Emerging Trends in Transfusion Science

Rajendra Chaudhary, MD, DNB
SGPGI, Lucknow
Figure 1.5. Direct transfusion by arteriovenous anastomosis via the two-pieceed cannula of Bernheim. (From Bernheim BM. Blood transfusion: hemorrhage and the anaemias. Philadelphia: JB Lippincott, 1917.)
Transfusion medicine, relatively new branch of medicine, has evolved rapidly in recent years thanks to:

- Improvement in knowledge on spread of infections transmissible by blood transfusion, notably HIV; Hepatitis viruses;
- New transfusion techniques and practices;
- Awareness of risks associated with transfusion
Giant Strides in TM

- Discovery of blood groups
- Antihuman globulin test
- Plasticizers
- Blood components – leukofiltered / irradiated
- Apheresis technology
- Blood safety – NAT testing
- Recombinant Technology – antisera / growth factors
- Plasma fractionation
- Stem cells- Regenerative medicine
- Hemovigilance
# 1-Improving Components

- Use of better preservative / anticoagulants
  - Additive solutions

- Improved methods of component preparation
  - Leukofiltration of products
  - Rapid freezing of plasma
  - Pooled products

- Pathogen inactivation technology
### ADDITIVE SOLUTIONS

<table>
<thead>
<tr>
<th>FIRST GENERATION</th>
<th>SECOND GENERATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAG</td>
<td>BAGP-M</td>
</tr>
<tr>
<td>SAG-M</td>
<td>PAGGS-M</td>
</tr>
<tr>
<td>AS-1</td>
<td>PAGGGG-M</td>
</tr>
<tr>
<td>AS-2</td>
<td>Erythrosol-1 &amp; 2</td>
</tr>
<tr>
<td>AS-3</td>
<td>Erythrosol-81</td>
</tr>
<tr>
<td>EAS-64</td>
<td></td>
</tr>
</tbody>
</table>
Advantages of Additives

- Increase level of ATP & 2,3 DPG - red cell viability is enhanced
- Increase in shelf life of red cells to 42 days
- Better inventory control
- Extraction of more plasma
  1. platelet rich plasma for optimal production of platelets,
  2. factor VIII yields and FFP
- Ease of administration – controlled Hct
Platelet Additive Solutions (PAS)

- PAS are synthetic mediums introduced to replace plasma volume in a PC
- Reduction of allergic reactions, febrile transfusion reactions & TRALI
- Facilitates ABO incompatible platelet transfusions
- Extended storage of platelets
- Supernatant plasma diverted for fractionation
Improving Components

- Use of better preservative / anticoagulants
  - Platelet additive solutions

- Improved methods of component preparation
  - Leukofiltration of products
  - Rapid freezing of plasma
  - Pooled products

- Pathogen inactivation technology
Clinical Effects of Contaminating WBCs

- **HLA alloimmunization**
  - FNHTR  Platelet  Graft
  - refractoriness rejection

- **Viral transmission**
  - CMV, HTLV, EBV, nvCJD

- **Immune Suppression**
  - Post op  Cancer  Viral
  - Infection  recurrence  reactivation
Outcome of ULR

- Vancouver General Hospital, Canada
- Retrospective analysis of platelet transfusion in 13,902 acute leukemia patients
- Comparison of pre Vs post ULR

<table>
<thead>
<tr>
<th>Clinical problem</th>
<th>Pre-ULR</th>
<th>Post-ULR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA Alloimmunisation</td>
<td>19 %</td>
<td>7 %</td>
</tr>
<tr>
<td>Platelet refractoriness</td>
<td>14 %</td>
<td>4 %</td>
</tr>
<tr>
<td>Patients needing HLA matched platelets</td>
<td>14 %</td>
<td>5 %</td>
</tr>
</tbody>
</table>

Seftel MD et al, Blood, 2004
Improving Components

- Use of better preservative / anticoagulants
  - Platelet additive solutions
- Improved methods of component preparation
  - Leukofiltration of products
  - Rapid freezing of plasma
  - Pooled products
- Pathogen inactivation technology
Rapid Freezing of Plasma

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Rapid Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII (IU/unit)</td>
<td>189</td>
<td>290</td>
</tr>
<tr>
<td>FN (mg/unit)</td>
<td>235</td>
<td>320</td>
</tr>
</tbody>
</table>
Improving Products

- Use of better preservative / anticoagulants
  - Platelet additive solutions
- Improved methods of component preparation
  - Leukofiltration of products
  - Rapid freezing of plasma
  - Pooled products
- Pathogen inactivation technology
Unwelcome Visitors
Pathogen Inactivation Methodology

- Solvent-detergent (SD plasma)
- Methylene blue (MB, for plasma)
- Psoralens (S-59, Amotosalen)
- Riboflavin (vitamin B2)
- S-303 (for RBCs, Amustaline)
- Other dyes
- UVC (under investigation for platelets)
Disrupting Viral Nucleic Acid

Amatosoraleen

Riboflavin
Riboflavin Treatment

1. Transfer Whole Blood unit to illumination bag
2. Add 35 mL Riboflavin
3. Illuminate
4. Transfuse or transfer to storage bag
2- Improving Processes to Make Products

- Multi component collection
- Red cell antibody screening / platelet cross match
- Molecular diagnostics / Micro-array
- Improved TTI screening technology
  - NAT testing
  - Bacterial detection systems
Advantages of MCC

- Avoid unnecessary exposure to different donors
- Standardized blood components - consistent yields and volume
- Maximize collection from a limited pool of donors
- In-line leukofiltration
- Improved quality control
- Minimal ‘lesion of collection’, k/as ‘citrate shock’
- All advantages of apheresis
  - can be adapted to physical characteristics of donor
  - Lesser vasovagal reactions which are of main concern for the donor
## MCC protocols

<table>
<thead>
<tr>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2RCC, RCC+FFP, RCC+PC, PC+FFP</td>
</tr>
<tr>
<td>RCC+FFP+PC, PC+FFP</td>
</tr>
<tr>
<td>2RCC, RBC+PC, PC+FFP</td>
</tr>
<tr>
<td>2RBC, RCC+PC, PC+FFP</td>
</tr>
<tr>
<td>RCC+FFP</td>
</tr>
</tbody>
</table>
Double product (2PC)-SGPGI experience

<table>
<thead>
<tr>
<th>Wt/ht (kg/cm)</th>
<th>Hct (%)</th>
<th>Pre count</th>
<th>yield $(10^{11})$</th>
<th>Procedure time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72/167*</td>
<td>52.7</td>
<td>236</td>
<td>5.7</td>
<td>69</td>
</tr>
<tr>
<td>74/165</td>
<td>42.5</td>
<td>265</td>
<td>6.2</td>
<td>49</td>
</tr>
<tr>
<td>70/170</td>
<td>40.7</td>
<td>271</td>
<td>5.25</td>
<td>62</td>
</tr>
<tr>
<td>65/175</td>
<td>46.0</td>
<td>276</td>
<td>5.9</td>
<td>74</td>
</tr>
<tr>
<td>74/165</td>
<td>41.4</td>
<td>219</td>
<td>5.3</td>
<td>60</td>
</tr>
<tr>
<td>72/167*</td>
<td>49.7</td>
<td>238</td>
<td>6.2</td>
<td>64</td>
</tr>
<tr>
<td>72/167*</td>
<td>44.3</td>
<td>276</td>
<td>6.9</td>
<td>84</td>
</tr>
</tbody>
</table>

Total 13 procedures done for BMT patient; out of which 7 were double products; spend Rs 97,500 besides TTI

Saved Rs 46500, besides TTI testing and others cost involved
Improving Processes to Make Products

- Multi component collection
- Leukofiltration
- Advanced in immunohematology
  - Improved antibody screening
  - Platelet cross matching
- Molecular diagnostics / Micro-array
- Improved TTI screening technology
  - NAT testing
  - Bacterial detection systems
Advances in Immunohematology

- Techniques
  - Tile → Tube → Microplate → CAT

- Antiserum
  - Polyclonal → Monoclonal

- Definitions of Rh D
  - Partial D → DVI
Improving Processes to Make Products

- Multi component collection
- Leukofiltration
- Red cell antibody screening / platelet cross match
- Molecular diagnostics / Micro-array
- Improved TTI screening technology
  - NAT testing
  - Bacterial detection systems
Serology Can Not.............

- Determine paternal RhD zygosity
- Type fetus from amniocytes / maternal plasma
- Type multi transfused patient
- Type RBC positive for DAT
- Screen donor units for antigens for which no reagents available
  - Dombrock, Jsa, Kpa, Coa, Yta
- Detect altered D antigen
  - Weak D, partial D
- Resolve antibody identification
  - Allo or Auto
DNA Based Assays

- Blood group antigens are inherited – genes have been identified / cloned
- Similar to disease gene markers, many result from SNPs
- Identified by PCR amplification method
- Can do some things serology can not
- In some situations, superior to serology
DNA based assay in antenatal serology - Family I

O positive

O neg

ICT positive 1:32

Death on D5

Hydrops at 36 weeks

Hydrops at 30 weeks
Husband in family I and II are different.

At molecular/ genetic level

DNA based assay in antenatal serology - Family II

O positive

O neg
ICT positive 1:32

O positive

Death on D5

Hydrops at 36 weeks

Healthy child
A high throughput technology that allows detection of thousands of genes simultaneously

Based on the principle of base-pairing hybridization

Two types
- Protein array
- Antibody array
B) MICROARRAY-BASED

All markers tested simultaneously in each donation

Donation

DNA array  Protein array

1 instrumental platform

Repeat testing

Multiple probes per target would eliminate need for repeat testing

RESULTS

Blood
Blood Components

RELEASE
Improving Processes to Make Products

- Multi component collection
- Leukofiltration
- Red cell antibody screening / platelet cross match
- Molecular diagnostics / Micro-array
- Improved TTI screening technology
  - NAT testing
  - Bacterial detection systems
Window Period

- **Window Period**
  - Time from infection to lab detection of organism

- **Eclipse Phase**
  - Time from entry into the cell until the appearance of new virus within the cell
  - No detectable evidence of infection

- **Incubation period**
  - Time from exposure to the appearance of symptoms
<table>
<thead>
<tr>
<th>Marker</th>
<th>3rd Generation ELISA</th>
<th>4th Generation ELISA</th>
<th>ID NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>20.6 day</td>
<td>13.7 day</td>
<td>5.6 day</td>
</tr>
<tr>
<td>HCV</td>
<td>58.3 day</td>
<td>9.4 day</td>
<td>4.9 day</td>
</tr>
<tr>
<td>HBV</td>
<td>36.3 day</td>
<td>24 day</td>
<td>20.6 day</td>
</tr>
</tbody>
</table>
Benefits of Implementing NAT

- Decreases WP infection unlike serological assays
- Increased sensitivity and specificity
- Monitor dynamics of viremia in early phase
- Earlier donor counseling and patient care
- Characterization of genotype is possible.
- Future application may include additional viruses e.g. parvo virus B-19, CMV, Dengue etc
## NAT: Indian Scenario

<table>
<thead>
<tr>
<th>Study</th>
<th>Samples tested</th>
<th>Type of NAT</th>
<th>Yield</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makroo et al, 2008</td>
<td>12,224</td>
<td>ID</td>
<td>8</td>
<td>(1/1108) 1 HIV, 1 HIV-HCV, 6 HBV</td>
</tr>
<tr>
<td>Jain et al, 2012</td>
<td>23,779</td>
<td>MP</td>
<td>8</td>
<td>(1/2972) 8 HBV</td>
</tr>
<tr>
<td>Agarwal et al, 2013</td>
<td>73,898</td>
<td>ID</td>
<td>121</td>
<td>(1/610) 1 HIV, 37 HCV, 73 HBV, 10 co-inf</td>
</tr>
<tr>
<td>Koshy et al, 2013</td>
<td>52,083</td>
<td>ID</td>
<td>47</td>
<td>(1/1108) 43 HBV, 3 HCV, 1 HIV</td>
</tr>
</tbody>
</table>
How big is the problem?

Bacterial contaminated blood components cause of >10% (77/694) of recipient fatalities reported to FDA from 1985-1999

American Red cross reports
- 1: 15000 prior to strategy introduction
- 1: 50,200 after intervention
Strategies for prevention of sepsis
<table>
<thead>
<tr>
<th>Study</th>
<th>SDP</th>
<th>RDP</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perez 2001</td>
<td>31.8</td>
<td>71.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Kuehnert 2001</td>
<td>9.8</td>
<td>10.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Ness 2001</td>
<td>74.5</td>
<td>67.0</td>
<td>ND</td>
</tr>
</tbody>
</table>
Rational use of blood
Forming and adaptation of existing guidelines
Education and networking
Patient blood management
Blood conservation
Use of technology to reduce blood usage - TEG
Use of TEG in bleeding patient
# 4- New Uses of Existing Products

- Autologous platelet gel
- Fibrin sealants
- Cellular therapy
  - Use of platelet rich plasma for hair growth or intra-articular injections in Osteoarthritis
  - Use of stem cells in regenerative medicine
Utility of Platelet Growth Factors from allogenic Platelet-Rich Plasma (PRP) for clinical improvement in split-thickness skin graft

**Sonker A**, Dubey A, Bhatnagar A, Chaudhary R K
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow (UP) India
Results

Total of 20 patients (6 PBC, 5 BU, 4 AVM, 5 others) were included.

Allogeneic from apheresis donors was applied to half of the affected part (study group) and half untreated (control).

All the treated part showed complete uptake of graft (100%),

while there was a complete or partial loss of graft in 55% cases of non-applied part.
Stem cells are undifferentiated cells that have many potential scientific uses:

- Cell based therapies
  - Often referred to as regenerative or reparative medicine
- Therapeutic cloning
- Gene therapy
- Cancer research
- Basic research
Potential of Adult Stem Cells

Diagram showing the potential of adult stem cells in various tissues and applications:
# 5 - Common heart aches 

- A wrongly reported group
- A wrongly identified sample
- A wrongly interpreted crossmatch
- Variations in grading of reactions
- Carry over from a previous sample – pipette not changed
- Improper red cell suspension
- Wrong reagent added – wrong interpretation
- Wrongly transcribed group
With even the “best”

- Errors continue to occur

- Automation can reduce the errors as it reduces variability in testing and greater standardization
Errors in Transfusion Chain

- Misadventures: 4 (1.5%)
- No harm event: 10 (3.5%)
- Near miss events: 271 (95%)

N=285

Actual events
N=14
Use of information technology

- Blood Bank softwares
- Bar coding
- Use of RFID technology
- Electronic gadgets to reduce patient / donor identification errors
  - Wrist bands
  - Electronic “Lock”
Bright Future of Transfusion Science