Blood and blood component therapy in neonates

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ABSTRACT
Blood component therapy is a very common intervention practiced in newborns; nearly 85% of extremely low birth weight (ELBW) babies get transfusions during their hospital stay. However, there are no set guidelines for transfusion of blood component therapy in newborns. This protocol includes available types of blood components , their methods of preparation, indications and side effects of transfusion, in relation to newborns.

Keywords: Transfusion, packed red cells, platelets, newborn

INTRODUCTION

Blood components used in modern day practice include, apart from whole blood, a variety of other products, like red blood cell components, platelet concentrates, and plasma. Blood component transfusion has been considered to be a safe and low risk procedure. In the last few decades there has been recognition of hazards of transfusion of blood and its products. It is no longer considered to be a low or no risk procedure, and consequently an increasing need for stricter guidelines for transfusing blood products has been recognized, not just to check infections, but also to minimize other side effects of transfusion. Preterm neonates comprise the most heavily transfused group of patients, and about 85% of extremely low birth weight newborns receive a transfusion by the end of their hospital stay.[1,2]

RED BLOOD CELL PRODUCTS

Red cells and their products include packed red blood cells (PRBCs) and modified blood products used for specific situations including:

1. Leukocyte reduced RBCs
2. Irradiated RBCs
3. Washed RBCs
4. RBCs with low CMV risk

Indications for PRBC transfusion in neonatal practice

PRBCs are the most commonly used blood product in neonatal transfusions.[3] Indications for transfusion of PRBCs are mainly resolution of symptomatic anemia and for improvement of tissue oxygenation. Tissue oxygenation depends on cardiac output, oxygen saturation and hemoglobin concentration. Once cardiac output and oxygen saturation are optimal, tissue oxygenation can only be improved by increasing the hemoglobin level. The guidelines for transfusion of PRBC vary according to age, level of sickness and hematocrit (Table 1).[3]

Table 1: Guidelines for packed red blood cells (PRBCs) transfusion thresholds for preterm neonates[3]

Less than 28 days of age and 1. Assisted ventilation with FiO_2 more than 0.3: Hb 12.0 gm/dL or PCV less than 40% 2. Assisted ventilation with FiO_2 less than 0.3: Hb 11.0 g/dL or PCV less than 35% 3. CPAP: Hb less than 10 gm/dL or PCV less than 30%
More than 28 days of age and 1. Assisted ventilation: Hb less than 10 gm/dL or PCV less than 30% 2. CPAP: Hb less than 8 gm/dL or PCV less than 25%

Any age, breathing spontaneously and

1. On FiO_2more than 0.21: Hb less than 8 gm/dL or PCV less than 25%
2. On Room Air: Hb less than 7 gm/dL or PCV less than 20%

Packed Red Blood Cells (PRBCs)

Most RBC components available today are derived from the collection of 350 to 450 mL of whole blood into sterile plastic bags containing citrate-phosphate-dextrose (CPD) anticoagulant. The whole blood is spun to sediment out the RBCs, and most of the plasma is removed by pushing it into a pre-attached satellite bag. Generally, 100 to 110 mL of a nutrient additive solution is added back to the packed RBCs, creating an "additive RBC" product that has a final hematocrit of 55% to 60%. A variety of additive solutions are in use today, each of which contains a particular mix of glucose, adenine, and mannitol. These solutions prolong the shelf life of the RBC product from 21 days (packed RBCs in CPD) to 42 days (additive RBCs). A transfusion of 10 mL/kg of additive RBCs would be expected to raise the newborn's hematocrit by 7% to 8%. Red cells collected in CPDA-1 are kept as packed RBCs, that is, additive solution is not added. CPDA-1 packed RBCs have a hematocrit of approximately 75% and a shelf life of 35 days. An infusion of 10 mL/kg of CPDA-1 packed RBCs would be expected to raise the patient's hematocrit by 9% to 10%.

Modified RBC products

1. Leukocyte reduced RBCs

Leukocyte depletion or reduction has been defined as achieving a concentration of less than 5 x 10^6 leukocytes per unit of RBCs. Leukocyte reduction is important in neonatal transfusion. It helps in preventing non-hemolytic febrile transfusion reactions (NHFTR), HLA alloimmunization, transmission of leukotropic viruses (CMV, EBV and HTLV-1), transfusion related GVHD, and transfusion related acute lung injury (TRALI).[4]

Methods of leuko-reduction include the following: [4]

a. Centrifugation and removal of buffy coat (pre-storage leukoreduction)
b. Use of leukocyte filters (pre or post storage)
c. Washing of RBCs with saline
d. Freezing and thawing of red cells

2. Gamma irradiation

Gamma irradiation of blood components including RBCs, platelets and white blood cell products is done to inactivate donor T cells, and the associated risk of transfusion associated graft versus host disease (TA-GVHD), which may occur in immunosupressed patients, very small babies, in large volume transfusions and during intrauterine transfusions.[5]

Irradiation causes an increase in the rate of leakage of potassium out of RBCs during storage, and irradiated RBCs have a shortened shelf life of only 28 days. Irradiation does not adversely affect the function or viability of platelets.[6] While most blood banks use a dose

of 1,500 cGy, the selection of an appropriate dose of gamma irradiation remains an issue.[7]

3. Washed RBCs

Saline washing is mainly done to remove plasma from the RBCs. It may also be done to reduce potassium in stored RBCs in large volume transfusions. Isotonic saline is added to blood components, and centrifugation is done followed by removal of supernatant, and resuspension of the cells in saline. Washed products must be used within 4 hours of processing, if stored at room temperature or within 24 hours, if stored in the refrigerator.

4. CMV reduced RBCs

CMV reduced RBCs are used to reduce the risk of transmitting CMV, which may be a cause of considerable concern in newborns. CMV reduction can be achieved by either leukoreduciton of blood components, or by pre-selecting donors who are CMV negative. Providing CMV reduced blood is important in preterm infants, who have a more severe form of CMV infection than term newborns.

5. Whole blood versus PRBC

Overriding indication for whole blood transfusion is when there is need for concurrent replacement of volume and coagulation factors (only fresh blood will supply coagulation factors).

6. Reconstituted whole blood:

It is obtained by resuspension of PRBCs, which is frozen and deglycerolized. Red cells are frozen with glycerol and stored at -80 ^{o}C in vapor phase of liquid nitrogen. RBCs can be suspended in compatible but not necessarily group-specific plasma. Reconstituted blood can be used in case of rare blood groups or when neonate has multiple antibodies from previous transfusions so that compatible blood is difficult to procure.

Practical Issues

1. *Amount of transfusion to be given*: It has been seen that transfusion with PRBC at a dose of 20 mL/kg is well tolerated and results in an overall decrease in number of transfusions compared to transfusions done at 10 mL/kg. There is also a higher rise in hemoglobin with a higher dose of PRBCs.[8]
2. *Properties of RBC products used in neonatal transfusion*:
   a. RBCs should be freshly prepared and should not be more than 7 days old. This translates into a high 2, 3-DPG concentration and higher tissue extraction of oxygen.

Other concerns with old RBCs are hyperkalemia, and a reduced RBC life span.

b. In small and sick neonates, where it is anticipated that blood component therapy may be needed more than once, it may help to have aliquots from a single donor given as sequential transfusions.[9] This is done practically by reserving a bag of fresh PRBC for up to 7 days for a newborn and withdrawing small aliquots required repeatedly from that bag under laminar flow using a sterile connecting device, into a fresh blood bag. The PRBC bag is immediately resealed under the laminar flow, and can be reused for withdrawing similar small quantities of blood for up to 7 days.

3. *Choosing the blood group for neonatal transfusions*:[4]

a. It is preferable to take samples from both, mother and the newborn, for initial testing prior to transfusion. Mother's sample should be tested for blood group and for any atypical red cell antibodies.

b. ABO compatibility is essential while transfusing PRBCs. Though ABO antigens may be expressed only weakly on neonatal erythrocytes, neonate's serum may contain transplacentally acquired maternal IgG anti-A and/or anti-B.

c. Blood should be of newborn's ABO and Rh group. It should be compatible with any ABO or atypical red cell antibody present in the maternal serum.

d. In exchange transfusions for hemolytic disease of newborn, blood transfused should be compatible with mother's serum. If the mother's and the baby's blood groups are the same, use Rh negative blood of baby's ABO group. In case mother's and baby's blood group is not compatible, use group O and Rh negative blood for exchange transfusion.

4. *Volume and rate of transfusion*:

a. Volume of packed RBC = Blood volume (mL/kg) x (desired minus actual hematocrit)/ hematocrit of transfused RBC

b. Rate of infusion should be less than 10 mL/kg/hour in the absence of cardiac failure.

c. Rate should not be more than 2 mL/kg/hour in the presence of cardiac failure.

d. If more volume is to be transfused, it should be done in smaller aliquots.

5. *Expected response*: Each transfusion of 9 mL/kg of body weight should increase hemoglobin level by 3 g/dL. Meticulous monitoring

of input, output and vital signs are mandatory during blood transfusion.

PLATELET TRANSFUSION

Thrombocytopenia is defined as platelet count less than 1.5 lakh/cubic mm.[10] Presence of thrombocytopenia leads to an increase in risk of bleeding. Dysfunctional platelets in the presence of normal platelet counts may also cause bleeding tendency. Thrombocytopenia has been observed in 1–5% of newborns at birth.[11-13] Severe thrombocytopenia defined as platelet count of less than 50,000/cubic mm may occur in 0.1–0.5% of newborns.[13-14] In NICU, there is a higher incidence; with thrombocytopenia being observed in up to 22–35% of all babies admitted to NICUs and in up to 50% of those admitted to NICUs who require intensive care. Significant proportions (20%) of these episodes of thrombocytopenia are severe.[15-16] Thus a large number of neonates are at risk for bleeding disorders in NICU.

Immune thrombocytopenia:

a. ***Neonatal alloimmune thrombocytopenia (NAIT)***

The best choice of platelet transfusion is human platelet antigen (HPA) compatible platelets, which are generally maternal platelets, meticulously washed and irradiated. The aim is to maintain the platelet count above 30,000/ cubic mm.[17] However; HPA compatible platelets are not easily available. In the absence of immunologically compatible platelets, random donor platelet transfusions may be an acceptable alternative, and has been shown to increase platelet counts above 40,000/cubic mm in most of the transfused patients.[18]

An alternative approach is the use of intravenous immunoglobulin (IVIG) (1 g/kg/day on two consecutive days or 0.5 g/kg/day for four days), alone or in combination with random donor platelet transfusion.[19]

b. ***Neonatal autoimmune thrombocytopenia***

The goal is to keep the count above 30,000/cubic mm. IVIG is given if counts are less than the acceptable minimum at a dose of 1 g/kg/day on two consecutive days. [20]

Nonimmunologically mediated thrombocytopenia

Low platelet count occurring at less than 72 hours of age is caused most commonly by placental insufficiency, maternal PIH, early onset sepsis (EOS), and perinatal asphyxia. EOS and asphyxia may, in particular, lead to severe thrombocytopenia. Thrombocytopenia occurring beyond the initial 72 hours is most commonly caused by sepsis and necrotising enterocolitis. Other infrequent causes include intrauterine infections, metabolic errors and congenital defects in platelet production.[10, 16] Indications for platelet transfusion in

nonimmune thrombocytopenia depend on the level of sickness of newborn[3] (Table 2)

Table 2: Indications for platelet transfusion in nonimmune thrombocytopenia in newborn

1. Platelet count less than 30,000/cubic mm: transfuse all neonates, even if asymptomatic
2. Platelet count 30,000 to 50,000/cubic mm: consider transfusion in a. Sick or bleeding newborns b. Newborns less than 1000 gm or less than 1 week of age c. Previous major bleeding tendency (IVH grade 3-4) d. Newborns with concurrent coagulopathy e. Requiring surgery or exchange transfusion
3. Platelet count more than 50,000 to 99,000/cubic mm: transfuse only if actively bleeding

Types of platelets available

Random donor platelet (RDP)

Each unit of a random donor pool is obtained from a single whole blood unit. Multiple such units from many donors can be pooled together or each such unit can be given separately. PRBC is separated from the platelet rich plasma, which is then respun at a higher speed to make a concentrated platelet button

Single donor platelet (SDP)

SDP units are obtained by a process called plateletpheresis. Here, from a single donor itself multiple platelet units are separated. This is achieved by returning RBCs and platelet poor plasma to donor's circulation after plateletpheresis. The procedure is repeated 4 to 6 times, yielding 4 to 6 units of platelets from one individual. It is especially useful to prevent alloimmunization in multiply transfused patients. Both SDPs and RDPs are irradiated, It is more cost effective to screen the larger SDP units for bacterial contamination, than to screen individual random donor units.[21] The concentration of platelets is more in SDP than in RDP, with SDP having a platelet concentration of $3x10^{11}$/unit and RDP having a concentration of $0.5x10^{10}$ per unit. In neonatal transfusion practice, RDP is generally adequate to treat thrombocytopenia. SDP is required only if prolonged and severe thrombocytopenia is anticipated, requiring multiple platelet transfusions.

Platelet storage: Platelets are stored at 20°C to 24°C using continuous gentle horizontal agitation in storage bags specifically designed to permit O_2 and CO_2 exchange to optimize platelet quality. The storage time from collection to transfusion of platelets (RDPs) is 5 days. SDPs can be stored for up to 7 days.

Practical Issues:

1. Platelets should never be filtered through a micropore blood filter before transfusion, as it will considerably decrease the number of platelets.
2. Female Rh-negative infants should receive platelets from Rh-negative donors to prevent Rh sensitization from the contaminating red blood cells.
3. The usual recommended dose of platelets for neonates is 1 unit of platelets per 10 kg body weight, which amounts to 5 mL/kg. The predicted rise in platelet count from a 5-mL/kg dose would be 20 to 60,000/cubic mm.[15] Doses of up to 10-20 ml/kg may be used in case of severe thrombocytopenia.[9]

PLASMA DERIVATIVES

Plasma contains about 1 unit/mL of each of the coagulation factors as well as normal concentrations of other plasma proteins. Labile coagulation factors, like factors V and VIII, are not stable in plasma stored for prolonged periods at 1–6° C; therefore plasma is usually stored frozen at –18° C or lower. Fresh frozen plasma (FFP) is stored within 8 hours of collection. It contains about 87% of factor VIII present at the time of collection and must contain at least 0.70 IU/mL of factor VIII.[22]

Fresh frozen plasma

FFP has traditionally been used for a variety of reasons, including volume replacement, treatment of disseminated intravascular coagulopathy (DIC), during the treatment of a bleeding neonate, for prevention of intraventricular hemorrhage, and in sepsis.[3] It has not been shown to have any survival benefits in most of these conditions and currently the only valid indications for transfusing FFP in a newborn include

1. Disseminated intravascular coagulopathy
2. Vitamin K deficiency bleeding
3. Inherited deficiencies of coagulation factors

Other rare indications include patients with afibrinogenemia, von Willebrand factor deficiency, congenital antithrombin III deficiency, protein C deficiency and protein S deficiency when specific factor

replacement is not available. It is also used for reconstitution of blood for exchange transfusion.

Cryoprecipitate
It is prepared from FFP by thawing at 2 – 4° C. Undissolved cryoprecipitate is collected by centrifugation and supernatant plasma is aseptically expressed into a satellite bag.
Cryoprecipitate contains about 80 to 100 U of factor VIII in 10-25 mL of plasma, 300 mg of fibrinogen and varying amounts of factor XIII. It is stored at a temperature of -20° C or below.

Indications for use of cryoprecipitate:
1. Congenital factor VIII deficiency
2. Congenital factor XIII deficiency
3. Afibrinogenemia & dysfibrinogenemia
4. von Willebrand disease

Practical Issues:[9]
1. FFP should be group AB, or compatible with recipient's ABO red cell antigens
2. Volume of FFP to be transfused is usually 10–20 mL/kg
3. Volume of cryoprecipitate to be transfused is usually 5 mL/kg

TRANSFUSION ASSOCIATED RISKS

Blood transfusion reactions may be broadly classified as
1. Infectious
2. Non-infectious
    a. Acute
        i. Immunologic
        ii. Non-immunologic
    b. Delayed

Infectious complications
In India, it is mandatory to test every unit of blood collected for hepatitis B, hepatitis C, HIV/AIDS, syphilis and malaria.[23] However, transfusion transmitted infections are still a considerable risk, because of the relative insensitivity of screening tests, and several other organisms besides those tested for, which may be transmitted through blood.

1. ***Viral infections:*** Transmissible diseases can be caused by viruses like human immunodeficiency virus (HIV), hepatitis B and

C viruses (HBV & HCV), and cytomegalovirus (CMV). Other uncommon viruses like hepatitis G virus, human herpes virus-8 and transfusion-transmitted virus have also been detected. Viral infections contaminate platelet products more commonly than RBC products due to a higher temperature used for storage of platelet products.[24] Though screening for HIV, HBV and HCV is mandatory in blood banks, other viruses still present an unaddressed problem. Insensitivity of pathogen testing is also an issue, and risk of viral infections with blood transfusions remains real. Risk of post transfusion hepatitis B/C in India is about 10% in adults despite routine testing because of low viraemia and mutant strain undetectable by routine ELISA.[25] HIV prevalence among blood donors is different in various parts of the country.

CMV: Transfusion related CMV infections in newborns were initially identified in the year 1969, and since then transfusion associated CMV transmission is a well known entity. It has been reported that there is a seroconversion rate of 10-30% in preterm newborns transfused with CMV positive blood. Leukodepletion and selection of CMV negative donors decreases the risk of transfusion transmitted CMV.[26]

2. ***Bacterial infections:*** Bacteria in donor blood are derived from either asymptomatic bacteraemia in the donor, or from inadequate skin sterilization leading to bacterial contamination of the blood. Platelets are at a higher risk of causing bacterial infection than other blood components, as they are stored at room temperature, leading to rapid multiplication of infectious organisms. The highest fatality is seen when the contaminating organism is a gram-negative bacteria. In case of a febrile non-hemolytic reaction post transfusion, bacterial contamination always remains a possibility. It generally causes a higher rise in temperature than other febrile transfusion reactions.
3. ***Parasites***: Plasmodium, trypanosome, and several other parasites may be transmitted through blood, depending on the endemicity of the area. Transfusion transmitted malaria is not uncommon in India, and may occur in spite of blood bag testing, as the screening tests for malaria are insensitive.[25]
4. ***Prions* :** Variant Cruetzfold Jacob Disease (v CJD) is an established complication of blood transfusion and has been reported since 2004. It is thought to have an incubation period of approximately 6.5 years. There is no easy test as yet to detect the presence of prions. It is not very clear whether leuko-reduction prevents transmission of CJD[24]. Restricted transfusions and avoidance of transfusions unless essential, are the only ways currently to prevent transmission.

Noninfectious complications: These can be further sub classified as immune mediated and nonimmune mediated reactions, and as acute and delayed complications.

Acute immune mediated reactions

1. ***Immune mediated hemolysis***

   Acute hemolytic transfusion reactions are a common cause of transfusion related fatality in adult patients, but these are rare in neonates. Newborns do not form red blood cell (RBC) antibodies; all antibodies present are maternal in origin.

   (1) Newborns must be screened for maternal RBC antibodies, including ABO antibodies if non-O RBCs are to be given as the first transfusion.

   (2) If the initial results are negative, no further testing is needed for the initial 4 postnatal months.

   Infants are at a higher risk of passive immune hemolysis from infusion of ABO-incompatible plasma present in PRBC or platelet concentrates. Smaller quantities of ABO-incompatible plasma (less than 5 mL/kg) are generally well tolerated. Newborns do not manifest the usual symptoms of hemolysis that are observed in older patients, such as fever, hypotension, and flank pain. An acute hemolytic event may be present as increased pallor, presence of plasma free hemoglobin, hemoglobinuria, increased serum potassium levels, and acidosis. Results of the direct antiglobulin (Coombs) test may confirm the presence of an antibody on the RBC surface. Treatment is mainly supportive and involves maintenance of blood pressure and kidney perfusion with intravenous saline bolus of 10 to 20 mL/kg along with forced diuresis with furosemide. Enforcing strict guidelines for patient identification and issue of blood; and minimizing human error is essential in preventing immune mediated hemolysis.

2. ***TRALI (Transfusion related acute lung injury):*** It refers to noncardiogenic pulmonary edema complicating transfusion therapy. It is a common and under-reported complication occurring after therapy with blood components. It has been associated with all plasma-containing blood products, most commonly whole blood, packed RBCs, fresh-frozen plasma, and platelets. It has also been reported after the transfusion of cryoprecipitate and IVIG. The most common symptoms associated with TRALI are dyspnea, cough, and fever, associated with hypo- or hypertension. It occurs most commonly with in the initial 6 hours after transfusion. The presence of anti-HLA and/or anti-granulocyte antibodies in the plasma of donors is implicated in the pathogenesis of TRALI. Diagnosis requires a high index of suspicion, and confirmation of donor serum cross-reacting antibodies against the recipient. Treatment is mainly supportive in this self-limiting condition. [27-28]

3. ***Febrile nonhemolytic transfusion reactions (FNHTR)*** are suspected in the absence of hemolysis with an increase in body temperature of less than 2°C. For reactions associated with a temperature rise of greater than 2°C or with hypotension, bacterial contamination also should be suspected and a Gram stain and microbial culture performed on the remaining blood product.

4. ***Allergic reactions***

Allergic reactions are caused by presence of preformed immunoglobulin E antibody against an allergen in the transfused plasma, and are a rare occurrence in newborns. In some cases, release of residual cytokines or chemokines (eg, RANTES) from stored platelets also may cause allergic reactions. These reactions are generally mild, and respond to antihistaminics. Severe anaphylactic reactions are rare.

Acute non immune reactions

1. ***Fluid overload***: Neonates are at increased risk of fluid overload from transfusion because the volume of the blood component issued may exceed the volume that may be transfused safely into neonates. Care should be taken to ensure that, in the absence of blood loss, volumes infused do not exceed 10 to 20 mL/kg. There is no role for routine use of furosemide while transfusing newborns.

2. ***Metabolic complications[29]*:** These complications occur with large volume of transfusions like exchange transfusions.

   a) *Hyperkalemia*: In stored blood, potassium levels tend to be high. It has been seen that after storage for around 42 days, potassium levels may reach 50 meq/L in a RBC unit.[30] Though small volume transfusions o not have much risk of metabolic disturbances, large volume transfusions may lead to hyperkalemia. Washing PRBCs before reconstituting with FFP before exchange transfusion helps in preventing this complication.

   b) *Hypoglycemia:* Blood stored in CPD blood has a high content of glucose leading to a rebound rise in insulin release 1-2 hours after transfusion. This may lead to hypoglycaemia and routine monitoring is necessary, particularly after exchange transfusion, after 2 and 6 hours, to ensure that this complication does not occur.

c) *Acid- base derangements*: Metabolism of citrate in CPD leads to late metabolic alkalosis. Metabolic acidosis is an immediate complication occurring in sick babies who cannot metabolize citrate.

d) *Hypocalcemia and hypomagnesemia* are caused by binding of these ions by citrate present in CPD blood.

Delayed complications

1. ***Alloimmunization:*** Alloimmunization is an uncommon occurrence before the age of 4 months, and is caused by transfusion of blood products with are mismatched for highly immunogenic antigens like Rh. [31]

2. ***Transfusion associated graft versus host disease (TA-GVHD):*** Newborns are at risk for TA-GVHD if they have received intrauterine transfusions, exchange transfusions, or are very small, or immunocompromised. Unchecked donor T cell proliferation is the cause of TA-GVHD, and it can be effectively prevented by leukoreduction of the transfused blood products in at risk patients.

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