Neonatal management and outcome in red cell alloimmunization

Smits-Wintjens, V.E.H.J.

11

General discussion and future perspectives

Discussion

Fetal and neonatal red cell alloimmune hemolytic disease results from alloimmunization to red cell antigens, for which mother and fetus are incompatible. Production of maternal IgG antibodies directed against the fetal red blood cells occurs when fetal red blood cells positive for a certain antigen, pass into the blood circulation of a mother lacking that particular antigen. These maternal IgG antibodies may then cross the placenta into the fetal circulation and cause a wide scale of symptoms in the fetus, ranging from mild to severe hemolytic anemia and fetal hydrops.[1] Maternal immunization to the Rh D-antigen is the most common cause of severe fetal and neonatal disease.[2] However, more than 50 other (non-Rh D) red cell antigens have been reported to be associated with HDFN. Anti-Rh c and anti-Kell antibodies constitute the major causes of severe fetal and neonatal disease, whereas anti-Rh E, e, C, Cw and a few rare other antibodies are seen less frequently.[2-4] Non-Rh D-immunizations mostly result from incompatible red blood cell transfusions, if not precautionary measures e.g. Kell-matched transfusions for female in (pre)fertile age are applied.[2]

In the Netherlands, the post-delivery Rh D prophylaxis program (introduced in 1969) resulted in a decline of new Rh D immunizations from 3.5% in 1969 to 0.6% in 1995.[5] These rates were comparable with international studies on this subject.[6] In the Netherlands, around 170 pregnancies are affected each year with Rh D immunization and 380 with non-Rh D immunization. In approximately 30 of these cases severe fetal hemolytic disease will occur, requiring antenatal treatment with IUT in the LUMC.

In this chapter we summarize the recent evidence and opinions on management and outcome of HDFN due to red cell alloimmunization and discuss future research perspectives.

Management

HDN due to red cell alloimmunization can lead to severe hyperbilirubinemia, acute bilirubin encephalopathy and subsequently chronic bilirubin encephalopathy, also known as kernicterus.[7] Prevention of kernicterus is considered to be the primary goal of postnatal management of red cell alloimmune HDN.[8] Treatment of hyperbilirubinemia consists primary of intensive phototherapy and ET.[1] Phototherapy lowers serum bilirubin levels through photo-oxidation and converts bilirubin to a water-soluble substance.[7] Phototherapy was first introduced in the late 1950s, when white light was the mainstay of treatment.[9] Since then significant improvements have been made and it has become clear that the efficacy of phototherapy is dependent on a number of factors, including spectral quality of the delivered light, irradiance (intensity of light), surface area receiving phototherapy, distance from the light to the skin, skin pigmentation, total serum bilirubin concentration at the start of phototherapy and duration of exposure.[1,7,10,11] In HDN due to red cell alloimmunization, prompt and intensive phototherapy should be started immediately after birth (as bilirubin can rise sharply after birth), in order to reduce the need for ET.[1] Intensive phototherapy implies the use of (1) emission of light in the blue-to-green range that overlaps the plasma bilirubin absorption spectrum (460-490 nm), (2) irradiance of at least 30 μW/cm^2/nm and (3) illumination of maximal body surface (diaper should be removed).[1,8,12] An exception on this intensive phototherapy regime is Kell alloimmunization, in which anemia is more prominent than hyperbilirubinemia. In Kell alloimmunization, anemia results mainly from reduced erythropoiesis by destruction of progenitor red blood cells rather than hemolysis of erythrocytes.[13] Consequently, only minimal phototherapy is required, despite severe anemia. Studies reporting on adverse effects of phototherapy are limited. In neonates with cholestasis, phototherapy can cause the bronze baby syndrome, in which skin, urine and serum evolve a greyish-brown discoloration.[7,14] The pathogenesis of this disorder is not fully understood, but it resolves spontaneously when phototherapy is discontinued. Recent reports from Swedish research groups have suggested an association between phototherapy and type 1 diabetes and childhood asthma.[15-17] The mechanism behind this association is unknown. However, effects of phototherapy on the neonatal gut and gut immune response have been suggested. Other studies have been reported on blue light phototherapy as a risk factor for melanocytic nevus development.[18,19] Additional prospective, multicenter studies are warranted to investigate the long-term adverse events of (intensive) phototherapy.

In case of failure of phototherapy, ET is used to remove bilirubin from the circulation. ET has the additional benefits of removing maternal antibodies (and consequently limiting further hemolysis) and correcting associated anemia.[1] Another favourable effect of ET is a decrease in plasma ferritin and iron levels.[20] ETs are performed with double volume transfusion (160 ml / kg) using irradiated, leucocyte-depleted compatible erythrocytes via an intravenous catheter, usually an umbilical vein. The rate of neonates with HDN requiring treatment with ET varies from 20 to more than 70%.[21] In 2004 more restrictive ET guidelines were published by the American Academy of Pediatrics[8] and which led to a decrease in the use of ET.[22] This reduction in ET has led to an increased need of top-up transfusions due to ongoing hemolysis and remaining antibodies.[22] Our center adopted the new guidelines in 2005 and the incidence of neonates with red cell alloimmunization requiring treatment with ET dropped significantly from almost 70% to less than 20% thereafter.[23]

After introduction in the late 1940s,[24-26] neonatal treatment with ET became one of the most frequently performed neonatal procedures. However, ET remains a procedure with a significant risk of adverse effects. The current mortality rate is reported to be less than 2%, whereas rates of morbidity and ET-related adverse events can reach 74%.[27-34] Reported adverse events include mainly catheter-related complications (malposition, sepsis), complications linked to the use of blood products (thromboembolization, graft versus host reactions, infection), metabolic derangements (acidosis, disturbance of serum levels of sodium, calcium, potassium and glucose) and cardio-respiratory reactions (including cardiac arrhythmias, cardiac arrest and apnea).[27-34] Our study on morbidity after ET demonstrates that treatment with ET in neonates with HDN is associated with a 6-fold increased risk of sepsis (incidence 8% in the ET-group versus 1% in the no-ET-group, odds ratio (OR) 6.3, 95% confidence interval (CI) 1.7-22.9), a 25-fold increase in leukocytopenia (incidence 88% (versus 23%), OR 24.7, 95% CI 13.4-45.5), a 21-fold increase in severe thrombocytopenia (incidence 63% (versus 8%), OR 21.4, 95% CI 11.5-39.7), a 29-fold increase in hypocalcemia (incidence 22% (versus 1%), OR 29.1, 95% CI 6.8-124.5) and an increased risk of hypernatremia (incidence 8% (versus 0% in the no-ET-group)). Treatment with ET was not associated with neonatal death in our study population. The remarkably lower incidence of ET-related morbidity and mortality in our study compared to previous studies can be explained by methodological differences (different sizes of the various study cohorts) and differences in disease-severity between the studied cohorts (premature neonates in previous studies versus (near) term-age neonates in our cohort).[28,29,31] Another explanation could be that in the Netherlands treatment for intrauterine and postnatal red cell alloimmunization is centralized in one tertiary center. Subsequently almost all severely affected neonates with HDN due to red cell alloimmunization are born and treated in our center. As a result, ET is a frequently performed and standardized procedure in our unit and part of routine practise. We speculate that in experienced hands severe permanent sequelae due to ET-procedures can be kept at a minimum. We therefore advocate a centralized management of neonatal red cell alloimmunization.

Neonatal treatment with IVIg has been suggested as an alternative therapy for ET in HDN due to red cell alloimmunization.[8] In many Western countries, including the Netherlands, IVIg is widely used.[35] In a few small RCTs, IVIg reduced the need for ET and duration of phototherapy in neonates with red cell alloimmunization.[36-39] However, these studies were restricted by several important methodological limitations.[23] In 2002 a Cochrane review concluded that further well-designed trials are needed before routine use of IVIg can be recommended.[40] In the last decade, two other study-groups performed a RCT on this topic, favouring the use of IVIg. However, these studies were flawed due to with important methodological restrictions related to unclear randomization and blinding procedures.[41,42] In contrast, our double-blind, placebo-controlled RCT on the prophylactic use of IVIg in neonatal red cell alloimmunization demonstrated that IVIg does not reduce the need for ET nor the rates of other adverse neonatal outcomes.[23] Recently, a research group from Brazil finalized a similar RCT and also found no difference between both groups on the rate of ET.[43] A possible explanation for the lack of effect of IVIg in our study could be that treatment with intensive and prophylactic phototherapy, starting immediately after birth, reduces the risk of severe hyperbilirubinemia.[23] In view of the absence of beneficial effects and because of rare but potential adverse effects,[44-46] we do not recommend the use of IVIg in HDN due to red cell alloimmunization.[23] A new meta-analysis of all recently published RCTs is needed to determine the efficacy and safety of IVIg in neonatal red cell alloimmunization.

In the past, various other treatment strategies for hyperbilirubinemia in neonatal red cell alloimmunization have been investigated, including treatment with albumin, phenobarbital, metalloporphyrines and clofibrate.[47] Administration of *albumin* before ET might increase the efficacy of ET, because more bilirubin will be mobilized and excreted from tissue to blood.[1,48] In 2009 Shahian et al. performed a RCT to determine the role of administration of intravenous albumin prior to ET in term, otherwise healthy neonates. They observed that infusion of 20% albumin one hour prior to ET significantly reduced the post-ET total serum bilirubin level and duration of phototherapy.[48] However, evidence that albumin infusion increases long-term outcome in infants with red cell alloimmunization is not available and thus routine use of albumin is not recommended.[1] *Phenobarbital* increases bilirubin uptake, conjugation and excretion[1] and its potential effect on hyperbilirubinemia has been studied for decades.[49-53] A recent retrospective study by Trevett et al. showed that antenatal maternal administration of phenobarbital significantly reduces the need for ET in neonates affected with HDN due to red cell alloimmunization. The incidence of ET in neonates with and without antenatal phenobarbital administration was 9% versus 52%, respectively ($p < 0.01$).[54] Further study in a randomized controlled trial is necessary to confirm these results. Recentlt, Chawla et al. performed a meta-analysis to evaluate the role of phenobarbital in the management of unconjugated hyperbilirubinemia during the first two weeks of life in preterm neonates. The authors reported that phenobarbital reduces peak serum bilirubin, duration and need of phototherapy and need of ET in preterm very low birth weight neonates. These impressive findings warrant further studies to evaluate adverse effects and neurodevelopmental outcome.[55] *Metalloporphyrins,* synthetic heme analogs*,* are competitive inhibitors of heme oxygenase, the rate-limiting enzyme in bilirubin production.[1,56] Their use has been proposed as an alternative strategy for treating severe hyperbilirubinemia by preventing the formation of bilirubin.[56] However, a recent Cochrane review suggests that placebo-controlled RCTs are required to report on outcomes such as severe hyperbilirubinemia, kernicterus, ET and long-term neurodevelopmental impairment.[52,56-58] Finally, a few studies report beneficial effects of *clofibrate* on hyperbilirubinemia. This drug activates peroxisome proliferator-activated receptors and increases bilirubin conjugation and excretion. One single dose of clofibrate has been reported to be effective, safe and cost-effective in view of reducing duration of admission.[59] However, long-term clofibrate treatment has been associated with serious adverse effects and therefore more research is needed to clarify its safety.[47]

Short-term outcome

In the past, various postnatal complications in neonatal red cell alloimmunization have been reported, including hematological complications (anemia, thrombocytopenia and leucopenia) and cholestatic liver disease.[1,20]

Anemia

Anemia in red cell alloimmunization results from hemolysis of fetal red blood cells by maternal IgG antibodies. Maternal antibodies usually persist in the infants circulation for several months after birth, causing prolonged hemolysis. Anemia in HDN due to red cell alloimmunization can be divided into early onset anemia (within 7 days after birth), caused by antibody dependent hemolysis of red blood cells, and late onset anemia (from 1 week until 3 months after birth). Late onset anemia may be secondary to either ineffective erythropoiesis due to suppressed bone marrow ('late hyporegenerative anemia') and/or persistent hemolysis ('late anemia of hemolytic disease').[20,60] Late anemia is a common problem in infants with red cell alloimmunization and we therefore advocate that a full work-up, including invasive diagnostic tests to exclude other causes of anemia is generally not necessary. Treatment of anemia exists of top-up transfusions, which can be necessary up to the third month of life. Infants must therefore be checked for the rate of hemoglobin fall once a week until three months of age. Approximately 80% of infants treated with IUT require at least one top-up transfusion for late anemia in the first three months of life, compared to around 65% of infants without IUT.[1,22,60] International guidelines for top-up transfusions in the first months of life including transfusion triggers are not available. In our center transfusion triggers include: haemoglobin level < 8 g/dL (5 mmol/L) or < 9.6 g/dL (6 mmol/L) when clinical symptoms of anemia are present (need of extra oxygen, poor feeding, tachycardia, tachypnea).[1,20] Generally, top-up transfusions given to neonates with HDN consist of 10-20 mL/kg irradiated, ABO/Rh type-specific and antigen-negative red blood cells.[20]

Erythropoietin (EPO) can be used to prevent late anemia and reduces the need for top-up transfusions. However, there is insufficient evidence to recommend routine use of EPO in HDN due to red cell alloimmunization.[61-69] Larger RCTs are needed to study this topic.

Various supplements, including folic acid and iron could theoretically support erythropoiesis. However, evidence on optimal dosage and side effects is lacking. Nevertheless, in our center we routinely administer folic acid 0.05 mg/day orally to infants with HDN during the first three months of life.[1,20] Iron supplementation is sporadically used to support erythropoiesis in anemic neonates with HDN. However, the vast majority of neonates usually do not lack iron, due to multiple intrauterine and/or postnatal transfusions. With each top-up transfusion, iron is transfused as well. Iron overload can cause damage to the liver, heart and other organs.[70] Therefore iron supplementation should be withheld, especially in transfused infants. More studies are needed to define indications for chelation therapy, which is sporadically used to treat iron overload in HDN.[71]

Thrombocytopenia

Limited studies have shown that fetuses with red cell alloimmunization are at increased risk of thrombocytopenia.[72-74] We investigated this topic in our study population and found that 26% of neonates with red cell alloimmunization had thrombocytopenia ($<150 \times 10^9$ / L) at birth.[75] Thrombocytopenia at birth was independently associated with IUT treatment, small for date and lower gestational age at birth. Etiologic factors contributing to thrombocytopenia in red cell alloimmunization include decreased production, increased destruction and dilution.[20] Moreover, thrombocytopenia is a well known complication of ET due to platelet-poor blood and/or catheter-related thrombosis.[76-78]

Leucopenia

Only scarce data are available on leucopenia in neonatal red cell alloimmunization. It appears that the incidence of neutropenia increases if HDN is more severe, but little is known about incidence and morbidity this complication deserves further scrunity.[79,80]

Liver disease

Cholestatic liver disease may occur in HDN due to red cell alloimmunization and has been associated with iron overload due to multiple IUTs.[81-84] However, data on incidence and severity is limited and little is known about pathogenesis, risk factors, neonatal management and outcome. In our study on this topic we found that cholestasis occurs in 13% of neonates with red cell alloimmunization and is independently associated with IUT treatment and Rh D type of alloimmunization. Extensive investigations were performed to rule out other causes of cholestasis, but all tests were normal. Cholestasis resolved spontaneously within 1 week to 3 months after birth in almost half of the patients. One patient was treated with iron chelation therapy due to a prolonged and severe course of hyperferritinemia. We suggest that a full work-up to exclude other causes of cholestasis in a child with red cell alloimmunization treated with at least one IUT, is not necessary, provided that no other factors are involved and monitoring of ferritin, liver enzymes and conjugated bilirubin levels is guaranteed during the first 3 months of life.

Long-term outcome

Before the LOTUS study, only a few small studies have reported on the long-term neurodevelopmental outcome after IUT with incidences of adverse outcome ranging from 4.5 to 12%.[85-92] In the LOTUS study, a large national cohort study designed to evaluate long-term neurodevelopmental outcome in children treated with IUT for red cell alloimmunization at our center, we examined 291 children at a median age of 8.2 years. The overall incidence of neurodevelopmental impairment (NDI) was low, 4.8% (including cerebral palsy, severe developmental delay and bilateral deafness). Several factors were associated with increased risk for NDI, including fetal hydrops, number of IUTs and severe neonatal morbidity. The high rate of intact survival proves the success of antenatal IUT-treatment. Whether reducing the incidence of risk factors (in particular severe hydrops) will also reduce the incidence of long-term neurodevelopmental outcome needs to be investigated in future studies.

Future perspectives

During the last decades, a significant evolution in prenatal and postnatal care strategies for patients with red cell alloimmunization has occurred. New management options have led to a remarkable decrease in perinatal mortality and morbidity. However, several questions are still unanswered. This paragraph focuses on future research perspectives.

Neonatal management

- Prospective, multicenter trials are warranted to investigate the long-term adverse events of (intensive) phototherapy, including bronze baby syndrome, asthma, type 1 diabetes and melanocytic nevus development.
- Prospective, double-blinded RCTs are necessary to evaluate benefits, adverse effects and neurodevelopmental outcome of administration of albumin, phenobarbital, metalloporphyrines and clofibrate in neonatal red cell alloimmunization.
- A new meta-analysis of all recently published RCTs on IVIg is needed to definitively establish the efficacy and safety of IVIg in neonatal red cell alloimmunization.
- Larger well-designed trials are needed to recommend on the use of EPO in neonates with HDFN to reduce the number of top-up transfusions.
- Studies on the use of folic acid are needed to determine if and in which dosage this therapy could be beneficial (although this is of minor importance compared to the issues mentioned here above).

Short-term outcome

- More studies are needed to determine the incidence and risk factors of iron overload in infants with HDN treated with and without IUT and to define indications for chelation therapy in infants with red cell alloimmune hemolytic disease.
- Large prospective follow-up studies are required to determine the exact course of cholestasis in neonates with red cell alloimmune hemolytic disease.
- Further research is required to study the prevalence and clinical significance of neutropenia and thrombocytopenia in relation to red cell alloimmunization and ET.

Long-term outcome

- Further studies are warranted to reduce the incidence of risk factors, including fetal hydrops, associated with adverse long-term outcome in children treated with IUT.
- More research is required to determine the effect of factors such as phototherapy, ET, IVIg and iron overload on the immune system and the risk of diabetes, allergy and asthma.

References

1. Smits-Wintjens VE, Walther FJ, Lopriore E. Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. *Semin Fetal Neonatal Med.* 2008;13:265-271.
2. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. *Semin Fetal Neonatal Med.* 2008;13:207-214.
3. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de HM. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. *Transfusion.* 2008;48:941-952.
4. Moran P, Robson SC, Reid MM. Anti-E in pregnancy. *BJOG.* 2000;107:1436-1438.
5. van Dijk BA, Hirasing RA, Overbeeke MA. Hemolytic disease of the newborn and irregular blood group antibodies in the Netherlands: prevalence and morbidity. *Ned Tijdschr Geneeskd.* 1999;143:1465-1469.
6. Urbaniak SJ. The scientific basis of antenatal prophylaxis. *Br J Obstet Gynaecol.* 1998;105 Suppl 18:11-18.
7. Maisels MJ, McDonagh AF. Phototherapy for neonatal jaundice. *N Engl J Med.* 2008;358:920-928.
8. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004;114:297-316.
9. Cremer RJ, Perryman PW, Richards DH. Influence of light on the hyperbilirubinaemia of infants. *Lancet.* 1958;1:1094-1097.
10. Murray NA, Roberts IA. Haemolytic disease of the newborn. *Arch Dis Child Fetal Neonatal Ed.* 2007;92:F83-F88.
11. Roberts IA. The changing face of haemolytic disease of the newborn. *Early Hum Dev.* 2008;84:515-523.
12. Phototherapy to Prevent Severe Neonatal Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation. *Pediatrics.* 2011;128:e1046-e1052.
13. Rath ME, Smits-Wintjens VE, Lindenburg IT, Brand A, Van Kamp IL, Oepkes D, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. *Vox Sang.* 2010;100(3):312-316.
14. Kopelman AE, Brown RS, Odell GB. The "bronze" baby syndrome: a complication of phototherapy. *J Pediatr.* 1972;81:466-472.

15. Dahlquist G, Kallen B. Indications that phototherapy is a risk factor for insulin-dependent diabetes. *Diabetes Care.* 2003;26:247-248.
16. Aspberg S, Dahlquist G, Kahan T, Kallen B. Is neonatal phototherapy associated with an increased risk for hospitalized childhood bronchial asthma? *Pediatr Allergy Immunol.* 2007;18:313-319.
17. Aspberg S, Dahlquist G, Kahan T, Kallen B. Confirmed association between neonatal phototherapy or neonatal icterus and risk of childhood asthma. *Pediatr Allergy Immunol.* 2010;21:e733-e739.
18. Brewster DH, Tucker JS, Fleming M, Morris C, Stockton DL, Lloyd DJ, et al. Risk of skin cancer after neonatal phototherapy: retrospective cohort study. *Arch Dis Child.* 2010;95:826-831.
19. Csoma Z, Toth-Molnar E, Balogh K, Polyanka H, Orvos H, Ocsai H, et al. Neonatal blue light phototherapy and melanocytic nevi: a twin study. *Pediatrics.* 2011;128:e856-e864.
20. Rath ME, Smits-Wintjens VE, Walther FJ, Lopriore E. Hematological morbidity and management in neonates with hemolytic disease due to red cell alloimmunization. *Early Hum Dev.* 2011;87:583-588.
21. De Boer I, Zeestraten EC, Lopriore E, Van K, I, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. *Am J Obstet Gynecol.* 2008;198:54.e1-54.e4.
22. Rath ME, Smits-Wintjens VE, Lindenburg I, Brand A, Oepkes D, Walther FJ, et al. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. *Vox Sang.* 2010;99(1):65-70.
23. 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;127:680-686.
24. Wallerstein H. Treatment of severe erythroblastosis by simultaneous removal and replacement of the blood of the newborn infant. *Science.* 1946;103:583.
25. Wiener AS, Wexler IB, Grundfast TH. Therapy of erythroblastosis fetalis with exchange transfusion. *Bull N Y Acad Med.* 1947;23:207-220.
26. Diamond LK, Allen FH, Jr., Thomas WO, Jr. Erythroblastosis fetalis. VII. Treatment with exchange transfusion. *N Engl J Med.* 1951;244:39-49.
27. Jackson JC. Adverse events associated with exchange transfusion in healthy and ill newborns. *Pediatrics.* 1997;99:E7.
28. Patra K, Storfer-Isser A, Siner B, Moore J, Hack M. Adverse events associated with neonatal exchange transfusion in the 1990s. *J Pediatr.* 2004;144:626-631.
29. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal exchange transfusions and its effect on exchange-related morbidity and mortality. *Pediatrics.* 2007;120:27-32.
30. Badiee Z. Exchange transfusion in neonatal hyperbilirubinaemia: experience in Isfahan, Iran. *Singapore Med J.* 2007;48:421-423.
31. Keenan WJ, Novak KK, Sutherland JM, Bryla DA, Fetterly KL. Morbidity and mortality associated with exchange transfusion. *Pediatrics.* 1985;75:417-421.
32. Ip S, Chung M, Kulig J, O'Brien R, Sege R, Glicken S, et al. An evidence-based review of important issues concerning neonatal hyperbilirubinemia. *Pediatrics.* 2004;114:e130-e153.
33. Hosseinpour SS, Gharehbaghi MM. Exchange transfusion in severe hyperbilirubinemia: an experience in northwest Iran. *Turk J Pediatr.* 2010;52:367-371.
34. Davutoglu M, Garipardic M, Guler E, Karabiber H, Erhan D. The etiology of severe neonatal hyperbilirubinemia and complications of exchange transfusion. *Turk J Pediatr.* 2010;52:163-166.
35. New HV, Stanworth SJ, Engelfriet CP, Reesink HW, McQuilten ZK, Savoia HF, et al. Neonatal transfusions. *Vox Sang.* 2009;96:62-85.
36. Rubo J, Albrecht K, Lasch P, Laufkotter E, Leititis J, Marsan D, et al. High-dose intravenous immune globulin therapy for hyperbilirubinemia caused by Rh hemolytic disease. *J Pediatr.* 1992;121:93-97.
37. Gottstein R, Cooke RW. Systematic review of intravenous immunoglobulin in haemolytic disease of the newborn. *Arch Dis Child Fetal Neonatal Ed.* 2003;88:F6-10.
38. Alpay F, Sarici SU, Okutan V, Erdem G, Ozcan O, Gokcay E. High-dose intravenous immunoglobulin therapy in neonatal immune haemolytic jaundice. *Acta Paediatr.* 1999;88:216-219.

39. Dagoglu T, Ovali F, Samanci N, Bengisu E. High-dose intravenous immunoglobulin therapy for rhesus haemolytic disease. *J Int Med Res.* 1995;23:264-271.
40. Alcock GS, Liley H. Immunoglobulin infusion for isoimmune haemolytic jaundice in neonates. *Cochrane Database Syst Rev.* 2002;CD003313.
41. Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. *Saudi Med J.* 2006;27:1827-1830.
42. Elalfy MS, Elbarbary NS, Abaza HW. Early intravenous immunoglobin (two-dose regimen) in the management of severe Rh hemolytic disease of newborn-a prospective randomized controlled trial. *Eur J Pediatr.* 2011;170:461-467.
43. Santos MC, Sa CA, Gomes SC, Camacho LA, Moreira ME. High-dose intravenous immunoglobulin therapy for hyperbilirubinemia due Rh hemolytic disease: a randomized clinical trial. Pediatric Academic Societies-annual meeting-Vancouver 2010 [E-PAS2010:2851.333], 143. 2010.
44. Walsh S, Molloy EJ. Towards evidence based medicine for paediatricians. Is intravenous immunoglobulin superior to exchange transfusion in the management of hyperbilirubinaemia in term neonates? *Arch Dis Child.* 2009;94:739-741.
45. Kumar A, Teuber SS, Gershwin ME. Intravenous immunoglobulin: striving for appropriate use. *Int Arch Allergy Immunol.* 2006;140:185-198.
46. Figueras-Aloy J, Rodriguez-Miguelez JM, Iriondo-Sanz M, Salvia-Roiges MD, Botet-Mussons F, Carbonell-Estrany X. Intravenous immunoglobulin and necrotizing enterocolitis in newborns with hemolytic disease. *Pediatrics.* 2010;125:139-144.
47. Cuperus FJ, Hafkamp AM, Hulzebos CV, Verkade HJ. Pharmacological therapies for unconjugated hyperbilirubinemia. *Curr Pharm Des.* 2009;15:2927-2938.
48. Shahian M, Moslehi MA. Effect of albumin administration prior to exchange transfusion in term neonates with hyperbilirubinemia-a randomized controlled trial. *Indian Pediatr.* 2010;47:241-244.
49. Boreus LO, Jalling B, Wallin A. Plasma concentrations of phenobarbital in mother and child after combined prenatal and postnatal administration for prophylaxis of hyperbilirubinemia. *J Pediatr.* 1978;93:695-698.
50. Trolle D. Decrease of total serum-bilirubin concentration in newborn infants after phenobarbitone treatment. *Lancet.* 1968;2:705-708.
51. Trolle D. Decrease in the mortality rates for low-birth-weight infants after phenobarbitone treatment. *Acta Obstet Gynecol Scand.* 1976;55:13-20.
52. Greenough A. Rhesus disease: postnatal management and outcome. *Eur J Pediatr.* 1999;158:689-693.
53. Valdes OS, Maurer HM, Shumway CN, Draper DA, Hossaini AA. Controlled clinical trial of phenobarbital and-or light in reducing neonatal hyperbilirubinemia in a predominantly Negro population. *J Pediatr.* 1971;79:1015-1017.
54. Trevett TN, Jr., Dorman K, Lamvu G, Moise KJ, Jr. Antenatal maternal administration of phenobarbital for the prevention of exchange transfusion in neonates with hemolytic disease of the fetus and newborn. *Am J Obstet Gynecol.* 2005;192:478-482.
55. Chawla D, Parmar V. Phenobarbitone for prevention and treatment of unconjugated hyperbilirubinemia in preterm neonates: a systematic review and meta-analysis. *Indian Pediatr.* 2010;47:401-407.
56. Stevenson DK, Wong RJ. Metalloporphyrins in the management of neonatal hyperbilirubinemia. *Semin Fetal Neonatal Med.* 2010;15:164-168.
57. Hansen TW. Recent advances in the pharmacotherapy for hyperbilirubinaemia in the neonate. *Expert Opin Pharmacother.* 2003;4:1939-1948.
58. Suresh GK, Martin CL, Soll RF. Metalloporphyrins for treatment of unconjugated hyperbilirubinemia in neonates. *Cochrane Database Syst Rev.* 2003;CD004207.
59. Fallah R, Islami Z, Lotfi SR. Single Dose of 50 mg/kg Clofibrate in Jaundice of Healthy Term Neonates: Randomised Clinical Trial of Efficacy and Safety. *Indian J Pediatr.* 2011.
60. Al-Alaiyan S, al OA. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. *J Perinat Med.* 1999;27:112-115.

61. Aher S, Ohlsson A. Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst Rev.* 2006;3:CD004868.
62. Aher SM, Ohlsson A. Early versus late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst Rev.* 2006;3:CD004865.
63. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst Rev.* 2006;3:CD004863.
64. Ovali F, Samanci N, Dagoglu T. Management of late anemia in Rhesus hemolytic disease: use of recombinant human erythropoietin (a pilot study). *Pediatr Res.* 1996;39:831-834.
65. Dhodapkar KM, Blei F. Treatment of hemolytic disease of the newborn caused by anti-Kell antibody with recombinant erythropoietin. *J Pediatr Hematol Oncol.* 2001;23:69-70.
66. Manoura A, Korakaki E, Hatzidaki E, Saitakis E, Maraka S, Papamastoraki I, et al. Use of recombinant erythropoietin for the management of severe hemolytic disease of the newborn of a K0 phenotype mother. *Pediatr Hematol Oncol.* 2007;24:69-73.
67. Pessler F, Hart D. Hyporegenerative anemia associated with Rh hemolytic disease: treatment failure of recombinant erythropoietin. *J Pediatr Hematol Oncol.* 2002;24:689-693.
68. Zuppa AA, Alighieri G, Calabrese V, Visintini F, Cota F, Carducci C, et al. Recombinant human erythropoietin in the prevention of late anemia in intrauterine transfused neonates with Rh-isoimmunization. *J Pediatr Hematol Oncol.* 2010;32:e95-101.
69. Zuppa AA, Maragliano G, Scapillati ME, Florio MG, Girlando P, Noia G, et al. Recombinant erythropoietin in the prevention of late anaemia in intrauterine transfused neonates with Rh-haemolytic disease. *Fetal Diagn Ther.* 1999;14:270-274.
70. Siddappa AM, Rao R, Long JD, Widness JA, Georgieff MK. The assessment of newborn iron stores at birth: a review of the literature and standards for ferritin concentrations. *Neonatology.* 2007;92:73-82.
71. Yilmaz S, Duman N, Ozer E, Kavas N, Oren H, Demircioglu F, et al. A case of rhesus hemolytic disease with hemophagocytosis and severe iron overload due to multiple transfusions. *J Pediatr Hematol Oncol.* 2006;28:290-292.
72. Saade GR, Moise KJ, Jr., Copel JA, Belfort MA, Carpenter RJ, Jr. Fetal platelet counts correlate with the severity of the anemia in red-cell alloimmunization. *Obstet Gynecol.* 1993;82:987-991.
73. Van den Akker ES, de Haan TR, Lopriore E, Brand A, Kanhai HH, Oepkes D. Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies. *Am J Obstet Gynecol.* 2008;199:387-4.
74. Van den Akker ES, Klumper FJ, Brand A, Kanhai HH, Oepkes D. Kell alloimmunization in pregnancy: associated with fetal thrombocytopenia? *Vox Sang.* 2008;95:66-69.
75. Rath ME, Smits-Wintjens VE, Oepkes D, van Zwet EW, Van Kamp IL, Brand A, et al. Thrombocytopenia at birth in neonates with red cell alloimmune haemolytic disease. *Vox Sang.* 2011; Epub ahead of print.
76. Petaja J, Johansson C, Andersson S, Heikinheimo M. Neonatal exchange transfusion with heparinised whole blood or citrated composite blood: a prospective study. *Eur J Pediatr.* 2000;159:552-553.
77. Gharehbaghi MM, Hosseinpour SS. Exchange transfusion in neonatal hyperbilirubinaemia: a comparison between citrated whole blood and reconstituted blood. *Singapore Med J.* 2010;51:641-644.
78. Samsom JF, Groenendijk MG, van der Lei J, Okken A. Exchange transfusion in the neonate, a comparison between citrate-, heparinized- and reconstituted whole blood. *Eur J Haematol.* 1991;47:153-154.
79. Koenig JM, Christensen RD. Neutropenia and thrombocytopenia in infants with Rh hemolytic disease. *J Pediatr.* 1989;114:625-631.
80. Segal N, Leibovitz E, Juster-Reicher A, Even-Tov S, Mogilner B, Barak Y. Neutropenia complicating Rh-hydrops fetalis: the effect of treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF). *Pediatr Hematol Oncol.* 1998;15:193-197.
81. Lasker MR, Eddleman K, Toor AH. Neonatal hepatitis and excessive hepatic iron deposition following intrauterine blood transfusion. *Am J Perinatol.* 1995;12:14-17.
82. Aygun C, Tekinalp G, Gurgey A. Increased fetal iron load in rhesus hemolytic disease. *Pediatr Hematol Oncol.* 2004;21:329-333.
83. Berger HM, Lindeman JH, van Zoeren-Grobben D, Houdkamp E, Schrijver J, Kanhai HH. Iron overload, free radical damage, and rhesus haemolytic disease. *Lancet.* 1990;335:933-936.

84. Nasrat HA, Nicolini U, Nicolaidis P, Letsky EA, Gau G, Rodeck CH. The effect of intrauterine intravascular blood transfusion on iron metabolism in fetuses with Rh alloimmunization. *Obstet Gynecol.* 1991;77:558-562.
85. Janssens HM, de Haan MJ, Van Kamp IL, Brand R, Kanhai HH, Veen S. Outcome for children treated with fetal intravascular transfusions because of severe blood group antagonism. *J Pediatr.* 1997;131:373-380.
86. Doyle LW, Kelly EA, Rickards AL, Ford GW, Callanan C. Sensorineural outcome at 2 years for survivors of erythroblastosis treated with fetal intravascular transfusions. *Obstet Gynecol.* 1993;81:931-935.
87. Hudon L, Moise KJ, Jr., Hegemier SE, Hill RM, Moise AA, Smith EO, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for the treatment of fetal hemolytic disease. *Am J Obstet Gynecol.* 1998;179:858-863.
88. Harper DC, Swingle HM, Weiner CP, Bonthius DJ, Aylward GP, Widness JA. Long-term neurodevelopmental outcome and brain volume after treatment for hydrops fetalis by in utero intravascular transfusion. *Am J Obstet Gynecol.* 2006;195:192-200.
89. Grab D, Paulus WE, Bommer A, Buck G, Terinde R. Treatment of fetal erythroblastosis by intravascular transfusions: outcome at 6 years. *Obstet Gynecol.* 1999;93:165-168.
90. Farrant B, Battin M, Roberts A. Outcome of infants receiving in-utero transfusions for haemolytic disease. *N Z Med J.* 2001;114:400-403.
91. Weisz B, Rosenbaum O, Chayen B, Peltz R, Feldman B, Lipitz S. Outcome of severely anaemic fetuses treated by intrauterine transfusions. *Arch Dis Child Fetal Neonatal Ed.* 2009;94:F201-F204.
92. Stewart G, Day RE, Del PC, Whittle MJ, Turner TL, Holland BM. Developmental outcome after intravascular intrauterine transfusion for rhesus haemolytic disease. *Arch Dis Child Fetal Neonatal Ed.* 1994;70:F52-F53.