Haemopoietic Progenitor Cell (HPC) Sources & Collection

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Acknowledge Elizabeth Newman
Topics for Discussion

• Types of Stem Cells
• What is an HPC?
• Types of HPC collections
• HPC preferences
• Mobilisation of HPC’s
Types of Stem Cells

Stem cell types include:
1. Fertilised egg
2. Embryonic stem cells
3. Somatic stem cells
What is an HPC?

Hematopoietic progenitor stem cells (HPCs) are stem cells that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells).
Where do we find HPC’s?

HPC’s are found in the bone marrow of adults and children, which includes femurs, hip, ribs, sternum, and other bones – and cord blood.
Types of HPC collections?

- Umbilical Cord Blood or HPC, Cord immediately post delivery.

- Bone Marrow Harvest or HPC, Marrow direct removal from the hip with needle and syringe.

- Haematopoietic Progenitor Cell Collection via Apheresis or HPC, Apheresis after HPC mobilisation via a procedure involving the separation and collection of a certain type of white blood cell from the peripheral blood.
Which type of HPC for which transplant?

- Use of donor HPC for rescue of the blood and immune cell production is well established
- Which type of HPC is now regarded as ‘ancillary’ and are now used interchangeably
- Very dependent on what donor source is available
Benefits & Challenges of CB

- No collection risk for mum and babe donors
- High degree of HLA disparity
- Reduced incidence of GVHD
- Delayed neutrophil and platelet engraftment
- Increased risk of serious infections
- Limited volume leading to double or even triple cord use/ small volume may be of benefit
- Less cost effective compared to MB and PB
- Lower risk of transplant related mortality
Benefits & Challenges of HPC(A)

- Possibility of less tumour cell contamination in the collection
- No general anaesthesia
- Less painful for the donor
- Less manipulation of cells
- Cost effective
- Grade 2 – 4 acute GVHD and mortality higher
- Side effects of colony stimulating factors
- Adverse events of the collection procedure
- Rapid neutrophil engraftment
Benefits & Challenges of BM

- General anaesthesia required
- Donor requires at least a week off from work
- Pain in the hip areas
- May require blood transfusion
- Transplant specialist preference
- Donor preference
- Reduced GVHD
- Rapid neutrophil engraftment
Umbilical Cord Stem Cells

- Blood that is left in the placenta and the umbilical cord after the delivery.
- Rich in HPC’s.
- Collected from the umbilical cord vein
- Average volume is 90mls
- No risk to mother or baby
- May be kept for over 20 years without loss of potency of the HPC’s
**Umbilical Cord Stem Cell**

- Cord blood stem cells do not need as rigorous matching as bone marrow.
- These HPC’s are more innocent than ‘educated’ stem cells from adult bone marrow.
- Transplant from cord blood produces far less immunological complications than adult bone marrow.
- Thus matching is less critical and it is often easier to find a cord match than an adult bone marrow.
- Cord Banks depend on having UCB donated and stored from a representative mix of all ethnicities that make up the Australian community.
Umbilical Cord Stem Cell

- Only 30% of patients have a matched bone marrow donor.
- 30,000 cord blood transplants over the last 25 years in both children and adults worldwide.
- More than 1240 performed in Australia to date.
- Cord blood transplantation is a curative treatment for diseases such as:
  - Immune deficiency
  - Haematological malignancies
  - Aplastic and Fanconi Anaemia
  - Metabolic Storage diseases
  - Thalassaemia
Umbilical Cord Stem Cell

Two methods:

1/ Whilst the placenta is still in-utero
   - immediately after delivery and UCB cord cut the collector will venepuncture the umbilical cord vein and drain the blood from the placenta in to a bag.
   - Takes several minutes.
   - Once the placenta is expelled more blood is then collected from the umbilical vein.

2/ Once placenta is expelled from the uterus.
   - UCB is extracted away from the delivery room
Directed UCB Donation

Donation of directed UCB where there is a family member with a disease such as leukaemia and which is in need of a bone marrow transplant. Sometimes referred to as a ‘Saviour Sibling’.

Arrangements for this type of donation are made through the transplant physician via the transplant centre.
Private Commercial Donation

• Little social or medical justification.
• Very low likelihood of requiring autologous UCB later in life.
• No proven benefit as yet for tissue repair or replacement in degenerative disorders such as diabetes and Parkinson’s disease – or is there?
• Collection fee varies.
• Storage is around $500.00 a year for 18 years.
• Not bound by TGA requirements.
Bone Marrow Harvest

• Patient may be required to donate 1 – 2 units of blood about a month prior to the donation for autologous use post donation of bone marrow - dependent upon organisational requirements

• Patient requires general anaesthesia.

• Marrow is collected from the cavities in the hip bones.

• Bone marrow trephine needle used to make multiple punctures through the skin into the hip bone cavity on both sides.

• Donor is rolled face down onto a frame shaped a bit like the Harbour Bridge which is strapped to the operating room table.
Bone Marrow Harvest

• Procedure takes about two hours.

• Usually collect between one and two litres of bone marrow for an adult or approximately 20mL/donor weight.

• Bone marrow is kept anticoagulated with ACD-A. Ratio is usually 100mls ACD-A:500mLs bone marrow.

• Donor may be given a bolus Heparin prior to commencement, 50 units/donor kilo.
Bone Marrow Harvest

• Will probably require an overnight stay.
• Requires pain relief, bruising evident, discomfort in lower back region, no sutures.
• May resume normal activities after two to three days.
• Bone marrow replacement occurs rapidly in about four weeks.
• Bone marrow may be filtered at the time of collection in order to remove skin plugs, fat clots and bone spicules
• Bone marrow is generally intravenously transfused to the patient just like a blood transfusion
HPC, Apheresis collection

Blood & Stem Cells go out one arm

Blood minus Stem Cells goes back in the other arm

PBSC are collected by machine

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HPC, Apheresis collection

An HPC, Apheresis collection is performed with a medical device called a blood cell separator, which uses a centrifuge to separate and collect mononuclear cells, including HPC’s, from the blood.
HPC Mobilization

Very small percentage of HPC’s in the peripheral circulation.

GCS-F is generally administered twice daily for four - five days to initiate HPC growth.

In autologous BMT chemotherapy may be utilised prior to G-CSF.
Mobilisation strategies

Varied and is dependent on the disease, stage or preference of physician

No stimulation – steady state (research collections)
G-CSF alone
Cyclophosphamide
Plerixafor
GDP, RICE, ICE, most regimens we may now attempt to mobilise from
Patient education

G-CSF administration
Medication importance and delivery
Patient assessment routine
Indications for sepsis
Need for admission – inpatient or outpatient
What happens if admitted
Patient protocol/clinical pathway
CRGH transplant booklet/BMT autologous book
Patient Education includes:

Role of chemotherapy
Side effects of chemotherapy
Responsibilities of the patient with home care
Blood count importance
Role of the reinfusion
Complications of reinfusion
G-CSF:

1. Promotes CD34+ cell proliferation
   - CD34+ cells retained by local adhesion molecules: VCAM-1/VLA-4

2. Protease release from neutrophils (Elastase, Cathepsin G, MMPs)
   - Degradation of VCAM-1/VLA-4 and SDF-1α/CXCR4 interactions

3. SDF-1α retention signals degraded, CD34+ cells migrate to bloodstream
   - CD34+ cells retained by local adhesion molecules: VCAM-1/VLA-4 and SDF-1α/CXCR4 interactions

Connective tissue

Capillary

Stromal cells

Endosteum

Bone

Osteoblast
Precautions

Aching bones and muscles
Redness or swelling around injection site
General allergy reactions
Spleen Rupture
ARDS
Sickle Cell
24hrs of chemotherapy
Scotland documents that 30% of attempted mobilisations fail - much less in Australia

Options to consider:

Mobilise again with chemo, G-CSF & another colony stimulating factor

Or

Do we attempt a rescue!!!!!
Mozobil inhibits SDF-1$\alpha$/CXCR4 binding and releases CD34$^+$ cells into circulation.
Dosage and Administration

Each vial contains 24 mg Mozobil in 1.2 mL solution. The patient's actual body weight is used to calculate the volume of Mozobil to be administered.

\[0.012 \times \text{patient's actual body weight (in kg)} = \text{dose to be administered (in mL)}\]

Required Concomitant Medications
Daily morning doses of G-CSF 10mcg/kg for 4 days prior to the first dose of Mozobil and on each morning prior to apheresis

Dose Modification
Renal Impairment – for patients with moderate and severe renal insufficiency (creatinine clearance of 20-50ml/min), decrease dose by one-third to 0.16 mg/kg
Precautions

Potential for tumour cell mobilisation in patients with lymphoma and multiple myeloma: effect of tumour cell reinfusion is unknown

Tumour cell mobilisation in leukaemia patients: may mobilise leukaemic cells and should not be used in leukaemia patients

Haematological effects: leukocytosis and thrombocytopenia

Potential effect on spleen size: evaluate patients who report left upper abdominal and/or scapular or shoulder pain

Renal impairment: should be used with caution in patients with moderate or severe renal dysfunction

Pregnancy: should not be used during pregnancy unless potential benefit justifies the potential risk to the foetus
When do we know to collect?

CD 34 antigen enumeration
IRF result
WCC

Different organisations utilise differing parameters in order to initiate an HPC-A
What is a CD34?

A protein that is expressed on the surface of the HPC that is able to be recognized and isolated with immunofluorescent testing in the flow laboratory.
CD34 Enumeration

The aim of CD34+ cell enumeration is to provide a measure for the number of haemopoietic stem cells present in peripheral blood or the enumeration in the collect bag which gives a total collected that may be intended for transplant.
What is an IRF?

IRF – Immature reticulocyte fraction. An increase in immature reticulocyte fractions precedes the presence of circulating CD34+ cells by about 2 days in patients mobilized with chemotherapy and growth factors.
Stem Cell Collection

Generally takes between 4 - 6 hours
May take more than one collection
Patient may experience adverse events
Slide of Blood Elements and Specific Gravities
OPTIA Collection

Utilised in 12 of the 14 BMT apheresis units in NSW
Takes between 3 – 6 hrs
Requires good venous access
Process = > 3 TBV
May retrieve prescribed dose of HPC’s in one collection
Adverse events
Large Volume Leukopheresis

\[
\text{Weight (kg)} \times A \\
\text{PB CD 34 (/µL)}
\]

\[A = \text{CD34 prediction score.}\]

Prediction score is based on collection efficiency data calculated by Annette Trickett from the BMT Network.
Before commencement

Venous assessment.
Vital signs
Pharmaceutical history.
Serology's.
Usual Bloods.
Medical Assessment.
Documentation.
Height/weight/haematocrit.
Patient education.
Comfort.
**Venous Assessment**

Peripheral or CVAD.

Collection efficiency will be compromised if the right access is not chosen.

Many different types of access options available.

AV Fistula needles and IV cannulas.

Vascaths and Apheresis Hickmans.
Extremely important:

To determine if the patient is on Anti-hypertensives – particularly ACE inhibitors.

Alternative anti-hypertensives must be prescribed until after collection is completed.
Documentation

Dose prescription
Referral
Procedure sheet
Laboratory requests
Electronic medical records
Progress notes
Other organisational requirements
Adverse Events

Poor venous access.
Needle phobia.
Citrate toxicity.
Anxiety.
Extracorporeal issues: clotting, etc.
The Arm With No Vein

There’s a terrible curse that all of us know
You stick in the needle, but the blood just won’t flow:
There’s nothing I know that causes such strain.
As that dreaded condition. The Arm With no Vein

The donor is willing, the doctor is tense,
The team disappears, if they have any sense,
You turn to the left arm, and try once again,
Then break down and tell him “Your arm has no vein!”
The Arm With No Vein

There’s a gloom o’er your day when you make a big bruise
And, ‘the end one is dripping’ is hardly good news.
But it’s nothing compared to the heartache and pain,
When you stand and look down at the Arm With no Vein.

Oh donors who faint are a problem it’s true.
And some get quite ‘Cut” when their blood is not blue.
But the thing that drives nurses and doctors INSANE
Is the terrible curse of the Arm With no Vein.

Dr Joy Bearup, Plasmapheresis Medical Officer, Red Cross, Sydney
Questions?
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