M, Jiao PY. Clinical study of early interventions for ABO hemolytic disease of the newborn. Nan Fang Yi Ke da Xue Xue Bao [Journal of Southern Medical University] 2006;26(9):1350–1.

Liu 2016 *[published data only]*
Liu YF, Zou CC, Yang HQ, Lou LJ. Comparison of therapeutic efficacy for neonatal ABO hemolytic disease treated with intravenous immunoglobulin G by different modes of administration. Zhongguo Shi Yan Xue Ye Xue za Zhi [Journal of Experimental Hematology] 2016;24(6): 1842–5.

Pishva 2000 *[published data only]*
Pishva N, Madani A, Homayoon K. Prophylactic intravenous immunoglobulin in neonatal immune hemolytic jaundice. Iranian Journal of Medical Sciences 2000;25(3 & 4):129–33.

Rübo 1996 *[published data only]*
Rübo J, Wahn V. Influence of high dosage immuno- globulin therapy on hyperbilirubinemia in rhesus-hemolytic disease. A cooperative study [Kooperative Studie zum Einfluß einer hochdosierten Immunglobulintherapie auf die Hyperbilirubinämie bei Rhesusinkompatibilität]. Monatsschrift fur Kinderheilkunde 1996;144:516–9.

Spinelli 2001 *[published data only]*
Spinelli SL, Otheguy LE, Larguia MA. Postnatal use of high-dose intravenous immunoglobulin therapy in rhesus hemolytic disease treatment. Journal of Perinatal Medicine 2001;29(Suppl 1):683.

Voto 1995 *[published data only]*
Voto LS, Sexer H, Ferreiro G, Tavosnanska J, Orti J, Mathet ER, et al. Neonatal administration of high-dose intravenous immunoglobulin in rhesus hemolytic disease. Journal of Perinatal Medicine 1995;23(6):443–51.

Wang 2002 *[published data only]*
Wang MQ, Guo YX, Yu SQ. Curative observation of ABO HDN treated by immunogbulin. Shanxi.Medical Journal 2002;31:239–40.

ADDITIONAL REFERENCES

Shown in general reference list at the end of this thesis.

REFERENCES TO OTHER PUBLISHED VERSIONS OF THIS REVIEW

Alcock 2002

Alcock GS, Liley H. Immunoglobulin for alloimmune hemolytic disease in neonates. Cochrane Databaseof Systematic Reviews 2002, Issue 3. DOI: 10.1002/ 14651858. CD003313

CHARACTERISTICS OF INCLUDED AND EXCLUDED STUDIES

Available through the Cochrane Database of Systematic Reviews.

DATA AND ANALYSES

The main comparison 'Intravenous immunoglobulin plus phototherapy versus phototherapy' (Comparison 1) and the forest plots of the primary outcome (use of exchange transfusion, Analysis 1.1, and Exchange transfusions per infant, Analysis 1.2) are shown in this chapter. The remainder of the 'Data and Analysis' section is available through the Cochrane Database of Systematic Reviews.

WHAT'S NEW

Available through the Cochrane Database of Systematic Reviews.

HISTORY

Available through the Cochrane Database of Systematic Reviews.

CHAPTER 7

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In addition to analysis of all included studies, analyses were performed of all placebo-controlled studies. The search method, inclusion criteria and criteria to measure risk of bias were more extensively described in the review than in the protocol.

For the 2017 update, we updated the search and included seven new studies. We updated the background to include contemporary literature. The eligible participants were further specified (from "Neonates with isoimmune hemolytic disease" to "Neonates with alloimmune HDN due to either Rh or ABO blood group antibodies with or without any other blood group antibodies." The primary and secondary outcomes were adjusted to more relevant outcomes in the current era. Previously, the subgroup analysis for timing of IVIg treatment was divided in 'prophylactic use' and 'treatment of established jaundice'. For the current review, this was changed to within 12 hours of life or later. Due to the lack of definitions and the possibility of incomplete reporting in regard to adverse events, the adverse events of individual trials were stated in the current review, rather than combined raw outcomes of all included studies.

A sensitivity analysis for risk of performance or detection bias (or both) was added. Furthermore, we incorporated the GRADE criteria and added a 'Summary of findings' Table for the most important outcomes.