Immunohematology
Blood Banking and Transfusion Medicine

• A science that deals with the principles of blood collection, storage, and transfusion

• It covers the areas of:
  ✓ Donor selection and blood donation
  ✓ Screening of blood for blood borne pathogens
  ✓ Separation of blood into its components
  ✓ Compatibility testing
  ✓ Transfusion reactions
Historical Background

- People have always been fascinated by blood: ancient Egyptians bathed in it, aristocrats drank it, authors and playwrights used it as themes, and modern humanity transfuses it.

- The road to an efficient, safe, and uncomplicated transfusion technique has been rather difficult, but great progress has been achieved.

- In 1492, blood was taken from three young men and given to the stricken Pope Innocent VII in the hope of curing him; unfortunately, all four died.

- That was the first attempt at using blood for therapeutic use.
Historical Background

• 1665: First animal to animal—Richard Lower

• 1667: Jean Baptiste Denys, first successful IV transfusion of blood from animal to human

• 1818: James Blundell First to transfuse human to human.

• Several attempts of unsuccessful transfusion were documented
Historical Background

- Clotting was the principal obstacle to overcome.

- Braxton Hicks in 1869: Sodium phosphate.

- Karl Landsteiner in 1901: Discovered the ABO blood group system.

- Hustin in 1914: An unprecedented accomplishment in blood transfusion; the use of sodium citrate as an anticoagulant.

- Transfusion became more practical and safer.

- Rous and Turner in 1916: Added sugar and introduced the citrate-dextrose preservative.
Historical Background

- Wiener and Landsteiner in 1939: discovery of the Rh blood group system

- 1941: establishment of the first blood bank in USA

- Loutit and Mollison in 1943: ACD

- Blood transfusion became routine only after WWII as the demand increased and the major blood group systems were discovered

- Many blood group systems were discovered in the 1940’s and 1950’s

- Gibson in 1957: CPD
The ABO System

• Most important of all blood groups in transfusion practice

• Four major groups A, B, AB, and O

• Frequency varies, but most common groups are O (45%) and A (41%), followed by B (10%) and AB (4%)

• Normal individuals have naturally occurring IgM antibodies against the missing ABO antigen
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Blood-group phenotype</th>
<th>Antigens on erythrocytes (agglutinins)</th>
<th>Serum antibodies (isohemagglutinins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA or AO</td>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>BB or BO</td>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>A and B</td>
<td>None</td>
</tr>
<tr>
<td>OO</td>
<td>O</td>
<td>None</td>
<td>Anti-A and anti-B</td>
</tr>
</tbody>
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Inheritance of the ABO Blood Groups

- The A, B, or O genes are present on chromosome 9
- The O gene is an amorph
- The A and B genes are codominant in expression
- The genotypes are AA, AO, BB, BO, OO, and AB
Formation of the ABH Antigens

- ABO genes code for specific glycosyltransferase that add sugars to a basic precursor substance on the RBC.

- The precursor substance is composed of glucose, galactose, N-acetylglucosamine, and galactose attached to ceramide on the RBC membrane.

- The addition of fucose to the terminal galactose creates the H substance which is catalyzed by fucosyltransferase that is encoded by the H gene which is inherited independent of the ABO genes.
Formation of the ABH Antigens

• The H substance is the O phenotype and it is necessary for the expression of the A and B antigens.

• Those who are hh represent a rare phenotype; the Bombay phenotype.

• Different immunodominant sugars are added to the H substance creating the blood group.

• The addition of N-acetylgalactoseamine to the terminal galactose creates the A blood group, whereas the addition of galactose creates the B blood group.
Formation of the ABH Antigens

• Individuals of the AB genotype attach both immunodominant sugars but the B gene product outcompetes the A gene product.

• The ABH antigens are expressed as early as the 37th day of gestational life, but newborn erythrocytes carry only 25-50% of adult antigenic sites.

• The ABO system interacts at the genetic level with the Lewis, Ii, and P blood group systems.
Formation of the ABH Antigens

- Another gene, the Zz gene is necessary for the expression of the ABO system on RBCs.
- The ABH antigens are expressed in all organs of the human body.
- ABH antigens are present in secretions as glycoproteins and this is under the control of the secretor, Sese, gene.
ABO Subgroups

• A1 (80%), A2 (20%)

• A1 reacts with anti-A and anti-A1

• A2 reacts with anti-A only

• Difference is both quantitative and qualitative

• A1 genes creates about $10^6$ antigen sites, whereas A2 creates about 250,000 antigen sites
ABO Subgroups

- The qualitative difference is believed to be conformational in nature.

- Most group A infants appear to be A2 at birth.

- A1B and A2B.

- There are several less important rare subtypes.

- There are weak A and B groups that are rare and insignificant from a transfusion point of view.
ABO Discrepancies

• Weak reacting or missing antibodies

• Weak reacting or missing antigens

• Proteins or plasma abnormalities that lead to rouleaux formation or pseudoagglutination

• Miscellaneous problems like polyagglutination, cold reactive antibodies, warm autoantibody, and others
The Rh System

- A complex blood group system that is currently composed of nearly 50 antigenic specificities

- The chemical structure and the mode of inheritance of the Rh system still elude scientists

- Two terminology systems are in common usage and two others are not commonly used

- The Fisher-Race (DCE) and the Wiener (Rh-Hr) terminologies

- Most important in HDN and transfusion reactions
Rh Antigens

• The Rh antigen is protein in nature

• Rh antigens are transmembrane polypeptides and are an integral part of the red cell membrane

• The Rh antigens are inherited as codominant alleles and the genes are very closely linked, so much so that crossing over is an extremely rare event

• The d gene is an amorph

• Antigenicity D> c> E> C> e
Variants of the Rh\(_0\) (D) Antigen

- **Weak D (Du):** Weakened expression, the antigen is complete but few in number. Reacts in the antiglobulin phase of the tube testing.

- **C Trans:** Weakened expression as a result of position effect by C antigen due to steric effect where the allele carrying D is in trans to the allele carrying the C (Dce/dCe as opposed to DCe/dce).

- **D Mosaic or Partial D:** One or more parts of the D antigen is missing.
The rare $\text{Rh}_{\text{null}}$ individuals lack all Rh antigens.

They demonstrate a mild compensated hemolytic anemia, reticulocytosis, stomatocytosis, a slight to moderate decrease in hemoglobin and hematocrit, an increase in hemoglobin F, a decrease in serum haptoglobin, and possibly an elevated bilirubin.

Severity is highly variable.

The $\text{Rh}_{\text{mod}}$ individuals exhibit features similar to $\text{Rh}_{\text{null}}$ but symptoms are usually less severe and rarely clinically remarkable.
DEVELOPMENT OF ERYTHROBLASTOSIS FETALIS (WITHOUT RHOGAM)

1st Pregnancy
- Placenta
- Maternal circulation
- RBCs with Rh antigen

Delivery
- Rh-specific B cell
- Memory cell

Mother
- Plasma cells
  - IgM
  - Anti-Rh

PREVENTION (WITH RHOGAM)

Mother (treated with Rhogam)
- B cell
- Rhogam

Prevents B-cell activation and memory cell formation

2nd Pregnancy
- Memory cell
- Plasma cells
  - IgG

IgG anti-Rh Ab crosses placenta and attacks fetal RBCs causing erythroblastosis fetalis
Other Blood Group Systems

- The Lewis System

  - This system is encoded by a single locus with two antigens, $Le^a$ and $Le^b$.
  
  - These antigens do not form an integral part of the red cell membrane, but are soluble antigens which may be present in body fluids and secretions.
  
  - They are adsorbed on to the surface of red cells if they are present in the plasma in sufficient amounts.
  
  - There are only three phenotypes: $Le^{(a-b-)}$; $Le^{(a+b-)}$; and $Le^{(a-b+)}$

- Lewis phenotypes may change during pregnancy.
Other Blood Group Systems

• The Lewis System

✓ Le \(^{(a-b^+)}\) phenotype is only transient.

✓ Lewis antibodies are only found in Le \(^{(a-b^-)}\) individuals, and are almost entirely IgM.

✓ They are the only blood group antibodies which have never been implicated in HDN (hemolytic disease of the newborn.)
Other Blood Group Systems

- The P System
  ✓ This system was also discovered by injecting animals with human red cells.
  ✓ P1 is the most common antigen which has variable strength of expression.
  ✓ Anti-P1 may be naturally occurring. It is most often an IgM antibody.
Other Blood Group Systems

• The MNSs System

✓ This system was discovered by injecting animals with human red cells.
✓ There are two loci: M/N and S/s.
✓ The antigens are M, N, S, and s.
✓ There are naturally occurring (IgM) antibodies to all of these antigens.
✓ Anti-S and anti-s commonly develop immune characteristics (IgG class) as a result of pregnancy or transfusion.
Other Blood Group Systems

- **The Kell System**
  - In this system there are four antigens at two loci: K (Kell) and k (cellano), and Kp\(^a\) and Kp\(^b\).
  - The Kp\((a+)\) phenotype and the Kp\((a-b-)\) phenotype are both rare.
  - The K\(_{\text{null}}\) phenotype K- k- Kp\((a-b-)\) is associated with chronic granulomatus disease (CGD).
  - Antibodies to Kell system antigens are IgG.
  - Named for the family of the antibody producer Mrs. Kellacher.
Other Blood Group Systems

• The Lutheran (Lu) System

✔ This system is a single locus system, with two antigens Lu\(^a\) and Lu\(^b\).

✔ The Lu\(^a\) negative phenotype is very rare.

✔ Antibodies to Lutheran antigens are IgG.

✔ The genes of the Lutheran group are linked to the genes responsible for the secretion of ABH substances.
Other Blood Group Systems

- The Duffy System
  ✓ The Duffy system is also a single locus with two antigens, \( Fy^a \) and \( Fy^b \).

  ✓ The only rare phenotype is \( Fy^{(a-b-)} \), which has a higher frequency in countries where there is a high incidence of *Plasmodium falciparum* malaria.

  ✓ This phenotype gives a degree of immunity to the disease because the malarial parasite requires Duffy antigens to enter the red cells.

  ✓ Duffy antibodies are almost exclusively IgG.

  ✓ This system is named after the family of the antibody producer, Duffy.
Other Blood Group Systems

• The Kidd (Jk) System

✓ Another single locus system, two antigen system (Jk\textsuperscript{a} and Jk\textsuperscript{b}).

✓ There are four possible phenotypes: Jk\textsuperscript{(a-b-)}; Jk\textsuperscript{(a+b-)}; Jk\textsuperscript{(a-b+)}; Jk\textsuperscript{(a+b+)}.

✓ Jk\textsuperscript{(a-b-)} is a rare phenotype.

✓ Antibodies to the Kidd antigens are almost exclusively IgG.

✓ Incompatible transfusion or pregnancy can lead to the formation of antibodies to all these blood groups, if the recipient/mother lacks the relevant antigen.
Blood preservation and storage

• Within 4 hrs of collection, Whole Blood (WB) should be separated

• WB: 63 ml preservative (HCT 36-40%)
  – CPD- A (citrate, phosphate, dextrose, adenine) shelf-life 35 days @ 1-6°C

• Packed RBC: (HCT 60%)
  – CPD-A
  – ADSOL (adenine, dextrose, saline, mannitol) shelf-life 42 days
Blood Component Therapy

• Effective transfusion therapy depends on the availability of many different blood components

• These components, used separately or in various combinations, can adequately meet most patient transfusion needs while keeping the risks of transfusion to a minimum

• Component transfusion therapy has the added benefit of using a limited natural resource more effectively by providing needed therapeutic material to several patients from a single donation
Red Cell Transfusion

• The primary indication for the transfusion of RBCs is to restore or maintain an adequate supply of oxygen to meet tissue demands.

• Since the body demand for oxygen varies greatly, a single laboratory measurement (Hb, Hct) cannot accurately assess the need for transfusion.

• **Fresh whole blood**: no solid indication, usually not available, and should be interpreted as a need for consultative help.
- Stored whole blood

- Rarely available and deficient in platelets and factors V and VIII

- WB-modified is prepared by returning plasma to RBCs after removal of platelets and cryoprecipitate

- It is indicated for active bleeding with a loss of more than 30% of blood volume

- Can lead to dilutional coagulopathy

- WB less than 7 days may be beneficial for neonatal exchange or cardiac surgery
• **Packed RBCs:** The component of choice and its use should not be dictated by a single hemoglobin trigger.

• **Leukocyte-Reduced RBCs:**
  - A unit of blood contains $1-3 \times 10^9$ WBCs and should be reduced to $< 5 \times 10^6$
  - WBCs are responsible for:
    - Febrile reactions
    - Sensitization to HLA antigens
    - Transmission of leukotropic viruses
    - GVHD

• **Washed RBCS**
Platelet Transfusion

- To stop established bleeding or to prevent spontaneous bleeding due to thrombocytopenia or abnormal platelet function

- The need is growing rapidly but not effective in ITP, TTP, and untreated DIC

- ABO compatibility is important but availability is the major consideration

- D-negative females of child-bearing potential should receive platelets from a D-negative donor or should be given anti-D

- Bacterial contamination is a major concern
Fresh Frozen Plasma

- FFP is indicated in bleeding patients with multiple coagulation factor deficiency

- It is the component of choice in TTP and HUS

- Deficiencies of multiple coagulation factors include:
  - Vit. K deficiency
  - Liver disease
  - Dilutional coagulopathy
  - DIC
  - Deficiency of protein C, protein S, or anti-thrombin III
Cryoprecipitated anti Hemophiliac Factor-CRYO (AHF)

• The cold insoluble portion remaining after FFP has been thawed between $1^\circ$ and $6^\circ$ c

• It contains approximately 50% of factor VIII, 20-40% of fibrinogen, some of factor XIII, factor vWF, and fibronectin

• **Indications:**
  - Von Willebrand Syndrome
  - Fibrinogen abnormalities
  - Factor VIII deficiency (hemophilia)
  - Topical plug (fibrin glue)
Other Components

- Liquid plasma
- Factor VIII concentrate
- Factor IX concentrate
- Albumin
- Immunoglobulins
- Anti-D
- Other plasma protein fractions
Pretransfusion Testing

- Positive identification of recipient and recipient sample
- Review of transfusion records for previous testing of recipient
- Test donor blood for ABO, Rh, and infectious agents
- Type and screen of recipient blood (ABO, Rh, and antibody screen)
- Selection of appropriate component
- Cross matching
- Labeling of component
- Electronic cross-matching before release
Blood Transfusion Reactions

Febrile Reactions

- **Cause**: recipient antibodies reacting with white cell antigens or white cell fragments in the blood product or due to cytokines which accumulate in the blood product during storage.

- **Management**: Symptomatic (paracetamol)

- **Investigation**: Fever can be the initial sign in more severe transfusion reactions (haemolytic or bacterial sepsis) and should be taken seriously.

- **Prevention**: leucocyte filtration (either bedside or pre-storage).
Urticarial (Allergic) Reactions

- **Cause**: Seen in approximately 1% of recipients and caused by foreign plasma proteins.

- **Management**: If urticaria occurs in isolation (without fever and other signs), slow the rate or temporarily stop transfusion. Consider administering an antihistamine before restarting the transfusion. If associated with other symptoms, cease the transfusion and proceed with investigation.

- **Investigation**: In the case of mild urticarial reactions with no other signs or symptoms, it is not necessary to submit blood specimens for investigation.
Severe Allergic (Anaphylactic) Reactions

• **Cause:** In some cases patients with IgA deficiency who have anti-IgA antibodies can have these reactions.

• **Management:** Immediately stop transfusion, supportive care and adrenaline may be indicated.

• **Investigation:** IgA levels and anti-IgA antibodies.

• **Prevention:** Patients with anti-IgA antibodies require special blood products such as washed red blood cells and plasma products prepared from IgA deficient donors.
Acute Haemolytic Reactions

• **Cause:** ABO incompatible blood. Most haemolytic reactions are the result of human error

• **Symptoms:** Chills, fever, pain (along IV line, back, chest), hypotension, dark urine, uncontrolled bleeding due to DIC.

• **Management:** Immediately stop transfusion. Notify hospital blood bank urgently (another patient may also have been given the wrong blood!).

• **Prevention:** Proper identification of the patient from sample collection through to blood administration, proper labelling of samples and products is essential.
Bacterial Contamination

• **Cause:** Bacteria may be introduced into the pack at the time of blood collection. Bacteria may multiply during storage. Gram positive and Gram negative organisms have been implicated. Platelets are more frequently implicated than red cells.

• **Symptoms:** Very high fever, rigors, profound hypotension, nausea and/or diarrhoea.

• **Management:** Immediately stop the transfusion and notify the hospital blood bank.

• **Prevention:** Inspect blood products prior to transfusion. Transfusions should not proceed beyond the recommended infusion time (4 hours).
Transfusion-Related Acute Lung Injury

TRALI is characterised by acute respiratory distress and bilaterally symmetrical pulmonary edema with hypoxemia developing within 2 to 8 hours after a transfusion.

Cause: It occurs secondary to cytokines in the transfused product or from interaction between patient white cell antigens and donor antibodies (or vice versa).

Management: Symptomatic support for respiratory distress includes oxygen administration and may require intubation and mechanical ventilation. Symptoms generally resolve over 24-48 hours.
Volume Overload

- **Cause:** Patients with cardiopulmonary disease and infants are at risk of volume overload especially during rapid transfusion.
- **Management:** Stop the transfusion, administer oxygen and diuretics as required.
- **Prevention:** Avoid unnecessary fluids and use appropriate infusion rates.

Hypothermia

- **Cause:** Rapid infusion of large volumes of stored blood. Infants are particularly at risk during exchange or massive transfusion.
- **Prevention and Management:** Appropriately maintained blood warmers should be used during massive or exchange transfusion.
- **Citrate toxicity**
  - **Cause:** Rapid administration of large quantities of stored blood. This can result in myocardial depression or coagulopathy.
  - **Management:** Slowing or temporarily stopping the transfusion allows citrate to be metabolised.

- **Potassium Effects**
  - **Cause:** Stored red cells leak potassium proportionately throughout their storage life. It can occur during rapid, large volume transfusion of older red cell units in small infants and children.
  - **Prevention:** Blood less than 7 days old is generally used for rapid large volume transfusion in small infants (eg cardiac surgery, exchange transfusion)
Delayed Hemolysis

A delayed haemolytic reaction occurs when a patient develops an antibody directed against an antigen on transfused red cells.

- The antibody may cause shortened red cell survival, with clinical features of fever, jaundice and lower than expected haemoglobin following transfusion.

- Most delayed haemolytic reactions produce few symptoms and may go unrecognised, however there are reports of serious consequences in critically ill patients.

- An antibody screen is performed as part of pre-transfusion testing.
Transfusion associated Graft Versus-Host Disease (Ta-GVHD)

- Ta-GVHD occurs when donor lymphocytes in cellular blood products engraft in a susceptible transfusion recipient.

- The usual onset is 8-10 days post transfusion, with a longer interval between transfusion and onset of symptoms in infants.

- The most commonly reported setting for Ta-GVHD is immunocompetent recipients of blood from biologically related or HLA identical donors.

- Gamma irradiation of cellular blood products (whole blood, red blood cells, platelets, granulocytes) for at risk patients.
INFECTIONOUS COMPLICATIONS

• Hepatitis B
• NANB and Hepatitis C
• HIV
• HTLV I, II
• CMV
• CJD (and variant CJD)