Recommendations for transfusion therapy in neonatology

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Introduction and aims

The indications for transfusion therapy in the neonatal period are based on specific knowledge of various aspects of this particular period of life, such as the dynamic interaction of the mother-placenta-foetus/neonate, the pathophysiological changes in the perinatal and neonatal periods and the profound haematological modfications that are characteristic of the first weeks of life. Furthermore, the physiological immaturity of various organs and systems can expose neonates, in particular those with a very low birth weight (VLBW) (Appendix I), to metabolic alterations following the transfusion of various blood components and the additives in them, and to infectious and immunological risks, such as Graft-versus-Host disease (GVHD).

This implies the need for close and continuous collaboration between paediatricians-neonatologists and transfusion medicine specialists in order to obtain "dedicated" blood components, both with regards to quality and quantity, able to meet the particular needs of the neonate, especially considering the now increased survival of extremely low birth weight (ELBW) babies.

ELBW and "critically ill" neonates are categories of patients with high transfusion needs, even though the number of transfusions given to premature neonates has progressively decreased over the last decade. It is, however, essential to establish appropriate transfusion criteria for these subjects.

The scientific contributions on transfusion medicine in the neonatal period derive predominantly from consensus of opinions rather than controlled studies and the lack of clear scientific evidence makes it difficult to formulate high-grade recommendations based on solid levels of evidence. Furthermore, it should be appreciated that neonatal transfusion medicine is, like all other scientific fields, a continuously evolving discipline. These Recommendations, which represent the opinions of the authors and include evidence-based data, when available, have been formulated to facilitate the implementation of uniform transfusion practices. They are not intended to provide absolute indications, but aim to be a "guide" which nevertheless guarantees individual healthcare professionals freedom of choice in the various different clinical situations.

This document deals with pre-transfusion tests, indications for the transfusion of blood components, characteristics of the blood components and methods of their administration for neonates. Details on the levels of evidence and strengths of the recommendations are provided in Appendix II.

This document does not consider the indications for the use of blood derivatives and some highly specialised, life-saving techniques used in particular emergencies, such as extracorporeal membrane oxygenation and cardiopulmonary bypass.

General criteria

Blood donors and blood components

The choice of donor may contribute to reduce the risk of transmission of infectious diseases; it is, therefore, recommended that only blood components obtained from repeat blood donors are used, as set out in current legislation in Italy[1-4].

Leucodepletion

The use of leucodepleted blood components has the now undisputed advantages of:

- preventing non-haemolytic febrile reactions;
- reducing the risk of alloimmunisation;
- lowering the risk of transmission of cytomegalovirus (CMV) infection.

For this reason, all cellular blood components used in the neonatal period, except granulocytes, which cannot currently be considered a standard therapy outside clinical studies, must be leucodepleted (white blood cells $<1\times10^6$/unit), preferably at the time of collection (pre-storage)[5,6] **(Level of evidence IV, Grade of recommendation C)**.

Prophylaxis of cytomegalovirus infection

The subjects at greatest risk of transfusion-transmitted infections are: the foetus, the neonate weighing ≤1,500 g at birth and/or born at a gestational age of ≤30 weeks (independently of maternal serology), neonates with congenital or acquired immunodeficiency and those who receive haematopoietic stem cells. It is, therefore, recommended that CMV-safe blood components are used in the following circumstances:

- intrauterine transfusion of red blood cells (RBC) and platelets;
- neonates weighing ≤1,500 g at birth and/or with a gestational age ≤30 weeks;
- neonates with congenital or acquired immunodeficiency;
- seronegative candidates for or recipients of allografts;
- pregnant women.

Blood components can be considered CMV-safe if they have been obtained from CMV-negative donors or contain $<5\times10^6$ leucocytes/unit. Thus, leucodepleted blood components (white blood cells $<1\times10^6$/unit) can be considered CMV-safe **(Level of evidence IIb, Grade of recommendation B)**. However, neither donation from CMV-negative donors nor leucodepletion, nor indeed the combination of strategies, is able to completely eliminate the risk of transmission of CMV infection, because of the possible, occasional cases of viraemia in the initial stage of the infection[7].

Fresh-frozen plasma (FFP) does not transmit CMV infection and can be administered without regard to the donor's serological status. Passive acquisition of antibodies can cause false positive results, giving rise to a patient's pseudo-seroconversion.

Prophylaxis of Graft-versus-Host disease

In order to prevent Graft-versus-Host disease, RBC and platelets (but not FFP) must be irradiated in the following circumstances[8-10] (**Level of evidence III, Grade of recommendation B**):

- intrauterine transfusion of RBC and platelets;
- transfusion of RBC (including exchange transfusion [ET]) and platelets after intrauterine transfusion;
- transfusion of RBC and platelets in neonates weighing ≤1,500 g at birth and/or with a gestational age ≤30 weeks;
- donated blood from a first or second degree relative or human leucocyte antigen (HLA)-like relative, although donation from a relative must be an exceptional event, to be discouraged;
- neonates with congenital or acquired immunodeficiency;
- recipients of haematopoietic stem cells.

The blood components must be irradiated with a dose ranging between 25 and 50 Gray (2,500-5,000 rad). Units destined for transfusion to neonates must be chosen from those collected within the preceding 5 days. Once irradiated, the RBC must be transfused within 24 hours; if that is not possible, they must be washed with physiological saline in order to remove any excess of potassium and, possibly, in a closed circuit to limit the risk of bacterial contamination. Once washed, the RBC must be transfused as soon as possible and, in any case, not later than 24 hours after preparation. It is good transfusion practice to irradiate the blood component immediately prior to transfusion[1].

Irradiation does not change the expiry data of platelet concentrates[1].

In the case of transfusion of small volumes, it is good practice to irradiate only the fraction destined for transfusion, rather than the whole unit **(Level of evidence IV, Grade of recommendation C)**.

The remaining sub-units should be irradiated within a maximum of 14 days of collection of the parent unit[1].

In order to guarantee optimal transfusion support, the turnover of irradiated units should be rapid, reserving the freshest units irradiated the least time previously (preferably on the same day as transfusion) for neonates.

In the exceptional case of administration of granulocyte concentrates, these must always be irradiated and transfused as soon as possible, but in any case within 24 hours[1] **(Level of evidence III, Grade of recommendation B)**.

Pre-transfusion tests

Serological investigations

The initial tests should include the following.

1) Tests to perform on the mother (if a blood sample is available):
   - determination of ABO/Rh phenotype;
   - screen for irregular erythrocyte antibodies with an indirect antiglobulin test.
2) Tests to perform on the neonate:
   - determination of ABO/Rh phenotype (to be confirmed on a second sample);
   - a direct antiglobulin test and, if positive, elution and identification of the eluted antibody;
   - screen for irregular erythrocyte antibodies.

However, it is good practice to search for and identify antibody specificities in maternal blood; thus, the use of these tests in the neonate should be

limited to cases in which a sample of maternal blood is not available.

Pre-transfusion compatibility tests

It is recommended that maternal serum/plasma is used for the search for irregular erythrocyte antibodies and/or cross-matching at the first transfusion[1]; when maternal serum/plasma is not available, the pre-transfusion tests can be performed only on the neonate's serum/plasma although, if the direct antiglobulin test is positive, it is preferable to use the eluate obtained from the RBC rather than the neonate's serum/plasma[11]. In cases in which the direct antiglobulin test and/or search for irregular antibodies are positive, cross-matching must always be performed, through the indirect antiglobulin test, using the mother's serum/plasma (at the first transfusion) and/or eluate of the neonate's RBC and/or the neonate's serum/plasma **(Level of evidence IV, Grade of recommendation C)**.

If the maternal serum contains a clinically relevant antibody, the neonate must be transfused with red blood cells lacking the antigen to which the antibody is directed. This practice must be maintained until the antibody disappears from the neonate's circulation[12].

The cross-match is mandatory in the case of transfusions following a first one, even when the direct antiglobulin test and/or search for irregular antibodies was initially negative. In this case the neonate's serum/plasma must be used.

Precautions and considerations

In the neonatal period, as in every other period of life, all measures must be taken to avoid errors in identification of the units of blood components and of the recipient, exchanges of samples, and labelling errors.

ABO phenotype determination in neonates is based only on the identification of RBC antigens, because anti-A/-B isoagglutinins are absent.

Errors of blood group typing can derive, albeit rarely, from the weak expression of erythrocyte antigens on the neonate's RBC or because of the presence of maternal antibodies capable of masking the corresponding antigens (RhD haemolytic disease of the foetus and neonate, HDFN)[11].

Intrauterine foetal transfusion

In the last decade there has been a gradual decline in the use of this technique. A survey carried out by Italian Society of Transfusion Medicine and Immunohaematology in 2010 in about 60% of the Transfusion Services active in Italy showed that intrauterine foetal transfusion is now only practised rarely in centres specialised in Mother and Child Medicine[13].

Plasmapheresis associated with the infusion of intravenous immunoglobulins (IVIG) seems to offer an effective, alternative antenatal treatment in cases of severe HDFN[14,15]. However, intrauterine foetal transfusion appears to be the most effective transfusion practice for a quick recovery from severe foetal anaemia[16].

Intrauterine foetal transfusion with packed red cells is mainly indicated for correcting foetal anaemia secondary to the haemolytic action of alloantibodies against blood group antigens present on foetal erythrocytes (the antigens most frequently involved are: D, c, E, K, Fy^a, Jk^a). Other, less common indications are foetal anaemia following Parvovirus B19 infection, homozygous alpha-thalassaemia, "massive" foeto-maternal transfusion or foeto-foetal transfusion between twins[11,17].

At present the indication for intrauterine foetal transfusion is provided non-invasively by the ultrasound evaluation of middle cerebral artery peak systolic velocity, which enables a moderate or high risk of anaemia to be determined in relation to gestational age[18].

Intrauterine foetal transfusion can be performed in two ways: intravascular transfusion or intraperitoneal transfusion.

Intravascular transfusion is currently the technique of choice, in particular in foetuses with hydrops, in whom a survival of greater than 70% has been reported[19].

Under ultrasound guidance a needle is passed transabdominally into the umbilical vein close to the site of entry of the cord in the placenta (cordocentesis). A small quantity of foetal blood is collected through this needle and used to evaluate the degree of anaemia, and then packed red cells are infused. The first intravascular transfusion can be performed from the 18th week of gestation, although the risk of foetal death decreases if the technique is used after the 20th week[20].

The risk of foetal death within 48 h of the procedure is about 2%[16].

With **intraperitoneal transfusion** red blood cells are infused directly through a needle placed in the peritoneal cavity of the foetus and reabsorbed slowly into the blood circulation through the subdiaphragmatic lymphatic vessels. This technique, which was widely used in the past, only allows gradual correction of the anaemia and, in the presence of ascites, the passage of transfused red cells can be altered or decreased. This procedure can, however, still be used in the case of failure of intravascular transfusion. Considering that an approximately one percentage point decrease in the haematocrit (Hct) can be expected daily, the procedure should be repeated at intervals of about 2-3 weeks until the time of the planned delivery.

In the case of very severe foetal anaemia, generally associated with foetal-placental hydrops, the post-transfusion Hct should not be more than four times higher than the initial Hct; in fact, a brusque increase in blood viscosity could compromise the cardiovascular system.

The delivery, to be performed by Caesarean section, is usually planned from the 34th week of gestational age, if the intrauterine transfusion has been successful, following induction of lung maturity. The use of intrauterine transfusion enables up to 80% of foetuses with severe HDFN to be treated successfully. Lower percentages are reported in cases of severe foetal-placental hydrops[19].

Intravascular transfusion of platelet concentrates currently has few indications. It was recently used in a case of severe foetal thrombocytopenia associated with anaemia due to Parvovirus B19 infection[21]. This technique is no longer approved for the treatment of alloimmune foetal-neonatal thrombocytopenia because of the high risk of bleeding following cordocentesis and the good results obtained with non-invasive treatment, based on the use of IVIG and corticosteroids[22].

The characteristics of the blood components and procedures for intrauterine foetal transfusion

Packed red cells

These must:

- be group O Rh (D) negative in the case of HDFN due to anti-D alloantibodies. RBC of the same blood group as the foetus can be used when this blood group is known and there is no ABO/Rh incompatibility with the mother;
- be compatible with maternal serum and, therefore, lacking the antigen against which the mother has produced alloantibodies: this is, of course, true for both HDFN due to anti-D and HDFN due to alloantibodies other than anti-RhD;
- be prepared within 5 days of collection;
- be leucodepleted/CMV-safe;
- be irradiated;
- have a Hct ~80%.

The volume to transfuse is calculated using the following formula:

$$\frac{\text{desired Hct} - \text{foetal Hct}}{\text{RBC unit Hct}} \times \text{foeto-placental blood volume (150 mL/kg)}$$

The desired Hct is about 40-45%.

The transfusion is given at a rate of about 5 mL/min and even more slowly in the case of a hydropic foetus (2-3 mL/min)[11].

Transfusions in the neonatal period

Exchange transfusion

Indications

The most frequent indication for exchange transfusion (ET) is marked hyperbilirubinaemia, since this technique quickly lower the levels of bilirubin considered responsible for neurological damage (kernicterus). The guidelines of the American Academy of Pediatrics supply indications on the levels of total bilirubin at which ET is advised in the case of lack of response to phototherapy. These levels depend primarily on post-natal age and the possible presence of numerous other risk factors such as HDFN, glucose-6-phosphate deficiency, prematurity, sepsis, acidosis, and hypoalbuminaemia[23].

Other extremely rare indications are accumulation of endogenous toxic metabolites and drug overdoses. It was also reported recently that ET was an effective treatment in a case of neonatal haemochromatosis[24,25].

The main indication for ET is, however, HDFN, even if recourse to this treatment is ever more uncommon because of the considerable decrease in the incidence of HDFN due to anti-D, the use of IVIG and the efficacy of modern phototherapy techniques[26,27]. The main aim of ET in HDFN is to remove the alloantibodies that are free in the serum or attached to RBC in order to simultaneously correct both the hyperbilirubinaemia and the anaemia resulting from antibody-mediated haemolysis.

"Early" exchange transfusion (within 9-12 h of birth) in haemolytic disease of the foetus and neonate

The criteria for early ET are based on the finding of:

- haemoglobin (Hb) values in the cord blood ≤8 g/dL;
- or levels of total bilirubin in cord blood >5.0-5.5 mg/dL and an increase in total bilirubin values ≥0.5-1.0 mg/dL/hour, despite the use of intensive phototherapy[11].

In the days following birth, the indications for ET are based on the levels of total bilirubin, as recommended by the guidelines of the American Academy of Pediatrics[23].

Methods of performance and recommendations

ET is performed using reconstituted whole blood with a "push-pull" technique, through a single vascular access, which is usually the umbilical vein. In exceptional cases, when this vein cannot be used, the ET can be performed with a technique involving two vascular accesses through which the reconstituted whole blood is contemporaneously removed and introduced. In these cases, two operators are necessary.

The volume exchanged each time should be about 5 mL/kg and the rate should not exceed 2-3 mL/kg/min, in order to avoid rapid fluctuations of intracranial pressure[11] **(Level of evidence IV, Grade of recommendation C)**.

Further details on the technique of ET can be found in other publication[28].

The total volume of reconstituted whole blood (mL) to exchange ("double volume" exchange) is 160 mL/kg for neonates born at term and 200 mL/kg for those born prematurely.

With "double-volume" exchange, about 80-90% of the neonate's RBC are removed and there is a reduction of approximately 50% of the pre-ET intravascular bilirubin level[29]. About 4 hours after the procedure there can be a rebound of about 60% of the total bilirubin level.

ET can be complicated by a series of side effects, such as thrombocytopenia, metabolic changes (hypocalcaemia, hyper- or hypo-glycaemia, hypernatraemia, hyperkalaemia) and thrombosis of the umbilical vein or lead to necrotising enterocolitis[30]. The platelet count must be controlled at the end of the exchange procedure because of a possible wash-out effect.

Furthermore, depending on the amount of calcium in the blood, it may be necessary to administer calcium gluconate **(Level of evidence III, Grade of recommendation B)**. Despite the risks associated with this procedure, the reported mortality rate is <0.6%, although it may be higher in preterm babies and in those with severe pathologies[11].

Characteristics of the reconstituted whole blood

The whole blood is obtained from reconstituting red cell concentrates with fresh-frozen plasma. The reconstitution procedure must only be performed in Transfusion Services, using appropriate formulae to obtain the desired Hct.

Characteristics of the red blood cells:

- the same blood group or ABO/Rh compatible with the neonate and the maternal plasma. In particular:
  - Rh(D) negative in cases of HDFN Rh(D);
  - O phenotype in cases of ABO incompatibility neonatal haemolytic disease;
- lacking the antigens against which any irregular antibodies are directed (HDFN due to anti-c, anti-K, etc.) identified in the mother's or neonate's serum/plasma;
- fresh (collected within the preceding 5 days). In cases in which fresh blood (collected within the preceding 5 days) is not available, products that have been stored for longer can be used, provided they are compatible with irradiation which, by law, must be performed within 14 days of donation, with an additional washing procedure to remove storage residues;
- cleaned of any additive or preservative, before reconstitution;
- leucodepleted, CMV-safe;
- heated to 37 °C, if specific equipment is available.

Characteristics of the plasma:

- safe FFP is used (quarantined or inactivated);
- AB phenotype.

The final product must:

- have a Hct between 0.50 and 0.60;
- be irradiated;
- be transfused within 24 hours from the preparation.

The product has the same metabolic and haemostatic characteristics as fresh, whole blood but lacks platelets.

"Partial" exchange transfusion

This is used in cases of severe anaemia at birth associated with congestive heart failure (HDFN with hydrops; chronic foeto-maternal or foeto-foetal post-haemorrhagic anaemia), in order to correct the anaemia without increasing blood volume.

The product used for partial ET is packed red cells with a Hct of about 0.70. The amount is determined from the following formula:

$$\text{Volume (mL)} = \frac{\text{desired Hct} - \text{observed Hct}}{\text{packed red cells Hct} - \text{observed Hct}} \times \text{neonate's blood volume}$$

The neonate's blood volume is estimated to be ~80 mL/kg and ~100 mL/kg for a preterm baby.

Partial ET is more commonly used in the treatment of polycythemia or hyperviscosity syndrome. This develops when the Hct of a venous sample is >0.65-0.70 and is characterised by symptoms such as tachypnoea, hypotonia, tremor, seizures, cardio-circulatory compromise and renal failure. In such cases it is advisable to perform partial ET in order to lower the Hct to about 0.50. There are no clinical data demonstrating either a short-term or long-term benefit of partial ET used as a treatment for polycythemia (Hct>65%), when performed in neonates in a stable clinical condition or with few symptoms[31]. A potential disadvantage of partial ET is the possibility of side effects, including a higher risk of necrotising enterocolitis. Data concerning long-term neurological development are vitiated by the inadequate follow-up and, consequently, indications on the benefits and risks cannot be formulated.

The Hct is corrected by using crystalloid solutions instead of plasma or albumin, which were widely used in the past **(Level of evidence Ib; Grade of recommendation A)**[32].

The volume of crystalloid solution to exchange is calculated using the following formula:

$$\text{Volume (mL)} = \frac{\text{observed Hct} - \text{desired Hct*}}{\text{observed Hct}} \times \text{neonate's blood volume}$$

*usually ~0.50.

Recent studies evaluating the long-term outcome of neonates with symptomatic polycythemia undergoing partial ET have not found clear benefits with regards to neurocognitive development[33].

Transfusion of packed red cells

The use of packed red cells in the anaemia of very low birthweight babies

For various reasons VLBW babies have lower Hct and Hb levels at birth and in the first weeks of life than babies born at term (Table I)[34]. This particular haematological profile, called anaemia of prematurity, can further worsen the clinical course of a premature baby, which is often complicated by cardiorespiratory, metabolic and haemorrhagic disorders.

Thus, VLBW and, in particular, ELBW, babies form a class of neonates more frequently administered transfusion therapy and, precisely because of the extreme immaturity of their various organs and systems, may be predisposed to more side effects of the blood transfusion[35].

Advances in the last 20 years in Transfusion Medicine have led to drastic reductions in infectious risks and adverse events related to blood transfusion[35]. Furthermore, the particular attention given to this vulnerable category of patients has led to further improvements, including a decrease in side effects and, above all, a reduction in the number of donors to which each neonate is exposed[36,37].

Studies carried out since the early 1990s aimed at evaluating the efficacy of recombinant human erythropoietin (rHuEPO) in anaemia of prematurity have contributed to both the production and application of specific transfusion protocols, promoting better use of blood transfusions in neonatal intensive care units. It has been demonstrated that transfusing according to pre-established criteria limits both the number of neonates given a transfusion and the number of donors to which each neonate is exposed[38-40]. For this reason it is recommended that individual neonatal intensive care units adopt transfusion protocols "dedicated" to this particular category of neonates (**Level of evidence Ib, Grade of recommendation A**).

The ever more restrictive transfusion practices adopted in recent years have led to the need to evaluate both the short-term and long-term outcomes of reduced transfusion regimes[41]. Most studies are concordant in not showing statistically significant differences in immediate outcomes, such as mortality, or long-term ones (auditory, visual or psychocognitive deficits) in neonates managed with a restricted transfusion regime compared to neonates managed according to more "liberal" criteria[42,43]. Other studies which have evaluated outcomes in school age have even found better neurocognitive development in neonates managed with the restricted transfusion regime, compared to those who received more blood transfusions[44].

The possible association between blood transfusions and adverse events, such as intraventricular haemorrhage and necrotising enterocolitis, frequently reported in recent years[45-48], justifies the current tendency to adopt ever lower Hct (or Hb) thresholds for blood transfusions[49].

The transfusion criteria used for VLBW babies are based more on consensus of opinions of "experts" than on scientific evidence. In any case, the diagnostic means that we have make it currently difficult, if not impossible, to formulate motivated indications (for example, reduced tissue oxygenation) on the need to give a blood transfusion. In the first few weeks of life, VLBW neonates generally have cardio-respiratory disorders and may have to undergo major surgical interventions, meaning that they need transfusions to maintain the levels of Hb>12g/dL. In contrast, a less aggressive transfusion approach is recommended in neonates in a stable clinical condition, particularly during the phase of recovery of body growth. The finding of an absolute reticulocyte count >75-100×10^3/mL is indicative of a rapid increase in Hb values, so, in the presence of a stable clinical condition, the decision to transfuse can be deferred[50].

Table I - Concentration of haemoglobin (g/dL) in prematurely born babies in the first 16 weeks of life.

Age (weeks)	Weight at birth	
	1,000-1,500 g	*1,501-2,000 g*
2	16.3 (11.7-18.4)	16.8 (11.8-19.6)
4	10.9 (8.7-15.2)	11.5 (8.2-15.0)
8	8.8 (7.1-11.5)	9.4 (8.0-11.4)
12	9.8 (8.9-11.2)	10.2 (9.3-11.8)
16	11.3 (9.1-13.1)	11.3 (9.1-13.1)

From Lundstrom U *et al.* 1977[34].

Table II - Indications for transfusion of packed red blood cells in VLBW neonates according to haemoglobin levels (g/dL)*.

Age (days)	Type of sample	Neonates receiving respiratory aid**	Neonates not receiving respiratory aid
1-7	Skin prick	≤11.5	≤10.0
	Central	≤10.4	≤9.0
8-14	Skin prick	≤10.0	≤8.5
	Central	≤9.0	≤7.7
≥15	Skin prick	≤8.5	≤7.5
	Central	≤7.7	≤6.8

Modified from Kirpalani et al. *2006*[42].
* These recommendations are not valid in the case of major surgery, sepsis, shock, haemorrhage or symptoms suggestive of anaemia (tachycardia, tachypnoea).
** Includes assisted ventilation, continuous positive-pressure ventilation, and administration of free-flowing oxygen.

The indications listed in Table II are given with the purpose of providing "threshold" values of Hb drawn from the most recent studies, leaving large decisional power to each professional on the appropriate choice to make faced with different, specific clinical situations[42].

Use of packed red cells in post-haemorrhagic and haemolytic anaemias and anaemia due to reduced or altered red blood cell production

Anaemia present at birth and in the first week of life

In cases of severe anaemia (Hb<8g/dL) with hypovolaemic shock (loss of blood volume >20%) following bleeding from a placenta previa, abruptio placentae, ruptured cord, etc., the intravascular volume must be restored quickly and the anaemia corrected in the ways reported in Table III **(Level of evidence IV, Grade of recommendation C)**.

In cases of anaemia that develop during the first week of life, for which the Hb values are moderately lower than the reference values for post-natal age (Table IV)[51] and the neonate is clinically stable, it is justifiable to wait for recovery of erythropoietic activity (evaluated from the reticulocyte count). Transfusion therapy is, however, necessary in the presence of severe cardiorespiratory difficult or surgery to maintain the Hct >0.35.

Late-onset neonatal anaemias (after the first week of life)

When evaluating these forms of anaemia it is essential to consider the reference ranges for Hb (or Hct) in the post-natal period (Table IV)[51] and the presence of any symptoms suggesting inadequate tissue oxygenation, such as apathy, difficulty in suckling, poor growth, tachycardia, and tachypnoea. It is also very important to evaluate the degree of reticulocyte response, in that a reticulocyte count $>100\times10^3/\mu L$ is an indicator of effective bone marrow compensation. Neonates who undergo ET in the first week of life can tolerate even very low levels of Hb (~6-7 g/dL) in the following weeks because of the high proportion of HbA and consequent increased release of oxygen to tissues.

Characteristics of packed red blood cells

It is considered good practice to give VLBW babies small aliquots (pedi-packs), obtained *via* a connecting device, of a single unit of packed red cells. The unit should be stored for the same neonate, when it is predicted that more than one transfusion will be necessary within a certain period of time, in order to reduce exposure of the neonate to different donors **(Level of evidence III, Grade of recommendation B)**.

The packed red cells must:

- be the same ABO/Rh group or a group compatible with the neonate and the mother's serum/plasma (Tables Va and Vb);

Table III - Use of packed red cells at birth in the case of acute post-haemorrhagic anaemia with hypovolaemic shock.

Correction of hypovolaemia: urgent transfusion (20 mL/kg) of physiological saline, a "volume expander" or reconstituted blood (if available).

Correction of anaemia: (unless reconstituted blood has been used to correct the hypovolaemia, restore the haematocrit [Hct] to about 0.35 without giving more than 20 mL/kg) transfuse packed red cells (PRC) according to the following formula:

$$\text{PRC (mL)} = \frac{\text{desired Hct} - \text{observed Hct}}{\text{PRC Hct}} \times \text{NBV}$$

NBV: neonate's blood volume.

Table IV - Red blood cell parameters in neonates born at term in the first 6 months of life.

Age	Hb g/dL		Hct %		RBC 10^{12}/L		MCV fL		MCH pg		MCHC g/dL	
	Mean	*−2 SD*	*Mean*	*−2 SD*	*Mean*	*−2 SD*	*Mean*	*−2 SD*	*Mean*	*−2 SD*	*Mean*	*−2 SD*
Cord	16.5	13.5	51	42	4.7	3.9	108	98	34	31	33	30
1-3 days	18.5	14.5	56	45	5.3	4.0	108	95	34	31	33	29
1 week	17.5	13.5	54	42	5.1	3.9	107	88	34	28	33	28
2 weeks	16.5	12.5	51	39	4.9	3.6	105	86	34	28	33	28
1 month	14.0	10.0	43	31	4.2	3.0	104	85	34	28	33	29
2 months	11.5	9.0	35	28	3.8	2.7	96	77	30	26	33	29
3-6 months	11.5	9.5	35	29	3.8	3.1	91	74	30	25	33	30

Modified from Dallman PR et al. *1977*[51].
Hb: haemoglobin; Hct: haematocrit; RBC: red blood cells; MCV: mean cell volume; MCH: mean corpuscular haemoglobin; MCHC: mean cell haemoglobin concentration; SD: standard deviation.

Table Va - Choice of ABO group of blood components to administer to a neonate ABO-compatible with the mother.

ABO blood group of the neonate		ABO blood group that can be transfused		
		Red blood cells	*Platelets*	*Plasma*
O	First choice	O	O	O
	Second choice	-	AB or A or B	AB or A or B
A	First choice	A	A	A
	Second choice	O	AB	AB
B	First choice	B	B	B
	Second choice	O	AB	AB
AB	First choice	AB	AB	AB
	Second choice	O or A or B	Plasma-free A or B	-

Table Vb - Choice of ABO group of blood components to administer to a neonate ABO-incompatible with the mother.

ABO blood group of the neonate	ABO blood group of the mother	ABO group that can be transfused		
		Red blood cells	*Platelets*	*Plasma*
O	A or B	O	O	O or AB or A or B
A	O or B	O	Plasma-free O	A or AB
B	O or A	O	Plasma-free O	B or AB
AB	O	O	Plasma-free O	AB
	A	O or A	Plasma-free A or O	
	B	O or B	Plasma-free B or O	

- lack the antigens against which any irregular antibodies found in the maternal or neonatal serum/plasma are directed; thus they must be negative in a cross-match test with maternal or neonatal serum/plasma;
- have a final haematocrit of about 0.70;
- be leucodepleted/CMV-safe;
- be irradiated, if indicated;
- be used within 14 days of collection if irradiation is necessary and preferably transfused immediately after irradiation.

In premature babies the volume of packed red cells to administer varies from 10 to 20 mL/kg or can be calculated using the following formula:

$$\text{Volume (mL)} = \frac{\text{desired Hct} - \text{observed Hct}}{\text{packed red cells Hct}} \times \text{neonate's blood volume}$$

It is not necessary to warm small quantities of red cell concentrate before its administration; the transfusion must be completed within 3-4 hours[11].

Transfusion of fresh-frozen plasma

Proof of the efficacy of FFP in neonates is extremely limited[52]. The use of this blood component in sepsis or as a volume expander in the neonate with hypotension is not considered appropriate[53]. Furthermore, the administration of FFP as a strategy to prevent intracranial haemorrhage has not been shown to be beneficial and is not, therefore, indicated[54] **(Level of evidence Ib, Grade of recommendation A)**.

The administration of FFP is, however, recommended for bleeding associated with coagulopathy (Tables VI and VII). It should be noted that the longer clotting times in the neonate than in the adult do not correlate with an increased risk of bleeding[55-59]. This is all the more the case in premature neonates; thus, isolated changes in clotting tests, in the absence of bleeding, are not indications for the transfusion of FFP (Table VII).

FFP can be used in the treatment of congenital deficiencies of single clotting factors for which the relative blood derivative is not available (Table VI).

In the cases in which FFP is advised (Table VI), it should be transfused at a dose of about 15-20 mL/kg **(Level of evidence IV, Grade of recommendation C)**.

Table VI - Indications for the transfusion of fresh-frozen plasma.

- Neonates with ongoing bleeding and significant coagulopathy
- Neonates with significant coagulopathy who must undergo invasive procedures
- Congenital deficiency of clotting factors when the specific clotting factor is not available.

Significant coagulopathy means prothrombin time (PT) and partial thromboplastin time (PTT) above normal limits or fibrinogen levels below the lower limit of normal for gestational age and post-natal age (Table VII).

Table VII - Definition of coagulopathy in premature neonates and neonates born at term, at birth (A) and in the post-natal period (B), and recommended interventions with level of evidence and strenght of recommendation.

(A) At birth			
Category	*Fibrinogen Lower limit*	*PT Upper limit*	*PTT Upper limit*
Neonate <28 weeks (1)	<71 mg/dL	>21 sec.	>64 sec.
Neonate 28-34 weeks (1)	<87 mg/dL	>21 sec.	>57 sec.
Neonate 30-36 weeks (2)	<150 mg/dL	>16 sec.	>79 sec.
Neonate at term (3)	<167 mg/dL	>16 sec.	>55 sec.
Recommended intervention			
In neonate without bleeding	Observation (IIb/B)	Observation (IIb/B)	Observation (IIb/B)
In neonate with bleeding or to undergo an invasive procedure	Cryoprecipitate* 5-10 mL/kg (IV/C)	FFP 15-20 mL/kg (III/B)	FFP 15-20 mL/kg (III/B)
(B) Post-natal period			
Category	*Fibrinogen Lower limit*	*PT Upper limit*	*PTT Upper limit*
Neonate 30-36 weeks (2) - Post-natal age			
Day 5	<160 mg/dL	>15 sec.	>74 sec.
Day 30	<150 mg/dL	>14 sec.	>62 sec.
Day 90	<150 mg/dL	>15 sec.	>51 sec.
Neonate at term (3) - Post-natal age			
Day 5	<162 mg/dL	>15 sec.	>60 sec.
Day 30	<162 mg/dL	>14 sec.	>55 sec.
Day 90	<150 mg/dL	>14 sec.	>50 sec.
Recommended intervention			
Neonate without bleeding	Observation (IIb/B)	Observation (IIb/B)	Observation (IIb/B)
Neonate with bleeding or to undergo an invasive procedure	Cryoprecipitate* 5-10 mL/kg (IV/C)	FFP 15-20 mL/kg (III/B)	FFP 15-20 mL/kg (III/B)

Reference ranges drawn from: (1) Christensen RD *et al.* 2014[55]; (2) Andrew M *et al.* 1988[56]; (3) Andrew M *et al.* 1992[57].
Product not easily standardised and subject to donor- and manual preparation-dependent variation, with a very wide range of fibrinogen concentrations. Fibrinogen is currently available as a plasma-derivative, but there is insufficient experience with its use in the neonatal period.
PT: prothrombin time; PTT: partial thromboplastin time; FFP: fresh-frozen plasma.

Characteristics of fresh-frozen plasma

It is recommended that the Transfusion Service divides a single unit of plasma, possibly produced by apheresis, into several fractions of suitable volume, prior to freezing, to be reserved for an individual neonate.

The FFP must be:

- ABO compatible or AB phenotype;
- "safe", that is, quarantined or subjected to pathogen inactivation.

Transfusion of platelet concentrates

Thrombocytopenia is common in premature neonates (occurring in up to 73% of neonates weighing <1,000 g and up to 85-90% in neonates weighing <750 g) and is associated with a risk of severe intraventricular haemorrhage[60].

The incidence of thrombocytopenia varies according to the definition used; new reference ranges were proposed recently, taking into consideration the effect of gestational age at birth and post-natal age on the platelet count (Figure 1 and Figure 2)[61].

In the light of these new reference ranges, it has been suggested that the classification of thrombocytopenia into mild, moderate and severe should be abandoned, except to assign a preliminary risk to the thrombocytopenia because platelet counts of 5,000/μL and 45,000/μL cause very different clinical problems, despite both being included in the same category of "severe thrombocytopenia".

The administration of platelets in the case of moderate thrombocytopenia (50,000-100,000/μL) does not, in any case, seem to reduce the severity of bleeding[62].

In the absence of randomised, controlled clinical studies, the indications for transfusing platelets in this category of children is based on clinical experience[63,64].

In healthy neonates born at term, the risk of bleeding is low if the platelet count is kept above 20,000-30,000/μL. Higher levels are recommended for premature neonates, particularly in the first few days of life, a period in which the risk of intraventricular haemorrhage is high, or when a concomitant coagulopathy is present. It is, therefore, recommended that the platelet count is kept above 50,000/μL in premature neonates (weight

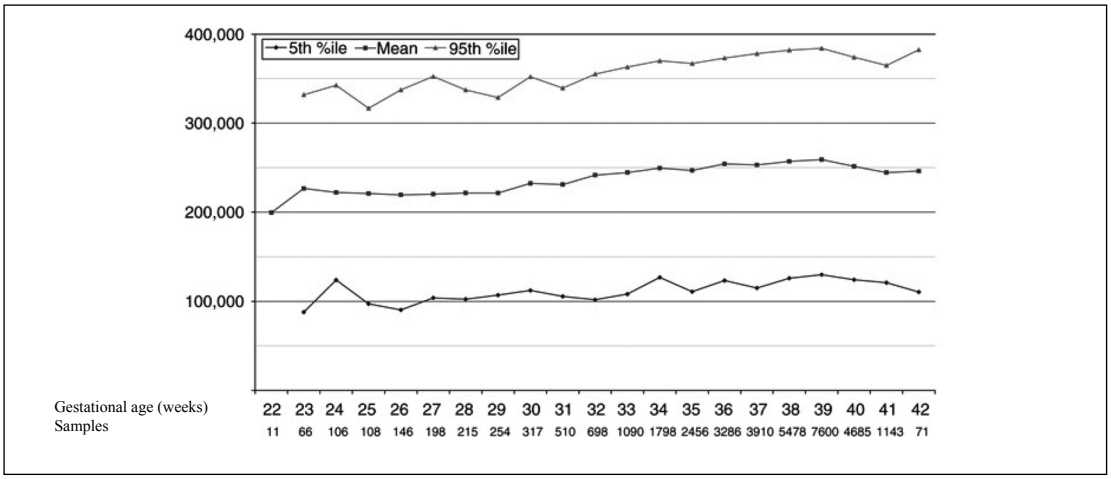

Figure 1 - Reference range of platelet counts at birth in neonates with a gestational age between 22 and 42 weeks. *Modified from Wiedmeier SE* et al., *2009*[61].

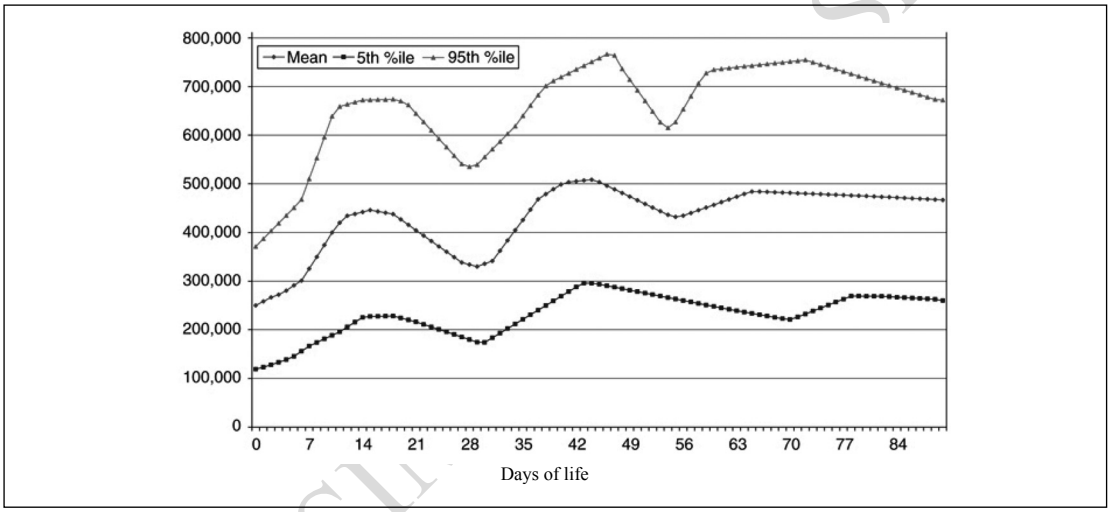

Figure 2 - Reference ranges of platelet counts in the first 90 days of life in neonates born at a gestational age of 22 to 42 weeks.
Modified from Wiedmeier SE et al., *2009*[61].

<1,000 g; gestational age <28 weeks) in the first week of life, in critically ill neonates (with sepsis or fluctuating blood pressure) or in the case of invasive procedures. In neonates who are bleeding, the platelet count should be maintained above 100,000/µL (**Level of evidence IV, Grade of recommendation C**).

In order to determine the risk of bleeding, the thrombocytopenia should be evaluated taking into consideration the mean platelet volume, the haematocrit, gestational age, post-natal age, use of drugs, stability of blood pressure, the presence of other comorbid conditions such as patent ductus arteriosus, sepsis and pulmonary hypertension[65,66].

Table VIII summarises the indications for platelet transfusion while Table IX summarises the practical aspects of these transfusions.

Characteristics of the platelet concentrates

There is no evidence that the increase in platelet count is greater when 15-20 mL/kg is transfused compared to 10 mL/kg[67]; the rate of infusion should be 5-10 mL/kg/h (**Level of evidence III, Grade of recommendation B**).

The increase in platelet count can be measured from 10 minutes to 3 hours after transfusion[68].

The platelets must be:
- of an identical or compatible ABO phenotype;
- human platelet antigen (HPA)-compatible in the case of alloimmune thrombocytopenia;
- leucodepleted/CMV-safe;
- irradiated, if indicated.

In the case of alloimmune thrombocytopenia, compatible platelets must be searched for as soon as possible. The product must have the following characteristics:

Table VIII - Indications for the transfusion of platelet concentrates.

- Platelet count <30×10⁹/L - Consider a transfusion in all cases.
- Platelet count 30-49×10⁹/L - Consider a transfusion in the following cases:
  - in the first week of life in neonates weighing ≤1,000 g at birth;
  - prior grade 3 intraventricular haemorrhage/intraparenchymal bleeding (in preceding 48-72 h);
  - concomitant coagulopathy;
  - critically ill neonate (with sepsis or fluctuating systemic blood pressure);
  - during invasive procedures.
- Platelet count 50-99×10⁹/L in neonates with bleeding.
- Do not transfuse if the platelet count ≥100×10⁹/L.

Table IX - Transfusion of platelet concentrates: practical aspects.

- Platelet concentrates must be collected from the Transfusion Service immediately before use.
- The transfusion must be started immediately after the arrival of the product in the ward.
- Platelet concentrates must not be stored in the ward's refrigerator.
- A specific venous line must be used for the infusion.
- The neonate's vital parameters must be monitored before the start of the transfusion and during it.
- The infusion must be started slowly; the rate can then be gradually increased, if there are no reactions, in order to complete the transfusion within 1 hour.
- Pre-transfusion drug treatment is not routinely indicated.
- A blood count should be performed 1 hour and 24 hours after completion of the transfusion in order to evaluate the efficacy of the transfusion.

- lack human platelet antigens (HPA) against which the mother has produced specific antibodies: in the absence of donors typed for the main HPA, maternal platelets can be used; these should be obtained by apheresis, washed to remove the plasma containing the antibodies, and irradiated;
- in the absence of HPA-compatible platelets, IVIG should be administered together with platelet concentrates from "random" donors[69].

Granulocyte concentrates

The transfusion of granulocyte concentrates has been proposed in the past for neonates with severe neutropenia who have severe sepsis resistant to antibiotic treatment. The data available so far do not, however, seem to justify such a practice and so, at present, there are no precise indications in this regard.

A meta-analysis performed in the early 2000s showed that there were no statistically significant differences in morbidity or mortality between neonates treated with granulocyte concentrates or those managed with "standard" treatment[70].

Thus, considering the potential serious side effects (transmission of infections), it is current practice to use recombinant granulocyte growth factors (recombinant granulocyte colony-stimulating factor, recombinant granulocyte-monocyte colony-stimulating factor) (**Level of evidence IV, Grade of recommendation C**).

Granulocyte concentrates must be ABO/Rh-compatible with the neonate and must be irradiated before being transfused. Consult specific instructions for information on the dose and methods of administering recombinant granulocyte growth factors.

Particular conditions

T activation

The main red blood cell membrane glycoproteins, glycophorins A, B and C, contain oligosaccharides (tetrasaccharides) conjugated with sialic acid. If the molecules of sialic acid are removed, an antigen named T is exposed. This phenomenon is called T activation.

RBC exposing this antigen can be polyagglutinated by anti-T IgM antibodies, which are naturally and constantly present in the plasma of adults[71]. These naturally occurring antibodies seem to be produced by exposure to intestinal bacterial flora containing structures antigenically similar to the red cell "crypto-antigens".

The T antigen on RBC can be activated when the erythrocytes come into contact with some enzymes (neuroaminidases) produced by aerobic and anaerobic bacteria (*Clostridium* spp.), able to remove the sialic acid residues. This phenomenon has been described in Gram-negative neonatal sepsis and in particular during necrotising enterocolitis[72].

The passive transfusion of anti-T antibodies with FFP, packed red cells and/or platelet concentrates in T-activated subjects can induce a haemolytic transfusion reaction of variable severity. This phenomenon must be suspected in neonates when the expected post-transfusion increase in Hb is not achieved (unexpected increase in transfusion requirements) and in the presence of a haemolytic transfusion reaction with haemoglobinuria due to intravascular haemolysis.

All patients with necrotising enterocolitis and/or systemic infection who develop haemolysis must be investigated to determine the cause of the haemolysis, taking into consideration the possibility of T activation[73]. The treatment includes the use of carefully washed cellular blood components and industrially pathogen-inactivated plasma[74].

Each Centre should establish a protocol for the diagnosis and treatment of this situation (**Level of evidence IV, Grade of recommendation C**).

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Appendix I

Definitions

Neonate: baby ≤28 days of life

LBW: low birthweight neonate, <2,500 g

VLBW: very low birthweight neonate, <1,500 g

ELBW: extremely low birthweight neonate, <1,000 g

Appendix II

The definitions of the levels of evidence and grades of recommendation used in these guidelines are those from the US Agency for Health Care Policy and Research.

Levels of evidence

- **Ia** Evidence obtained from meta-analysis of randomised, controlled clinical studies (RCT).
- **Ib** Evidence obtained from at least one RCT.
- **IIa** Evidence obtained from at least one well-designed, not randomised, controlled trial.
- **IIb** Evidence obtained from at least one other well-designed type of study.
- **III** Evidence obtained from well-designed descriptive studies, such as case-controlled studies, cohort studies and case studies.
- **IV** Evidence obtained from reports of expert commissions or opinions and/or clinical experience of authoritative persons.

Strength of the recommendations

A (Levels of evidence Ia, Ib)

Requires at least one RCT as part of a set of literature of overall good quality and consistency which suggests specific recommendations.

B (Levels of evidence IIa, IIb, III)

Requires availability of well-conducted clinical studies, but not RCT, on the object of the recommendation.

C (Level of evidence IV)

Requires evidence obtained from reports of expert commissions or opinions and/or clinical experience of authoritative persons. Indicates the lack of good quality,