



How to harvest bone marrow for transplantation

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Introduction

Over the past decade bone marrow transplantation (BMT) has graduated from being an experimental treatment to having an established role in the first line management of patients with life threatening haematological disorders. The therapeutic principle involved is that the administration of myelotoxic treatment (preparative protocol), at doses beyond the threshold normally set to guarantee haematological recovery, will be effective in eradicating the abnormal pathology in the patient. A second requirement is that, where an allogeneic donor is the source of marrow, the preparative protocol will immunosuppress the host sufficiently to ensure that the marrow will successfully graft. There are preclinical data,¹ and some circumstantial evidence in man to suggest that the mechanisms by which the underlying disease in the host is eliminated, may not be solely due to the intensity of the preparative protocol but augmented by an immunologically mediated effect from the donated marrow, referred to as the "allogeneic effect".^{2,3} There are now several conditions to which bone marrow transplantation has been successfully applied (table).

The bulk of this experience has been of transplantation from an HLA matched, mixed lymphocytic culture (MLC), non-reactive sibling donor. The capacity of this form of treatment to eradicate the underlying disease more effectively than conventional approaches is clear and has encouraged investigators to explore means by which transplantation can be made available to those who lack a suitable matched sibling. This has led to the use of the patients' own marrow collected earlier when in remission and, following storage, used as the source of haematological rescue to support high dose treatment (autologous BMT). More recently the organisation of registries of sufficient size has permitted a more systematic approach to using HLA phenotypically matched but unrelated donors as a source of stem cells.

Selection and preparation of the marrow donor

The availability of an HLA matched, sibling

donor is an important factor in deciding between an allogeneic and an autologous transplant for a given patient. Thus the siblings of a potential allogeneic transplant recipient must be screened to identify any who are HLA matched. In the absence of a related donor a search of volunteer donor registers may locate an HLA matched unrelated donor. Alternatively an autograft may be preferred.

When a donor is found, preliminary investigations constitute a full medical examination to establish that they are fit for anaesthetic. Routine haematological and biochemical parameters are checked. The donor must be hepatitis B, hepatitis C, and human immunodeficiency virus (HIV) negative. If indicated, a chest x ray picture and an electrocardiogram are taken.

Written informed consent must be obtained.

The donor is admitted the night before harvest and the best method of anaesthesia discussed. In our unit we recommend a general anaesthetic but a regional block by spinal or epidural anaesthesia is an alternative.

Personnel required for bone marrow harvest

- Anaesthetist
- Operators to harvest marrow
- Haematology technician
- Scrub sister
- Theatre staff

Diseases that may be treated by bone marrow transplant

- (1) Bone marrow failure
 - Severe aplastic anaemia
 - Severe paroxysmal nocturnal haemoglobinuria (PNH)
 - Fanconi's anaemia
- (2) Leukaemia
 - Acute lymphoblastic leukaemia
 - Acute myeloid leukaemia
 - Chronic myeloid leukaemia
 - Acute malignant myelocytosis
 - Myelodysplasia
- (3) Lymphoma
 - Hodgkin's disease
 - Non-Hodgkin's lymphoma
- (4) Immunodeficiency syndromes
 - Severe combined immunodeficiency
 - Wiskott-Aldrich syndrome
- (5) Other genetic disorders
 - Thalassaemia
 - Gaucher's disease
 - Hurler's syndrome
 - Infantile osteopetrosis

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Equipment required for bone marrow harvest sterile equipment

- Jamshidi trephine needle
 - Two modified Rosenthal marrow harvest needles or equivalent
 - One sternal aspiration needle
 - Eight 30 ml plastic disposable syringes
 - Two 2 ml plastic disposable syringe
 - 100 ml Waymouth solution (Gibco) with preservative free heparin (Leo laboratories added at 50 units per ml)
 - Two blood donor (Tuta) 500 ml bags
 - Two sampling site couplers (Fenwal)
- Sterile drapes, swabs and towel clips
Sterile solutions for cleansing operation site
Stand for marrow collection bag

Additional non-sterile equipment

One light microscope
Modified Neubauer cell counting chamber

Technique of bone marrow harvest

The anaesthetised patient is positioned prone on the operating table with pelvis supported to make the iliac crests prominent. The skin is cleansed and drapes applied to leave the posterior iliac crests exposed.

The exact technique of harvesting varies between centres but is unlikely to differ greatly from that described by Thomas.⁴

The choice of harvest needle is one of personal preference as a range are commercially available which differ little except in the design of the gripping handle. Some have holes along the lateral aspects of the shafts but these clog easily and are of little value.

The needle with trochar is inserted through the skin and into the bone, the trochar is withdrawn, and marrow blood aspirated into a 30 ml syringe. The syringe is immediately detached and discharged into a collecting receptacle. The syringes are flushed with Waymouth solution with the addition of heparin and recycled. We use eight syringes for each harvest.

In some centres donors are anticoagulated to reduce the risk of the aspirated marrow clotting, but we have found that flushing the syringes with anticoagulant between each aspiration and regular mixing of the marrow in the collection bag makes this unnecessary.

It is our impression that the yield is optimised by inserting the needle superficially, and two aspirates taken at that level, and then the needle advanced and the aspiration repeated. It is usually possible to take marrow at several different depths from one hole. The needle is then withdrawn and re-sited, samples being taken as widely as possible along the posterior iliac crest. Most marrow will be obtained at the initial part of "the pull," thereafter the volume will largely be peripheral blood. We therefore restrict each pull to 8–10 ml.

If an inadequate cell count is obtained from the posterior iliac crests the patient should be turned over and further aspirates taken from the anterior iliac crests and the sternum. This is sometimes necessary for autologous bone marrow transplant where the patient has been heavily pretreated with radiotherapy or cytotoxic drugs, but in most cases the posterior crests are sufficient.

It is quite common for aspirated marrow to be first discharged through a stainless steel mesh into a sterile beaker to remove fat aggregates, clots, or bone spicules. The marrow may be exposed to airborne contamination if this technique is used and we prefer a closed system where the syringe is emptied directly into a 500 ml blood donor bag which contains citrate-phosphate-dextrose (CPD) (Tuta) as anticoagulant. The bag is held in a stand and is manually agitated.

The marrow bag can easily be sampled with a 2 ml syringe to allow a cell count to be performed as the harvest proceeds. We normally take 500 to 1000 ml of marrow at a harvest. If inadequate counts are obtained it is repeated at a later date.

Marrow dose

There are different ways to calculate the dose obtained during a harvest. Some simply take 10 ml/kg recipient body weight but in most centres a nucleated cell count is performed while the harvest is in progress to determine when an adequate harvest has been obtained.

The marrow cell count can be calculated by subtracting the donor's peripheral white cell count from the marrow count but we simply count the marrow nucleated cells in a Neubauer counting chamber and use this as a guide to marrow yield.

The absolute amount of marrow needed is not known and current clinical practice varies widely, but some principles apply. Successful re-engraftment is related to tissue compatibility such that autografts, twins, HLA matched T replete siblings; HLA matched unrelated and HLA mismatched donations require a progressively larger graft to increase the chance of graft take.

The "dose" of nucleated cells is expressed per kilogram of recipient body weight. An



Figure 1 Theatre trolley laid up for a bone marrow harvest. (a) aspirating syringes (30 ml); (b) needles; (c) flushing fluid (Waymouth); (d) collection bag on stand; (e) bag cap + coupler; (f) syringe for sampling bag.

historical target figure for matched sibling allografts is 3×10^8 nucleated cells per kg. This was the threshold that ensured engraftment in sensitised patients with aplastic anaemia.⁵ In unsensitised HLA matched patients who receive total body irradiation a dose of $1.5 - 2.0 \times 10^8/\text{kg}$ is probably sufficient. We aim to have a minimum of $1 \times 10^8/\text{kg}$ for autologous grafts.

Postoperative care

The harvest procedure lasts about 40 minutes and after recovering from the anaesthetic the patient is transferred back to the ward. It is mandatory to replace volume lost during the procedure with crystalloids or plasma expander and ideally red cells are also replaced. It is desirable to avoid exposing a volunteer donor to the risk of allogeneic blood transfusion however slight. Two options exist: either autologous blood is taken a week or more prior to the procedure or the red cells are separated from the marrow by centrifugation.

In an autologous harvest replacement from a community donor may be required and it is usual to have 2 units of matched irradiated blood available. Irradiation destroys lymphocytes that survive in the donated blood and in theory could contaminate aspirated marrow, and when returned to the patient, set up graft versus host disease. Delaying transfusion until after the harvest abolishes the risk unless a second harvest is needed in the future. We routinely have 2 units of irradiated blood available but these are very rarely needed.

The donor is retained overnight after the harvest to allow full recovery from the anaesthetic and red cell replacement.

Complications for the marrow donor

Postoperative pain is modest, easily controlled, and transient. With care any risk to the donor is minimal. Serious complications occurred in 0.27% of 3000 cases from Seattle and the international bone marrow transplant register reviewed by Bortin and Buckner.⁶ Five complications related to the anaesthetic, three to septicaemias secondary to infection at the aspiration site, and one was a cerebral infarction but this occurred many hours after the harvest when the patient had fully recovered. The risks are similar to that for other minor operative procedures.

In vitro manipulation

ALLOGENEIC

For allogeneic transplantation the harvested marrow can be taken and immediately infused intravenously into the patient. However, for specific reasons, some form of in vitro processing or treatment may be required before it is given. In the autograft setting in vitro treatment aimed at reducing occult tumour cell contamination or preparation for storage may be needed. It is not the purpose of this article to discuss the pros or cons of any of these manipulations as they affect clinical outcome.

In two circumstances in relation to allogeneic transplantation it may be necessary to remove selectively the mononuclear cell fraction which is known to contain the repopulative stem cells. First, density separation, which results in a suspension with a very low haematocrit, is required where there is a significant blood group incompatibility between donor and host. Second, if removal of T cells is required, as a strategy to prevent graft-versus-host disease, then it is convenient to reduce the volume of the donation before starting incubation with monoclonal antibodies. A convenient way to do this is the automated procedure on a Cobe 2991 blood cell washer, as originally described by Gilmore *et al.*⁷

The Cobe 2991 can provide a buffy coat, or ficoll-hypaque can be introduced to permit on-line density separation to produce the mononuclear cell fraction (MNC). An equivalent result can be obtained using different cell separators. Incubation with antibody and complement to destroy T cells can be easily achieved in the system, and the subsequent washing steps all carried out in a closed semiautomated system.

Autologous marrow

Autologous marrow can be similarly separated to leave a buffy coat or further processed to a mononuclear cell fraction which can be "purged" or stored. The most popular method aimed at elimination of occult leukaemia from autologous marrow in acute myeloid leukaemia is by incubation with the cyclophosphamide metabolite (4 hydroxy-per-cyclophosphamide, 4 H-C).⁸ An important variable in the technique of purging with this method is the haematocrit of the starting marrow suspension, so it is helpful to start with a standard cell preparation. When an immunological method of purging is used, which is most readily applicable in acute lymphoblastic leukaemia, the sequence of events is similar to that described above for T cell depletion of allogeneic marrow. There are several different methods of treating marrow in vitro with the MNC as the starting point.

Bone marrow storage

Some form of storage is always required for autologous BMT. Where the preparative protocol is short then cryopreservative is not required. We have demonstrated satisfactory reconstitution from a total body irradiation (TBI) based, myeloablative protocol, using marrow stored as it was collected in anticoagulant, or as an MNC fraction, at 4°C for up to 54 hours.⁹ In vitro survival of committed haemopoietic precursors (CFU-GM, BFU-E) suggest liquid storage for seven to 10 days might be feasible, but this has not been tested clinically in a patient given an ablative protocol.

Where prolonged treatment regimens are used, or further treatment of the patient is envisaged autologous marrow must be cryopreserved. Here the initial preparation of an

MNC fraction may also have major advantages. The neutrophil content of an MNC is small and neutrophils freeze poorly which may result in clumping. The final volume of an MNC fraction can easily be kept to less than 100 ml from a 1 litre harvest, which allows even freezing through the cell suspension, and reduces the absolute volume of cryoprotectant which will be returned to the patient. The reduction in volume also saves a considerable amount of expense in liquid nitrogen storage capacity.

Having reduced the initial marrow volume to 50–70 ml resuspended in the patient's autologous plasma, an equal volume of dimethyl sulphoxide (DMSO) at 20% in autologous plasma can then be added at 4°C. In theory glycerol is the best cryoprotectant, but requires to be eluted from the cells when thawed and then be washed off, which is impractical. DMSO at a final concentration of 10% has come to be the accepted cryoprotectant, having the important advantage that it rapidly diffuses in and out of the cells. It is, however, toxic to cells at room temperature and it is important to introduce it to the marrow suspension at 4°C, and on thawing, to waste no time in returning the marrow to the patient. One of the advantages of the low bulk of an MNC is that it is not necessary to wash the small amount of DMSO out of the thawed suspension because it can safely be given directly to the patient. Large volumes of DMSO are toxic when given intravenously.¹⁰ Any *in vitro* purging is invariably done before preparation for freezing begins. There is no important evidence to suggest that marrows which have been treated *in vitro* are any more susceptible to damage during cryopreservation.

There are several factors which are thought to affect the quality of freezing. These include the cellular component, the protein content of the suspending fluid, the choice and concentration of cryoprotectant, the rate of freezing and the storage conditions. These aspects have been the subject of a helpful review.¹¹ It has been generally accepted that a controlled freezing rate is mandatory (around 1–2°C a minute) at least until the eutectic point, but this has recently been challenged.¹² Storage in the liquid phase of nitrogen is probably preferable to the vapour phase which is vulnerable to variation caused by periodic opening of the storage container.

Assessment of *in vitro* manipulation and storage

One of the current difficulties is that there is no direct laboratory measurement which correlates with the regenerative capacity of the graft. In general most storage techniques, particularly when initially introduced, should be monitored using assays of the committed haemopoietic precursors (CFU-GM and BFU-E). Marrows can be successfully regenerated after these populations have been removed, but it is reasonably safe to accept their presence before and after manipulation as

an indication that the repopulative potential has been retained. These precursor populations are not a direct measure of the pluripotent stem cell population.

We have found that samples taken from the harvest occasionally yield a positive microbiological culture usually with a skin commensal. We have noted no clinical consequences as a result of this.

Administration of the bone marrow

Thawing of stored marrow in a water bath at 37°C, and the immediate reinfusion to the patient via the central venous line, usually passes uneventfully. From time to time some patient reaction ranging from nausea and vomiting, to rigors or anaphalaxis (rarely) is seen. The frequency of such episodes is not, in our experience, related to whether or not a particular laboratory manipulation was involved or any blood group disparity existed. Such episodes can be alarming and medical supervision of the reinfusion is recommended.

Administration through a standard blood administration set ensures that any aggregates are removed. The small amount of DMSO administered is expired over the following 24 hours without clinical sequelae.

New developments

Use of stem cells taken from peripheral blood by leucapheresis is becoming fashionable.^{13,14} It was initially felt that this approach would have the advantage of avoiding any potential tumour cells which may be contaminating the bone marrow—and in particular would allow an autograft option to be available to patients known to have an infiltrated marrow. There is no evidence to support the view that the peripheral blood is a purer source of stem cells, but the thoughtful use of cytokines to mobilise peripheral blood stem cells (PBSC) has meant that adequate numbers can be obtained with one or two leucaphereses, making it more practical. Particularly attractive are the accumulating data that the pace of haemopoietic reconstitution—particularly of platelets—is much quicker with PBSC alone or with bone marrow than with marrow alone.

Technical developments now suggest that positive selection of a stem cell population from marrow (as defined by a CD34 positive phenotype) is becoming a practical possibility. It has been known for some time that such a population can reconstitute irradiated patients, but the techniques involved (column immunoadsorption) gave poor cell yields. More efficient techniques will be validated in the near future. This approach would represent a universal approach to purging and also segregate a target cell population for *in vitro* genetic manipulation.

Dr Jones is a Leukaemia Research Fund Clinical Fellow.

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