INTRODUCTION

Bone marrow is a tissue which is indispensable to life, since it is where blood cells and the cells of the immune system are formed. The bone marrow is also where the **haematopoietic stem cells** are housed. These cells are capable of producing all of the cells of which the blood is comprised:

- The white blood cells or **leukocytes,** which help you fight infection. There are various different types of white blood cells: Myeloid cells (neutrophils, monocytes, basophils, and eosinophils) and lymphoid cells (T and B cells).

- The red blood cells or **erythrocytes/haematids** are responsible for transporting oxygen to the body's tissues and bringing back carbon dioxide from these tissues to the lungs, where it can be expelled. Erythrocytes give your blood its characteristic red colour.

- The *platelets* or **thrombocytes** contribute to the clotting of blood when a blood vessel is ruptured.

Many illnesses are due to the excessive, insufficient, or abnormal production of a certain type of cell derived from stem cells. A **bone marrow transplant** enables such illnesses to be cured by replacing the defective cells with normal cells from a healthy donor. In the case of many of these illnesses, a transplant is the only treatment option.

Stem cells can be retrieved from bone marrow, circulating (peripheral) blood, and the blood in the umbilical cord at birth. In other words, transplants can take the form of **bone marrow, peripheral blood, or cord blood**. For this reason, when talking about transplants in general terms we use the term **haematopoietic stem cell transplantation**.

COMPATIBLE DONORS

In order to carry out a haematopoietic transplantation, a donor who is compatible with the patient is required. "Compatible" means that the cells of the donor and of the patient must be sufficiently similar to enable the donor cells to live indefinitely in the recipient. This is because all cells carry a series of proteins on their surface - **human leukocyte antigens (HLAs)**. These differentiate them from the cells of another organism. The **lymphocytes** in our blood can detect the presence of cells with HLAs which differ from their own and can destroy such cells. This defence mechanism is responsible for the **rejection of organs and grafts** in the case of transplants between incompatible people.

Due to the high number of lymphocytes in the bone marrow or peripheral blood transplanted during haematopoietic stem cell transplantations, rejection can take place in both directions: **(a)** Rejection of the transplanted cells by the recipient and **(b)** rejection of the recipient by the transplanted cells. The latter reaction is called **graft-versus-host disease (GvHD)** and is one of the most serious complications of a haematopoietic stem cell transplantation.

Since the human leukocyte antigens (HLAs) of each individual are different to those of another person (except in the case of identical twins) and since each person inherits half of their antigens from their father and the other half from their mother, the best chance of finding a compatible donor is to investigate the siblings of the patient or the patient's first-degree relatives (parents and children). Sadly, due to the natural laws of inheritance, the probability of a brother or sister being compatible with another sibling is just **25%**.

The probability of finding a match within the wider family is just **5%**. If a patient requiring a transplant is unable to find a compatible donor within his or her family, the only possibility of a cure is through finding a **compatible, unrelated voluntary donor**.

REGISTERS OF VOLUNTARY DONORS

Since there are millions of possible combinations of different HLAs, it is difficult to find an unrelated donor who is compatible with a specific patient. Fortunately, in order to carry out a successful transplant, it is not necessary for the HLA systems of the donor and recipient to be absolutely identical. Despite this, the only way to find a donor who is *sufficiently compatible* with a patient is to collect information on the HLAs of a large number of possible donors. To help these donors to be found, **registers of voluntary donors** have been created in all of the developed countries. In Spain, this work has been entrusted to the **Spanish Bone Marrow Donors Registry (REDMO)**, which was created in 1991 by the International José Carreras Foundation with the aim of finding unrelated donors for Spanish patients. In 1994 REDMO was named as the national registry for searching for bone marrow donors for Spanish and foreign patients and for recruiting and recording the details of voluntary donors of haematopoietic stem cells from amongst the population of Spain.

VOLUNTARY DONORS

Characteristics

Any person in a **good state of health** and aged between **18 and 55** can donate haematopoietic stem cells as long as they agree to the following:

- To provide basic personal information (age, address, telephone number, and a **brief medical history**)

- To undergo the **extraction of a blood sample** so that histocompatibility can be tested and to consent to the storage of a small quantity of blood at a laboratory to enable tests to be furthered if necessary in the future without requiring more blood to be extracted if a possible compatible patient is found

- To consent to the entry of his or her basic data and compatibility data into the REDMO database and - once the data has been encrypted to protect the donor's identity (Organic Law 13/1999) - to allow the entry of information on compatibility into the global register of voluntary donors of haematopoietic stem cells

Issues preventing donation

The donation of haematopoietic stem cells is **strictly contraindicated** in the case of people with certain diseases. For this reason, before registering, each possible donor must check that he or she does not suffer from/have any of the following:

- Uncontrolled arterial hypertension
- Insulin-dependent *diabetes mellitus*
- Any cardiovascular, pulmonary, hepatic, haematological disease or any other disease which gives rise to an added risk of complications during the donation process
- Any malignant tumoral, haematological, or autoimmune disease which involves a risk of transmission to the recipient
- A viral infection of hepatitis B or C, Acquired Immune Deficiency Syndrome (AIDS), or any other potentially contagious agent
- Any AIDS risk factor (positive anti-HIV antibodies, an intravenous drug habit, multiple sexual relationships, a relationship with anyone who has any of the previously mentioned risk factors, haemophilia, a relationship with anyone who has haemophilia, or injury involving exposure to contaminated material within the last year)
- A permanent ban on acting as a blood donor

In addition to the contraindications mentioned above, the following contraindications apply to the **donation of stems cells from peripheral blood**:

- The donor must not have a history of ocular inflammatory disease (iritis, episcleritis) or fibromyalgia.
- The donor must not have a history of or risk factors for deep vein thrombosis or pulmonary embolism.
- The donor must be undergoing lithium treatment.
- The donor must not have a platelet count below 120,000 per mL.

Temporary contraindications:

- Pregnancy; following delivery, a woman can donate once she has finished breastfeeding

- Anticoagulant or antiaggregant treatments (with aspirin, dipyridamole, or similar); no donation for the duration of treatment

There are many other circumstances not included in the lists above which can make donation more difficult (morbid obesity, deformities of the neck or spinal column, allergies to anaesthetics, and genetic enzyme deficiencies, amongst other things). For this reason, we recommend that all candidates find out about their particular situation before registering as a donor, especially since some disorders preclude the donation of bone marrow but not that of peripheral blood, and vice versa.

Becoming a donor

If you meet the prerequisites stated above and want to register as a voluntary donor, please proceed as follows:

1) Ring the **Donor Referral Centre** ("Centro de Referencia de Donantes") of your autonomous community (see the provided list) to arrange a day and time when you can go along to find out more about the donation process and undergo the compatibility tests.

2) If you require **further information** on the donation process prior to your appointment, you can ask your referral centre or you can request more information from the José Carreras Foundation by ringing 93 414 55 66, sending an e-mail to donantes@fcarreras.es, or via the foundation's Website (www.fcarreras.org). You must carefully read the **document of informed consent** (see appendix) and the back of the **donor registration document** (see appendix).

3) On the day of your appointment at your referral centre, you must:

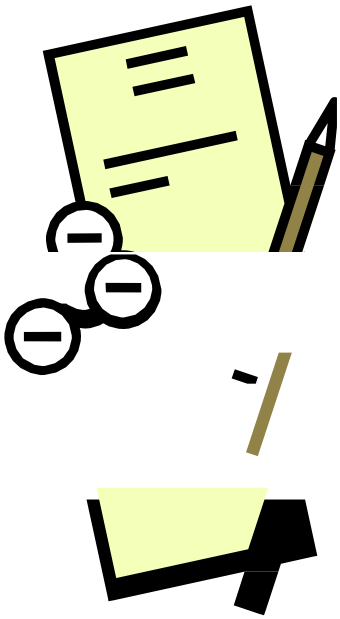
- Expand upon your knowledge about the donation process and clarify any questions you might have

- Fill out all parts of the donor registration document clearly and in capitals

- Sign the donor registration document

- Get a witness (relative, friend, or other person present at the moment of signing) to witness your signing of this document

- Allow a small **sample of blood** to be extracted (no need to fast beforehand) to enable compatibility tests to be carried out



If you are called upon as a donor

The probability of finding a patient who is compatible with a donor is very low. However, if this should happen, you will be called upon by your **referral centre** for the following**:**

a) The taking of a further sample of blood to **extend the study of your HLA system**, only if the sample mentioned above has not been kept at the lab or;
b) The taking of a further sample of blood to carry out compatibility testing at the hospital where the transplant is going to be carried out. This sample will also be used to analyse a series of parameters which clarify whether the donor has or has had an infectious/contagious illness and which provide information on how his or her main organs (liver, lungs, and kidneys) are working. Before this blood sample is taken, the donor will again be asked if he or she wishes to go through with the donation.

Once the donor has been confirmed as the best match, he or she will be informed of the **type of stem cell donation** required by the patient (bone marrow or peripheral blood; see below). The donor is then asked for the last time whether he or she wishes to go ahead. Any donor can back out at any time, but it is important to note that once the patient has started treatment to prepare him or her for the transplant, **the cancellation of the donation will result in the death of the patient**!
The decision as to whether to use bone marrow or peripheral blood depends exclusively on the needs of the patient; in certain illnesses and clinical circumstances, one method might be preferable to the other. It is possible to restrict the donation to stem cells from peripheral blood only if the donor has a problem which prevents him or her from having a general anaesthetic or epidural.

Features of the donation

It is important to point out that all donations of haematopoietic stem cells must comply with the stipulations of **Royal Decree 1301/2006**, which regulates the use of human tissue. The most noteworthy features of all donations are as follows:

1) Confidentiality: Under no circumstances will information which would enable the identification of the donor and/or recipient be provided or divulged.

2) No payment for donation: Under no circumstances may payment be received by the donor; no payment may be requested from the recipient.

3) Therapeutic purpose: The acquisition of haematopoietic stem cells may take place only for therapeutic purposes.

4) The **acquisition of stem cells** may only take place in duly authorised hospitals/clinics located as near as possible to the place of residence of the donor and on national territory.

Obviously, the fact that donors are not allowed to be paid for their donation does not exclude donors from being **compensated for all costs** arising from the donation (travel, accommodation for the donor and a friend or

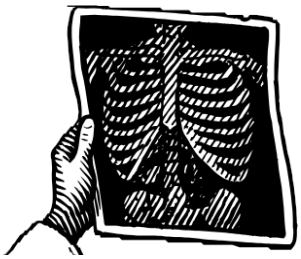
relative, and expenses if the extraction takes place in a town or city other than the donor's place of habitual residence) and, if necessary, for lost working days.

DONATING BONE MARROW

1. Before donating

If a donation is to take place and the patient requires stems cells from bone marrow, the following must take place during the month prior to the donation:

- Blood will be taken once or twice for use in the subsequent auto transfusion process (see below).



- Various **tests** (X-ray of the thorax, electrocardiogram, tests of respiratory function) and a **complete medical revision** to determine whether the donor can be anaesthetised without risk will take place. All of these tests take place at the hospital or clinic where the extraction is going to take place. In all cases, attempts will be made to use a hospital or clinic as near to the donor's home as possible and with a high level of experience in this type of procedure. Following further information on the process from the doctor responsible for the extraction, a decision will be made as to whether the extraction will take place under **general anaesthetic or with an epidural** (see below).

- The donor will then sign the **informed consent form** to consent to being anaesthetised and to authorise the extraction (see the appendix).

2. The donation itself

Bone marrow can be extracted under general anaesthetic or with an epidural. However, even though the risks are higher than for an epidural, general anaesthetic is normally used because it is more comfortable for the donor.

General anaesthetic

General anaesthetic is administered through a vein in one of the donor's arms. This sends the donor to sleep and relaxes him/her. Whilst the donor is anaesthetised, his/her breathing must be maintained artificially by means of a tube inserted into his/her mouth. This tube provides oxygen to the lungs. Normally, the donor is anaesthetised without any noteworthy issues. However, there are possible **side effects** like the following:
- Discomfort in the mouth or throat in the hours following the anaesthetic due to the breathing tube (this is a common complication but is minor and temporary).
- Feeling of nausea and instability in the hours following the anaesthetic. For this reason, the donor is advised to remain in hospital for 24 hours following the donation.
- An allergic reaction to any of the medications used (this is a rare complication, occurring less than once per 50,000 anaesthetics)

Epidural

This involves anaesthetising the donor's body from the waist down. It is done by injecting the anaesthetic into the space between two vertebrae in the lumbar area (lower back). It is extremely rare for this type of anaesthetic to have **side effects**, but the following can occur:

- The anaesthetic affects the central nervous system and the donor has to have a general anaesthetic anyway.

- It is not possible to correctly anaesthetise the area to be punctured and the donor has to have a general anaesthetic anyway.

- The epidural can cause pain in the donor's head or shoulders (easily controllable using mild painkillers) in the days following the donation.

Bone marrow aspiration

The procedure takes place in an **operating theatre** and is subject to the same conditions of asepsis (hygiene measures) as any other surgical intervention. The **anaesthetic** is administered and the donor is placed into the **prone position** (face down) on the operating table. Once the skin covering the **posterior iliac crests** (the bony prominences in the posterosuperior region of the pelvis) has been disinfected, two members of the extraction team - one on each side of the operating table - puncture these crests with needles designed especially for this procedure. Each puncture obtains around **5 ml of medullary blood**. This blood contains the haematopoietic stem cells. Once the blood has been obtained, it is placed in a bag with heparin (which prevents it from coagulating) and nutrient media (to prevent the deterioration of the stem cells).

Even though multiple punctures are made, only 1 or 2 holes will be seen in the skin covering each iliac crest once the aspiration has finished. This procedure can take between **one and two hours.** The **auto transfusion** (see below) is administered during the aspiration or immediately afterwards. After the aspiration, the donor is taken to the anaesthesia recovery area, where he or she will be observed for the next 2-3 hours; afterwards, the donor is taken back to his or her room. The duration of the donor's hospital stay should be between **24 and 36 hours**; usually, the donor is admitted the night before the aspiration and is allowed to go home the morning afterwards.

Auto transfusion

When bone marrow is extracted, a mixture of stem cells and **medullary blood** (blood which nourishes the bone marrow) is obtained. Normally, 15 – 20 ml of blood is obtained per kilo of the donor's weight, which means that a donor weighing 70 kilos would produce around **1000 - 1400 ml** of medullary blood. The **loss of this volume of blood** in the short amount of time that the extraction process lasts (1-2 hours) can give rise to **side effects** in the donor (drop in blood pressure, dizziness and tiredness, and other symptoms). This can result in the need for a blood transfusion, which - as is always the case - poses a risk of the transmission of infectious diseases.
An auto transfusion solves this problem.

During the 2-3 weeks before the donation takes place, **1 or 2 units of 300 - 450 mL of blood** are extracted from the donor. This blood - properly labelled to ensure that it can be identified without error - is kept refrigerated until the time of the bone marrow transplant, when it is transfused into the donor. As a result, the loss in blood volume is never so great that symptoms occur. The **oral administration of iron** from the time of the extraction of blood for the auto transfusion and for up to two months after the donation of the bone marrow encourages the donor's body to quickly recover the donated volume of blood.

Side effects of donating bone marrow

The only noteworthy side effect is tenderness at the puncture sites. This pain is brought under control quickly using common painkillers such as paracetamol and normally disappears within 48 hours. We recommend a few days of **comparative rest** to encourage this to abate. For this reason, donors are provided with the documentation necessary for their family doctor to authorise **sick leave** for 4 to 5 days. However, there is no medical reason why a donor who feels fine cannot return to his or her normal work straight away.

Other unusual **side effects** are as follows:

- **Fever** (sometimes a slight fever for the first few hours after donation)
- Slight **bleeding** at the puncture site; this complication is not serious at all and only requires the use of a compressive bandage

- **Dizziness**, especially when sitting up; this is a consequence of the slight residual anaemia which follows any donation and the only precaution to be taken is to sit up slowly

- **Infection** at the puncture site (rare)

With regard to blood tests, donating bone marrow can result in a moderate **reduction in the number of red blood cells and of haemoglobin** (anaemia). This resolves itself within a few weeks.

DONATING PERIPHERAL BLOOD

1. Before donating

If a donation is to take place and the patient requires stems cells from peripheral blood, the following must take place during the month prior to the donation:

- The donor is given an **electrocardiogram (ECG) and full medical check-up** to ensure that the donation can take place without risk to the donor. These tests take place at the hospital or clinic where the extraction is going to take place. In all cases, attempts will be made to use a hospital or clinic as near to the donor's home as possible and with a high level of experience in this procedure. The doctor responsible for the extraction will again explain the procedure to the donor to clarify any doubts.

- The donor signs the **informed consent form** to authorise the extraction (see the appendix).

2. The donation itself

Obtaining the stem cells

During normal conditions, stem cells are located in the bone marrow; it is rare to see them in the blood. However, there are ways of **mobilising** these stem cells so that they move into the donor's circulating blood for subsequent collection. The simplest method of achieving this is the administration of substances known as **haematopoietic growth factors**. These temporarily cause stem cells to move from the bone marrow into the peripheral blood. 4 to 5 days after receiving these factors **subcutaneously**, the donor has a sufficient number of stem cells in his or her peripheral blood for them to be retrieved by means of a procedure called **cytapheresis.**

Collecting the stem cells

Cytapheresis is carried out by drawing blood from a vein in the donor's arm and passing it through **cell separation apparatus**. The apparatus collect the stem cells. The rest of the blood is returned to the donor by means of a vein in the donor's other arm.
This procedure takes between 3 and 4 hours. The donor is able to lie stretched out comfortably on a supportive couch.
With a single procedure, the same amount or more stem cells can be obtained than in bone marrow aspiration.

Moreover, because the process can be repeated the next day, many more haematopoietic stem cells can be collected overall than by means of a bone marrow aspiration process.

The process of obtaining stem cells from peripheral blood is normally possible as an outpatient procedure. However, if a venous catheter is required (see below), a hospital stay might be planned in order to make the donor as comfortable as possible.

Side effects

Usually, the administration of growth factors is tolerated well. The main side effect is **generalised pain** in the bones and muscles (similar to when you have the flu). This symptom can be alleviated using paracetamol. The following side effects are less common: **Headaches, anxiety, non-specific thoracic pain, nausea, vertigo, and night sweats**. Long-term side effects have not been described (see below).

Side effects due to cytapheresis are infrequent. However, the most common of these are **short-term cramps and tingling** due to the use of citrate to ensure that the blood circulates through the inside of the cell separation apparatus without coagulating.

From an analytical point of view, the administration of growth factors results in an **increase in the number of leukocytes.** Following cytapheresis, there is a **reduction in the number of platelets and leukocytes** which - although it can persist for 1 or 2 weeks - never reaches levels which are dangerous to the donor.

Advantages and disadvantages of peripheral blood

For the donor, the main **advantage** of using peripheral blood is that it is easy to obtain and avoids the need for a general anaesthetic, multiple punctures, and auto transfusion. It has the following **disadvantages**:

(1) It requires the administration of growth factors, which - in the past - some thought posed a risk of altering the normal production of blood in the long term. Today, it is generally believed that the administration of such factors for 4 to 6 days is completely innocuous. The following observations support this belief: **(a)** These factors exist naturally in our bodies anyway. **(b)** They are manufactured using technologies which ensure that the produced substance is identical to the naturally one. **(c)** Although leukaemia has been observed occasionally in children with congenital neutropaenia who have been treated for several years using these products, it is believed that this complication is directly due to the underlying illness itself, and not to the growth factors. This is proven by the fact that other patients with similar characteristics who suffer from severe chronic neutropenia and have also been treated for several years with these products do not develop this complication. **(d)** The monitoring of a large number of voluntary donors over the course of years has not detected any anomaly. For these reasons, the National Committee for Haematopoietic Transplantation authorised the use of these factors to obtain stem cells from voluntary donors in Spain as of November 2002.

(2) 5% of donors do not have veins large enough to enable this procedure. This scenario can be detected in advance, which gives the donor the chance to authorise the use of a central venous catheter or to opt for the donation of bone marrow, instead. Despite this, in some cases this situation can become known only at the time of the actual donation.

The insertion of a central venous catheter does pose certain risks since it involves penetrating a vein in the neck, clavicle, or groin. The most frequent complication is a haematoma at the puncture site, but more serious complications can occur in 1% of cases. For this reason, the insertion of a central venous catheter is avoided whenever possible.

SECOND DONATION

Once several weeks have passed following the donation, the function of the donor's bone marrow has fully recovered and the blood analytics have returned to normal; a second donation could take place without any problems. However, current legislation clearly distinguishes between two situations:

1. Following a donation of bone marrow

a) A donation for a different patient: At least **1 year** must have passed since the previous donation. In fact, donors who have already made one donation are asked whether they wish their name to remain on the list of possible voluntary donors. If they choose to remain on the register, they are not asked to donate again until this time period has elapsed. Furthermore, they are not asked to donate if an alternative donor is available for the patient in question.

b) Another donation for the same patient: There are two different situations here:

- The second donation takes place **a few weeks after** the first donation because the donation was rejected. In this case, peripheral blood stem cells are usually requested. Since a greater quantity can be harvested than from bone marrow, this can solve the problem.

- The second donation takes place **months or years after** the first donation because **the recipient's leukaemia has recurred**. Today, we know that in certain circumstances the administration of **lymphocytes from the donor** can be used to control the recurrence of leukaemia. These blood cells are sometimes capable of eliminating the patient's leukaemia cells. The method used for the donation of lymphocytes differs depending on the required quantity. If only a few are required, a simple donation of 300 - 500 mL of blood is sufficient; however, if a greater quantity is required, cytapheresis must take place (but without the need for growth factors).

2. Following a donation of peripheral blood

After this kind of donation, the donor is removed from the registry and will not be asked to donate again unless the same patient requires a second transplant. In this case - if the donor agrees to donate again - the repeat donation will always take the form of the donation of bone marrow. The donation of lymphocytes is also allowed.

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