First Annual SSPH & PGTI Update

SOUVENIR 2017

Paediatric Transfusion Medicine Practice

Sunday, August 6, 2017

"Accredited with UP Medical Council for 4 CME hours"

Super Speciality Paediatric Hospital and Post Graduate Teaching Institute (SSPH & PGTI), Noida

(An Autonomous Body under Govt. of UP)

ISTM

INDIAN ACADEMY OF PEDIATRICS NOIDA (U.P.)

First Annual SSPH & PGTI Update on "Pediatric Transfusion Medicine Practice"

Patron

Dr. A.K. Bhatt, Director

Advisor

Dr Jyotsna Madan (Dean) Dr DK Singh (CMS) Dr Shekhar Yadav (MS)

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Dr. Seema Dua

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Dr Nita Radhakrishnan Dr Satyam Arora

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NOIDA (U.P.)

सुपर स्पेशियलिटी बाल चिकित्सालय एंव स्नातकोत्तर शिक्षण संस्थान

सेक्टर- ३० नोएडा-२०१३०३

SUPER SPECIALITY PAEDIATRIC HOSPITAL & POST GRADUATE TEACHING INSTITUTE

SECTOR-30 NOIDA-201303

डा० ए० के० भट्ट
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Ref No. SSPH&PGTI Noida/DIR/121 / 2017

Date:- 21 July, 2017

It gives me immense pleasure that Department of Transfusion Medicine and Department of Pediatric Hemato-oncology are going to organize a CME on- Pediatric Transfusion Medicine Practice in this institute on 6th August 2017. To mark the occasion souvenir is also being brought out.

Transfusion Medicine and Clinical Haematology constitute important segment of medical science & health care services. Tremendous advancements have been observed in the field in recent years. Such CME's prove extremely productive exercise where participants learn & share the current trends.

It is a pleasure to note that eminent faculty members from Transfusion Medicine; Pathology and Pediatric Haematology specialties, from all over India, have gathered and will enrich the delegates with their knowledge and experiences.

Such CME's will enable our institute to establish link with distinguished faculty and academicians across the country.

My Best wishes and whole hearted support to the CME and I wish the CME to be a grand success.

(Prof. A. K. Bhatt)
Director
21/7/17

To

Dr. Satyam Arora
Organizing Secretary
Department of Transfusion Medicine

To

Dr. Nita RadhaKrishnan
Organizing Secretary
Department of Transfusion Medicine

सुपर स्पेशियलिटी बाल चिकित्सालय एंव स्नातकोत्तर शैक्षणिक संस्थान
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Dr Jyotsna Madan
Dean & Professor, Pathology
SSPH&PGTI, Noida

REF.NO.SSPH&PGTI/DEAN/ 58
Date: 22 / 07/ 2017

MESSAGE

It gives me immense pleasure to say that Department of Transfusion Medicine and Pediatric Hemato-oncology, SSPH& PGTI are organizing First Annual Update on Pediatric Transfusion Medicine Practice on 6th August 2017.

I whole heartedly welcome all the guest faculty & delegates who are participating in this CME.

Transfusion Medicine is evolving at a very fast pace and such CME's play a very important role to update the specialists of concerned speciality with the latest developments. I firmly believe deliberations by eminent guest faculty will enlighten the delegates towards newer concepts in emerging subspeciality of Pediatric Transfusion Medicine.

I wish the CME a great success.

(Dr. Jyotsna Madan)

सुपर स्पेशियलिटी बाल चिकित्सालय एंव स्नातकोत्तर शैक्षणिक संस्थान
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Sector-30, NOIDA (U.P.)
Phone/Fax: +91-120-2455552; **E-mail:** sspginoida@gmail.com; **W:** www.ssphpgtinoida.com

It is a matter of great pleasure that Department of Transfusion Medicine and Pediatric Hemato-oncology, SSPH& PGTI have taken an initiative to organize First National CME on Paediatric Transfusion Medicine Practice, scheduled on 6th August 2017.

It is appreciable to note that galaxy of distinguished faculty from Transfusion Medicine, Pediatrics and Pediatric Hemato-oncology will provide an excellent platform for exchange of ideas, their experiences and knowledge in a comprehensive manner. I believe this CME will be beneficial to all pediatricians, transfusion medicine specialists and blood bank personnel.

I extend my warm welcome to all guest faculty and delegates and wish the CME a great success.

Dr D.K Singh,
Professor, Pediatrics
Chief Medical Superintendent

C. M. S.
Super Speciality Paedia[illegible] Hospital & Post Graduate Teaching Institute Sec-30, Noida

To,
Dr. Satyam Arora,
Organizing Secretary,
Dept. of Transfusion Medicine,

To
Dr. Nita Radha Krishnan
Organizing Secretary,
Dept. of Pediatric Hemato-oncology,

ISTM

Indian Society of Transfusion Medicine

Message

I am delighted to know that Department of Transfusion medicine and Pediatric Hemato-oncology is organizing a CME on Pediatric Transfusion Medicine Practice at SSPH & PGTI on 6th August 2017.

Transfusion medicine has made exponential progress in last 2 decades with the advent of cellular therapy, and therapeutic role of apheresis. Blood safety has considerably improved with introduction of the NAT and Pathogen inactivation in the developed world. We periodically keep on updating ourselves with conferences CME's, seminars and workshops. I congratulate the organizers for coming up with the idea of organizing a National CME on Pediatric Transfusion Medicine Practice at SSPH & PGTI to discuss and resolve the issues related with pediatric transfusion.

I am fully confident that deliberations by faculty members will open newer avenues to tackle clinical immunohematological conditions and tackle transfusion related issues with paediatrics, besides keeping our young postgraduates abreast with the recent developments.

I wish the CME a grand success.

Dr (Prof) R N Makroo
President, Indian Society of Transfusion medicine,
Director & Senior Consultant,
Dept. of Transfusion medicine, Molecular Biology and Transplant Immunology,
Indraprastha Apollo Hospital, New Delhi

Academy of Pediatrics

Noida Branch

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E-mail: priyankajainashish@gmail.com

Dear Iapians & fellow academicians

Greetings from IAP, Noida

I am happy to see that department of transfusion medicine and department of Paediatric hemato oncology, SSPH and PGTI is organizing first annual update on Paediatric transfusion medicine practice.

I am sure and fully confident that this update shall serve as a mean of upgrading the knowledge of practicing and in training pediatricians, transfusion specialist as well as pathologist and medical officer from blood bank.

IAP Noida and myself are personally thankful to the Organizers

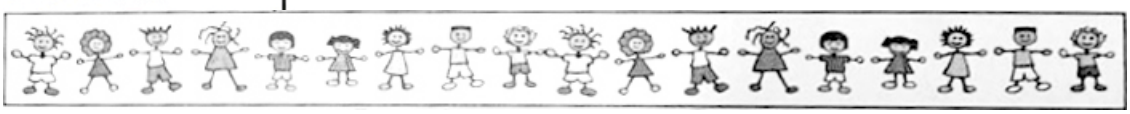
At the end I wish the organizing team, Good luck

DR. INDU PRAKASH

President IAP, Noida

Sr. consultant deptt. of pediatrics

Fortis Hospital Noida



Beti Bachao - Beti Padhao

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Message from the Organizing Team

Greetings of the day!! We, the Department of Transfusion Medicine and Department of Paediatric Hemato-oncology, extend our warm welcome to all distinguished faculty and delegates who have come to participate in this CME.

Transfusion Medicine for paediatric patients has been an important area and since long has not been given due importance. We at Super Speciality Paediatric Hospital and Post Graduate Teaching Institute (SSPH & PGTI) have made a humble beginning to develop country's first transfusion medicine department focusing purely on paediatric and neonatal group of patients. As a part of our journey we have collaborated with department of hemato-oncology to initiate an annual academic meeting with a *'dream and mission'* of creating high level of awareness over this newly emerging subspecialty of transfusion medicine and issues revolving around it. We are happy to have you all in the first chapter of the series.

We are privileged to have amongst us well renowned faculty from eminent institutes all across the country to enrich us during scientific sessions. We are grateful to these stalwarts who have spared some time for us and graced the occasion. We are thankful to our patron and advisors from SSPH & PGTI for their continuous support and encouragement.

'We do not remember days but we remember moments'. We hope this CME becomes one of those memorable moments with lots of take home messages which will help us to do better for our patient care in our respective fields.

Dr Seema Dua
Organising chairperson

Dr. SEEMA DUA
Assoc. Prof & Head
Deptt. of Transfusion
Medicine & Blood Bank
S.S.P.H. & P.G.T.I., Sec-30, Noida

Dr Satyam Arora
Organising Secretary

Dr. SATYAM ARORA
Assistant Professor
Department of Transfusion Medicine
(Blood Bank)
S.S.P.H. & P.G.T.I., Sec-30, Noida

Dr Nita RadhaKrishnan
Organising Secretary

Dr. Nita Radhakrishnan
Asst. Professor
Dept. of Pediatric Hemetology Oncology
S.S.P.H. & P.G.T.I. Sec-30, Noida

First Annual Update on Paediatric Transfusion Medicine Practices
Theme: "Introduction to Paediatric Transfusion Medicine"

INDEX

Scientific Program

08:00 - 08:30 Registration

08:30 - 09:00 Inauguration and Lamp Lightning by the Director, SSPH&PGTI, Noida
Release of souvenir book

09:00 - 11:00 Session 1: Nuts and Bolts in Transfusion Medicine

Chair Person

Dr AK Bhatt
Director
SSPH & PGTI
Noida

Dr Jyotsna Madan
Professor Pathology & Dean
SSPH & PGTI
Noida

Dr RN Makroo
ISTM President
Director & Head, Transfusion Medicine
Indraprastha Apollo Hospital, Delhi

09:10 - 09:30 INTRODUCTION : Paediatric Transfusion Medicine as a sub-speciality of Transfusion Medicine in India

Highlight Present scenario and unmet needs in India, NIH 2016 Priorities, Future prospects for India

Speaker Dr Neelam Marwaha, Professor and Head, Transfusion Medicine, PGIMER, Chandigarh

09:30 - 09:50 IMMUNO-HEMATOLOGY : Application of Immuno-Haematology for Fetus, Neonates and Paediatric patients

Highlight Sample requirements for IH testing; Concepts of IAT & DAT; Antibody Screening
Rh and extended Phenotyping; Pre transfusion testing; Concepts of HDN (both ABOi and Rhi)

Speaker Dr RK Chaudhary, Professor and Head, Transfusion Medicine, SGPGIMS, Lucknow

09:50 - 10:10 TRANSFUSION TRANSMITTED INFECTIONS (TTI) : Screening for Transfusion Transmitted Infections: Safe transfusion and Minimizing the risk

Highlight NAT vs Anti HBcAg Indian context; CMV and other new agents (Zika, Dengue) screening for Paediatric Patients; Bacterial contamination

Speaker Dr Prashant Pandey, Consultant and Head, Transfusion Medicine, Jaypee Hospital, Noida

10:10 - 10:30 CLINICAL TRANSFUSION PRACTICES : How to Transfuse a Neonate or Paediatric Patient?

Highlight When to order, How much to order, How to Transfuse and How to monitor; Liberal Vs Restrictive Policies; Aliquoting of blood; Additive solutions (SAGM) & Age of blood

Speaker Dr Naveen Agnihotri, Senior Consultant & Head, Transfusion Medicine, Nayati Medicity, Mathura

10:30 - 10:45 Discussion followed by Tea & Snacks Break

11:00 - 13:30 Session 2: Everyday Practice "One Size Will Never Fit All"

Chair Person

Dr DK Singh
Prof Paediatrics and CMS,
SSPH & PGTI
Noida

Dr Indu Prakash Sharma
President IAP Noida,
Consultant Paediatrics
Fortis Hospital, Noida

Dr Meenu Bajpai
Addl Prof., Transfusion Medicine,
ILBS
New Delhi

11.00 -11.30 UPDATE TO TRANSFUSION GUIDELINES FOR FETUS, NEONATES AND PAEDIATRICS

Highlight BCSH and JPAC guidelines; IUT; NAN; NAIT; Top up transfusions; Massive transfusions, Erythropoietin use

Speaker Dr Amita Mahajan, Senior Consultant & Head, Paediatric Hemato-Oncology, Apollo Hospital, Delhi

11.30 -12.00 SPECIAL SCENARIO IN PAEDIATRIC TRANSFUSION

Highlight Nutritional Anemia, Dengue, Paediatric Surgery, Peds ICU, ECMO Hemoglobinopathies, Platelets Refractiveness, AIHA, Peds Oncology

Speaker Dr Tulika Seth, Professor, Clinical Hematology, AIIMS, Delhi

12.00-12.20	COMPONENT THERAPY: What is New ? and What is the evidence ?
Highlight	Granulocytes; Buffy Coat; Irradiated products; Platelets Additive Solutions (PAS); When to use them and what is the evidence
Speaker	Dr Aseem Tiwari, Associate Director & Incharge, Transfusion Medicine, Medanta, Gurgaon
12.20-12.40	VENOUS ACCESS: Concerns for Transfusionists and Apheresis Physicians
Highlight	Vascular access in neonates and children for transfusion, central venous access for stem cell apheresis plasmapheresis & Plasma Exchange
Speaker	Dr Zainab Ahmad, Assistant Professor, Paediatric Anasthesia and Trauma, SSPH &PGTI, Noida
12.40-13.00	THERAPEUTIC APHERESIS AND THERAPEUTIC PLASMA EXCHANGE (TPE) IN PAEDIATRICS
Highlight	Indications, Differences from adults, Outcomes, Scope
Speaker	Dr RR Sharma, Professor, Transfusion Medicine, PGIMER, Chandigarh
13.00-13.30	PANEL DISCUSSION: Diagnosis and management of adverse reactions to transfusions
Highlight	Scenarios encountered in daily practice: Fever with rigors following transfusion, Anaphylaxis, High coloured urine, Respiratory distress following transfusion, Allosensitization
Panelist	Dr Veena Doda; Head (Retd), Transfusion Medicine, RML Hospital Delhi Dr Bharat Singh, Head, Transfusion Medicine, GTB Hospital, Delhi Dr Tulika Chandra, Addl Professor & Head, Transfusion Medicine, KGMU, Lucknow Dr VK Khanna, Senior Consultant and HOD, Paediatrics, Sir Gangaram Hospital, Delhi Dr Manas Kalra, Consultant, Pediatric Hemato-Oncology, Indraprastha Apollo Hospital, Delhi
Moderator	Dr Nita Radhakrishnan, Asst Professor, Pediatric Hemato-Oncology, SSPH & PGTI, Noida

13:30 - 14:30 Lunch Break

14:30 - 15:30 Session 3: Moving Forward to Cellular Therapy in Paediatrics

Chair Person

Dr (Brig.) Anil Khetrapal
Director, Transfusion Medicine
Artemis Hospital,
Gurugram

Dr Anil Handoo
Director, Lab Medicine
BLK Super Speciality Hospital
Delhi

14.30-15.00	ROLE OF TRANSFUSION MEDICINE SPECIALIST IN PAEDIATRIC BMT
Highlight	PBSC Donor selection, Mobilization and Stem Cells Harvest, Stem cell product manipulation
Speaker	Dr Rasika Setia, Senior Consultant and Head, Transfusion Medicine, BLK Super Speciality Hospital, Delhi
15.00-15.20	WHAT IS NEW IN PAEDIATRIC BMT AND CELLULAR THERAPY
Highlight	CAR T cells and T Cell Depletion
Speaker	Dr Satyendra Katewa, Head, Pediatric Hemato-Oncology and BMT, Manipal Hospital, Jaipur
15.20-15.30	VOTE OF THANKS & DISCUSSION
	Dr Satyam Arora, Asst Professor, Transfusion Medicine, SSPH & PGTI, Noida

15:30 - 17:00 Session 4: Seeing is Believing

15.30-17.00	VISIT TO BLOOD BANK, DEMONSTRATION OF IMMUNOHEMATOLOGICAL TESTS AND APHERESIS
Station 1	Blood Group Phenotyping; IAT and DAT, How to test a patient with suspected AIHA
Station 2	Demonstration of Concept- Apheresis; Plasma Exchange
Moderators	Dr Gopal Pathidar, Asst Professor, Transfusion Medicine, AIIMS, New Delhi; Dr Ravi Dara, Consultant and Head, Transfusion Medicine, Jaipur

17:00 onwards Tea and Snacks (Blood Bank)

Pediatric Transfusion Medicine as a sub-speciality of Transfusion Medicine

Prof. Neelam Marwaha, *MD; FAMS; FISHTM*

Head, Department of Transfusion Medicine

PGIMER, Chandigarh

Dr H.K.Dhawan, *MD*

Assistant Professor, Department of Transfusion Medicine

PGIMER, Chandigarh

Introduction

Children are not "little adults". They have important differences and hence the likelihood that transfusion challenges in pediatric patients might be different from those in adults. The physiology, developmental stage and the sequential transition from fetus to neonate to childhood to adolescence, the ever-changing weight, blood volume, post-transfusion life span, pathophysiology of diseases and spectrum of disorders requiring transfusion vary from those seen in adults. Pediatric transfusion medicine as a discipline, therefore, can be seen as ensuring the collection, processing, testing and availability of blood components and derivatives optimally suited to the variable needs of patients undergoing multi-organ growth and development and/or suffering from the effects of congenital disorders [1].

Building up the speciality

The Transfusion Medicine/Hemostasis Clinical Trials Network (TMH CTN) was created by the National Heart, Lung and Blood Institute (NHBLI) USA, in 2002 to perform trials in children and adults. It was observed that there were adequate numbers of pediatric hematologists to initiate research in their field, however few investigators focused on pediatric transfusion medicine. In 2005 the NHLBI formed a Working Group on Pediatric Transfusion Medicine (PTM) to identify research agenda in the field. The Group discovered gaps in training and lack of unified, comprehensive and accepted curriculum in PTM. Their recommendations resulted in curriculum development grants named the Pediatric Transfusion Medicine Academic Awardees (PTMAA) program. Another favorable change for the field of PTM was the American Board of Pathology's move to allow board eligible pediatricians to apply directly into TM fellowship after residency. [2]

In 2008 the PTMAA sponsored a working group to focus on clinical and translational research gaps and three major areas of concern were identified; (i) transfusion strategies; (ii) short-and long-term consequences of transfusion; and (iii) transfusion-transmitted disease as they relate to neonatal and pediatric patients. Subsequently after three more NHLBI sponsored meetings, a separate meeting was held in 2016 focused exclusively on developing research agenda specific to PTM. Six key areas were targeted:

- Neonatology and perinatology
- Oncology and transplant
- Chronic transfusion
- Devices and surgery
- Intensive care and trauma
- Teenage blood donation

Neonatal and Perinatal concerns

Hemolytic Disease of the Fetus and Newborn

Red blood cell (RBC) sensitization occurs in some women upon exposure to paternally derived RBC antigens during either pregnancy or consequent to a prior transfusion event. Once sensitized, the maternal alloantibodies may cause hemolytic disease of the fetus and newborn (HDFN). Alloantibodies against more than 50 non-ABO blood group antigens have been implicated in HDFN, the majority occurring against antigens in the Rh, Kell, Duffy, Kidd and MNS systems. Naturally occurring isohemagglutinins in O group mothers may cause fetal anemia if the fetal red cells have A and/or B blood group antigens. The diagnosis of alloantibody specificity and non-invasive fetal monitoring tools are well-defined. However, gaps in knowledge exist regarding alloimmunization risk factors, preventive therapies and treatment strategies. Generally maternal alloantibody titers are used to predict the risk to the fetus, however some fetuses are severely affected even with low titers while others are less severely affected with high titers. IgG subtypes and glycosylation patterns of the alloantibodies may determine fetal outcome more than total antibody titers. After birth the transfer of maternal IgG stops through the placenta, however persistence of alloantibodies has been seen for longer periods of time than predicted. RhIg is the most widespread immunoprophylaxis against RhD alloimmunisation. However, there is variation in the fucosylation patterns of the IgG, which may affect the antigen binding and hence its efficacy. Hence, there are research frontiers in the immunobiology of HDFN. [3]

Neonatal transfusions

Neonates are amongst one of the most highly transfused group. Yet there is no currently large neonatal RBC transfusion data set to adequately evaluate association between RBC transfusion practices and outcome. RBC storage lesions

are well recognized – decrease in pH, increase in plasma K^+, decrease in ATP and 2,3 – DPG, increase in percentage hemolysis and decrease in red cell viability and function. There is paucity of longitudinal data evaluating post transfusion outcomes such as neurodevelopment, growth and quality of life in relation to liberal versus restrictive transfusion policies. There are presently two large ongoing randomized trials – the Transfusion of Prematures (TOP) and Effects of Transfusion Thresholds on Neurocognitive outcome of ELBW infants (ETTNO) [4] which will compare neurodevelopment outcomes among infants receiving Liberal vs Conservative transfusion. Another cause of concern is the association between RBC transfusion and necrotizing enterocolitis in preterm neonates and evidence from observational studies and randomized trials is conflicting. Neonates generally require small volume top-up transfusions. What are the best methodologies, anti-coagulant preservative solutions and age of the RBCs for neonatal safety is still open to research. Availability and standardization of microsampling and microtechniques would help limit phlebotomy losses. Use of erythropoiesis stimulating agents might decrease the requirement of RBC transfusion. Plasma transfusions are given to almost 15% of patients admitted in NICU [5] due to various reasons; hypovolemia, abnormal coagulation tests with or without bleeding, sepsis, intra-operative bleeding and partial exchange for polycythemia. Use of plasma is associated with various risks; transfusion-related acute lung injury, transfusion-related volume overload, febrile reactions and hemolytic-reactions which may go unnoticed or under recognized in neonates. Two important concerns of plasma transfusion are (i) the association with venous thrombosis and (ii) limitations of coagulation testing in neonates. Platelet transfusions in neonates, like in adults are prophylactic or therapeutic but neonates are transfused at higher thresholds, due to hypofunctional platelets in neonates.

Oncology and transplant

Prior to hematopoietic stem cell transplant (HSCT) pediatric patients receive blood components as part of supportive care. These transfusions may lead to HLA sensitization and as the stem cell donor base expands from related to unrelated donors, cord blood or haploidentical donors, donor-specific HLA issues might become critical. Strategies other than leucoreduction which are being evaluated in adults might differ in outcome when applied to pediatric patients. Also adequate data on outcomes with major ABO incompatible HSCT in children is lacking. Evidences are also required to determine the transfusion thresholds for RBCs and platelets. The use of erythropoietin and G-CSF/GM-CSF to hasten recovery from cytopenias may not be without risk of malignancy after HSCT in pediatric patients. Post-HSCT transfusional hemosiderosis is also a concern. There are still unanswered questions regarding best transfusion practices in pediatric patients in the pre-transplant, transplant and post-transplant phases.

Chronic transfusions

Presently 36 blood group systems with 343 antigens have been characterized and there is significant diversity amongst population groups. Using the conventional red cell serological methods and ABO, Rh(D) matching, allo-antibodies to minor blood group antigens develop in chronically transfused patients – largely thalassemia and sickle cell disease. The prevalence of alloimmunisation with ABO/Rh(D) matching alone ranges from 18 – 75% in patients with sickle cell disease and 4-37% in patients with thalassemia major. The wide range of prevalence is due to variation in risk factors – age of starting transfusions, exposure frequency and heterogeneity between donor-recipient populations. Most of the allo-antibodies belonged to the Rh (C,c,E,e) and Kell blood groups. Some of

the transfusion centers in the developed countries are issuing ABO, D and extended Rh and Kell matched blood and have brought down the incidence of allo-immunisation. Serology based phenotype is not accurate in chronically transfused patients, hence molecular blood group assays have been introduced. DNA based assays are reliable for most of the blood group antigens, except A, B and Rh(D). Hence both serology and molecular typing will both be required and transfusion services will be challenged with changing technologies and policies.

Devices and Surgery

Extracorporeal life support (ECLS) or extracorporeal membrane oxygenation (ECMO) is used to treat critically ill patients with cardiac and/or respiratory failure. Hemostatic complications, both bleeding and thrombosis are common and include factors related to the circuit and the critically ill patient. Exposure of blood to artificial non-endothelialised surface of an EC circuit results in activation of coagulation and inflammation. Blood transfusion during ECMO is required for circuit priming, restoration of oxygen carrying capacity and maintenance of hemostasis. The circuit priming is done either with RBCs alone or in combination with FFP. For thrombotic complications, heparin and/or antithrombin has been administered. For intractable hemorrhage rFVIIa has been used. Increased blood products support has been reported to be associated with increased mortality in pediatric patients [6], however well-designed studies are required before definitive conclusions can be drawn.

Pediatric apheresis is a challenging procedure. There are concerns of small blood volumes, inadequate vascular access and inability of the child to communicate with the apheresis physician. The apheresis devices are not designed to run small volumes, hence there is a need to prime the instrument with RBCs and

albumin to avoid hemodynamic changes or anemia. Vascular access has to be through central venous lines (double –lumen). Significant differences from adults include the ASFA category of the disease, in adults it is largely category 1, but in children due to spectrum of disease prevalence it is largely category III/II.

Massive transfusion

Massive transfusion (MT) may be required in trauma, surgical complications, cardiac surgery and ECMO. In adults MT is defined as the transfusion of one blood volume or ≥10 RBC units in the first 24 hours of injury. In pediatric patients the above definitions will not work and MT has been defined as transfusion of ≥40ml/kg of blood products within the first 24 hours of injury [7], however this definition does not easily translate into a working clinical need. Moreover, there are as yet no evidences to indicate that a 1:1:1 ratio of PRBC, FFP and platelets that is recommended in adult trauma setting is applicable in pediatric trauma. A recent study in 907 pediatric trauma patients in Afghanistan and Iraq, those who received a greater than 0.8 FFP per unit RBC had a significantly higher mortality. [8]. In studies analyzing outcomes with fresh whole blood *vs* whole blood stored for 24 to 48-hour *vs* reconstituted whole blood (PRBCs, FFP, platelets) in children undergoing cardiopulmonary bypass have shown varying results and better designed trials are needed to define transfusion thresholds, volume transfused, products transfused and use of concentrated plasma or factor concentrates.

Teenage blood donation

In India, the minimum age at which blood donation is permitted as per rules is 18 years, however in some developed countries due to shrinking blood donor

base and increased requirements of blood products in aging population, the minimum age has been lowered to 16 or 17 years. Teenagers are still developing physically through adolescence and into early adulthood. Blood donation removes ~ 200-250mg iron per donation and recovery of iron stores takes several months. Myelin production in the brain requires iron and iron deficiency during adolescence might affect white matter integrity. Another concern is the increased risk of vasovagal reactions and syncope related injury.

Scenario in India

Recognition of pediatric transfusion medicine either as a sub-speciality of pediatrics or transfusion medicine is still lacking in India. However, there are some publications which have looked at the alloantibody specificities and outcomes in newborns, others at transfusion audits, aliquots of small volumes for top-up transfusions and recent studies on factors affecting efficacy of red cell transfusions in neonates. Alloimmunisation challenges have also been studied in children with thalassemia major and sickle cell disease. Observations from few of the published Indian studies are cited since inclusion of all studies is not possible. In a study by Wade et al [9] blood transfusion episodes in patients below 12 years of age were compared against internationally accepted guidelines. It was observed that out of 184 total number of episodes of transfusion 153(83.1%) were appropriate. Amongst the inappropriate transfusions highest number were seen with fresh frozen plasma transfusions (58%), followed by red cells (35.5%) and platelets (6.45%). In a study on alloimmunsation in thalassemics, 18 out of 319(5.64%) patients were alloimmunised. Antibodies to Rh and Kell system accounted for 80% of the alloantbodies [10]. This makes a case for extended phenotype matched blood for such patients and preliminary studies have been conducted in the

country. However, more data needs to be generated before making policy decisions. Another study evaluated change in hematocrit 15 min, 6 h and 24 h after PRBC transfusion in neonates and factors predicting this change. Among neonates receiving PRBC transfusion, pre-transfusion hematocrit was recorded and attempt was made to identify variables affecting change in hematocrit following transfusion. Eighty-one neonates received 119 PRBC transfusions (mean volume 16 ± 4 mL/kg). Hematocrit increased from 26 ± 5 to 41 ± 5% at 15 min after PRBC transfusion ($p = 0.001$) and remained stable till 6 h (41 ± 5%, $p = 0.11$). It decreased to 40 ± 5%, at 24 h post transfusion ($p < 0.001$). On linear regression analysis, baseline hematocrit of the baby, donor blood hematocrit and volume of PRBC transfusion were independent determinants of increase in hematocrit [11]. The feasibility of small volume transfusion with linked/dedicated donor units for the blood components was studied with the aim of reducing donor exposure in neonates. Dedicated donor units significantly reduced donor exposure and in planned surgeries this was feasible [12].

Thus in India there is already an awareness of the need to study various aspects on neonatal and pediatric transfusion practices. However concerted efforts are required amongst pediatric physicians, surgeons, intensive care providers, neonatologists, hematologists and transfusion medicine experts to spearhead the recognition of the sub-speciality, both for best transfusion practices and research.

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Immuno-hematological Testing in Neonates and Paediatric Patients

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Why Immunohematological practices in neonates & paediatric age group are different from an adult?

A. ABO antigens & antibodies

- ABO antigens are constructed of a series of carbohydrates attached to glycoproteins or glycolipids. These antigens are also in soluble forms in other body fluids, such as saliva, plasma, and tears, and are expressed by tissues. Glycosyltransferases attach sugars to the end of these carbohydrate chains to determine the ABO type.
- ABO antigens can be detected on red cells of embryos as early as 5 to 6 weeks of gestation. The quantity of ABO antigens on umbilical cord red cells is less than that of adults as the result of the immaturity of Type 2 chain precursors on cord red cells (due to decreased levels of branching enzyme).
- With increasing age, precursor chains become increasingly branched, thereby allowing more A and B antigen to be expressed. Adult levels of ABO expression are generally present by age 2 to 4 years.
- This sometimes results in weaker reactions with antisera, especially Group A. This decreased level of antigen could result in mixed field agglutination (two cell populations) being observed even though all of the red cells are Group A.

- A "naturally occurring" immune response is produced to the A and B antigens. Anti-A and anti-B are stimulated not by transfusion of incompatible blood but by exposure to environmental flora ("gut-associated" bacteria, plants, and other exogenous material) and are usually IgM, containing some IgG antibodies.
- In most cases, infants with HDN due to ABO incompatibility have Group O mothers. Anti-A and anti-B are not present at birth or, if present, are of maternal origin.
- Endogenous synthesis of anti-A and anti-B can develop as early as age 3 to 6 months, with nearly all children displaying the appropriate isohemagglutinins in their sera at 1 year of age.
- Titers of anti-A and anti-B continue to increase during early childhood and achieve adult levels within 5 to 10 years.

B. Immunological status

- Most of their humoral immunity (antibody protection) is provided by the mother starting early in pregnancy (approximately 12 weeks) through placental transfer of immunoglobulins. Between 20 and 33 weeks of gestation, fetal IgG levels rise significantly because of selective transport system maturation in the placenta.
- The breakdown of IgG occurs at a slower rate in the fetus than in the mother, enabling conservation of the transplacental maternal antibody during the neonatal period.
- Unexpected red cell alloantibodies of either IgM or IgG class are rarely produced by the infant during the neonatal period. So antibodies in new born or early infant (3-4 months) serum are actually transplacentally transmitted

maternal IgG antibodies on paternally inherited antigens present on fetal red cells, but absent on the maternal red cells.

- The antigen-negative mother may have naturally occurring antibodies or may have developed antibodies to fetal red cell antigens by exposure to the antigens by blood transfusion or, more often, by silent feto-maternal hemorrhage during pregnancy (iso- or alloimmunization).

Sample requirement

Pre-Transfusion testing is performed to prevent the transfusion of incompatible donor red cells that might result in an immune-mediated hemolytic transfusion reaction.

- When a pre-transfusion specimen is received in the laboratory, laboratory personnel must confirm that the information on the specimen label and the information on the pre-transfusion testing request are identical.
- Each laboratory should establish policies and procedures that define requirements for specimen ID information and how to document the receipt and management of mislabeled specimens.

For ABO Rh grouping

- Infants - only forward (cell) grouping is required. Serum grouping not necessary up to 1 year of age. However, before non-group O RBCs can be issued, testing of the infant's plasma or serum is required to detect passively acquired maternal anti-A or anti-B demonstrable with an indirect antiglobulin test.
    - Sample required- whole blood sample in EDTA vial.

- Children > 1year of age- Both forward and reverse or serum grouping is mandatory
    - Sample required- for forward grouping -whole blood sample in EDTA vial for Reverse grouping- Plasma or serum sample (preferably plasma sample)

Sample for Cross matching

1. Incompletely clotted serum specimens may contain small fibrin clots that trap red cells into aggregates and cause false-positive results with column agglutination technology.
2. Clotting may be incomplete in anticoagulated serum specimens, such as those from patients who have been treated with heparin.
3. At times, it may be necessary to obtain a pre-transfusion blood specimen from the same extremity in which there is an intravenous infusion. If this is the case, steps should be taken to avoid dilution of the specimen, which might result in a failure to detect unexpected red cell antibodies.

Specimen Age

- When pre-transfusion testing is performed for a patient who has been pregnant or transfused within the previous 3 months or when pregnancy and/or transfusion history are uncertain, the pre-transfusion sample used for testing must be no more than 3 days old at the time of the intended transfusion because a recent transfusion or pregnancy may stimulate production of unexpected antibodies.

- Although the use of 3 days is arbitrary, the 3-day requirement was created as a practical approach to ensure that the specimen used for testing reflects the recipient's current immunologic status.
- If the histories of transfusion and pregnancy are certain and if no transfusion or pregnancy has occurred in the previous 3 months, the length of time that a pre-transfusion sample is valid for testing should be based on the manufacturer's recommendations (in the package or instructional insert) for the test being performed.

ABO Rh Grouping and compatibility testing

ABO Rh Grouping

1. Forward groping: Cell/forward grouping is based on an agglutination reaction between A and B antigen present on RBC with commercial anti –A anti-B antisera respectively.
2. Reverse grouping: Serum / reverse grouping is based on an agglutination reaction between naturally occurring anti-A and anti-B antibodies in serum/plasma with reagent A or B cells respectively.

ABO Grouping discrepancies

Anomalous results in blood grouping testing i.e. where forward and reverse grouping fail to tally with each other.

Specific ABO discrepancies in infants:-

- *Type 1 ABO discrepancy (weak/missing antibody)*

  Resolution- For newborn, only forward grouping is done till 4 months of age. This discrepancy may occur in infants > 4 months of age.

- *Type 2 ABO discrepancy (weak/missing antigen)*

  This type discrepancy generally not seen in new born/infants (specially if using CAT) . But sometimes reaction strength may be poor due to low density of ABO antigens on RBC surface (specially in tube technique).

- *Type 3 discrepancy in cord blood sample-*

  This is due to Wharton's jelly in cord blood samples. This may give false positive agglutination.

Compatibility testing (cross match)

Purpose of compatibility testing

- Selection of safest blood components for transfusion
- With acceptable donor's red cell survival rates
- Without destruction of recipient's red cells

Pre-transfusion testing of neonates *(in less than 4 months old infants)*

1. Group ABO and Rh (D) group of infant cells should be determined by cell grouping only.
2. Direct AHG test on baby cells is performed.
3. Maternal serum should be screened for any irregular body, if mother's blood sample is available.
4. If the antibody screening and DAT is negative in mother's blood, and no evidence of HDN cross-matched blood of the same ABO and Rh(D)type as that of the infant using mother's serum (if ABO compatible and sample is available) or baby's serum. This is to exclude the possibility that incomplete antibody

from the mother may be in baby's serum in the absence of the antigen on the baby's red cells to that antibody.

5. If the antibody screening is positive, the direct AHG test is positive or HDN is present, the donors blood must be cross matched against maternal serum. The neonates serum can also be used (if mothers blood sample is not available).
6. When group O blood needs to be given to an infant who is group A and/or B, then red cell concentrates of units with low titre anti-A, or anti-B should be used. It is a good practice to use packed cells re-suspended in 1/3 rd volume of AB plasma (or A plasma or B plasma as appropriate).
7. The policy of the hospital transfusion service determines the frequency for re-evaluating the patient's antibodies. Once a negative antibody screening result is obtained, crossmatches and use of antigen-negative blood are no longer required in infants younger than 4 months because of their immature immunologic status.
8. Multiple observational studies have shown that alloimmunization to red cell antigens is rare during the neonatal period. For this reason, repeated typing and screening, which are required for adults and children older than 4 months, is unnecessary in younger infants and contributes to significant iatrogenic blood loss.

Role of Direct coombs test (DCT) in neonates and paediatric age group

- The direct antiglobulin test (DAT) is used to determine if red cells have been coated IN VIVO with immunoglobulin (Ig), complement, or both.
- The DAT is used primarily for the investigation of hemolytic transfusion reactions, haemolytic disease of the fetus and newborn (HDFN), autoimmune hemolytic anemia (AIHA), and drug-induced immune hemolysis.

- The DAT should be performed on every patient in whom the presence of hemolysis has been established to distinguish immune from nonimmune hemolytic anemia.

Sample requirement for DAT:

- Although any red cells may be tested, EDTA-anticoagulated blood samples are preferred.
- The EDTA prevents in-vitro fixation of complement by chelating the calcium that is needed for C1 activation.
- If red cells from a clotted blood sample have a positive DAT result due to complement, the results should be confirmed on red cells from freshly collected blood kept at 37 C or an EDTA-anticoagulated specimen.

Interpretation of DAT results

Poly AHG	Control	Anti-IgG	Anti-C3	Interpretation
Pos	Neg	Pos	Pos	Warm AIHA, Drug induced, DHTR
Pos	Neg	Neg	Pos	PCH, CAD
Pos	Neg	Pos	Neg	Warm AIHA
Pos	Neg	Neg	Neg	Repeat with another lot of AHG
Pos	Pos	Pos	Pos	Unable to report

Interpretation of DAT in neonates

DAT on neonate	Maternal Ab status	ABO in-compatibilty	Interpretation
Positive	Negative	Yes	+DAT due to ABO Ab
Positive	Clinically significant Ab	No	+DAT due to maternal alloantibody (RhD)
positive	Clinically significant Ab	Yes	+DAT due to maternal allo-Ab and/or ABO Ab
Negative	Negative	Yes	HDN due to anti-A, anti-B cannot be ruled out

Some common causes of Positive DAT results in neonates & paediatric patients

1. ABO HDFN (Most common cause of positive DAT IN Newborns)
2. Rh HDFN (Most common is anti- D mediated)
3. HDFN due to other transplacentally transmitted antibodies
4. Nonspecifically adsorbed proteins (eg, hypergammaglobulinemia, high-dose intravenous immune globulin, or modification of red cell membrane by some drugs)
5. Passively acquired alloantibodies (eg, from donor plasma, derivatives, or immunoglobulin)
6. Complement activation due to bacterial infection
7. Sickle cell disease, beta-thalassemia, multiple myeloma, autoimmune disorders, diseases associated with elevated serum globulin
8. Autoantibodies to intrinsic red cell antigens
9. Drug induced antibodies

Indirect Antiglobulin Test

- Indirect antiglobulin test is used to detect in-vitro sensitization of red cells
- Performed in 3 steps (1) sensitization of red cells (2) Reaction with anti human globulin (3) Centrifugation
- Applications of ICT

Application	Detection
Compatibility testing	Recipient antibodies with donor cells
Antibody screening(IgG)	Recipient antibodies with screening cells
Antibody identification	For antibody specificity
Antibody titration	Rh antibody titre
Red cell phenotype	Weak D test, Jk, Fy Antigens

HEMOLYTIC DISEASE OF FETUS AND NEWBORN (HDFN)

There are three main classes of alloimmune HDN based on the antigen responsible: Rh, ABO, and hemolytic disease due to other red cell antigens. Most frequently encountered antibody HDFN is anti D.

Pathogenesis

- Mechanism of immune HDFN can be summarized in four stages:
  (1) FMH induced alloimmunisation of the mother (against foetal antigens)
  (2) The placental transfer of IgG antibodies to the foetus;
  (3) The immune destruction of sensitized foetal red cells,
  (4) Clinical manifestations, secondary to the destruction of fetal red cells such as severe anaemia, heart failure, hydrops, hyperbilirubinemia and erythropoietic suppression.

ABOi Vs Rhi HDFN

Blood group	Rh	ABO
Mother	negative	O
Infant	positive	A OR B
Type of antibody	IgG1 &/OR IgG 3	IgG 2
CLINICAL ASPECTS		
Occurrence in first new born child	<5 %	40- 50 %
Predictable severity in next pregnancy	usually	No
Stillbirth & /or Hydrops	frequent	rare
Severe anaemia	frequent	rare
Degree of jaundice	+++	+
Hepatosplenomegaly	+++	+
LABORATORY FINDINGS		
Maternal antibodies	always present	Not clear cut
DCT (infant)	+++	+
Peripheral blood picture	nucleated red blood cells	microspherocytes
TREATMENT		
Antenatal measures	YES	Not indicated
Exchange transfusion frequency	In approx. 2/3 rd patients	rare
Donor blood group	O Rh(negative) if possible, or ABO group specific with Rh negative	Group O only
Incidence of Late Anaemia	common	rare

Screening for Transfusion Transmitted Infections: Safe Transfusion and Minimizing the Risk

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Transfusion practice in pediatric and neonatal age is altogether different from the adult transfusion practice. Unfortunately, the proportion of multi-transfused patients in pediatric age group is higher than the adult age group patients. Thus risks associated with multiple transfusion, are reportedly higher than the adult patients.

In India, where there is high prevalence of HIV, HBV and HCV in general population and where substantial proportion of blood donations are replacement donations, the biggest challenge for transfusion services is ensuring safe blood transfusion despite improvements in donor selection and transfusion transmitted diseases (TTDs) testing. In India, serology based Enzyme Linked Immunosorbent (ELISA)/chemiluminescence assays, are the only mandatory testing method for the viral screening of blood donors. To reduce the risk of transmission of TTDs, nucleic acid amplification testing techniques (NAT) have been implemented in several countries. Nucleic acid amplification testing of blood donors has remarkably reduced the window period of detection of viral infections and is able to detect both window-period infections and chronic carrier with low viremia. The NAT screening of the blood donors has demonstrated several fold reduction in risk of transfusion transmitted infections. In India, a multi-centric trial conducted through

2004–2005 observed that there was significant number of cases in which the serological assays were not able to detect the state of infection while the NAT test demonstrated the presence of viral genome in blood donors (NAT yield). This condition of sero-non-reactivity and NAT positivity is called "NAT yield". In the last decade many more blood centers have initiated NAT testing routinely and two have published data with variable NAT yield rate ranging from 1 in 1528 to 1 in 610.

Among the thalassemia patients in India, the prevalence HCV is estimated to be as high as 45%, while two other studies from the western parts of India has estimated its prevalence as 16.7% and 17.5%, respectively. The prevalence of transfusion transmitted HBV and HIV has been estimated as 2% each. A study from the northern part of India has estimated the prevalence of HBV infection among the thalassemia patients as high as 5.7%. In developed world, NAT testing and high proportions of repeat voluntary donations have considerably improved the blood safety. However, in India, prevalence of these viral infections in donor and patient populations is high due to the lack of regular repeat voluntary blood donations and sporadic presence of second tier nucleic acid testing (NAT) of the blood donors at pan India level.

Studies indicate that 0.5-1.0% of anti-HBc-reactive; HBsAg-negative donations contain very low HBV. DNA levels, which are unlikely to be detected by NAT. This scenario where serology test shows positive result whereas NAT test demonstrates negative result is called as "seroyield."

Simply, the reasons for not doing are the high prevalence of anti HBcore antibody in general population and unavailability of clear-cut guidelines for anti HBcore total positive blood donors. In some other parts of World where it is being done along with NAT has formulated their own guideline for re-entry of blood donor based on their anti-HBs titer. But, still we don't have any guideline. Thus,

anti HBcore antibody testing vs. NAT testing still remains an enigma for blood bankers.

The risk of CMV transmission in pediatric transfusion population is approx. between 1 to 3%. In addition, manifestation remains quite variable from asymptomatic sero-conversion to death. Symptomatic CMV infection is uncommon in seropositive mothers but risk is higher in multitransfused low birth weight infants born to seronegative mothers. For this reason, it is recommended that low birth weight infants born to CMV seronegative mothers receive CMV reduced risk blood for transfusion. Since, practically it is difficult to get CMV- seronegative blood it is recommended to transfuse Leukocyte depleted blood components. Leukodepleted blood components can be used effectively to reduce the risk of transfusion transmitted CMV infection.

Transfusion-transmitted infections have been documented for several arboviruses, including West Nile and dengue viruses. Zika virus, a flavivirus transmitted primarily by *Aedes aegypti* mosquitoes that has been identified as a cause of congenital microcephaly and other serious brain defects became recognized as a potential threat to blood safety after reports from a 2013–2014 outbreak in French Polynesia. Blood safety concerns were based on very high infection incidence in the population at large during epidemics, the high percentage of persons with asymptomatic infection, the high proportion of blood donations with evidence of Zika virus nucleic acid upon retrospective testing, and an estimated 7–10-day period of viremia. At least one instance of transfusion transmission of Zika virus has been documented in Brazil after the virus emerged there, likely in 2014. Rapid epidemic spread has followed to other areas of the Americas, including Puerto Rico. In February 2016, the US Food and Drug Administration (FDA) issued recommendations for donor screening, donor deferral,

and product management to reduce the risk for transfusion-transmitted Zika virus in the United States and its territories. In addition to behavioral- and health-risk questionnaires for blood donors in all areas, FDA recommends deferrals for donors in unaffected areas who recently lived in or visited an area with active mosquito-borne transmission of Zika virus. For establishments collecting blood in areas with active, local mosquito-borne transmission, such as Puerto Rico and other U.S. territories, the recommendations include discontinuing local blood collections and importing blood units from unaffected areas of the continental United States unless one of the following is implemented: 1) Zika virus screening of locally collected blood donations or 2) treatment of locally collected units with pathogen-reduction technology (FDA-approved only for plasma and apheresis platelets). In Puerto Rico, interventions initially were limited to importation of blood units from unaffected U.S. areas and to treatment of plasma and apheresis platelets with pathogen-reduction technology; no Zika virus screening test was available. On April 3, 2016, Zika virus screening of locally collected blood donations was implemented using a newly developed nucleic acid test (NAT) (cobas Zika, Roche Molecular Systems, Inc., Pleasanton, California) authorized by FDA under an investigational new drug application (IND) (*6*). As part of the IND, plasma samples from blood donors are screened individually, and specimens with reactive results are subjected to additional testing including an alternate NAT and immunoglobulin M serology. A blood donation with an initial reactive result by NAT is regarded as a presumptive viremic donor, indicating an infected donor, and is interdicted and removed from the blood supply.

Worldwide, over the last 20 years, astounding reductions in the risk of viral infection via allogeneic blood have been achieved. As a result of this success, bacterial contamination of blood products has emerged as the greatest residual

source of transfusion-transmitted disease. Unlike red cell or whole-blood components, which are stored at 1 to 6°C, platelets are stored at 20 to 24°C to preserve function and survival. Such storage makes them an excellent growth medium for a broad spectrum of bacteria. Multiple aerobic-culture surveillance studies have demonstrated that 1 in 1,000 to 2,000 platelet units are bacterially contaminated. Thus, clinical sepsis would be expected in at least 1 in 10 to 2 in 5 contaminated transfusions (200 to 1,600 cases). National passive-reporting studies from the United States, the United Kingdom, and France. Ness et al. from Johns Hopkins University reported a fatality rate of 1 in 17,000 with pooled whole-blood-derived platelets and 1:61,000 with single-donor-apheresis-derived platelets. Similarly, University Hospitals of Cleveland observed a fatality rate of 1 in 48,000 per random platelet unit. The French BACTHEM study documented a fatality rate due to bacterially contaminated platelets of 7 per 10^6 (1 in 140,000).

How to transfuse blood in neonates and pediatric patients

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Introduction

Blood transfusion (BT) requirements and practices in neonates and pediatric patients are dissimilar to those in adult patients. This dissimilarity is mainly due to the difference in blood volume, hematologic values, maturity of organ systems, maturity of the immune system and physiologic response to hypovolemia and hypoxia in pediatric and adult patients.

When to order a blood transfusion?

There are two inherent variables in this question – (a) whether to order at all? (b) What exact time to order a BT?

To have an optimum efficiency of blood transfusion orders in an institution – 'Utilization Survey' should be done. This survey provides historic transfusion probability and is able to predict the need for transfusion for specific category and location (e.g. emergency department, oncology, etc.) of patients. Such a data has the potential to reduce unnecessary perioperative blood sampling, testing and associated costs. One study found that the intraoperative transfusion rate for 8620 non-cardiac pediatric procedures was only 2.78% [1]. Similarly, most typed and cross-matched (arranged) blood in pediatric emergency department may never be used [2]. Therefore, order a blood transfusion, only when there is a high probability of transfusion. Measuring crossmatch to

transfusion (CT ratio) ratio as well as patient to transfusion ratio (PT ratio) helps immensely in knowing the clinical conditions requiring a blood transfusion. Every institution needs to have its own data.

Once it is decided to order a BT, determine the exact time when it will be required. Ordering too early may lead to unavailability of blood at the time of real need (because of release by blood bank, expiry etc of arranged blood unit). If some manipulation of the blood unit is required, for e.g. irradiation, timing the delivery of blood becomes more critical as unit may not be fit for transfusion after a stipulated time. Similarly, too late ordering may lead to waste of precious and critical time in arranging the blood unit(s). Communication with the blood centre is the key to timing such blood ordering. Know the turnaround time for arranging and issuing the blood and order accordingly. Any special treatment of the blood unit, like irradiation, leukodepletion, volume depletion, etc. may require additional time and should be known to the ordering clinician and treating team.

How much to order?

Blood volumes of pediatric patients vary with body weight and gestational age (in neonates). Practices which decrease the need for repeated phlebotomy in this category of patients should be encouraged, like:

- Knowing exact sample quantity for the diagnostic tests and blood compatibility tests
- Planning tests and blood transfusions in advance;
- Considering in-line, ex-vivo tests, thus avoiding blood loss, etc

It is not essential to replace the volume of blood lost ml for ml, by blood transfusions. Rather the **target should be to achieve a pre-determined**

hemoglobin value in certain clinical situation. Full-term and ill preterm neonates specifically benefit by this method as they achieve lower levels of circulating fetal hemoglobin and an increased level of adult hemoglobin and thus a better oxygenation at the tissue level. However, since newborn do not tolerate hypovolemia well, it is essential to maintain (near) normovolemia in these small sized patients.

How to transfuse and to monitor

- Nursing team involved in pediatric BT should be trained for small volume transfusion and should be aware of signs and symptoms of an adverse reaction in this age group.
- Patient should be well prepared before blood is issued from the blood centre. It means, having a patent IV access, consent from the parents taken and pre-medication (if required) administered. Following points cannot be overemphasized:
    - Positive patient identification – by at least 2 identifiers; these should match on the blood unit, the compatibility report and an ID attached to the patient (e.g. wrist band).
    - Do not store blood units in ward/ ICUs
    - Commence BT within 30 minutes of issue from the blood bank and finish within 4-hour maximum.
    - If patient condition requires, use in-line warmer. Avoid IV line tubing's exposure to UV light when the patient is undergoing phototherapy.
    - Final patient identity checks before commencing the BT. It should be by 2 independent users – 1 doctor and 1 nurse or any other combination as per institutional policy.

- Observe patient before, during and after the BT for:
    - Check vitals,
    - Note any significant change in baseline vital parameters.
    - Observe for any obvious sign of a BT reaction. Most of the serious and severe reactions occur in the initial 15 minutes of the BT.

Treating team involved in administering blood transfusions should take care of the following issues:

- *Hypothermia* – Although in bigger children and adults, up to 4 units (which have attained room temperature) can be given without much consideration, it is not so in the neonates. Hypothermia should be avoided by using in-line warmers in all exchange transfusions and transfusions in sick neonates. Hypothermia can lead to metabolic acidosis, hypoglycemia, apneic events leading to hypoxia, hypotension and cardiac arrest.
- *Metabolic complications* – Acidosis and/ or hypocalcemia is a potential complication with large volume transfusions (including exchange transfusion). Hyperkalemia, although not a problem with simple/ top-up transfusions, can be troublesome with large (>20 ml/kg) transfusions. Washing of RBC units before transfusion or using units stored for less than a week is helpful to avoid hyperkalemia in large volume transfusions.
- *Ta-GVHD* – Transfusion associated graft versus host disease carries a high mortality (>90%) in patients experience this complication of BT. Although rare in general pediatric BT recipients, it is a distinct possibility in those with depressed immunological status, e.g. congenital immunodeficiency or with impaired cellular immunity. Irradiation of blood units before transfusion is recommended for all such patients.

Liberal versus restrictive transfusion policies

All prospective, randomized trials and retrospective analyses published since year 2000, support the use of a restrictive packed RBC transfusion policy in most clinical conditions in children [3]. Neonatal transfusions guidelines however, rely largely on "expert opinion" rather than experimental data. New data indicate that using a hemoglobin transfusion threshold of >7 g/dL does not yield improved outcomes. Furthermore, smaller studies have suggested that pediatric intensive care unit patients may be at an increased risk for morbidity and mortality when undergoing transfusion [4]. Latest research shows that neonates undergoing surgery are at increased risk of surgical site infections (SSIs) if they receive BT preoperatively [5].

Aliquoting of blood

Advances in medical science and neonatology have permitted the survival of extremely premature infants. These very low birth weight and extremely low birth infants require very small volume of blood components and thus the need to aliquot the standard blood unit. Small volume transfusion aliquots are usually made for packed RBCs, however, platelet and plasma units may similarly be aliquotted. Technique used for this aliquoting should ideally maintain the sterility of the mother blood unit. Some of the ways to achieve this is - special pediatric bags, sterile connection device and aliquoting under laminar air flow. If the integrity of the mother blood bag is breached than the packed RBC unit should be used within 24 hours if kept at 4-6 degree Celsius. Aliquoting has the following advantages:

- Prevents circulatory overload in small infants

- Limits donor exposure and thus decreases the chances of donor related risks.
- Avoid wastage of precious blood units, especially in rare group blood components
- If done in sterile manner, mother blood unit can be used till original expiry and thus saves repeated cost to the patient.

Additive solution (SAGM)

Additive solutions (AS) are added to packed RBCs and platelets to extend the shelf life of these blood components. However, there were many 'theoretical' concerns with regards to the safety of adenine and mannitol in these additive solutions as both of these are known to be potential nephrotoxic substances. Mannitol in addition is also a potent diuretic and may alter the fluid dynamics in small infants especially preterm infants. Most evidences suggest that small volume (5-15 ml/kg) transfusions containing these AS are safe in neonates. Nonetheless, although there are no controlled studies and practices vary, it is recommended that for infants with renal and/ or hepatic insufficiency, the AS be removed from the packed RBC units. Similarly, safety of AS in large volume (15-20 ml/kg) transfusions is still not known. Recently a lot of metabolomics studies have been done inconclusively to understand the effect of AS on infants and therefore its use with caution is recommended.

Age of blood

Of late questions have been raised and studies done to address the issue of clinical consequences of transfusing packed RBC units that have been stored for 'some' time in blood bank, i.e. older blood units. There have been studies which

proved that older blood is deleterious for the recipient while some other studies found no difference in ‘fresher’ versus ‘older’ blood. While the debate is still going on, The Cochrane Database of Systematic Reviews published in 2015 that no clear differences in the primary outcome - death - were noted between 'fresher' and 'older' or 'standard practice' red blood cell units [6]. Several factors precluded firm conclusions about the clinical outcomes of transfusing red blood cell units that have been stored for different periods of time before transfusion, including differences in clinical population and setting, diversity in the interventions used, methodological limitations and differences in how outcomes were measured and reported. ARIPI trial (Age of Red blood cells In Premature Infants – a randomized controlled trial conducted in Canada) was also criticized for its poor validity measures and thus it can be said that a causal relationship is yet to be firmly established between morbidity and transfusion of older RBC units in infants.

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UPDATE ON TRANSFUSION GUIDELINES FOR FETUS, NEONATES AND PEDIATRICS

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Summary of the recent Guidelines from the British Committee for Standards in Haematology (BCSH) guidelinse on transfusion in neonates and older children and the JPAC: Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services

Much of the evidence about optimal transfusion practices in neonates, infants and children is extrapolated from adult trials considering the difficulties associated with conducting such trials in children and collecting the evidence base for optimal practice in this age group. Nevertheless, in the recent eras many areas of clinical practice in this area have become much clearer and refined.

The potential risks and benefits must always be considered when making the decision to transfuse children but there is a lack of high-quality research evidence on which to base guidelines. 74% of transfused patients received a single red cell component during their admission, suggesting that many transfusions might be avoidable. There is a higher incidence of serious adverse events related to transfusion in children.Randomised controlled trials suggest that restrictive Hb transfusion thresholds are safe in clinically stable neonates requiring small volume 'top-up' transfusions. Low platelet counts are common in sick neonates but the relationship of thrombocytopenia to serious bleeding and appropriate triggers for

platelet prophylaxis remain uncertain.A significant proportion of FFP transfusions in patients inNICUs) and PICUs are given to non-bleeding patients with minor abnormalities in coagulation parameters of uncertain significance.

INTRAUTERINE TRANSFUSIONS (IUT)

IUTs are invasive procedures with a risk of fetal death of 1-3% per procedure and up to 20% for hydropic fetuses, depending on the underlying etiology of the anemia. Red cell IUTs are performed for the treatment of fetal anemia, most commonly due to hemolytic disease of the fetus and newborn (HDN) caused by anti-D, -c or -K, or fetal parvovirus infection. IUT procedures may be required every 2-3 weeks, the frequency minimized by transfusing red cells of high Hct and the maximum volume. The aim of each transfusion is to raise the Hct to 0.45. After delivery, neonates with HDN following IUTs may become anaemic due to haemolysis or bone marrow suppression and require monitoring for several weeks post-delivery. Red cells for IUT are irradiated to prevent transfusion-associated graft-versus-host disease and have only a 24-hour shelf life following irradiation. Maternal blood should not be used for IUTs because of the significant risk of TA-GvHD

Intrauterine platelet transfusions are usually given to correct fetal thrombocytopenia caused by platelet alloimmunization (NAIT). In most cases fetal transfusion can be avoided by treating the mother with IVIg and/or corticosteroids. Compatible platelets should be available at the time of diagnostic fetal sampling for NAIT, in order to prevent fetal haemorrhage if severe thrombocytopenia is detected, the risk of which increases substntially with platelet counts $<50 \times 10^9$/l.

Transfusions to Neonates

Red cell transfusions

The majority of preterm neonates receive at least one red cell transfusion as they frequently become anemic, partly caused by phlebotomy. Neonatal transfusions are usually given as small-volume "top-up" transfusions. Potential benefits of transfusion need to be weighed against possible adverse outcomes. In addition to the standard risks associated with transfusion, necrotizing enterocolitis may follow neonatal transfusion, although a causal link has not been demonstrated. The use of pedipacks reduces donor exposure for these multiply transfused preterm infants.

Exchange transfusion is performed usually with group O, and should also be compatible with any maternal antibody, preferably irradiated and before the end of Day 5 following donation. With regards to partial exchange transfusion, there is no evidence of long-term benefit and the procedure has been associated with up to an11-fold increase in risk of NEC. Normal saline should be used if at all hemodilution is undertaken.

Small volume transfusion

The majority of red cell transfusions to neonates are top-up transfusions of small volumes given to replace phlebotomy losses in the context of anaemia of prematurity, particularly for preterm VLBW neonates. It seems prudent to use top-up transfusion volumes of 15 ml/kg for non-bleeding neonates in most cases. Three randomized studies addressing 'restrictive' vs 'liberal' transfusion thresholds for neonatal red cell transfusion in VLBW babies have been published. Overall there is no evidence that restrictive transfusion policies have a significant impact on

mortality or major morbidity. Suggested red cell transfusion thresholds for very preterm neonates are given in Table I. Erythropoietin (EPO) may reduce red cell transfusion requirements in neonates but its effect appears to be relatively modest whether given early or late and may be considered for preterm babies of parents who object to transfusion but may not prevent the need for transfusion

Platelet Transfusion

The use of platelet transfusions for neonates with thrombocytopenia and active bleeding is considered appropriate, but there is great variation in the wider use of platelet transfusions for prophylaxis in the absence of bleeding. Severely thrombocytopenic neonates with suspected Neonatal alloimmune thrombocytopenia (NAIT) should receive platelet transfusions at thresholds depending on bleeding symptoms or family history. The suggested threshold of 25 x 10^9/l in the absence of bleeding is the same as that for neonates without NAIT, but it is acknowledged that this is not evidence- based. If HPA-1a/5b-negative platelets are unavailable or ineffective in producing a platelet rise random donor platelets and/or IVIg may be used, which may reduce the need for platelet transfusions until spontaneous recovery in platelet count occurs 1-6 weeks after birth.

FFP & Cryoprecipitate

Prophylactic use of FFP, including prior to surgery, is of unproven benefit and uncertainty is compounded by the difficulty in defining a significant coagulopathy in this age group. A large RCT reported by the Northern Neonatal Nursing Initiative reported no benefit from prophylactic FFP given to neonates to prevent ICH

Infants and Children

More than half of pediatric transfusions are given to hematology/oncology patients. Other frequently transfused groups include those on PICU or undergoing cardiac surgery. Randomized controlled trials of different red cell transfusion policies have mostly been conducted in adults and children and systematic reviews indicate that liberal transfusion thresholds are not associated with benefit and may be associated with harm. Those on chronic transfusion regimens should have an extended red cell phenotype/genotype particularly those with haemoglobinopathies, but also those with congenital dyserythropoietic anaemia, aplastic anaemia and other bone marrow failure syndromes. This should be performed prior to, or as soon as possible after, commencing regular transfusions.

Red cell transfusion

Based on current evidence it is recommended to use an Hb threshold of 70 g/l pre-transfusion in stable non-cyanotic patients (1B). If the child is unstable or has symptomatic anaemia a higher threshold may be considered (2C). A threshold of 70 g/l may be insufficient in the long-term to support normal growth and development in non-haemoglobinopathy children with chronic anaemia. Practice is consensus-based, and for patients with Diamond-Blackfan anaemia, transfusion to keep the Hb above 80 g/l has been recommended. Tranexamic acid should be considered in all children undergoing surgery where there is risk of significant bleeding (1B). Red cell salvage should be considered in all children at risk of significant bleeding undergoing surgery and where transfusion may be required, providing there are appropriately trained staff (2C).

Platelet Transfusion

Given a lack of studies in paediatrics, recommendations for platelet transfusions in critically ill children or those with haematological/oncological malignancies who develop severe thrombocytopenia are drawn from the wider adult literature and recommendations (2C). As pragmatic guidance, it is suggested that for most stable children prophylactic platelet transfusions should be administered when the platelet count is below 10 x 10^9/l, excluding patients with immune thrombocytopenia, thrombotic thrombocytopenic purpura/haemolytic uraemic syndrome and heparin-induced thrombocytopenia who should only be transfused with platelets for life- threatening bleeding (2B).

FFP and cryoprecipitate may be administered either therapeutically for the management of bleeding or prophylactically. There is very little evidence of benefit from FFP administration in many settings where it is currently used. The major indications for cryoprecipitate transfusion in infants and children are DIC with bleeding, bleeding following cardiac surgery and major haemorrhage. There remains controversy over the fibrinogen transfusion threshold for cryoprecipitate transfusion. There is no evidence to alter the previously recommended fibrinogen threshold of 1.0 g/l outside the setting of major bleeding. When indicated transfuse FFP volumes of 15-20 ml/kg, using the higher volumes particularly in bleeding patients, and ensure monitoring of clinical outcome. Transfuse cryoprecipitate volumes of 5-10 ml/kg, Prophylactic FFP should not be administered to non-bleeding children with minor prolongation of the prothrombin time(2B)/activated partial thromboplastin time including prior to surgery, although it may be considered for surgery of critical sites (2C).

Table I. Suggested transfusion thresholds for preterm neonates

Postnatal age	Suggested transfusion threshold Hb (g/l)		
	Ventilated	On oxygen/ NIPPV†	Off oxygen
First 24 h	< 120	< 120	< 100
≤ Week 1 (day 1-7)	< 120	< 100	< 100
Week 2 (day 8 -14) ≥ week 3 (day 15 onwards)	< 100	< 95 < 85	< 75

Table II. Suggested thresholds of platelet count for neonatal platelet transfusion

Platelet count (x 10^9/l)	Indication for platelet transfusion
< 25	Neonates with no bleeding (including neonates with NAIT if no bleeding and no family history of ICH).
< 50	Neonates with bleeding, current coagulopathy, before surgery, or infants with NAIT if previously affected sibling with ICH
< 100	.Neonates with major bleeding or requiring major surgery (e.g. neurosurgery)

NAIT, neonatal alloimmune thrombocytopenia; ICH, intracranial haemorrhage

Table III Suggested thresholds of platelet counts for platelet transfusion in children

Platelet count ($x\ 10^9$/l)	Clinical situation to trigger platelet transfusion
< 10	Irrespective of signs of haemorrhage (excluding ITP, TTP/HUS, HIT)
< 20	Severe mucositis Sepsis Laboratory evidence of DIC in the absence of bleeding* Anticoagulant therapy Risk of bleeding due to a local tumour infiltration Insertion of a non-tunnelled central venous line
< 40	Prior to lumbar puncture**
< 50	Moderate haemorrhage (e.g. gastrointestinal bleeding) including bleeding in association with DIC Surgery, unless minor (except at critical sites) - including tunnelled central venous line insertion
< 75 -100	Major hemorrhage or significant post-operative bleeding (e.g. post cardiac surgery) Surgery at critical sites: central nervous system including eyes

* note: routine screening by standard coagulation tests not advocated without clinical indication; for laboratory evidence of DIC

** It is accepted that prior to lumbar puncture some clinicians will transfuse platelets at higher counts (e.g. 50 x 109/l) in clinically unstable children, non ALL patients, or for the first LP in newly-diagnosed ALL patients to avoid haemorrhage and cerebrospinal fluid contamination with blasts, or at lower counts (≤ 20 x 109/l) in stable patients with ALL, depending on the clinical situation These practices emphasize the importance of considering the clinical setting and patient factors.

SPECIAL SCENARIOS IN PEDIATRIC TRANSFUSION

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There are special issues related to pediatric transfusion in special case scenarios. Some problems related to these are due to the unique pathophysiology of a child and the fact that admitted children are more sick and complex, furthermore they have a long life ahead of them. This is compounded by lack of data as most guidelines have extrapolated from adult clinical trials. More studies on transfusion therapy for pediatric patients, and separately on neonates, are urgently required.

Some major differences related to transfusion in children and important case scenarios are-

- Metabolic concerns, -hyperkalemia, additive/preservative solutions
- Testing differences- newborn screen
- Smaller components/Aliquots
- Infectious -CMV, Zika etc
- Special situations- ECMO, PICU, Surgery, Hemoglobinopathies, HSCT and hem-onc cases, AIHA, exchange transfusions, and intrauterine transfusions

Metabolic issues:

Red blood cells (RBCs) lose viability and function with increasing duration of storage. Other biochemical changes include -decrease in pH, increase in plasma K+, decrease in ATP, an important decrease in 2,3-DPG and increased % of hemolysis. These metabolic factors play an important role particularly in newborns or in

infants and children receiving large volume transfusions as in exchange or massive transfusions for trauma. Solute load from anticoagulant solutions, result in osmotic diuresis. Alterations of cerebral microcirculation and resultant periventricular hemorrhage

The immunity of newborns or children with hemato-oncology conditions are compromised and care to provide irradiated and leuco depleted products is necessary to prevent transfusion-associated viral diseases and graft-versus-host-disease resulting from passive transfer engraftable lymphocytes.

Dengue:

In dengue patients' many misconceptions regarding need and necessity of platelet transfusions have been rampant. This was because the patho-physiology was not well understood. Massive bleeding may occur without prolonged shock due to -acetylsalicylic acid, ibuprofen, or corticosteroids. Bleeding may occur in patients due to peptic or duodenal ulcers. Acute liver and renal failure and encephalopathy may be present in severe shock; described even in the absence of severe plasma leakage or shock. Most deaths from dengue occur in patients with profound and prolonged shock resulting from plasma leakage & complicated by bleeding and/or fluid overload. Patients with severe plasma leakage may not have shock if prompt fluid replacement given. May manifest with respiratory distress due to massive pleural effusion and ascites, exacerbated by unguided intravenous fluid therapy. With new data the WHO has provided guidelines for those with severe dengue disease. A case of severe dengue is defined as a patient with either-

1. severe plasma leakage that leads to shock (dengue shock) and/or fluid accumulation with respiratory distress;
2. severe bleeding;
3. severe organ impairment.

Management- Mainstay is fluid management. The hematocrit provides a useful guide in deciding adequacy of fluid management, check for hypotension. In case of bleeding give packed red blood cells or whole blood to prevent shock.

"The action plan for the treatment of hemorrhagic complications is as follows: Attempts made to stop bleeding e.g. severe epistaxis nasal adrenaline packing. If blood loss can be quantified, should be replaced. If not, give aliquots of 5−10 ml/kg of –young packed red cells or 10−20 ml/kg of whole blood (FWB) at an appropriate rate and observe clinical response. Important that fresh whole blood or fresh red cells are given. Oxygen delivery at tissue level is optimal with high levels of 2,3 diphosphoglycerate (2,3 DPG). Stored erythrocytes lose 2,3 DPG, low levels of which impede the oxygen-releasing capacity of hemoglobin, resulting in functional tissue hypoxia. A good clinical response includes improving hemodynamic status and acid-base balance. repeat if overt blood loss or no appropriate rise in hematocrit Blood transfusion is only indicated in dengue patients with severe bleeding." (WHO-TDR)

There is no evidence that supports the practice of transfusing platelet concentrates and/or fresh-frozen plasma for severe bleeding in dengue observational studies show that transfusions of platelet concentrates, apheresis platelets and fresh frozen plasma in dengue were not able to sustain the platelet counts and coagulation profile. However, in the case of massive bleeding, they often exacerbate the fluid overload.

Pediatric ICU:

TRIPICU study results suggest that a threshold Hb of 7 g/dL can be safely applied to stable critically ill children. Recommendations of AABB regarding blood transfusion in children, are general and do not address the critically ill child. Many ICU transfuse to keep Hb >9-10g/dl, though paucity of data to support this.

However, this will depend on the case, hemodynamic, respiratory status, other disease, meds and whether intervention like surgery planned.

Hemoglobinopathies:

Baseline extended red cell phenotype/genotype particularly those with Sickle cell and Thalassemia (TM and TI) patients, but also those with congenital dyserythropoietic anemia, aplastic anemia and other bone marrow failure syndromes. This should be performed prior to, or as soon as possible after, commencing regular transfusions.

These patients should receive Phenotype matched blood-minor antigens-CEK. All children starting regular transfusions should be vaccinated against hepatitis B as early as possible. Thalassemia major patients (TIF guidelines)-need to be transfused to keep pre- transfusion hb>9g/dl. A threshold of 7.0 g/l may be insufficient in the long-term to support normal growth and development even in non-hemoglobinopathy children with chronic anemia. Practice is consensus-based for patients with Diamond-Blackfan anemia, transfusion to keep the Hb above 8.0 g/l has been recommended. For chronically transfused pediatric patients, monitoring growth and development are important outcome measures of efficacy.

Bleeding disorders DIC and inherited bleeding conditions:

Commonest reasons for FFP is for the correction of minor/moderate abnormalities of the PT/INR in non-bleeding patients (Stanworth et al, 2011), prior to surgery or other invasive procedures. Minor abnormalities of the PT or INR are poorly predictive of surgical bleeding and effect of FFP to normalize PT/INR is poor. Evidence this approach is incorrect and FFP exposes patients to unnecessary risk. Two studies in adults and children assessing the effect of FFP in patients with INRs 1.1–1.6 and 1.1–1.85 found that FFP failed to significantly improve the INR in the majority of cases and no relationship with bleeding Abnormalities of the PT or APTT

should however be appropriately investigated. Cryoprecipitate similarly should not be given to correct mild degrees of hypofibrinogenaemia in non-bleeding patients.

Data on blood product support in children with DIC limited largely extrapolated from adult practice. FFP useful in patients actively bleeding and /or prolonged PT/APTT (> 1.5 times midpoint of normal range) or a decreased fibrinogen (< 1.5 g/l). Need account rate of fall of fibrinogen and severity of bleeding. FFP contains all coagulation factors and fibrinogen, reserve cryoprecipitate for persistent hypofibrinogenaemia despite FFP.

May use cryoprecipitate as the initial treatment if fibrinogen is very low (e.g. 0.5 g/l), dropping rapidly, or major hemorrhage. FFP and cryoprecipitate should not be administered on basis of laboratory tests alone, but should be restricted to those with bleeding or prior to invasive procedures. Exception children with acute promyelocytic leukemia (APML). Thrombocytopenia co-exists with a coagulopathy in DIC, platelets should be administered to maintain a platelet count > 50 x 10^9/l.FFP and cryoprecipitate may be administered either therapeutically for the management of bleeding or prophylactically. There is little evidence of benefit from FFP administration in many settings where it is currently used and significant variation in practice is seen. The major indications for cryoprecipitate transfusion in infants and children are DIC with bleeding, post cardiac surgery and major hemorrhage. There is no evidence to alter the previously recommended fibrinogen threshold of 1.0 g/l outside the setting of major bleeding. Same threshold levels recommended for inherited hypofibrinogenemia (BCSH, 2014b) but where there is rapid consumption e.g. in DIC or major hemorrhage, higher target thresholds for therapy may be recommended.

Inherited bleeding disorder-Where a specific coagulation factor concentrates is available; these are the treatment of choice for patients with inherited bleeding disorders. FFP and cryoprecipitate should not be used (BCSH, 2014b). Some exceptions are -Factor (F) V deficiency is the only single factor deficiency where a factor concentrate does not currently exist; in this situation, pathogen-inactivated plasma, e.g. SD FFP is recommended. This can also be used together with FVIII concentrate in the management of combined FV & FVIII deficiency. Or while awaiting confirmation of a suspected inherited factor deficiency, FFP may be used for acute management. In suspected hemophilia (A or B, doses of 20 ml/kg are used.

FFP should not be used in the management of inherited factor deficiencies other than in a few exceptional circumstances where specific factor concentrates are not available (1B). Cryoprecipitate should not be used for congenital hypofibrinogenaemia unless fibrinogen concentrate is unavailable (1C). In India FX, XII XI, fibrinogen concentrates not available, hence FFP is used for many conditions including FXII deficiency**.**

Other special case scenarios will be discussed.

Important reading:

BCSH guidelines 2016

Pediatric Transfusion Practices

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Pediatric transfusion is a complex area of medicine covering a wide age range from intrauterine life to young adults. The prescriber must balance the risks and benefits of transfusion in each age group and be aware of the indications for special components. The routine transfusion practices include Red Blood Cell (RBC), Fresh Frozen Plasma (FFP), platelet and cryoprecipitate and special transfusion practices in this population would include granulocyte transfusion and transfusion of irradiated blood components.

Fetal Transfusion Practices [1-2]

The most common indications for intrauterine transfusion (IUT) are red cells for prevention and treatment of fetal anemia due to Hemolytic Disease of Fetus and Newborn (HDFN) or parvovirus infection and platelets for Neonatal Allo-Immune Thrombocytopenia (NAIT).

Intrauterine transfusion of RBC for HDFN

The objective of red cell Intra Uterine Transfusion (IUT) is to prevent or treat life-threatening fetal anemia (hydrops fetalis) and allow the pregnancy to continue to a stage where a viable baby can be delivered (ideally at least 36-week gestation). Good multidisciplinary communication is essential between fetal medicine units undertaking the IUTs, the hospital transfusion laboratory and their counterparts in the hospital where the baby will be delivered. To balance the

competing risks of fetal anemia and the hazards of invasive IUT procedures, the transfusion program is started as late as possible and the frequency of transfusion is reduced by giving the maximum safe volume of a special red cell component with a high hematocrit.

Special requirements of RBC include:

- Hematocrit 70-85%
- In citrate phosphate dextrose (CPD) anticoagulant (theoretical risk of toxicity from additive solutions)
- Leucocyte-depleted
- Less than 5 days old (to avoid hyperkalemia)
- Cytomegalovirus (CMV) antibody negative
- Sickle screen negative
- Irradiated to prevent Transfusion Associated- Graft versus Host Disease (TA-GvHD; shelf life 24 hours)
- Usually group O with low-titre hemolysins (or ABO identical with the fetus)
- RhD and Kell negative and red cell antigen negative for maternal alloantibodies
- Indirect anti-globulin test (IAT) crossmatch compatible with the mother's plasma

Intrauterine transfusion of Platelets for NAIT

The IUT of platelets is used in the treatment of severe fetal thrombocytopenia due to platelet alloimmunisation. Nearly all cases are caused by antibodies to HPA-1a (80–90% of cases), HPA-5b or HPA- 3a. Management is influenced by any history of previous fetal losses and their timing. Fetal blood sampling and platelet transfusion carry a significant risk of life-threatening

hemorrhage (suitable platelets should always be immediately available when fetal blood sampling is performed).

Special requirements of Platelets include:

- HPA-compatible with maternal alloantibody
- Hyper-concentrated (platelet count- at least 2000×10^9/L)
- Irradiated
- CMV negative

Red Cell Transfusion Recommendations [2-3]

1. Transfusion volumes of 15 ml/kg are generally recommended for non-bleeding neonates.
2. The routine use of EPO or darbepoeitin-alfa is not recommended in preterm infants to reduce transfusion
3. There is a risk of hyperkalemia following large volume transfusions, particularly if infused rapidly, so it is recommended that red cells for large volume neonatal and infant transfusions are used before the end of Day 5 following donation (and within 24 h of irradiation) in order to reduce the risk in the recipient.
4. All large volume transfusions should be given via a blood warmer to avoid the development of hypothermia and the core temperature should be monitored.
5. Use an Hb threshold of 7g/dl pre-transfusion in stable non- cyanotic patients. If the child is unstable or has symptomatic anemia a higher threshold may be considered. Patients with chronic anemia due to red cell aplasia may require an Hb threshold of 8g/dl.
6. Tranexamic acid should be considered in all children undergoing surgery where there is risk of significant bleeding

Platelet Transfusion Recommendations [3]

Suggested thresholds of platelet counts for platelet transfusion in neonates and children

Platelet count ($x 10^9$/ l)	**Indication for Transfusion**
Neonates	
<25	Neonates with no bleeding
<50	Neonates with bleeding, current coagulopathy, before surgery, or infants with NAIT
<100	Neonates with major bleeding or requiring major surgery (e.g. neurosurgery)
Children	
<10	Irrespective of signs of hemorrhage
<25	Anticoagulant therapy ; Sepsis
<50	Prior to lumber puncture; Moderate hemorrhage; Minor surgery
<100	Major hemorrhage or significant post-operative bleeding (e.g. post cardiac surgery)

FFP and Cryoprecipitate Transfusion Recommendations [3]

- Transfuse FFP volumes of 15–20 ml/kg, using the higher volumes particularly in bleeding patients, and ensure monitoring of clinical outcome. However, care should be taken to avoid volume overload, particularly in vulnerable patients.
- Transfuse cryoprecipitate volumes of 5–10 ml/kg, using the higher volumes particularly in bleeding patients, and ensure monitoring of clinical outcome and fibrinogen levels.
- Prophylactic FFP should not be administered to non- bleeding children with minor prolongation of the pro- thrombin time (PT) /activated partial thromboplastin time (APTT) including prior to surgery, although it may be considered for surgery to critical sites.
- Prophylactic cryoprecipitate should not be routinely administered to non-bleeding children with decreased fibrinogen including prior to surgery. It may be considered for fibrinogen <1 g/l for surgery at risk of significant bleeding or to critical sites (2C).
- FFP may be beneficial in children with DIC who have a significant coagulopathy (PT/APTT >1.5 times midpoint of normal range or fibrinogen <1.0 g/l) associated with clinically significant bleeding or prior to invasive procedures. Cryoprecipitate may be given if the fibrinogen is <1.0g/l despite FFP, or in conjunction with FFP for very low or rapidly falling fibrinogen.
- Urgent plasma exchange with SD FFP is indicated for TTP and some forms of atypical HUS.
- FFP infusion (in the acute phase) and intermediate purity Factor VIII should be used to treat congenital TTP.

Special pediatric transfusion practices

Irradiated blood component indications [4-5]

- All donations from first- or second-degree relatives and all HLA matched components should be irradiated, even if the patient is immuno- competent.
- Blood for intrauterine transfusion (IUT) and neonatal exchange transfusion (ET) if there has been a previous IUT should be irradiated.
- Routine 'top-up' transfusions of premature or term infants require irradiated blood components if either there has been a previous IUT, in which case irradiated components should be administered until 6months after the expected delivery date (40 weeks' gestation), or the donation has come from a first- or second-degree relative.
- Platelets transfused in utero to treat alloimmune thrombocytopenia should be irradiated
- All granulocyte components should be irradiated before issue and transfused with minimum delay.
- All recipients of allogeneic hemopoietic stem cell transplantation (SCT) must receive irradiated blood components from the time of initiation of conditioning chemo-radiotherapy. If chronic GvHD is present or if continued immuno-suppressive treatment is required, irradiated blood components should be given indefinitely.
- Allogenic blood transfused to bone marrow and peripheral blood stem cell donors 7 days prior to or during the harvest should also be irradiated.
- All children with Hodgkin lymphoma at any stage of the disease should have irradiated red cells and platelets for life.

Granulocyte Transfusion [6]

Why do we need Granulocyte Transfusion?

- Despite the use of broad spectrum antibiotics and colony-stimulating factors, frequent and prolonged periods of neutropenia
- Shifts in the emerging pattern of pathogens, antibiotic resistance and changes in host immune patterns has resulted in increased incidence of infection

Limitations

- Paucity of appropriate matched donors
- Availability of marrow stimulation cytokines like G-CSF

Granulocytes for transfusion are produced using one of two means: by apheresis or as a component derived from whole blood donations (Bashir et al, 2008). Granulocyte transfusions may be requested for use in neutropenic hematology/oncology/immunology patients with refractory infection or at high risk of developing severe infection. Most patients prescribed granulocyte transfusions are those with cancer-related neutropenia, who are receiving myeloablative chemotherapy with or without hemopoietic stem cell rescue. Recent studies with variable or promising, but overall inconclusive, results have been reported in children (Sachs et al, 2006). The exact role of granulocyte transfusions (whether derived from whole blood or collected by apheresis) therefore remains unclear.

Recommendation:

Granulocyte transfusions may be considered for treatment of refractory infections in children with:

- A resistant severe clinical infection (neutrophil count <0.2–0.5 $X10^9$/l) that has shown no response to aggressive antibiotic treatment with no recovery in neutrophil count expected for more than 7 days.

- Severe infections, e.g. systemic fungal infections/necrotizing fasciitis or severe neutropenic typhlitis progressing on appropriate anti-fungal or broad-spectrum antibiotics, (neutrophil count <0.2–0.5 $X10^9$/l), and no recovery in neutrophil count expected for more than 7 days.

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Therapeutic apheresis and therapeutic Plasma Exchange in Paediatrics

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Therapeutic apheresis is the procedure to remove pathological cellular or plasma components from the patient as a treatment modality in various clinical conditions. The few commonly performed procedures are plasma exchange, leukapheresis, erythrocytapheresis, peripheral blood stem cell harvest and thrombocytapheresis. Though these procedures are widely performed in adult patients however, they are indicated in various pediatric disorders as well. These procedures are technically difficult in pediatric patients because of poor patient co-ordination, low blood volume, increase volume shifts during procedures and thus, these procedures demand special considerations in pediatric patients.

Special considerations in application of therapeutic apheresis to paediatric patients

To ensure safe and effective treatment of pediatric patients, careful attention should be paid to four areas: technical/procedural, vascular access, anticoagulation and psychosocial aspects.

A. **Technical/Procedural Considerations**: Extracorporeal Blood Volume- Current apheresis equipments are generally designed for adults rather than infants and young children. To perform pediatric procedures safely, one must be familiar with the physical characteristics of apheresis instruments including

Extracorporeal blood volume (ECV), patient's clinical condition and Total blood volume (TBV). ECV varies depending on the type of equipment and even with the same equipment, may depend on the type of procedure. Regardless of the type of instrument, the ECV will represent a larger fraction of TBV in a child than an adult, thus resulting in a greater blood volume shifts in children. To estimate the degree of volume shift, the TBV of the child and ECV of cell separator should also be estimated.

In children ECV should not exceed 15% of TBV, if so priming saline may be infused to patient without diverting. The children who have low hematocrit level (less than 20%), red cell priming may be considered to prevent sudden hypoxic state due to decline in intravascular haematocrit.

B. **Replacement Fluid**-In therapeutic plasmapheresis (plasma exchange) procedure large volume of patient's plasma is retained and it has to be replaced with fluids to maintain adequate intravascular volume and oncotic pressure during and post procedure. Albumin (5%) with normal saline is generally preferred however, FFP is recommended in plasma exchange in patients with thrombotic thrombocytopenia (TTP) or hemolytic urinary syndrome (HUS).

C. **Calcium supplementation**- Can reduce chances of hypocalcemia. So, addition of 10% calcium gluconate (10 ml per litre of return fluid i.e 1% infusion) should be considered in TPE.

D. **Vascular access**- Apheresis procedures require high blood flow rates which can be achieved by peripheral venous access using one or two large bore needles (16-18 gauge) in adults. However, in children, central catheters are needed. A central venous catheter (CVC) should be inserted in subclavian, internal jugular or femoral vein. Internal jugular and subclavian catheter insertions carry increased risk of pneumothorax, hemothorax and air embolism whereas

femoral catheterization require less specialized skills and is relatively safe in pediatric patients.

E. **Anticoagulants**- Citrate, heparin or combination of both is used during apheresis to prevent coagulation in the extracorporeal circuit. Citrate prevents coagulation by binding ionized calcium which is required in coagulation cascade. Citrate is metabolized by liver, if liver functions are deranged or if citrate infusion exceeds its metabolic rate, transient hypocalcemia may occur which may present as mild paresthesia (perioral, distal extremities), gastrointestinal symptoms, hypotension or in most extreme cases, cardiac dysrhythmias can occur. Risk of citrate toxicity is more in patients where FFP is used as replacement fluid as it has four times more citrate than in 5% albumin.

Citrate is available in three forms- ACD-A with concentration of citrate as 113 mmol/l, ACD-B with citrate concentration of 68 mmol/ l and concentrate of Trisodium citrate having citrate as 136 mmol/l. ACD-A is commonly used. Heparin results in systemic anticoagulation. When used alone, can cause platelet aggregation during cytapheresis procedures. Heparin is required less, when used in combination with citrate as in LDL apheresis and large volume procedures like peripheral blood stem cell harvest or in patients with deranged liver function tests.

F. **Psychological aspects**- Use of distraction, involvement of child life specialists and Parental/familial involvement is required to make the child comfortable and co-operative during the procedure.

Common indications for therapeutic plasma exchange and apheresis in pediatric patients as per latest American Society of Apheresis guidelines are listed in table-1 & 2.

Table 1. Indications for Cytapheresis

CATEGORY I	CATEGORY II
• Acute /Chronic graft vs host disease: *Photopheresis* • Erythrocytosis/polycythemia vera: *RBC depletion* • Hyperparasitemia, babesiosis: *RBC Exchange* • Symptomatic Thrombocytosis: *Platelet depletion* • Familial hypercholesterolemia: *LDL Apheresis* • Hyperleukocytosis: *Leukocytapheresis* • Lipoprotein (a) hyperlipoproteinemia:*LDL Apheresis* • Phytanic acid storage disease (Refsum's disease): *LDL Apheresis*	• Cutaneous T-cell lymphoma: *Photopheresis* • Familial hypercholesterolemia: *Lipid absorption* • Sickle cell disease: *RBC Exchange* • Polycythemia vera; erythrocytosis: *Erythrocytapheresis* • Hereditary hemachromatosis: *Erythrocytapheresis*

Adverse Events

The commonly encountered adverse events are related to Patient, Cell Separator or Disposable kit. There are few data on the frequency of adverse effects in pediatric patients undergoing therapeutic apheresis; reported rates range widely from 4% to 55% of procedures. In study by Michon et al minor adverse reactions, were reported in 55% of procedures in 82% of patients. The most frequent complications were hypotension, symptomatic hypocalcemia, allergic reactions, catheter-related adverse effects, and severe anaemia. Hans et al has shown much

lower (7.69%) incidence of adverse events in 30 pediatric patients of atypical Hemolytic Uremic Syndrome (aHUS) and recommended early initiation of TPE in atypical HUS as reported earlier by Sharma et al from same centre.

Three types of complications appear to be more common and serious in children than in adults and are sometimes difficult to recognize at an early stage. These are hypovolemia, hypocalcemia and iron deficiency anemia resulting from chronic iatrogenic blood loss during long-term apheresis.

1. *Hypocalcemia*- In pediatric patient's hypocalcemia often manifest as acute episodes of abdominal pain and vomiting, agitation, pallor and hypotension. Hypotension can be managed by administration of a bolus of saline or colloid solution. To prevent ionized hypocalcemia during TPE, especially with an ACD-A infusion rate higher than 0.8mL/minute/L of TBV with the Spectra, 2 to 3 mL of 10% calcium gluconate can be added to each 250-mL bottle of 5% albumin.
2. *Hypovolemia*- Vasovagal reactions are common in children and can be managed by interrupting the procedure, elevating the legs and distracting the child's attention from the procedure; these manoeuvres usually allow resumption of the procedure. However, with new generation of cell separators which are used these days, procedures remain isovolemic.
3. *Iron deficiency anemia*- Small quantity of red cells remains in the disposable set at the completion of a procedure even after rinse back. In infants and smaller children sometimes rinse back cannot be done to prevent fluid overload. This loss may be significant for infants and smaller children who require several sessions of therapeutic apheresis procedure and may develop iron deficiency anemia. Iron supplementation (ferrous sulfate at 3-

4 mg/kg/day e when hematocrit falls more than 5% from baseline) and regular haematocrit monitoring is recommended in pediatric patients.

Other adverse events-

4. *Electrolyte imbalance*: Large volume apheresis like autologous peripheral blood stem cell collection, therapeutic leukapheresis and plasma exchange with 4e5% albumin can sometimes cause electrolyte imbalance such as hypokalemia. Pre-procedure electrolyte assessment and constant monitoring throughout procedure is required to prevent any such complication.
5. *Allergic reactions*- to replacement fluid.

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Table 2. Category and system-wise indications of therapeutic plasma exchange (TPE)

CATEGORY I				
Neurology	**Hematology**	**Renal**	**Connective tissue disorder**	**Others**
• AIDP/CIDP • Demyelinating polyneuropathy with IgG/IgA • Myasthenia gravis • Polyneuropat	• Atypical Hemolytic uremic syndrome-due to autoantibody to factor –H • Thrombotic thrombocytopenia purpura	• Antiglomerular basement membrane antibody disease • Recurrent focal glomerulosclerosis • Renal transplantation, ABO compatible	-	• PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) • Wilson's disease, fulminant • Familial

hy with IgM • Sydenham's chorea • Progressive multifocal leukoenchephalopathy • associated with natalizumab • NMDA receptor antibody encephalitis	• Hyperviscosity in monoclonal • Gammopathies	(Antibody mediated rejection Desensitization, LD) • Renal transplantation, ABO incompatible (Desensitization, LD) • ANCA associated Rapidly progressive glomerulonephritis (dialysis dependence)		hypercholesterolemia (Homozygotes with small blood volume)
CATEGORY II				
• Acute disseminated encephalomyelitis • Rasmussen's encephalitis • Neuromyelitis optica spectrum disorders (acute) • Lambert-Eaton myasthenic syndrome	• Severe cold agglutinin disease • Cryoglobulinemia • Hematopoietic stem cell transplantation, • HLA desensitization		• Vasculitis (HBV-PAN)	• Phytanic acid storage disease • Hashimoto's encephalopathy: Steroid responsive encephalopathy associated with autoimmune thyroiditis • Mushroom poisoning • Voltage-gated potassium channel Antibodies • Systemic lupus erythematosus
CATEGORY III				
• Guillain-Barre syndrome (After IVIG) • Chronic	• Aplastic anemia, pure red cell aplasia • Autoimmune hemolytic	• ANCA-associated rapidly progressive • glomerulonephritis (Granulomatosis • with polyangiitis;	• Scleroderma (systemic sclerosis) • Henoch-Scheonlein	• Acute hepatic failure • Atopic (neuro-) dermatitis • (atopic eczema), recalcitrant

focal encephalitis (Rasmussen • Encephalitis) • Neuromyelitis optica spectrum disorders (Maintenance) • Sydenham's chorea, severe	anemia; • WAIHA • Hematopoietic stem cell transplantation, • HLA desensitization • Coagulation factor inhibitors (Autoantibody) • Immune thrombocytopenia • Heparin induced thrombocytopenia • Post transfusion purpura • HLH/IAHS/MAS	and Microscopic • Polyangiitis) (Dialysis independence) • Anti-glomerular basement membrane • disease (Goodpasture's syndrome) (Dialysis dependence, no DAH) • Immunoglobulin A nephropathy	purpura	• Burn shock resuscitation • Cardiac neonatal lupus • Erythropoietic porphyria, liver disease • Hypertriglyceridemic pancreatitis • Lung transplantation (Antibody mediated rejection • Desensitization) • Pemphigus vulgaris • Sepsis with multi-organ failure
CATEGORY IV				
-	• Thrombotic microangiopathy, Shiga toxin mediated(Absence of severe • neurological symptoms) • Thrombotic microangiopathy, drug Associated (Gemcitabine • Quinine) • Coagulation factor inhibitors(Alloantibody)	• -	• Polymyositis or dermatomyositis • Amyloidosis , • systemic • Inclusion-body myositis	• Psoriasis • POEMS (plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes) syndrome • Stiff-man syndrome

ROLE OF TRANSFUSION MEDICINE SPECIALIST IN PAEDIATRIC HSCT

"*Children are not little adults*."

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Over 50 years have passed since the original reports by Thomas et al. on the use of hematopoietic stem-cell transplantation (HSCT) in children and adolescents made headlines. Since that time, there has been a considerable progress and success in hematopoietic stem cell transplantation in a large variety of pediatric malignant and nonmalignant conditions. Specialists in the field of Transfusion Medicine (Tm) have one grown from being mere observers in the field of cellular therapy to becoming active participants and leaders in hematopoietic stem cell transplantation (HSCT). Blood banks and transfusion services are well versed in regulations governing processing, labeling, storage, and quality control of blood components. Hematopoietic stem cells are the ultimate blood component, and it stands to reason that a well-organized transfusion service is a *'conditio sine qua non'* of a well-structured transplant program.

The role of a transfusion specialist begins from the pre-transplantation phase: starting from HLA typing of recipient and presumptive donors to performing plasma exchange procedures in recipients who have tested positive for donor specific antibodies (DSA) in haploidentical HSCT or ABO mismatch HSCT recipients with high iso-haemagglutinin titers.

TM specialist also plays vital role during the transplant phase by aiding bone marrow harvesting, depletion of red cells in case of ABO-incompatibility and bone marrow manipulation when T-cell depletion or purging procedures are required. Bone marrow has for long been the typical source for HSCTs from pediatric donors. Bone marrow harvest is a safe procedure with only to mild and transient side effects. Recently, a dramatically increased use of mobilized peripheral blood stem cells (PBSCs) in the autologous as well as allogeneic setting has been seen worldwide. Though the PBSC procedures in adult and pediatric donors/patients are technically similar, there are potential medical risks and complications of performing large volume leukapheresis in pediatric group of donors/patients. These include difficulty with vascular access with possible need for central venous catheter placement, need for anesthesia or sedation, granulocyte colony-stimulating factor (G-CSF) induced low platelet count, anemia, need for red blood cell priming of kit prior to apheresis, vasovagal complications, shifts in blood volume with resultant cardiovascular changes, hypocalcaemia due to citrate anticoagulant (or iatrogenic hypercalcemia due to supplementation) requiring an expertise of a Tm specialist to perform these procedures. Storage of hematopoietic cells in liquid nitrogen/by dump freezing and thawing is also a technique requiring support from transfusion services.

Myeloablative conditioning given to Allo-HSCT recipients results in pancytopenia and the patient is prone to infections, anemia and bleeding both before and after transplantation. Until red cell and platelet engraftment, the patient is usually transfusion dependent needing red cell and /or platelet components. The Tm specialist plays a vital role during this period, as knowledge of immmunohematological issues arising in ABO mismatched transplant helps in selection of blood components compatible with both the patient and donor. Providing blood components, irradiated and leukoreduced to avoid TA-GVHD is a

major job of a Tm Specialist. Platelets express both HLA class I antigens (HLA-A, HLA-B) and HPA antigens. Strong and broad-spectrum anti-HLA and anti-HPA antibodies induce refractoriness to platelet transfusions in a small group of HSCT recipients. A well-structured transfusion service can detect anti-HLA and/or anti-HPA antibodies in such patients and provide compatible platelets when platelet transfusion is indicated.

Bacterial and fungal infections continue to pose a major clinical challenge in HSCT patients with prolonged severe neutropenia. With the advent of granulocyte colony-stimulating factor (G-CSF) to mobilize neutrophils in healthy donors, granulocyte transfusions have been broadly used to treat life-threatening infections during this phase. Tm specialist can perform granulocyte collections from ABO compatible healthy donors to support such patients till resolution of sepsis or WBC engraftment.

The role of a TM specialist continues even in the post-transplant period. After allogeneic hematopoietic stem cell transplantation acute GVHD occurs in 20–50% of patients, and chronic GVHD occurs in 30–50% of engrafted survivors. GVHD is usually treated with steroids and immunosuppressive drugs. Extracorporeal photopheresis (ECP) has also been proven highly effective in steroid/immunosuppressive non- responsive GVHD patients. A Tm specialist can facilitate ECP procedures in such patients with refractory GVHD.

To conclude the key to running a successful HSCT program is to ensure safe and effective care in this vulnerable high-risk population and their treatment should be undertaken in a specialized unit by an experienced and fully dedicated multidisciplinary team. Tm specialists are well-positioned to play pivotal role in pediatric HSCT and setting of guidelines for supportive care in this evolving sub-specialty of hematopoietic stem cell transplantation.

Hematopoietic Stem Cell Transplant in children: What to expect from Haploidentical Transplants

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The field of "Bone marrow transplant" (BMT) now called as hematopoietic precursor cell transplant (HPCT) holds a history of more than 50 years. The first ever BMT was done in 1956 by Dr. E. Donnall Thomas in New York for a kid with acute leukemia (donor was an identical twin), which later in 1990 resulted in a Nobel Laureate for him. This was the time when very little was known about the graft (donor cells or organs) tolerance, and exercising a cell or an organ transplant was a farfetched idea. As many of the contemporary physicians considered Dr Thomas's treatment approach very risky, it took more than 10 years for the first ever human leukocyte antigen (HLA) matched sibling BMT to happen in a kid with severe combined immunodeficiency disease (SCID) at university of Minnesota. This was performed in 1968 by Dr Robert A. Good, who is known as the founder of modern immunology. Following this, rapid development of science related to HLA matching and graft tolerance facilitated the first ever matched unrelated donor bone marrow transplant in year 1973 and since then it's one of the most rapidly developing field in the history of medical science.

BMT is basically transfusion of healthy hematopoietic precursor cells in an attempt to repopulate a damaged or diseased marrow. When bone marrow (hematopoietic stem cells, mesenchymal stem cells and the whole

microenvironment) is used as a source of stem cells, then it is called a BMT and when we use hematopoietic precursor cells only (after peripheral stem cell harvest) the transplant is known as hematopoietic precursor cell transplant (HPCT). In either type of transplant, the most important cell to repopulate a marrow is hematopoietic stem cell and that's why the umbrella term for these transplants is known as hematopoietic stem cell transplants (HSCT). These cells can be patients own (autologous HSCT) or from an appropriately selected healthy donor (allogeneic HSCT). The basic steps of an allogeneic HSCT are shown in figure.1.

We will be focusing at allogeneic HSCT and in that too we will be discussing haploidentical HSCT (50% HLA matched bone marrow transplants).

A. Allogeneic HSCT

In allogeneic HSCT, the stem cells used are from non-self, known as donor derived. The prerequisite to be an optimal donor for an allogeneic transplant is good matching of human leukocyte antigen (HLA) between the donor and the patient. If patient's HLA is matched with one of the family member (sibling has 25% chance to be matched, and chances of other family members getting matched are<5%), we call it a matched related donor transplant but if HLA matches with someone outside the family, we call it matched unrelated donor(MUD) transplant. If the donor is from the family but not completely matched, then this transplant is known as mismatched related donor transplants and similarly for unrelated donors we call it as mismatched unrelated donor transplants. In nutshell whenever we think of an allogeneic HSCT the first thing to consider is a detailed HLA matching between patient and the potential donors. In the next paragraph we will discuss why HLA matching is important in allogeneic transplants.

Basics of HLA and its clinical significance:

MHC is a genetic region that has genes which code for individual's specific tissue antigens. In humans the MHC region is located on the short arm of chromosome 6 (6p) and is designated as HLA region. In a HLA region, most (but not all) of the genes regulates the immune responses in an individual. The HLA region has been divided into class I, class II, and class III regions, each containing numerous gene loci that encode a large number of polymorphic alleles. The importance of matching at HLA class I and II is well established. In a large retrospective study, it was shown that HLA matching all class I & II antigen and alleles is associated with lower GvHD, early immune reconstitution and overall better survival.

Antigens from class III are not thought to play a significant role in transplantation and therefore are not used to identify donor/recipient matching in the current practice. In a normal individual when the immune system develops, the cells of immune system are exposed to the self-antigens in a process called tolerance (central & peripheral) and only the tolerant immune cells are allowed to survive and they form a functional and healthy immune system. Whenever there is a break in the tolerance system the outcome is an autoimmune disease. In an allogeneic HSCT, hematopoietic stem cells from a donor establish a new immune system in the patient. The cells of this new immune system have not been exposed to the antigens of the patient and they are obviously not tolerant to these antigens. The severity of crosstalk/interaction between the cells of this new immune system and the patients' antigen (tissue) depends upon the matching of the HLA expression between the patient and the donor. The reaction of these new immune cells towards patients' antigen is known as graft versus host disease (GvHD), the most dreaded complication of

transplant. In other words, severity of the GvHD depends on how imperfectly a patient and donor are matched (lesser the matching more severe is the GvHD).

In an individual 50% of HLA is inherited from each parent and as per the Mendelian law of inheritance there is only 25% chance that the next sibling will inherit the same HLA from same parents. In other words, when we start looking for a HLA matched sibling in a family, in the present scenario (families with two kids' norm) the chances to find a matched sibling is just 25% which increases considerably with increase in the numbers of siblings.

It is very important to note that a 100% matched donor means matching at commonly done major antigens (HLA-A, -B, -C, -DR, -DQ) but doesn't mean that it would be a match at minor antigens (which we haven't tested) level as well. It is thought that the mismatch at minor antigen level incites GvHD even in 100% matched sibling transplants.

B. Haploidentical HSCT- The game changer

As we discussed before, that the chances that any patient will find a 100% matched sibling donor in family are just 25%, means a significant proportion of patients wouldn't have a chance to get the HSCT. To increase the donor pool then we start searching outside family in the donor registries and public cord blood banks and 15% more patients will have a chance to find a donor and thereby a HSCT. Unfortunately, majority still remains without a donor and these patients were and are left alone and they succumb to their diseases, had it been a malignant one.

This was the time when scientists started performing HSCT with 50% matched (haploidentical) donors. The one very good thing about these haploidentical transplants is that every patient will have a 50% matched donor available in family as we inherit 50% HLA from each of our parent. In earlier days,

these transplants had very high rate of GvHD (70%) and other complications leading to higher mortalities. Working on this approach, and to decrease the GvHD, the stem cell grafts were filtered out of T cells (cell causing GvHD) and then were infused. These transplants are called as T cell depleted HSCT (TCD HSCT). There were minimal GvHD in these patients but since T cells are very important in fighting against infection and also for engraftment, there was high mortality and morbidity secondary to infections and graft failure in these transplants. The large rejection rates were later overcome by a mega dose ($\geq 10x10^6$/kg $CD34^+$) of stem cells in these HSCT. Since the cost associated with these transplants was very high (separation kit for filtering out the GvHD causing cells costs around 12 lacs INR), these transplants did not become popular with transplant physicians particularly the ones from developing world. Since this TCD HSCT had an unusually high incidence of opportunistic infections and transplant related mortality other approaches (haploidentical HSCT with T cells replete graft) were tried and they were found to be encouraging.

Luznik et.al at John Hopkins showed that by using cyclophosphamide after bone marrow transplant in a T cell replete (we give all cells, GvHD or no GvHD causing) haplo HSCT, not only decreases the severe GvHD post-transplant but also decreases the infection rates. It was suggested that cyclophosphamide doesn't damage stem cells, kills the actively proliferating lymphocytes (GvHD causing cells) but preserves the silent lymphocytes (infection fighting cells). This approach has revolutionized the world of HSCT. For patients who do not have a matched sibling donor available, this approach gives them a dedicated donor in family to avail a HSCT. The most amazing thing about this transplant is that the cost is similar to a matched sibling transplant as patient doesn't have to spend a single penny to get the donor stem cells. We need more research to fine tune

these transplants as per patients need so that we have outcomes at par with matched sibling transplants.

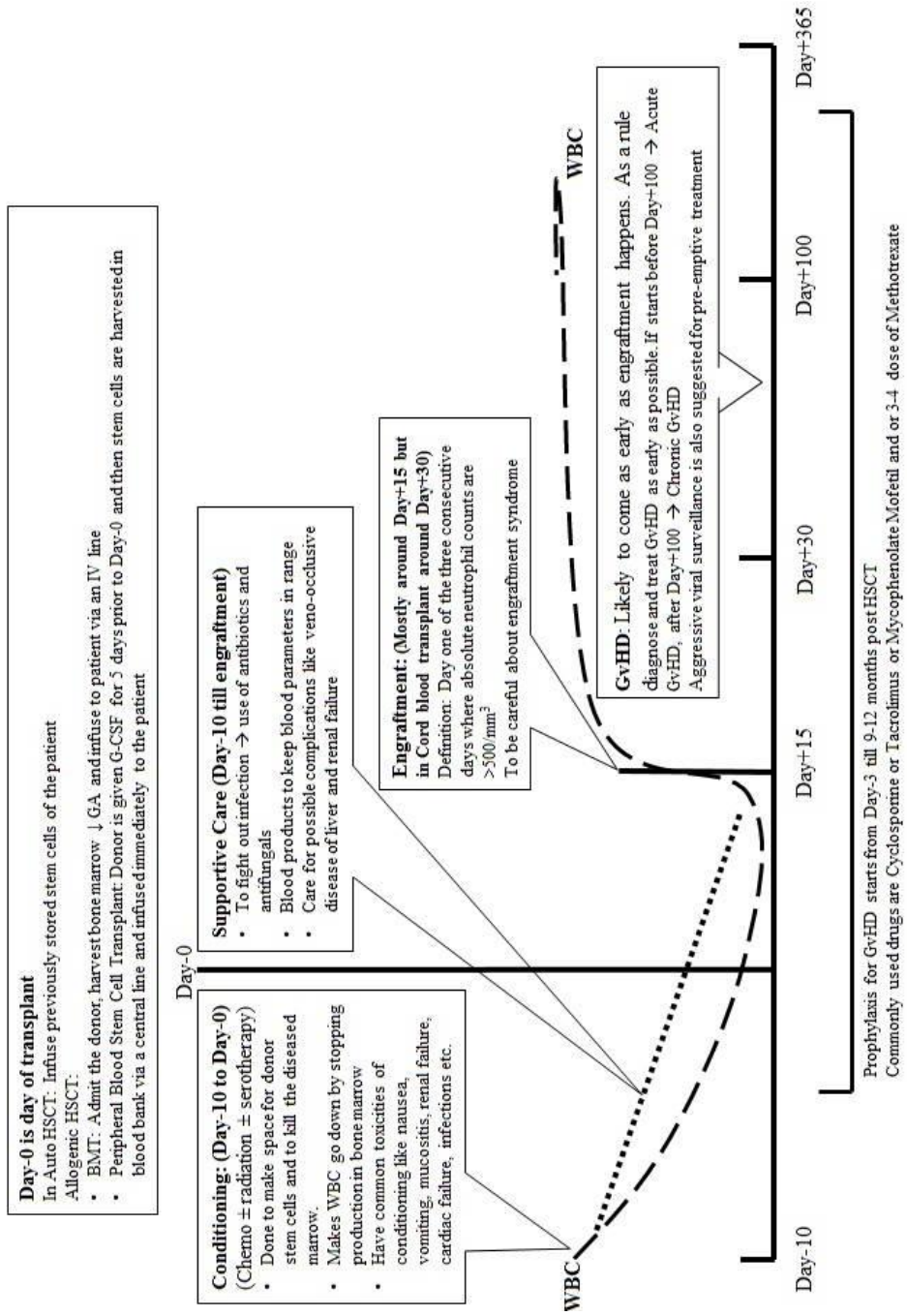
Recently a different way of haplo HSCT has been suggested by Dr Rupert Handgretinger from Germany, where only the GvHD causing T cells (T cell receptor αβ- TCR αβ) and B cells have been depleted from the graft and rest of the cells with stem cells are given to the patient. This approach has produced very encouraging results, though very early and small data. Unfortunately, at present this technique is coming at a very high cost but simultaneously providing a system that seems very safe and effective for haploidentical stem cell transplants.

In summary, the field HSCT has evolved very rapidly particularly in the last decade. Autologous HSCTs need to be done in an immunologically interactive way so that the chemo-radio intensity of these transplants can be toned down (less toxicity related deaths) without compromising on the results. Matched sibling transplants are already moving towards reduced intensity conditioning with good results and less transplant related mortality. Unrelated transplants, as of today are very costly and risky, and need more research to make them easy to perform and safe to exercise. With the rise of haploidentical transplants, suddenly HSCT world has become very interesting as every eligible patient now have a chance to avail HSCT and that too with good results. More research is needed to make these transplants less costly and toxic so that even small centers can provide these high end transplants to the children in the developing world.

Key Messages

- ✓ Pediatric bone marrow transplant is one of the most rapidly advancing field in modern medicine.
- ✓ The proven indications for an autologous bone marrow transplant are few but are increasing day by day based on the latest research.
- ✓ Allogeneic BMTs are changing the fortune of endless children suffering from benign and malignant haematological diseases.
- ✓ Allogeneic BMT is now practiced frequently and successfully for metabolic and primary immunodeficiency diseases.
- ✓ The most initial and important step in an allogeneic BMT is human leukocyte antigen matching between the patient and the potential donors (usually siblings).
- ✓ For a sibling to be 100% HLA match with the patient, chances are just 25%.
- ✓ Matched unrelated donor transplants (both cord blood and live unrelated donor transplants) are good costly options but the chances of finding a matched donor from registries depends on the racial representation of the patient in the searched registry.
- ✓ Mismatched related donor transplants ($\leq$100% matched transplants from parents or siblings) are the real game changer as every patient has a donor in family who would be at least 50% matched.
- ✓ More research would make these mismatched related transplants safer and effective.
- ✓ Next decade is likely to see bone marrow transplants where less of chemotherapy but more of immunotherapy would be used.

Figure 1: Basic framework of allogeneic HSCT



GvHD- Graft versus host disease, WBC- white blood cells

Immunohematological tests (DAT and IAT) in pediatrics

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Antihuman globulin test (AHG)

The antihuman globulin test, which is also referred to as the Coombs' test, is based on the principle that antihuman globulins (AHGs) obtained from immunized nonhuman species bind to human globulins such as IgG or complement, either free in serum or attached to antigens on red blood cells(RBCs). The antihuman globulin test (AGT) is an essential testing methodology when it comes to transfusion medicine; without its use, patients' well-being would be negatively impacted.

There are two major types of blood group antibodies: IgM and IgG. Because of their large pentamer structure, IgM antibodies bind to corresponding antigen and directly agglutinate RBCs suspended in saline. Some IgG antibodies are termed none agglutinating, or incomplete antibodies, because their monomer structure is too small to directly agglutinate sensitized RBCs. Adding AHG that contains anti-IgG to RBCs sensitized with IgG antibodies allows for hemagglutination of these sensitized cells. Antiglobulin tests detect IgG or complement-sensitized RBCs.

Principles of the Antiglobulin Test

The antiglobulin test is based on the following simple principles:

- Antibody molecules and complement components are globulins.
- Injecting an animal with human globulin stimulates the animal to produce antibody to the foreign protein (i.e. AHG). Serologic tests employ a variety of AHG reagents reactive with various human globulins, including anti-IgG

antibody to the C3d component of human complement, and polyspecific reagents that contain both anti-IgG andanti-C3d activity.

AHG reacts with human globulin molecules, either boundto RBCs or free in serum. Washed RBCs coated with humanglobulin are agglutinated by AHG.

Figure1. Figure illustrates complete antibody (Ig M)-because of their large pentamerstructure, IgM antibodies bind to corresponding antigen and directly agglutinate RBCs suspended in saline while incomplete antibody IgG- because their monomer structure is too small to directly agglutinate sensitized RBCsneeds AHG for allowing hemagglutination to occur.

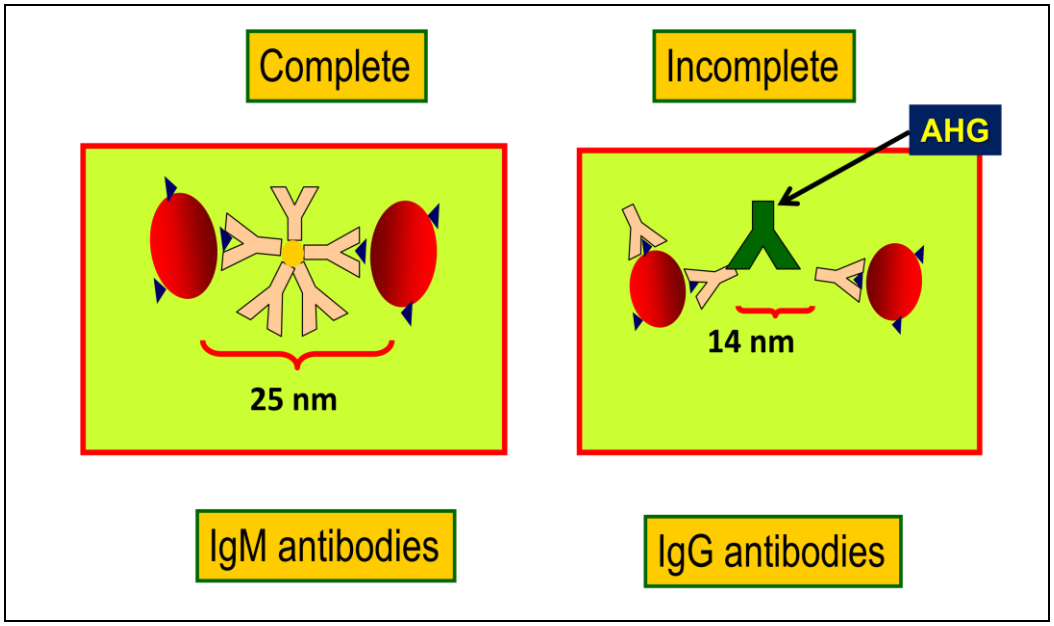
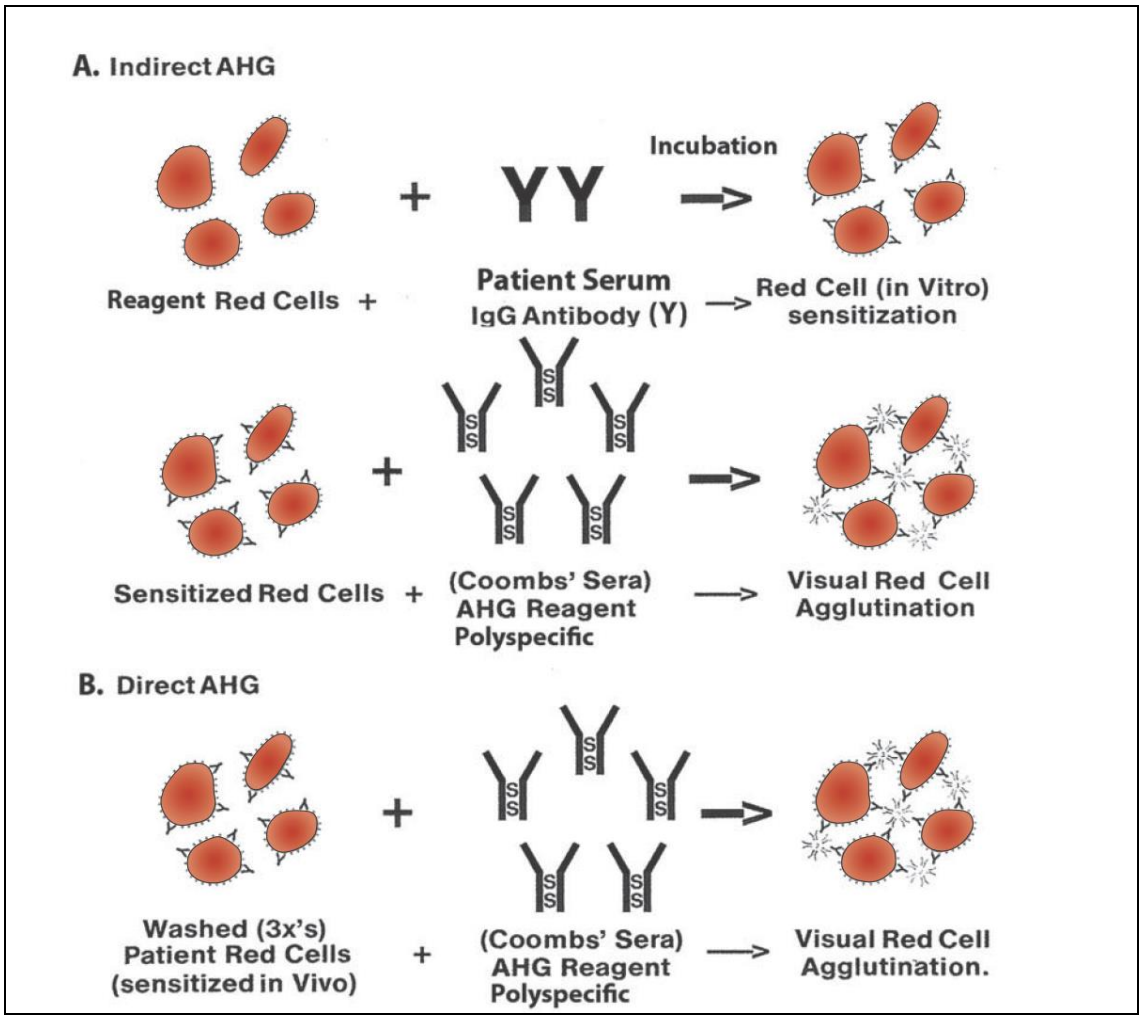


Figure2. Figure illustrates in vitro sensitization detected in the IAT and in vivo sensitization detected by the DAT


A. Indirect AHG
Incubation
YY
Reagent Red Cells +
Patient Serum
IgG Antibody (Y)
Red Cell (in Vitro)
sensitization
Sensitized Red Cells +
(Coombs' Sera)
AHG Reagent
Polyspecific
Visual Red Cell
Agglutination
B. Direct AHG
Washed (3x's)
Patient Red Cells
(sensitized in Vivo)
+
(Coombs' Sera)
AHG Reagent
Polyspecific
Visual Red Cell
Agglutination.

Direct Antiglobulin Test (DAT)

Principle and Application

The direct antiglobulin test (DAT) detects in vivo sensitization of RBCs with IgG or complement components. Clinical conditions that can result in in vivo coating of RBCs with antibody or complement are:

- Hemolytic disease of the newborn (HDN)
- Hemolytic transfusion reaction (HTR)
- Autoimmune and drug-induced hemolytic anemia (AIHA)

Clinical application	In vivo sensitization
Hemolytic disease of the newborn (HDN)	Maternal antibody is coating fetal RBCs
Hemolytic transfusion reaction (HTR)	Recipient antibody is coating donor RBCs
Autoimmune and drug-induced hemolytic anemia (AIHA)	Autoantibody is coating individual's RBC's

DAT testing

Initial DATs include testing one drop of a 3% to 5% suspension of washed RBCs with polyspecific (anti-IgG, anti-C3d) reagent. Positive results are monitored by a DAT panel using monospecific anti-IgG and anti-C3d to determine the specific type of protein sensitizing the cell. In an effort to save valuable tech time, some institutions run polyspecific and monospecific reagents at one time as well as a saline control. The saline control serves to detect spontaneous agglutination of cells or reactions occurring without the addition of AHG reagents.

In warm AIHA, including drug-induced hemolytic anemia, the RBCs may be coated with IgG or C3d, or both. Patterns of reactivity and the type of protein sensitization in AIHA are summarized below.

Control	Anti IgG	Anti C3	Interpretation
Negative	**Positive**	**Positive**	Mixed type AIHA (Warm and Cold), Drug induced HA
Negative	Negative	**Positive**	Cold AIHA (CAS,PCS)
Negative	**Positive**	Negative	Warm AIHA
Negative	Negative	Negative	Repeat with new lot of AHG
Positive	**Positive**	**Positive**	Unable to report

CAS = cold agglutinin syndrome; PCH = paroxysmal cold hemoglobinuria; WAIHA = warm autoimmune hemolytic anemia

Evaluation of a Positive DAT

Clinical consideration should dictate the extent to which a positive DAT is evaluated. Interpreting the significance of a positive DAT requires knowledge of the patient's diagnosis, drug therapy, and recent transfusion history. A positive DAT may occur without clinical manifestations of immune-mediated hemolysis.

The AABB Technical Manual states that "a positive DAT alone is not diagnostic. The interpretation of the significance of this positive result requires knowledge of the patient's diagnosis; recent drug, pregnancy, and transfusion history; and information on the presence of acquired or unexplained hemolytic anemia."

Answering the following questions before investigating a positive DAT for patients other than neonates will help determine what further testing is appropriate:

• Is there evidence of in vivo hemolysis?

• Has the patient been transfused recently? If so, did the patient receive blood products or components containing ABO-incompatible plasma?

• Does the patient's serum contain unexpected antibodies?

• Is the patient receiving any drugs?

• Is the patient receiving antilymphocyte globulin or antithymocyte globulin?

Table3: Below outlines the in vivo phenomena that may be associated with a positive DAT.

	Condition	Cause
Transfusion	Recipient alloantibody and donor antigen	Alloantibodies in the recipient of a recent transfusionthat react with antigen on donor RBC
	Donor antibody and recipient antigen	Antibodies present in donor plasma that react withantigen on a transfusion recipient's RBCs
Drug induced	Type I (hapten-dependent Ab)	Drug binds covalently tomembrane proteins and stimulates hapten-dependent Ab
	Type II (autoantibody)	Drug induces autoantibody specific for RBC membrane proteins through unknown mechanism; Ab reacts with normal RBCs in the absence of drug.
	Type III (drug-dependent Ab)	Drug induces Ab that binds to RBC only when drug ispresent in soluble form,

		unknown mech; Ab reactswith normal RBCs when soluble drug is present.
Autoimmune hemolytic anemia	WAIHA (IgG and/or C3)	Autoantibody reacts with patient's RBCs in vivo.
	CAS (C3)	Cold-reactive IgMautoagglutinin binds to RBCs in peripheral circulation (32°C). IgM binds complement as RBCs return to warmer parts of circulation; IgM dissociates, leaving RBCs coated only with complement.
	PCH (IgG)	The IgG autoantibody reacts with RBCs in colder parts of body, causes complement to be bound irreversibly to RBCs, and then elutes at warmertemperature.
Hemolytic disease of Newborn	Maternal alloantibody crosses placenta (IgG)	Maternal (IgG) alloantibody, specific for fetal antigen, coats fetal RBCs. DAT is reactive with anti-IgG.

Miscellaneous	Absorbed proteins; administration of equine preparations of antilymphocyte globulin and antithymocyte globulin	Heterophile antibodies that are present in ALG or ATGcoat recipient's RBCs. High levels of protein causingred cells to spontaneously agglutinate.
	Administration of high-dose IV gamma globulin and hypergammaglobulinemia	Non-antibody-mediated binding of immunoglobulin to RBCs in patients with hypergammaglobulinemia

Indirect Antiglobulin Test

The IAT is performed to determine in vitro sensitization ofRBCs and is used in the following situations:

- Detection of incomplete (non-agglutinating) antibodies topotential donor RBCs (compatibility testing) or to screeningcells (antibody screen) in serum
- Determination of RBC phenotype using known antisera(e.g., Kell typing, weak D testing)
- Titration of incomplete antibodies

Table4: Below lists the IATs and the in vitro sensitization detected for each application.

	Tests	In Vitro sensitization
Antibody detection	Compatibility testing	Recipient antibody reacting with donor cells
	Antibody Screening	Antibody Reacting with

		screening cells
Antibody identification	Antibody panel	Antibody reacting with panel cells
Antibody titration	Rh antibody titer	Antibody and selected Rh cells
RBC phenotype	RBC antigen detection (Phenotyped matched blood)	Specific antisera +RBCs to detect antigen

Case Discussion

A 32-year female gave birth to a 2.8 kg healthy male. The mother was an RhIg candidate in that her blood group was O, D-negative. A cord blood was sent down to the blood bank for ABO, Rh, and DAT. The baby blood group was identified as A negative, weak D-positive. The DAT was also positive with polyspecific AHG and monospecific anti-IgG. The laboratory realized the test for weak D could not be reported in the presence of a positive DAT and reported the blood group as **"A unknown"**.

Question

- Why is a weak test for the D antigen not performed in the presence of a positive DAT?
- What additional steps need to be performed?

Answer

Problems can arise in accurate D typing in the case of a newborn with a positive DAT. If the DAT is positive due to IgG and the immediate spin for D typing is negative, a test for weak D cannot be performed. The same is true for a patient with AIHA due to a warm IgG antibody coating the patient cells. The antibody

must be removed from the RBCs for accurate phenotyping. Other techniques can be used to **remove antibody from the patients RBCs**. These include chloroquinediphosphate, EDTA-glycine, and a method using murine monoclonal antibodies.

Summary

The antiglobulin test is used to detect RBCs sensitized by IgG alloantibodies, IgG autoantibodies, and/or complement components. AHG reagents containing anti-IgG are needed for the detection of IgG antibodies because the IgG monomeric structure is too small to directly agglutinate sensitized RBCs.

Polyspecific AHG sera contain antibodies to human IgG and the C3d component of human complement. Monospecific AHG sera contain only one antibody specificity: either anti-IgG or antibody to anti–C3b-C3d. The DAT detects in-vivo sensitization of RBCs with IgG and/or complement components. Clinical conditions that can result in a positive DAT include HDN, HTR and AIHA. The IAT detects in-vitro sensitization of RBCs and can be applied to compatibility testing, antibody screen, antibody identification, RBC phenotyping, and titration studies.

A positive DAT is followed by a DAT panel using monospecific anti-IgG and anti-C3d to determine the specific type of protein sensitizing the RBC. EDTA should be used to collect blood samples for the DAT to avoid in-vitro complement attachment associated with refrigerated clotted specimens.

Aphaeresis in Paediatric

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Introduction

The word “Aphaeresis” derived from greek word “Apherios” meaning “to take from”. Specifically, whole blood is separated into components during collection, the desired component is removed/ modified, and the remaining components are returned to the donor or patient. As per component name it can be platelet-aphaeresis for platelet removal, eryhtracyt-aphaeresis for erythrocytes, plasmapheresis for plasma, granulocyt-aphaeresis for granulocytes, etc. Therapeutic Aphaeresis symbolizes selectively removal of pathological cellular or plasma components from patient for treatment purpose. In therapeutic plasma exchange (TPE) patient’s plasma that contains pathological substances is exchanged with replacement fluid.

Extracorporeal Photopheresis (ECP) is a specialized procedure in which the buffy-coat layer is collected from peripheral blood, treated with 8-methoxypsoralen and ultraviolet A light, and re-infused into the patient. The treatment causes cross-linking of leukocyte DNA, which prevents replication and induces apoptosis. The procedure was developed for the treatment of cutaneous T-cell lymphoma, although it is increasingly used for other indications.

Selective Adsorption is also a specialised procedure in which selectively adsorb the pathological substances like antibodies in immuno-adsorption, and LDL in LDL aphaeresis.

Therapeutic aphaeresis and TPE are very often performed in adult patients, however, they are also indicated in paediatric patients.

Aim of the Therapeutic aphaeresis and plasma exchange

Therapeutic aphaeresis standardly deliberates for depletion of circulating pathological substance from peripheral blood. Three basic principles of therapeutic aphaeresis: 1. There should be firm evidence of circulating pathogenic substances, 2. There should be firm evidence that therapeutic aphaeresis can reduce these substances in an effective manner, 3. These evidences should be from clinical trials.

In TPE one volume exchange reduce approximately 60% of pathological substances, second cycle reduce additional 10%. Thus, it is generally recommended to exchange 1 to 1.5 patient plasma volumes per procedure in the majority of clinical conditions. TPE is more effective in IgM antibody mediated diseases as compared to IgG mediated. IgM antibodies are intravascular while IgG is extravascular, so IgG antibodies are rapidly re-equilibrated due to rapid synthesis.

Equipment for aphaeresis: **There are many automated equipment available for aphaeresis and plasma exchange procedures. Some equipments are specially designed for special components. Collection of aphaeresis from donor or patients can be single or double needle. Table 1 shows equipment designed for components.**

Table 1: List of equipment as per component collection:

S.No.	Equipment Name	Platelets	Red Cells	Plasma	TPE	Granulocyte
1	Fresenius ALYX	No	Yes	No	No	No
2	Fresenius Amicus	Yes	No	No	Yes	No
3	Fresenius COM-TEC	Yes	No	Yes	Yes	Yes
4	Terumo BCT (Cobe Spectra)	Yes	Yes	Yes	Yes	Yes
5	Terumo BCT (Trima Accel)	Yes	Yes	Yes	No	No
6	Terumo BCT (Optia)	Yes	No	No	Yes	Yes
7	Haemonetics MCS+	Yes	No	No	Yes	Yes

Special Consideration for therapeutic aphaeresis and TPE in paediatric patients

1. Technical consideration:

Most of the aphaeresis equipments are designed for adult patients, for paediatric patient's special considerations are required according to clinical condition and total blood volume (TBV). TBV can be calculated by multiplying the blood volume in ml per kg of body weight. Estimate TBVs for different age groups are in Table 2.

Table 2: Total blood volume estimate according to age group

Age Group	Total Blood Volume (ml/kg)
Premature Infants	90-105
Term Newborn infants	80-85
Infant >3 month to pre-school age	75-80
School age to puberty	70-75
After Puberty	70

Extra-corporeal blood volume (ECV) and Extra-corporeal red cell blood volume (ERCV) are, respectively, the volumes of whole blood and red cells that fill the instrument's centrifuge module and associated tubing during a procedure. ECV and ERCV are varies according to type of equipment. The fraction of TBV loss in ECV is greater in paediatric patients as compared to adults. In children ECV should not exceed 15% of TBV, if so priming saline may be infused to a patient without diverting. The children who suffer a low hematocrit level (less than 20%), red cell priming may be taken to prevent sudden hypoxic state due to decline in intravascular hematocrit.

For TPE plasma volume (PV) and red cell volume (RCV) are too important. A procedure that takes the plasma equal to the patient's plasma volume is called one-volume plasma exchange. A one volume plasma exchange reduces 60% of unwanted constituents of plasma. It is broadly recommended to exchange 1 to 1.5 patient plasma volume per procedure in the majority of clinical conditions requiring TPE.

Formulas for calculation of PV and RCV are shown beneath:

PV =TBV x (1-Hct) or TBV-RCV

RCV =TBV x Hct or TBV -PV

Hct- Hematocrit in decimal fraction (means 38% Hct is taken as 0.38)

2. **Replacement fluid:**

Albumin (5%) with normal saline is generally preferred as replacement fluid. Fresh Frozen Plasma (FFP) contains all the constituents of the removed plasma and is an optimal replacement fluid. FFP has the disadvantage that it may transmit transfusion transmitted diseases, can cause allergic reactions, ABO incompatibility or sensitization to plasma proteins. FFP is recommended in plasma exchange in patients with thrombotic thrombocytopenia (TTP) or haemolytic urinary syndrome (HUS).

3. Vascular access:

Central venous catheters (CVC) are recommended to achieve regular high flow in aphaeresis procedure in paediatric patients. Femoral catheterization is more preferred over subclavian, internal jugularvein because they carry increased risk of pneumothorax, hemothorex and air embolisation. A CVC should be flush with heparinised saline having heparin in a concentration of 100 units/ml to 5000 units/ml or with heplock flush having heparin in the concentration of 10 units/ml.

4. Anticoagulant:

Acid Citrate Dextrose (ACD) is using as anticoagulant during aphaeresis. In paediatrics patients to prevent citrate toxicity the ration of citrate and blood is kept higher as compared to adult patients (1:13 vs 1:9).

5. Psychological consideration:

Paediatric patients are more anxious and fearful as compared to adult patients. To reduce this anxiety, parents or caretakers are essential with them. Sedation of a child should be avoided whenever possible unless medically indicated, since sedation may interfere with early recognition of adverse effects of aphaeresis.

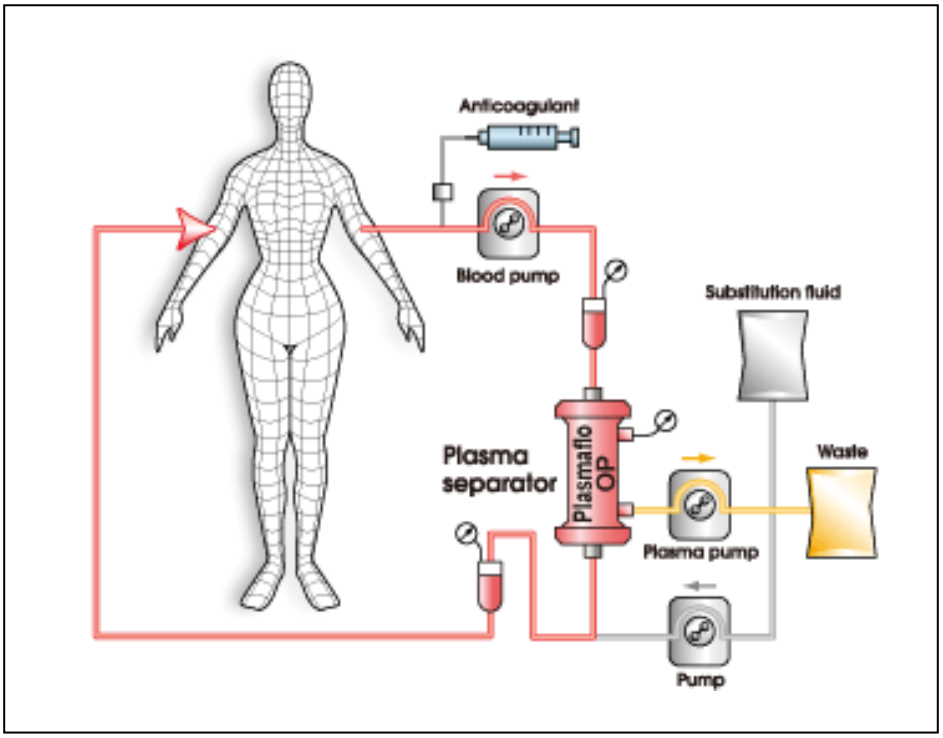
Adverse effect related to therapeutic aphaeresis:

1. **Hypocalcemia:** Hypocalcemia due to citrate toxicity can be more severe in paediatric patients as compare to adult. It can be appearing as mild symptoms like paresthesias, tingling and numbness to severe symptoms like tetany, seizures or cardiac arrhythmias, which are uncommon. For prevention of this reaction patient's pre-procedure ionized calcium should be evaluated and correct before starting procedure. During procedure also continuous

infusion of 10% calcium gluconate is practiced for prevention of citrate toxicity.

2. **Hypotension:** Significant hypotension with systolic blood pressure below 80 mm Hg is a common adverse effect in paediatric patients due to aphaeresis procedure. If it occurs, it should be treated by lowering the patient's head below trunk and leg level and infusing extra saline or colloid. This can prevent in pediatric patients in some instances by priming the instrument with blood.
3. **Allergic reaction:** If FFP is used as replacement fluid then there are more chances of allergic reaction due to plasma protein. Mild symptoms like fever, urticarial and rash on the body are observed, that can be easily treated by anti-histamine.

Flow diagram of TPE



Venous Access – Concerns for Transfusionists and Apheresis Physicians

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The art is long, Life is short, Experiments perilous, Decisions difficult - Hippocrates

Venous access is one of the most frequent procedures carried out in hospitalized patients for a variety of clinical indications. In fact, most of us often do not think of vascular access when we refer to a 'procedure'. The indication for and duration of vascular access should be carefully taken into account and it should be planned in a way so as to meet these needs in a rational and scientific manner. Venous access in children could be especially challenging because of their anatomy and physiology.

Difficult venous access is defined as a clinical condition in which multiple attempts and/or special interventions are anticipated or required to achieve and maintain peripheral venous access, examples of which could be equipment for enhanced vein visualization or expert help (intravenous team, anesthetist etc.) A number of approaches can be used to facilitate difficult venous access such as gentle tapping, tourniquet or blood pressure cuff, warming the limb. Topical application of nitroglycerin ointment alone or with a eutectic mixture of local anesthetics (EMLA) cream is a safe and effective way to induce local vasodilation,

improving the visibility of the veins of the hand and ease of cannulation. Trans-illumination using an infrared source of light can also improve the visualization of non-palpable, nonvisible veins in infants and young children. Other techniques such as ultrasound, fluoroscopy may also be useful.

Difficult IV access score (DIVA), was created by Yen and colleagues as an objective scale for scoring for difficult venous access in children. Multiple needle sticks increase patient anxiety, pain, and suffering, and also has a negative impact on health care workers including doctors and nurses. Good preparation, however, can significantly alleviate patient distress and enhance cooperation. A number of factors should be considered when taking into account alternative sites and other alternatives to peripheral cannulation such as PICC lines, central venous catheters etc. viz. severity of illness, emergency or non-emergency situation, immediate and future medical needs, staff time and resources, and the patient's pain and suffering.

Peripherally inserted central catheters (PICCs) are available in single-, double-, and triple-lumen configurations and in sizes ranging from 28 G catheters for use in premature neonates to 7

Fr triple-lumen catheters. By convention, single-lumen catheters are described by gauge, while multi-lumen catheters by French (Fr) size). The size of the PICC to be used is determined

by the size of the access vein and the therapy required and not simply by the age of the patient.

If blood sampling via the PICC is required, then at least a size 3 Fr will be needed. Advantages of PICCs in children are that they may be inserted and removed without a general anaesthetic in some children and have the lowest complication rate of central venous access devices.

Central venous catheters These devices are indicated for short-term to intermediate term therapy or when urgent access is required. They are available in configurations of up to five lumens. Common sites for access and techniques for insertion in children are as for adults, although the procedure carries a greater risk of complications in children with a lower success rate. The use of ultrasound to guide insertion of these catheters is becoming more common. The size of the catheter is determined by the size of the vein and the therapy required, size 4–5 Fr catheters are usually suitable for infants <6 months, size 5 Fr for those aged 6 months to 5 yr., and size 7 Fr for those over 5 yr. Generally, for internal jugular insertion, 5 cm lines may be used for children <15 kg, 8 cm lines for patients 16–40 kg, and 13 cm lines for those >40 kg.

Silicone central venous catheter (e.g., Hickman, Broviac) These catheters are placed in a central vein using either an open surgical cut-down technique or percutaneously utilizing the Seldinger technique. The catheter is then tunneled away from the vein insertion site to a skin exit site determined by comfort, patient preference, and safety considerations Mounted on the line within the tunnel is a Dacron® cuff into which subcutaneous tissue grows over a period of weeks. This stabilizes the line and may serve as a barrier preventing the ingress of micro-organisms along the line. Many types of cuffed central venous catheters (CVCs) are available including Broviac® and the larger Hickman® lines. Wide-bore lines are required for hemodialysis and plasmapheresis. These lines are generally preferred to PICC lines when the duration of therapy is likely to exceed 6–8 weeks.

Therapeutic apheresis procedures require two separate points of vascular access, one to remove blood and the second to simultaneously return the blood back. Vascular access can be achieved wholly or in combination from peripheral veins, central venous catheters, arterial catheters, arteriovenous (AV) fistulas, AV

grafts and high flow ports and options chosen will depend on how long the access will be required i.e. short or long term. Peripherally inserted central catheters (PICC) are unsuitable and contraindicated for apheresis procedures due to the high flows and pressures which can cause rupture of the PICC. Peripheral venous access is required in both arms as use of one arm can result in recirculation of blood and/or longer procedures.

VASCULAR ACCESS FOR APHERESIS

Peripheral venous access devices: Peripheral veins of children, except for adolescents, can rarely accommodate minimal inlet flows)20-40 ml/min) required for plasma exchange. Peripheral access may be possible for older children with adequate vein size. (18-gauge or larger steel needle placed in the antecubital vein for the draw and a 22-gauge or larger needle placed in peripheral vein of opposite arm for return). Suitable for short-term or intermittent procedures. Peripheral Needles/cannulae for returning blood may be sited in any part of the lower arm or hand as return flow can be accommodated by smaller veins, however use of peripheral leg veins is contraindicated in apheresis. Return needles/cannulae are best placed away from joints where

bending of the arm or wrist cause increased pressure and can occlude the return flow back to the patient.

Central venous access is often required in children. Vascular access is achieved by use of dual lumen central venous catheters or single lumen catheters in very small children. Body size and weight will dictate the length and French size of dual lumen central venous catheters, which can be placed in either the femoral vein or right jugular or subclavian vein. Femoral venous catheters are placed in urgent situations and are usually restricted for patients requiring only a few

procedures or for temporary use. Apheresis procedures are also carried out using mature **AV fistulas** and are an alternative option for some patients. Other types of **CVC e.g. triple lumen** are generally unsuitable. Return flow may be possible through some access/draw flow is not. **Tunneled catheters (ex. Hickman Lines)**

Not suitable for draw flow as small lumen diameter does not withstand high pressure and flow rates generated. Can be used for low return flow rates. **Implanted Intravascular Access Devices (PORT)**

Only special high flow dual (VORTEX) ports used with large bore non coring needles are suitable for apheresis. Standard Portacaths are not suitable for apheresis

In addition, the physician should have working knowledge of the vascular access device to avoid confusion and mishandling of the catheter.

Comparison of Options for Vascular Access in Children

Method	Duration of Use	Advantages	Disadvantages	Concerns for transfusion
Peripheral intravenous (IV) access	Short term	○ Ease of insertion ○ Low cost ○ Minimal complications	○ Easily occluded ○ Potential for local tissue injury ○ Use limited to certain antibiotics or medications	○ Safe with at least 24-25 G catheters
Peripherally inserted central catheter (PICC)	Short-to-long term Popular in NICU	○ Ease of insertion (bedside) ○ Can be used with variety of medications ○ Relatively safe and inexpensive	○ Potential for occlusion ○ Can be difficult to position in central vein	○ Not preferred for transfusions with less than 2.0 Fr. Line
CVC	can be maintained over the long term	○ Reliable method of infusing large volumes of fluid ○ frequently used for critical-care monitoring	○ Require expertise ○ Potential for infection	○ Very good aseptic technique and flushing technique is required
Silicone central venous catheter (e.g., Hickman, Broviac)	Long term	○ Less thrombogenic ○ Decreased infection rate ○ Safe with most medications	○ Increased cost ○ Requires surgical insertion (tunneled)	○ Preferred in patients who require transfusions regularly
Implantable vascular-access device (ports)	Long or permanent	○ Low visibility ○ Lowest rate of infection	○ Increased cost ○ Requires surgical insertion	○ Have to be accessed with special non coring needles

Intraosseous	Emergency access for children aged 6 years or less	○ Rapidly and easily inserted ○ Low complication rate ○ Safe with resuscitation medications	○ Not for long-term use ○ Potential for osteomyelitis	○ Large bore Enable administration of blood without lysing blood cells
Venous cut down	Emergency access	○ Direct exposure to vein	○ Increased rate of dislodgement and infection ○ Requires incision	○ Not suitable for long term

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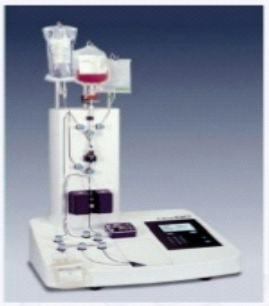

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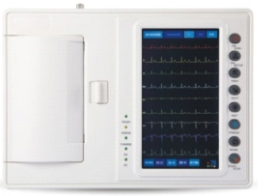

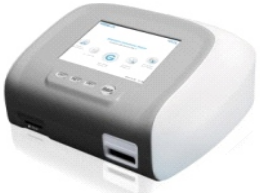

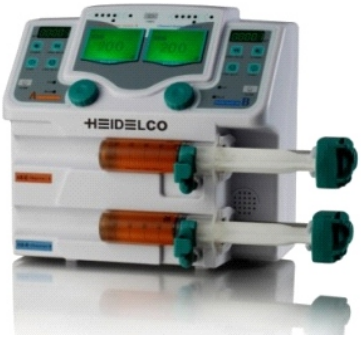

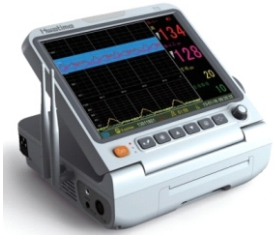

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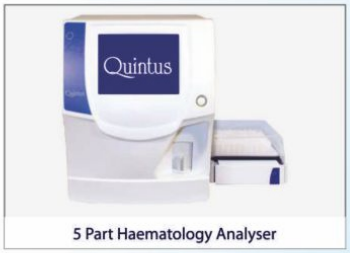
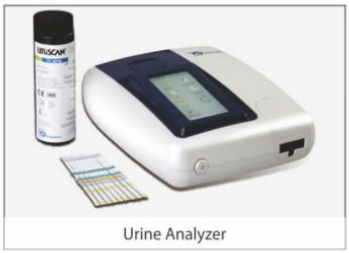
Fully Auto Bio-Chemistry Analyzer


3 Part Haematology Analyser



5 Part Haematology Analyser


Urine Analyzer


Electrolyte Analyzer


Slide Stainer


Osmometer

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