Dr Saeed Al Amoudi
Consultant Hematologist
Laboratory, Blood Bank & IPCD / Chairman
Specialized Medical Center
MAGIC BULLET
Cord Blood Banking
What is Cord Blood?
Cord blood is the blood left in the placenta and umbilical cord after a baby is born.
Cord blood may cure life threatening disease

- Leukaemias
- MDS
- Bone marrow failure syndromes
- Hereditary immunodeficiency states
- Metabolic disorders
Why is Cord Blood Important?
Advantages

- Effortless & risk free
- Rapid availability
- Used against the HLA barrier therefore there is potential to expand effective donor pool
- Low risk of GVHD
- Low risk of transmission of infections
- Risk of CMV in new born <1%
- High proliferative capacity.
Disadvantages

- Low dose of stem cell when used for adults
- Delay engraftment
- Patients vulnerable to infection
- May expose the patient to rare genetic disorders
- Donor cord blood stem cells is unavailable for extra cord blood donation if needed
- Access to DLI is not possible
• CBT was used first time in 1988 for boy with fanconi anemia

• Cord blood was collected at the birth of his sibling

• Patient alive and well to this date
Cord blood collection may not be advisable or possible
• Baby is premature
• Multiple pregnancy
• Cord around the neck
• Emergency C/S
• H/0 maternal medication or positive viral markers.
Cord blood banking
• The CB banks comprises of integrated team responsible for collection, processing, testing, banking, selecting and releasing of CB units.
1-collection.

- Eligible women are consented during the antenatal period to donate their babies' cord blood to public cord blood bank service.

- Provide information for women in form of leaflets and educational materials.
• Questionnaires for medical history and family history

• Permission to contact the donating women after six months to ask about her health and her baby.
**Techniques of collection:**

- In-utero (prior to placental delivery)
- Ex-utero (following placental delivery)
- Used in spontaneous vaginal deliveries & Elective caesarean section
Methods of collection:

1-Closed technique

- Most common method in clinical practice
- Similar to standard blood collection
• Trained staff

• Torolley in assigned room

 steril cover
 steril bowl
 steril wipes
 steril gloves
 steril forceps
 cord blood collection kit (sterile collection bag, blood test tubes and collection pot)

• Sealable envelope for paperwork

• Sealable bag for samples and paperwork

• CB unit collection bag and pre printed barcode labels provided by cord blood bank
• Following the deliveries the umbilical cord is clamped and cut
• Baby is separated from the placenta
• Placenta is delivered
• The product is placed in collection trolley
• The cord is wiped using the sterile wipes
• The trained staff cannulates the umbilical vein
• The cord can be milked
• The cannula can be removed and discarded into a sharp bin
• Labelling
  Collection bag
  Samples
  Cord pot
  Paper work
2. **Syringe method:**

- Contain an anticoagulant
- Syringe used to draw blood from umbilical cord
- Similar to drawing blood for test
• Collection bag, samples and cord pot are placed in a bag then in the fridge

• All paperwork, consent maternal notes and collection information are placed in sealable envelope then in the fridge
2-Processing of CB unit

• There are two type of CBB

1. Private
2. Public

• The cord blood units are transported into cord blood bank by courier in transfer boxes or shipping container

• Temp inside the shipping container 3-15°C

• The temp is monitor by small metallic piece placed inside the container

• The units, samples and paperwork are taken out of the boxes and scanned

• Woman's details entered into database
• The cord blood units and samples are transfer into the clean room

• The cord unit connected by automated sterile device to small tube through Which 1ml of blood is taken for initial testing

  - TNC (WBC count X volume)

  - CD34

  - CD45

  - viability test

• Processing is hold till the result is out
32-44hrs

- <500\times 10^6 \text{ cells}
  - Discarded

- >500-900 \times 10^6 \text{ cells}
  - Research use only

- <32\text{hrs} >900 \times 10^6 \text{ cells}
  - Clinical use
Barcelona (Banc De Sang)
TNC

TNC

<1100X10⁶ → Research

1100-2000X10⁶ → CD34

>2000X10⁶ → Process

CD34

>4X10⁶ → Process

<4X10⁶ → Research
• If the TNC more than the target value
• Viability >85%
• Processing start
  - Sepax (40 min)
  - unit connected to the kit

• The machine draw the blood into chamber
• Centrifuge
• Buffy coat (stem cells) is diverted into small bag
• Plasma into waste bag (Used for testing)
• RBC into original bag (Used for testing)
• Stem cell bag is placed in cooler to reduce the temp 3-4°C in order to add the DMSO (dimethyl sulfoxide) (15-20 min)

• DMSO is add by pump (10-15min)

• The unit is placed in CRF (control rate freezer) for gradual reduction of the temp

• Then the unit transfer into quarantine tank
3-Tasting of CB unit

A- maternal Testing

1- Serological (ELISA)

- HBsAg
- HBc Ab
- HCV Ab
- HIV I,II
- HTLV I,II
- Syphilis (T-pallidum)
- CMV- IgG & IgM
- Toxoplasmosis IgG

2- NAT

- HBV
- HCV
- HIV
**B- cord blood testing**

- **Plasma**
  1- Serological (ELISA) testing
    - HBs Ag
    - HBc Ab
    - HCV Ab
    - HIV I,II
    - HTLVI,II
    - Syphilis (T-pallidum)

- **2- NAT for clinical unit**
  - HBV
  - HCV
  - HIV
• RBC
  - HB electrophoresis
  - Blood grouping
  - HLA Typing
  - Bacteriological testing (Aerobic, Anaerobic & Fungus)
• After the processing of the cord blood unit is completed a samples is taken for:

• TNC

• CD34, CD45, and viability (7AAD)

• Recovery assessment

• >60%

• Units for clinical use
• Smear
  
  - NRBC to adjust the TNC count
  
  - Few data suggest faster engraftment is associated with high percentage of NRBC
• Testing after thawing of the cord blood unit
  
  - CD3
  
  - CD34 & CD45
  
  - Viability (7AAD & Trypan blue)
  
  - Chimerism
  
  - Bacteriological testing (Aerobic & Anaerobic) from the waste bag
- CFU
- Media containing, STF, cytokins and growth factors
- Placed in the incubator

- Temp 37°C
- Humidity 85-90%
- CO2 5.5-6.5%
- Two week
  - examined under the microscope for GM, E & mixed colony
- If the colony > 10% then the unit for clinical use
• Selected units for clinical use tested for
  - HLA typing
  - CFU
  - Gender
  - ABO

• Sample is obtained from unit segment
• Annoxin V by flow is under study

• Apoptotic Marker
4-Banking of CB unit

- After addition of DMSO
- Unit placed in CRF for gradual temp reduction
- Transfer into quarantine tank till viral markers result out then
- Transfer the unit to nitrogen tank for storage
5-Selection of CB units

- Unit selection criteria
- TNC dose
- HLA
- TNC dose frequently is given priority over donor-recipient HLA in CB unit selection
- Diagnosis should be considered
HLA typing

- HLA I
  - A,B,C intermediate resolution

- HLA II
  - DQ, DR(B3,B4&B5) intermediate resolution
  - DRB1, high resolution

- Partial matching
  - 4/6

- HLA I, A,B
- HLA II, DRBI
University of Minnesota have defined adequate dose of single unit

- $3 \times 10^7$ TNC/kg for 6/6 HLA matched unit

- $4 \times 10^7$ TNC/Kg for 5/6 HLA matched unit

- $5 \times 10^7$ TNC/kg for 4/6 HLA matched unit

- Unit with TNC > $2 \times 10^7$ / kg can be used
Study analyzed the effect of the TNC dose and HLA match on the outcome in 1061 patients

- Leukemia or MDS
- Myeloablative regimen
- Single cord blood unit

(ASH 2010)
Reference Group

- 1 HLA mismatch (MM)
- TNC 2.5 – 4.9×10^7/kg
- The best outcome for neutrophils and platelet engraftment, aGVHD, TRM, treatment failure and overall mortality was associated with O MM (6/6 HLA – matched) units regardless of TNC dose
Next best outcomes observed in recipient of

1 MM units with TNC dose of $2.5 \times 10^7$/Kg or greater
2 MM units with TNC dose of $5 \times 10^7$/Kg or greater

No increase in risk of relapse
• 3 MM or TNC < $2.5 \times 10^7$ /Kg did substantially worse
Findings support new unit selection criteria

Recommendation may prompt selection of a unit with lower TNC dose but better HLA match over lesser matched unit with a greater TNC dose
6-Releasing of CB unit

**Thawing of CBU**

- Selected unit is taken from the tank and transported to clean room by - Dry– shipper

- CBU placed in sterile plastic bag and immerse in sterile water bath at 37°C till it become liquid

- Addition of equal volume of Dextran and Human serum Albumin

- Then the bags placed in Sepax (washing program which take around 20 min )
Sepax
- Albumin & Dexran mixed with CB in the chamber
- Centrifuge
- Supernatant into waste bag
- Stem cell with albumin & Dextran into collecting bag
- Testing CD3, CD34, C45, Viability, CFU, chimerism and microbiological testing from the waste bag
• If Sepax system is not available

• Recommendation of NMDP for washing of CBU prior to infusion

• The mixture centrifuge at 400xg for 10 minutes

• Supernatant removed and sedimented cells resuspended in fresh Dextran and albumin solution
- Dilution of the CBU with Albumin / Dextran prior to infusion without washing associated with serious adverse events

- Reported cases to NDMP

- Heart failure, respiratory distress, hemoglobinuria, and renal failure

- Patient required dialysis, intubation, and mechanical ventilation
Spain card blood Banks

- 7 national CBB
- 50,000 units
- 14,000 units in Barcelona
- 835 units were used from 1995-2010
- 235 units in 2010
Thank you