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NHS Blood and Transplant

Blood Matters

Information for hospitals served by NHS Blood and Transplant

Inside

Editorial

This edition of ***Blood Matters*** is divided into two themes. The first considers the impact of new technologies in the world of Transfusion Medicine whilst the second discusses developments in organ and tissue banking, transplantation and regulation. To start with we have revisited intrauterine transfusions which, as **Helen New** and **Sheila McLennan** point out, are of great importance since red cells and platelets are administered to extremely vulnerable recipients in fetal medicine centres. These are a group in whom even remote long term risks of transfusion are important. Doppler ultrasound monitoring of the fetal cerebral circulation has now replaced more invasive techniques for detecting anaemia requiring transfusions and survival after red cell transfusion for non-hydropic fetuses is now greater than 90%.

Next we have articles that illustrate how powerful a tool molecular biology has become. **Geoff Daniels** describes how it is possible to define fetal blood groups (Rh D, c, C, E and Kell antigens) based on analysis of cell-free DNA present in the blood of pregnant women and how this helps in the management of pregnancies where there is blood group incompatibility between the mother and fetus. **Cristina Navarrete** and **Andrea Harmer** discuss how human leucocyte, neutrophil and platelet antigens are defined in the modern Histocompatibility and Immunogenetics Laboratory (once known to many of us as Tissue Typing!). Finally **Willem Ouwehand** and **Kerstin Koch** show us how the genetic architecture of common diseases is being defined in genome – wide association studies (GWAS). Here a large number of genes from cohorts of patients with coronary heart disease, diabetes and other disorders are being compared to those from healthy individuals. This approach will help individual risk prediction and give valuable insights into the cellular and molecular mechanisms of disease, with the prospect of developing new strategies for disease prevention and treatment.

Patients with leukaemia and other diseases which require intensive chemotherapy frequently develop life-threatening bacterial and fungal infections. In the absence of healthy neutrophils these may not get better. **Ed Massey** and colleagues update us on the use of granulocyte transfusions collected by apheresis or made from buffy coats which can play such a crucial role in these patients and point out that future clinical studies are needed. **Marc Turner** wrote an excellent article summarising how to manage the risk of transmission of vCJD by blood and tissues and this was published in the *Bulletin of the Royal College of Pathologists* recently. I am delighted that they have given us permission to reproduce it here so that we can all understand the challenges that abnormal prions pose in the provision of blood and tissues by NHSBT. Two years ago a whole issue of ***Blood Matters*** was devoted to Stem Cells and we thought that it was time for an update. Here **Suzanne Watt** has summarised recent developments that are helping to define the best ways of using stem cells and immune cells to make stem cell transplantation safer, how to collect more stem cells and how stem cells may also be used for tissue repair.

In the second section we have articles that address the supply of organs for transplantation. **Chris Rudge** and **Sue Falvey** tell us that the shortage of donated organs is as bad as ever and this at a time when success rates are high if a graft is available. They describe what is required to match supply to demand. **Elizabeth Buggins** chairs the Organ Donor Taskforce whose recommendations are currently being considered by the Department of Health and she highlights the barriers to donation. Better availability of donated organs could prevent as many as 1,000 deaths each year. We are all taught that kidney transplant patients who receive pre-transplant blood transfusions have better graft survival (the reverse had been expected!). This so called "blood transfusion effect" has been the subject of much discussion ever since, even though it is no longer routinely used. **Derek Gray** explains studies in both mouse and man that show how the effect works and tells us why it is no longer standard therapy in the 21st century.

Many of you will recall the very striking and powerful image of a mouse with an ear growing on its back! The ear in question was constructed from cartilage cells taken from the knee of a cow, grown on a biodegradable matrix and implanted onto the back of a mouse with immune deficiency that was not able to reject it. The use of matrix, cells and growth factors in tissue engineering is the subject of the article by **Paul Rooney** and **John Kearney** who work in Tissue Services at the NBS Centre in Liverpool. They describe the enormous potential of this approach. If there is ever a problem in freezing tissues or cells for transplantation then **David Pegg** usually can find the answer – and he's just done it again! Those of us who have worn out knee joints must have wondered why a new pad of cartilage can't just be implanted in the joint. Well there are good reasons; mainly that it doesn't freeze well. The new approach that he has discovered could have a big impact in the treatment of arthritic patients.

The Human Tissue Act and the European Union Directive on Tissues and Cells have had a big impact on the working lives of all who deal with organs, tissues and cells. **Adrian McNeil** of the HTA summarises how the authority has approached the task of regulating this complex field. A flexible approach has allowed altruistic organ donation to a stranger in one case.

This edition of ***Blood Matters*** appears in its new format. We hope that you like it and that you will give us the feedback that we need either by returning the enclosed questionnaire to us in the post or by completing it online. We do value your feedback and our aim is to make ***Blood Matters*** ever easier to read and as informative as possible. Please feel free to tell me directly what articles you would like to see in future editions.

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Intrauterine Transfusion in 2008

Introduction

Intrauterine transfusions (IUTs) are highly specialised procedures, performed only in a small number of fetal medicine centres in the UK. Intravascular IUT was pioneered in the early 1980s, and in recent years the number of procedures has decreased following the introduction of routine anti-D prophylaxis for Rh-D negative women. The components used are either red cells to treat fetal anaemia and prevent hydrops, or, more rarely, platelets when there is fetal alloimmune thrombocytopenia – approximately 400 red cell and 70 platelet IUT components have been issued annually in England over the last three years. Fetuses are amongst the most vulnerable recipients of blood transfusion and problems with long term side-effects of transfusion are likely to be greatest for this population as most will have a normal life-expectancy. Special components are available for IUT in order to minimise the risks *(BCSH guidelines on transfusion for neonates and older children, 2004)*.

Red cell transfusion

The commonest cause of fetal anaemia is haemolytic disease of the fetus and newborn, caused by transplacental passage of maternal IgG antibodies (particularly anti-D, anti-c, and anti-Kell) which bind to and destroy red cells carrying paternal antigens. Fetal anaemia may also occur following intrauterine infection with parvovirus B19, fetomaternal haemorrhage (when blood from the fetal circulation leaks into the maternal circulation in the placenta, as in placental abruption), or in congenital red cell aplasia. In severe cases the anaemic fetus develops subcutaneous oedema, ascites, pleural and pericardial effusions (hydrops fetalis) and may die *in utero*.

In the past, pregnancies at risk of haemolytic anaemia were monitored by serial measurements of amniotic fluid bilirubin levels. However this is invasive, and bilirubin levels are poor predictors of fetal anaemia where there is erythroid suppression (for example Kell alloimmunisation and B19 infection). This technique has now been supplanted by regular Doppler ultrasound monitoring of fetal middle cerebral artery peak systolic velocities (MCA-PSV). The MCA-PSV is raised in anaemic fetuses, and values greater than 1.5 MoM (Multiples of the Median) for the specific gestation are predictive of moderate or severe fetal anaemia. The technique shows 100% sensitivity and a false positive rate of 12%, is non-invasive, and the results correlate well with amniotic fluid bilirubin levels in haemolytic disease.

If the MCA-PSV suggests anaemia, confirmation is by ultrasound-guided fetal blood sampling from the umbilical vein, either at the placental insertion site or in the intrahepatic portion (*reviewed by Brennand and Cameron, 2007*). This procedure has a 1-2% risk of fetal loss; other complications include haemorrhage, cord haematoma, fetal bradycardia, emergency caesarean section, and increased maternal alloimmunisation (in up to 25% of women treated). Facilities for an immediate full blood count should be available so that if fetal anaemia is sufficiently severe (trigger level depends on the departmental unit policy, for example, a hematocrit of <30%, or less than two standard deviations for gestational age), IUT can proceed immediately, avoiding the risk of having to recannulate the umbilical vein. Suitable blood must be pre-ordered and crossmatched in readiness.

Transfusions are started as late in pregnancy as possible, but before the development of fetal hydrops (ideally after 18 weeks' gestation). They are usually given intravascularly, but in certain situations may be given intraperitoneally. The volume to be transfused is calculated by an accepted formula incorporating fetal and donor haematocrits and the fetoplacental blood volume (*BCSH 2004*), aiming for a post-procedure haematocrit of around 45% depending on the policy of the centre. Packed cells with a high haematocrit are used for IUT in order to reduce the risk of volume overload and to minimise the number of procedures. The timing of further procedures depends on ongoing weekly MCA-PSV monitoring, the presence of hydrops, and predictions of the rate of haemoglobin drop. As the fetal blood is gradually being replaced by transfused blood (antigen negative for the relevant antibody) however, the frequency of transfusion tends to decrease over time. Overall survival is about 85%, with greater than 90% survival for non-hydropic fetuses.

Red cell components for IUT

The requirements for blood components are agreed in close consultation between the Fetal Medicine Unit, Consultant Haematologist and Blood Centre. Red cells for IUT must be:

- Group O and RhD negative in most cases; negative for the relevant antigen(s) determined by maternal antibody status and IAT cross-match compatible with maternal serum
- Kell negative
- In CPD, not SAG-M
- Used within five days of collection
- Free from clinically significant antibodies including high-titre anti-A and anti-B
- CMV antibody negative
- HbS screen negative
- Gamma irradiated and used within 24 hours of irradiation
- Leucocyte depleted

The required haematocrit should be agreed with the Fetal Medicine Consultant, but > 0.7 L/L is recommended (BCSH guidelines state 0.7-0.85). They should not be transfused straight from 4°C storage.

Platelet transfusion

Fetal platelet transfusions are given for feto-maternal alloimmune thrombocytopenia. This occurs as the result of maternal alloimmunisation to fetal platelet antigens inherited from the father in approximately 1/1000 live births. The most common platelet antigens involved are HPA-1a (85%) and HPA-5b (10%). Affected fetuses have a 10-30% risk of antenatal or peripartum intracranial haemorrhage.

There is increasing consensus regarding antenatal management, which may include maternal treatment with intravenous immunoglobulin (IVIg), steroids, or intrauterine platelet transfusions. Depending on response, most centres start with IVIg, add in steroids, or give fetal platelet transfusions.

Concentrated platelets may be given weekly in order to maintain a platelet count above 30 x 10^9/l (more frequent than required for red cell transfusions because of the shorter life of platelets in the circulation). The NBS supply hyperconcentrated platelets for IUT, produced by apheresis, from a small panel of accredited donors who are individually called to donate for a particular patient. There must be close collaboration between clinicians, Blood Bank and Blood Centre. Practice varies between units regarding the number of transfusions per pregnancy.

The risks of intrauterine platelet transfusions are similar to those of red cell transfusion, with at least 1% risk of fetal loss.

Platelet components for IUT

- ABO group compatible with the fetus
- CMV negative, irradiated, leucodepleted (as for red cells)
- HPA compatible with maternal antibody (generally supplied from HPA1a, 5b negative donors)
- Concentrated to a platelet count of 2000-4000 x 10^9/l

Summary

IUT is a technique performed in only a few specialised centres. The only option for treatment of severely anaemic or thrombocytopenic fetuses prior to the development of this technique was early delivery which significantly increases the risk of morbidity and mortality. The procedure is risky, with at least a 1% fetal loss rate per procedure. However, with the introduction of reliable non-invasive fetal monitoring it is now possible to target pregnancies for which the benefits outweigh the risks. Blood components used in IUT are highly selected so as to reduce potential risks to the fetus. Close collaboration between clinician, Blood Bank and Blood Centre is essential in order to provide the correct specification of component at the right time.

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The Application of Molecular Genetics to Fetal Blood Grouping

Of the 33 genes encoding the 262 antigens of the 29 red cell blood group systems, all but one has been cloned and sequenced. The molecular bases for all clinically significant blood group polymorphisms and for numerous rare variants have been determined. This information makes possible the prediction of blood group phenotypes (i.e. blood groups expressed on the red cell) from tests on genomic DNA with a high degree of accuracy.

The most common application of blood grouping from DNA is for predicting the Rh (D) phenotype of the fetus of a pregnant woman with anti-D in her blood, to predict the risk of haemolytic disease of the fetus and newborn (HDFN). If the fetus is D-positive it is at risk and the appropriate management of the pregnancy can be arranged; if it is D-negative it is not at risk and unnecessary interventions can be avoided.

Before 2001 the usual sources of fetal DNA were amniocytes, obtained by needle aspiration of amniotic fluid during pregnancy (amniocentesis), and cells of the chorionic villi, obtained by chorionic villus sampling usually through the cervix but also done transabdominally. Both techniques are invasive and are associated with increased risks of spontaneous miscarriage. In addition, with amniocentesis there is a 20% risk of transplacental haemorrhage, which could boost the maternal antibody, increasing the risk of severe HDFN. A better source of fetal DNA, avoiding highly invasive procedures, is cell-free fetal DNA present in the blood of pregnant women. The fetal DNA, which originates from the placenta, represents between 3% and 6% of total cell-free DNA in the maternal plasma. The Fetal D phenotype can be predicted reliably from

fetal DNA in the plasma of D-negative pregnant women from the beginning of the second trimester.

The antigens of the Rh system are encoded by a pair of homologous genes, *RHD* and *RHCE*. D-positive individuals have at least one copy of *RHD*, whereas most D-negative white people are homozygous for a complete deletion of *RHD*. *RHCE* is almost universally present. Consequently, in people of European origin D phenotype can be predicted by determination of whether *RHD* is present in the DNA.

At the International Blood Group Reference Laboratory (IBGRL) in Bristol we provide fetal *RHD* genotyping as a routine service for all pregnant D-negative women with a significant level of anti-D (usually greater than 4 IU/mL). The technology we use is real time quantitative polymerase chain reaction (PCR) with Taqman chemistry with primers and probes that detect exons 4, 5, and 10 of *RHD*, but not *RHCE*.

A problem in all tests on fetal DNA derived from maternal plasma is that it is not possible to separate the small quantity of fetal DNA from the much larger quantity of maternal DNA. In the third trimester about 6% of the DNA is of fetal origin and about 94% of maternal origin. As mothers with anti-D are D-negative, if *RHD* is detected the fetus must be D-positive and if no *RHD* is detected the fetus is presumed to be D-negative. The presence of such a large quantity of maternal DNA present in the DNA preparation makes it very difficult to include suitable internal controls for the presence of a sufficient quantity of fetal DNA. Reactions for detecting the Y-linked gene *SRY* are included in the test, which will provide a positive result when the fetus is male, but if the fetus is apparently D-negative and female the results are reported with an explanation that the tests were not adequately controlled and a "false negative" result cannot be totally ruled out.

After anti-D, the most common causes of HDFN are anti-K of the Kell system and anti-c of the Rh system. IBGRL also provides a service for K and c testing on fetal DNA in maternal plasma, and also for Rh C and E, which occasionally cause severe HDFN. These tests are carried out by real-time quantitative PCR and Taqman technology, each test employing an allele-specific primer.

To prevent D immunisation during pregnancy it is policy in the UK to offer anti-D immunoglobulin prophylaxis to all D-negative pregnant women at 28 and 34 weeks' gestation. Because the D phenotype of the fetus is not known, about 40% of these women (in a predominantly white population) will be carrying an D-negative fetus and will receive this immunoglobulin therapy unnecessarily. Consequently, a high-throughput method suitable for routinely determining fetal D type from fetal DNA in maternal plasma in all pregnant D-negative women would be valuable. Application of such a test would be cost-effective, as it would save wastage of anti-D immunoglobulin, a valuable and expensive resource. It would also spare D-negative pregnant women with a D-negative fetus unnecessary therapy with blood products.

Large scale trials of mass screening for fetal D type have been carried out in Bristol and in Amsterdam. The results of both trials demonstrate clearly that this testing would be sufficiently accurate and cost-effective to be implemented routinely. It is likely that this technology will be made available for all D-negative pregnant women within the next few years.

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The H&I Laboratory in 2008

There are six histocompatibility and immunogenetics (H&I) laboratories within NHS Blood and Transplant (NHSBT) supporting a wide range of clinical activities including transfusion, transplantation and immunogenetic analyses of markers associated with diseases and susceptibility to drugs.

The work performed in the laboratories can be divided into two main areas of testing:

Tissue Typing. This involves Human Leucocyte Antigen (HLA) typing to determine a patient or donor's tissue type for matching for transfusion or transplantation or to identify HLA types associated with specific diseases or drug reactions. Human Platelet Antigen (HPA) typing is performed in patients or mothers and new-borns with thrombocytopenia requiring compatible platelets. Human Neutrophil Antigen (HNA - granulocyte) typing is also performed in selected cases. HLA typing is also performed on bone marrow donors and cord blood units available for patients undergoing haemopoietic stem cell (HSC) transplants and needing an unrelated matched donor.

Antibody testing. This involves the detection and identification of HLA antibodies (Abs), which may lead to the rejection of solid organ transplants, HLA & HPA Abs involved in platelet refractoriness or HLA & HNA Abs implicated in the development of serious transfusion reactions such as transfusion related acute lung injury (TRALI).

New Techniques

H&I laboratories new techniques across the UK have been at the forefront of the development and introduction of new techniques for the identification of both HLA antigens and antibodies. Many of the DNA based techniques now used in different laboratory disciplines were first developed and/or routinely used in H&I laboratories. These include PCR-SSP, PCR-SSOP* and DNA sequencing. UK laboratories were amongst the first to use flow cytometry for crossmatching for renal transplants. This test was introduced over 20 years ago.

Given this history of innovation and development it is not surprising that the NHSBT H&I laboratories are constantly updating their techniques and introducing the latest technologies. One of these is Luminex technology which is used for detection of HLA Abs and for HLA typing. Luminex analysers are a special type of flow cytometer which can distinguish between up to 100 different bead sets in a single tube. Each bead set can be coated with oligonucleotide probes for HLA typing or with HLA antigens for antibody detection and identification. This technology allows detailed analyses on large numbers of samples in a matter of hours. The ability to rapidly process these samples has allowed our laboratories to increase both the accuracy of the tests and their workload. This has allowed our laboratories to accommodate the growing demand for services to support HSC and solid organ transplant programmes. For HSC transplantation, where exact matching of patient and donor is critical, Luminex typing is supplemented by DNA sequencing providing the highest degree of accuracy possible.

NHSBT H&I laboratories are mostly using HLA DNA sequencing techniques that have been developed by staff within the service. As well as being more cost-effective than commercial kits, these have proved to be more flexible, allowing laboratories to respond rapidly to changes in the number of described HLA variants and in the increasingly stringent standards that apply to HSC transplantation. The work required to support HSC transplantation is the most challenging area of clinical work in terms of HLA typing. Finding suitable donors for patients takes a significant amount of work both in performing the tests and analysing the very complex set of results that DNA techniques, especially DNA sequencing generate.

In the solid organ transplantation setting all NHSBT laboratories are using Luminex. This has allowed the identification of antibodies with a degree of sensitivity not available before. Most recently this technology has been further improved by the introduction of beads coated with single antigens which improve the accuracy of antibody specificity identification.

Although Luminex is the most sensitive technique currently available for the detection of HLA antibodies, this degree of sensitivity and accuracy does however have drawbacks. There is increasing evidence that these techniques detect 'irrelevant' antibodies. This is probably due to its increased sensitivity resulting in the detection of low level antibodies which may not be clinically relevant. Therefore, there is an increasing need for H&I Consultants and other senior scientific staff to carefully analyse both patient history and the detailed antibody results in order to determine risk levels associated with different antibodies. This requires a very close working relationship with the hospital clinical teams responsible for these patients.

In addition, clinical transplant units performing renal transplants have, in the last few years, developed protocols for transplanting patients with donors who would previously have been judged unacceptable due to the presence of donor specific antibodies. The successful outcome of these transplants can be attributed to two main factors. Firstly at the clinical level, the development of reliable techniques for removing antibodies from the circulation combined with more effective immunosuppressive drug treatments. Secondly at the laboratory level, the ability to accurately identify antibodies, to monitor their removal and continue to monitor for resynthesis post-transplantation in order to rapidly assess if any intervention is required. The increasing numbers of antibody incompatible transplants, especially using living donors, is one of the factors that may lead to increases in overall transplant numbers - a key element of the UKT strategy.

Summary

By establishing summary close working relationships with the clinical units they support, the H&I laboratories are able to contribute to the growth in transplant rates, to the improved platelet support for immunologically refractory patients and also to accuracy in the diagnosis of diseases associated with the HLA or HPA markers. The experience and training of the H&I staff plays a major role in the ability to develop, validate and then introduce new techniques and also in the contribution that H&I laboratories can make to the diagnosis and treatment of patients.

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* PCR-SSP: Polymerase chain reaction (PCR)-based amplification of DNA sequences for analysis using sequence specific primers (SSP).

PCR-SSOP: PCR-based amplification of target DNA which is then analysed using sequence-specific oligonucleotide probes (SSOP)

For further information see Navarrete CV, Human leucocyte antigens, Practical Transfusion Medicine (2005) (2nd Edn). Eds. M.F. Murphy & D.H. Pamphilon. Blackwell Science

Genome Wide Association Meets Systems Biology

Discovering genes implicated in the common diseases

Many of the common diseases which afflict hundreds of thousands of NHS patients have a heritable component. The past year has marked a new era in defining the genetic architecture of common diseases using so-called genome-wide association studies (GWAS). For the first time ever the whole landscape of genomic sequence variation can be surveyed by testing hundreds of thousands of genetic markers simultaneously. The NHS Blood and Transplant (NHSBT) research team at the University of Cambridge has been amongst the first to engage in this fast moving field, and has contributed significantly to the success of the Wellcome Trust Case Control Consortium (WTCCC) study.

This study is the largest GWAS to date, analysing a total of 17,000 DNA samples on the Affymetrix GeneChip Mapping Array Set for 500,000 single nucleotide polymorphisms (SNPs). Two thousand DNA samples each from 7 cohorts of NHS patients with coronary artery disease (CAD), hypertension, type 2 diabetes, type 1 diabetes, rheumatoid arthritis, Crohn's disease and bipolar disorder were compared to two DNA collections of healthy individuals of 1,500 samples each, the so-called 'shared controls'.

Appreciating the significance of this project for improvement of patient care, NHSBT under the leadership of Willem Ouwehand joined the study effort from its inception. Offering its organisational framework and resources, it collected more than 3,000 DNA samples from consenting blood donors for use as 'shared controls' in the WTCCC study, together with the Blood Services of Scotland and Wales. Two panels of reference DNA samples have been created, of which the first one was genotyped as part of WTCCC, and the second one has been used in WTCCC replication studies.

For all diseases novel genetic markers were discovered and a total of 24 new strong association signals were identified (Burton *et al*, 2007), many of which have been confirmed in further replication studies. In addition, for each of the diseases hundreds more SNPs have been identified and a substantial fraction of these will be true associations. This discovery will have an impact on the face of future healthcare in many ways. First, it will contribute to the development of novel algorithms, which will include genotyping results for disease risk prediction. Second, it has already created new insights into the cellular and molecular mechanisms of several of the common diseases, and for one disease trials with a new therapeutic agent have commenced. Finally, the allele frequency tables for the 500,000 SNPs are freely available from the WTCCC website and genotyping results at the level of the individual DNA sample can be obtained via the data access committee (www.wtccc.org.uk). This unprecedented openness of study results will facilitate similar projects by other groups worldwide.

Many of the genetic markers discovered held surprises and most are in non-coding regions of the genome, e.g. SNP rs1333049 on chromosome 9p21 shows a strong association (P value 1.16×10^{-13}) with CAD and is outside a locus. The second phase of the WTCCC study is concerned with so-called fine mapping of the strongest association signals and it is hoped that this will shed light on some of the genetic mechanisms underlying the observed associations between sequence variation and disease risk.

However, this is by no means the end of the story. Many more treasures are hidden in the results of the WTCCC project. Again, CAD is a good example: Besides the very strong association signal on chromosome 9, hundreds more SNPs showed associations with less robust P values but still warranting further investigation. Using this enormous wealth of data, the NHSBT Platelet Biology and Genetics group at the University of Cambridge is in the process of identifying further risk genes for thrombosis and myocardial infarction.

The group has over the last four years pursued a platelet systems biology study as part of the Bloodomics project (www.bloodomics.org). The Bloodomics Consortium is a multi-disciplinary effort of 14 research teams across Europe and supported by a grant from the European Commission. The aim of the project is to discover genetic markers for the prediction of thrombus formation in CAD. It was based on the notion that the variation in platelet function between individuals in the normal population is to a large extent genetically controlled. It was therefore reasoned that the discovery of genes which control platelet function may also modify the risk of arterial thrombosis. They first catalogued all genes transcribed in the megakaryocyte (the bone marrow cell from which platelets are derived) and the erythroblast (precursor of red blood cells), using whole-genome expression arrays (Jones *et al*, 2007). In parallel, the function of platelets in a cohort of 500 blood donors was defined and the extreme responders identified (Macaulay *et al*, 2007). The transcriptome landscapes from the most and least active platelets were then compared and this identified 68 gene transcripts which showed significant correlation with platelet function. In the most recent effort to identify and confirm genes implicated with CAD, the results from the Bloodomics platelet systems biology study and the WTCCC GWAS were integrated. This revealed another two genes which confer risk for CAD.

Summary

In conclusion, the long awaited expectation that the sequencing of the human genome would move the frontiers of medical genetics has been fulfilled. It is however early days and many more new developments will emanate from currently ongoing projects. With the recent introduction of ultra high-throughput sequencing it has become feasible to sequence the genome of an individual within a week, opening the door to many more studies on the relation between sequence variation and disease risk.

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Granulocyte Therapy

Background

Granulocytes are the major phagocytic white cell in the blood and are crucial for the control of bacterial and fungal infections. Granulocyte transfusions are used as treatment for patients who are severely neutropenic and have infections that fail to respond to standard anti-microbial drugs. They are also transfused prophylactically to prevent the development of severe infection in patients at high risk (Kerr *et al*, 2003; Oza *et al*, 2006). Most patients prescribed granulocyte transfusions have neutropenia associated with bone marrow failure, due to underlying disease of the bone marrow or from treatment with intensive chemotherapy. Requests for granulocyte components for transfusion have steadily increased in England and Wales during the last five years. This may have been driven by recent publications suggesting some success for either *therapeutic* indications or for *secondary prophylaxis*, in patients who have had severe bacterial or fungal infections previously but who require a further cycle of intensive chemotherapy with or without haemopoietic stem cell rescue.

There has also been a general resurgence of interest in granulocyte transfusion therapy over the last decade as a consequence of using granulocyte colony stimulating factor (G-CSF) and steroids to 'prime' donors for apheresis (where cells are collected using a cell separator), permitting the collection of significantly greater yields of granulocytes for transfusion. These higher yields are considered clinically important and their transfusion is associated with definite post-infusion increments and appropriate localisation in vivo at sites of infection.

However, the granulocyte component available for transfusion has not to date been evaluated for efficacy in a large prospective randomised controlled trial, and the exact clinical role for granulocyte transfusions (whether derived from whole blood or collected by apheresis) therefore remains unclear. Potential efficacy including a dose-dependent effect has been raised by systematic reviews/meta-analyses (Stanworth *et al*, 2004), and in animal studies. The existing literature is, perhaps not surprisingly, dominated by case reports and small case series, with a significant risk of publication bias. However, anecdotal evidence of benefit in selected patients continues to be reported, including one larger study based on biological randomisation - although this trial was too small to detect a reduction in mortality (Oza *et al*, 2006).

Methods of collection in UK

In the UK, granulocytes for transfusion are produced:

- by apheresis, from stimulated or unstimulated donors,
- as a component derived from whole blood donations.

The administration of G-CSF and steroids to donors increases the circulating granulocyte count before apheresis, promoting greater yields of granulocytes for transfusion. However, The UK Blood Services have made a decision not to permit G-CSF and steroid administration to volunteer unrelated donors for the purpose of collecting granulocytes (Guidelines for UK Transfusion Services), to ensure the absolute safety of volunteer donors.

In some hospitals in the UK granulocyte collections are obtained from *directed* G-CSF and/or steroid-stimulated donors who are 'family and friends' of patients. But this process involves multiple steps, and there are a number of potentially important constraints that can limit provision of apheresis products on a regular and timely basis. For example, hospitals managing granulocyte collections by apheresis now have a requirement for meeting 'blood establishment status' according to EU legislation, enacted in the UK as the Blood Safety & Quality Regulations 2005. Requests for granulocytes are unpredictable and can conflict with other commitments such as pre-booked stem cell collections in busy apheresis units. It may be difficult to ensure that 'family and friends' of patients are given time and adequate explanation of the small risks they are exposed to by both taking specific drugs (steroids and G-CSF) to mobilise granulocytes into the peripheral blood (Bennett *et al*, 2006; Goldman *et al*, 2006) and by undergoing an apheresis procedure. Appropriate privacy for counselling and screening are required to offset significant personal and familial pressure to donate.

An alternative source of granulocytes is the buffy coat layer, derived from whole blood donations. It has the immediate advantages of availability but a lower yield, and the component has not been evaluated in any detail yet. These donations are commonly described as "buffy coats" as they are derived from the 'buff' layer between red cells and plasma in centrifuged whole blood. The main disadvantage of this source of granulocytes is the lower yield, by comparison to apheresis collections. Given that buffy coats are typically transfused for an adult dose, risks of buffy coat granulocyte transfusions include alloimmunisation and transfusion transmitted infection associated with multiple donor exposure. Such risks include vCJD.

Recent work in the National Blood Service Components Development Laboratory (CDL) has reported the characterisation of a purer pooled granulocyte component derived from whole blood donations known as Optimised Granulocyte Component (OGC; see Table). The method involves the addition of platelet additive solution but without the need for hydroxyethyl starch or dextran to sediment red cells during processing (Bashir *et al*, 2008). The findings for pH, viability and a range of *in vitro* tests for neutrophil function indicate well-maintained results during storage up to (and over) 24 hours.

The OGC is not yet available in the NBS portfolio as it is undergoing clinical study but it retains the potential advantages of ready availability for transfusion on a daily basis. This may be clinically important given that there is some evidence that provision of granulocytes very soon after the onset of severe infection may be critical (Sachs *et al*, 2006). In addition, by providing a standard adult component derived from twenty donations, a consistent daily cell dose of around 2 x 10^{10} cells may be transfused to patients, which is considered by many physicians a clinically 'meaningful' yield for transfusion.

Summary

Despite uncertainty about their effectiveness, requests for granulocyte transfusions continue to be received. A larger multi-centred trial of apheresis granulocytes may be starting in the US in the near future, although results will not be available for many years (Price *et al*, 2006). A small safety study of the optimised component derived from whole blood has started in the UK, and a total of five patients (four adults and one child) have been recruited to date. Preliminary data on the 36 doses of granulocytes issued in total to patients in this study has indicated no transfusion reactions, HLA alloimmunisation has occurred in one patient; future clinical studies will need to evaluate how best to use this additional component derived from whole blood, given the current constraints on regular provision of apheresis granulocytes in the UK.

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Properties of Different Granulocyte Concentrates

(Data provided by the National Blood Service: Rebecca Cardigan, Saber Bashir, Fred Goddard)

	Single buffy coat (n=21) (mean, SD)	**10 buffy coats** (dose typically transfused for adults)	**Pooled granulocytes from whole blood, in development** (n=13) (mean SD)	**Unstimulated apheresis collection** (n=20) (mean, SD)	**Stimulated apheresis collection** (n=5) (median, range)
Volume (ml)	59 (3)	590	250 (10)	279 (46)	299 (214-333)
Neutrophils (10^{10}/U)	0.105 (0.04)	1.05	0.88 (0.14)	0.54 (0.2)	6.37 (3.69 – 8.47)
Haematocrit (%)	45 (6)	45	21 (2)	23 (7)	9 (7-20)
Lymphocytes (10^9/U)	0.88 (0.41)	8.80	6.72 (0.75)	8.97 (14.0)	N/A
Monocytes (10^9/U)	0.18 (0.07)	1.80	1.22 (0.37)	0.95 (0.39)	N/A
Platelets (10^9/U)	70 (22)	700	344 (96)	111 (25)	160 (82 – 293)
Red cells (10^{12}/U)	0.27 (0.04)	2.70	0.57 (0.06)	0.71 (0.23)	3.0 (2.8 – 6.1)

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Managing the Risk of Transmission of vCJD by Blood and Tissues

Variant Creutzfeldt-Jakob disease (vCJD) was first described in 1996. It differs from the sporadic form of the disease in a number of important respects, including a younger age at onset (median 28 years, range 14-74 years), an unusual clinical presentation consisting of behavioural disturbance, dysaesthesia and cerebellar ataxia followed by more generalised neurological deterioration, and a prolonged clinical phase (median 14 months, range 6 to 48 months). The epidemiological, clinical, neuropathological and experimental data all point to vCJD being the same strain of prion disease as Bovine Spongiform Encephalopathy (BSE).

Incidence and prevalence

To date there have been 166 definite or probable cases of vCJD in the UK, four in the Irish Republic, three in the USA, two in the Netherlands and Portugal, and one in each of Canada, Italy, Japan, Saudi Arabia and Spain. Two of the Irish and US cases, along with those from Canada and Japan, are thought to have been infected in the UK, whilst the third US case is believed to have been infected in Saudi Arabia. The other cases have thought to have been infected in their country of origin.

Although the number of clinical cases in the UK is falling, there is an increase in geographical spread and an important discrepancy between the number of clinical cases now projected (maximum likelihood estimate 70, 95% confidence interval 10–190) and the apparent prevalence of abnormal prion protein accumulation in a retrospective study of tonsils and appendices of 3 per 12,674. On the basis of these data, current mathematical models suggest a maximum likelihood estimate of 3,000 infected people (95% confidence interval 520-6,810) suggesting a possibility of sub-clinical infection of 0.93 (95% confidence interval 0.7-0.97). This would equate to a prevalence of sub-clinical disease in the UK donor population in the order of 1/4,000 to 1/20,000. It should be remembered that these estimates are based on a small amount of data and that further large-scale prospective epidemiological studies are in progress.

Infectivity and transmissibility

Extrapolation from animal studies suggests that there are around 10 infectious prion doses /ml of whole blood, of which approximately half is associated with leucocytes and half with plasma. It is now apparent that, despite our inability to detect the abnormal conformer of prion protein (PrP^{TSE}) or infectivity by bioassay in the peripheral blood of patients with vCJD, the disease is transmissible from blood donated during the pre-clinical stages of disease.

The first probable transmission occurred in 1996, the blood donor was well at the time but went on to die of vCJD in 1999. The recipient was diagnosed with vCJD in 2003. The second probable transmission was described in July 2004, the patient received blood in 1999 from a donor who developed symptoms of vCJD 18 months later. The recipient died from unrelated causes five years after the transfusion with no evidence of neurological disease, but was found to have evidence of prion accumulation in the spleen and one cervical lymph node on post-mortem examination.

A third transmission was reported in February 2006. The patient developed symptoms eight years after receiving a transfusion from a donor who themselves developed evidence of vCJD around 20 months after donating blood. The most recent (fourth) transmission was reported in January 2007. Again, the patient developed symptoms just over eight years from receiving a blood transfusion from a donor whose symptoms of vCJD appeared 17 months after donating blood. This donor was also the source of one of earlier transmissions.

It, therefore, appears that vCJD can be transmitted up to three years before the development of clinical vCJD and that it might take six to eight years thereafter for the recipient to develop vCJD, although clearly longer incubation periods may not yet have come to light. All four transmissions were via non-leucodepleted red cell units. The combination, therefore, of a cohort of sub-clinically infected individuals in the donor population with evidence of the transmissibility by blood transfusion gives rise to continuing concern around the likely efficacy of current risk management measures.

Donor selection

In the UK it is not possible to identify sub-groups of the population at significantly higher risk of vCJD apart from those considered 'at risk for public health purposes' by the CJD Incidents Panel. In addition, UK Blood Services exclude donors who themselves have received blood or tissue in order to reduce the risk of prolonging the vCJD outbreak through tertiary and higher order transmissions.

Other countries have taken steps to exclude blood donors who have spent a specified cumulative period of time in the UK and some other western European countries over the period of highest risk of dietary exposure (1980-1996). Such policies are likely to have some mitigating effect on the risk of vCJD transmission but in some cases have led to significant damage to the donor base.

Although plasma from non-UK donors is used for product manufacture and also for clinical plasma for patients under the age of 16 years or those who are exposed to large volumes of plasma (e.g. through undergoing plasma exchange for thrombotic thrombocytopenic purpura), it is not practical to import most other blood components and tissue products in sufficiently large amounts from non-remunerated donors outside the UK due to availability and concerns relating to the quality, safety and shelf-life of the products.

Blood component processing

The estimated concentration and distribution of infectivity noted above suggests that whilst plasma reduction is likely to be beneficial in terms of reducing the overall level of infectivity, sufficient infectivity would remain in individual components to effect transmission.

Universal leucodepletion was implemented in the UK 1999, as a precautionary measure. Overall the experimental data suggests that leucodepletion removes 40-70% of infectivity in whole blood but has little or no impact on plasma infectivity. These data again suggest that whilst leucodepletion is likely to reduce infectivity it is unlikely to be sufficient to impact on overall transmissibility.

A number of companies are now developing prion reduction devices which offer the potential of a further three to four log reduction in infectivity which, if achievable, would be more likely to impact on transmissibility. There is the need, however, for independent evaluation of the efficacy of these devices and concerns around ensuring the quality and safety of the blood components which have been processed in this way. Prion reduction devices would also be likely to represent a further significant increase in the unit cost of blood components.

Donor screening

10-12 peripheral blood assays are now under development worldwide, most of which rely on detection of PrP^{TSE} through a variety of approaches including monoclonal antibodies, affinity ligands and proteinase digestion. Evaluation of such assays is problematic given the small number of patients with clinical vCJD and the difficulty in extrapolating from studies using human brain homogenates and animal blood.

Sensitivity has proved a significant challenge but perhaps of more concern is the likelihood that first generation assays will have relatively poor specificity leading to large numbers of false positives results. In the absence of confirmatory assays, such individuals will need to be informed and excluded from the donor base despite the absence of clarity on the import of a positive assay. The detrimental psychological and social impact on the donor, the direct negative impact on the donor base of excluding false positives and the indirect impact represented by the unwillingness of people to continue to donate are all areas of serious concern.

Plasma product manufacture

Since 1999 plasma products manufactured in the UK have used non UK sourced plasma. In addition, much of the experimental data suggests that the plasma fractionation process is likely to reduce infectivity in therapeutic products. Nevertheless it has been felt prudent to identify and notify individuals who have been exposed to implicated plasma product batches in order that appropriate public health measures can be taken.

Cellular and tissue products and organ transplantation

It is thought that given the mass of tissue involved in transplantation of cellular therapies (such as haemopoietic stem cells), tissues (such as cornea, bone and heart valves) and solid organs, it is likely the transmission would occur should the donor be infected. Importation of such products has proved problematic with the possible exception of skin. Studies are ongoing to explore the possibility of reduction in the infectivity through tissue processing and a feasibility study of cadaveric tonsil testing is ongoing.

Conclusions

The uncertain prevalence of sub-clinical vCJD amongst donor populations coupled with the clear demonstration of transmission by red cell components gives rise to continuing concern with regard to the risk of secondary transmissions by blood and tissue products. A number of precautionary donor selection and component processing policies have been put in place but it seems unlikely that these will obviate this risk entirely. Several new technologies including prion reduction filters and prion assays are in development, however these bring important issues of evaluation, cost and potential negative impact on the donors or the quality and safety of products. It is likely that managing the risk of transmission of vCJD will continue to be highly problematic for the foreseeable future.

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Recent Developments in Stem Cells

Over the past five years, there has been a great deal of excitement about new developments in stem cell research and the potential therapeutic use of stem cells or their products for treating degenerative diseases and for tissue repair and replacement. It is sometimes forgotten, however, that the term stem cell was coined over a century ago, and that, shortly after this, Pappenheim hypothesised that haemopoietic stem cells generated blood cells, while Neumann and Bizzozero identified the bone marrow as the site of blood production after birth (Watt & Forde, 2008). It must be remembered also that stem cell therapies in the form of bone marrow transplants have been used successfully to treat haematological malignancies and other disorders of the blood for over 30 years, and that they now form a part of mainstream medicine in the treatment of these diseases. Indeed, haemopoietic stem cells for transplantation are now sourced from peripheral blood after administration of mobilising agents such as G-CSF and from umbilical cord blood obtained at birth, as well as from bone marrow, with each having particular advantages in treating patients with haematological cancers. In the UK where around 65 haematological malignancies are diagnosed each day, these transplants have saved and continue to save many lives. Furthermore, the haemopoietic stem cell itself has been used as a paradigm for defining and studying other tissue specific stem cells. Although there have been many developments in stem cell research, only a few will be highlighted here.

Haemopoietic Stem Cells for Transplantation

One of the key challenges is to optimise the outcome of haemopoietic stem cell transplantation. Factors affecting clinical outcome include the source of the haemopoietic stem cells, the number and quality of the stem cells used for transplantation, the engraftment potential of the stem cells, the occurrence of acute or chronic graft-versus-host disease, HLA and cytomegalovirus (CMV) matching between donor and recipient, infectious disease complications and the eradication of the cancer cells (Austin *et al*, 2008). Examples of advances in these areas include the development of novel T cell therapies for preventing CMV infections, which have been undergoing clinical trials in Birmingham with excellent results, and clinical trials with mesenchymal stem cells to ameliorate treatment resistant graft-versus-host disease. Major advances have also come about in part through basic research which is defining the intrinsic and extrinsic regulatory networks that control the fate of haemopoietic stem cells within the local bone marrow environmental niches in which they reside. One example is the chemokine receptor, CXCR4, and its ligand, CXCL12, which have been shown to play a central role in haemopoietic stem cell trafficking to and retention in the bone marrow. Clinical trials using CXCR4 antagonists to effectively mobilise, in a matter of hours compared to days, haemopoietic stem cells for transplantation from the bone marrow of myeloma and lymphoma patients who are refractory to the most often used mobilising agent G-CSF, as well as in normal allogeneic donors, appear promising. The outcome of other potential uses of these antagonists, such as promoting acute myeloid leukaemic cell chemosensitivity and enhancing the engraftment of cord blood haemopoietic stem cells are awaited with interest (Watt & Forde, 2008). Other challenges relate to providing sufficient cord blood haemopoietic stem cells for transplantation into adults where the numbers of such cells required for transplantation are limiting. Current clinical trials are using double cord blood transplants with promising results. Other studies have identified factors which when added to haemopoietic stem cells can significantly expand their numbers *ex vivo* or regulate their survival, longevity or lifespan. The angiopoietin-like proteins, and the HOXB4, NOV and forkhead or FoxP transcription factors are of particular note (Watt *et al*, 2008) and further studies on their safety and efficacy in clinical situations are awaited. Interestingly, members of the forkhead transcription factor family may also regulate the lifespan of red blood cells.

Stem Cells for Tissue Repair

Advances in haemopoietic stem cell research and therapeutic use have led the way in defining stem cells which are now thought to exist in almost all tissues (e.g. heart, skin, the central nervous system etc) and to contribute to the repair of the tissues from which they arise. Stem cell based therapies to repair or replace tissues offer an alternative promising approach to organ transplantation and significant advances are expected in this area in the coming years. One of the most intriguing developments recently has been in the generation of patient-specific induced pluripotent stem cells or iPS cells by reprogramming skin fibroblasts. Of 24 genes analysed, 3-4 of them, namely Oct-3/4, Sox-2 and Klf-4 with or without c-Myc, when ectopically over-expressed, could reprogramme human fibroblasts to a pluripotent-like state, i.e. a state where they can give rise to multiple tissues. The safe clinical use of such cells to repair and regenerate damaged tissues and organs is one ultimate aim in regenerative medicine, but this awaits a much more detailed understanding of the mechanisms which underlie this reprogramming at the basic scientific level.

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Organ Donation: Matching Supply To Demand

For as long as organ transplantation has been the preferred, or only, form of treatment for patients with end-stage organ failure there has been a shortage of suitable organs for transplants. The problem, however, is worse now than it has ever been, and this is the result of two factors. Firstly, the very success of modern transplantation means that an ever-increasing number of patients could benefit from a transplant. For kidney, liver, heart, lung and pancreas transplant patients, approximately 90% or more of them will be alive and well one year after the transplant. Although there is a small failure rate in each subsequent year, many patients can expect 10-20 years of normal life and there are an increasing number of patients who remain well more than 30 years after the transplant. The overall waiting list in the UK for an organ transplant currently stands at 7,609 patients, and this figure is rising by 8% every year. Even this does not truly reflect the need for transplants – many clinicians are reluctant to list more patients than are realistically likely to be able to receive a transplant, and the true need is estimated to be 2-3 times greater. Even with the current situation, around 1,000 patients listed for a transplant die each year before a suitable organ becomes available. In contrast, the number of deceased organ donors has remained static over the past 10 years, during which time there has been a fall in the number of heartbeating donors (patients certified dead after neurological tests of the brain stem), only compensated in part by a rise in the number of non-heartbeating donors (patients certified dead after cardio-respiratory arrest). Overall transplant numbers have only been able to increase through a dramatic rise in the number of living donors for kidney transplants. As a result of these two factors the gap between the number of patients waiting for a transplant and the number of organs available is widening rapidly.

Organ donation depends on a complex series of actions and events that occur primarily within hospital critical care units. Patients with catastrophic brain injuries – from trauma, strokes, sub-arachnoid haemorrhage and other acute events – may progress, despite all possible treatment, to the stage at which all functions of the brain stem are irreversibly destroyed. These patients may then be tested formally, according to clearly defined clinical criteria commonly known as the brain-stem death criteria. It is very important to emphasise that these tests should be performed because they are in the patient's best interests – not simply because the patient may be a suitable organ donor. However, if the patient **is** suitable, it is then essential that referral is made to the donor transplant co-ordinator, who together with the clinical staff caring for the patient will assess the potential donor, identify which organs could be donated, and – crucially – will approach the family to obtain consent for organ donation. The NHS Organ Donor Register (ODR) is a valuable, permanent record of the individual's wish to donate organs after death and should always be consulted. Almost 15 million people are now on the ODR and when the wishes of the individual are known, they are paramount. This relieves the donor's family from the responsibility of giving consent and gives great reassurance that organ donation was indeed what the individual wished. Unfortunately, many relatives do not give consent – about 40% – and this figure is much higher when the donor comes from the Black and Minority Ethnic (BME) communities.

All the stages of organ donation – the identification of potential donors, their notification to the donor transplant co-ordinator network, consent and indeed the arrangements for surgical retrieval of organs – currently fall short in the UK. Very many critical care clinicians are extremely supportive of organ donation – without them there would be no donation – but improvements could still be made. Not all patients are identified as potential donors and referred. The UK has far fewer donor transplant co-ordinators than are needed, many of them are currently working unacceptably long hours and there is variation in their employment arrangements. It is a tribute to their commitment that the current system works as well as it does, but it is not sustainable and must be improved. Finally, the public have a clear role – because consent is often not given at present, four out of every 10 suitable organs are not made available for transplantation.

A realistic assessment is that all steps of this process – and many other issues surrounding donation – can in fact be improved significantly if the UK commits to a fundamental overhaul of the donation system. This will inevitably take longer than we all would wish but if donation could be increased by 50% over the next five years, we would be very much closer to meeting the demand and to preventing well over 1,000 early deaths each year.

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The Department of Health Review of Organ Donation

Organ transplantation is one of medicine's great success stories, saving or transforming thousands of patients' lives every year. The UK played a major part in the development of transplantation and yet, over the past fifteen years, we have slipped from being a leader in terms of access to transplantation for our population to 14th in Europe (Figure 1). As a direct consequence nearly 1,000 people die every year either while on the transplant waiting list or after having been removed because their condition has deteriorated.

The need for transplants is growing. The number of patients on the active transplant waiting list in the UK at the end of October 2007 was 7,512, an increase of 6.22% compared with the same month in 2006. At the same time, the total number waiting, including those suspended amounted to 9,595 – an increase of 8.19% compared to the same period last year. (Figure 2)

Figure 1
Deceased organ donor rates for Europe and US, 2006

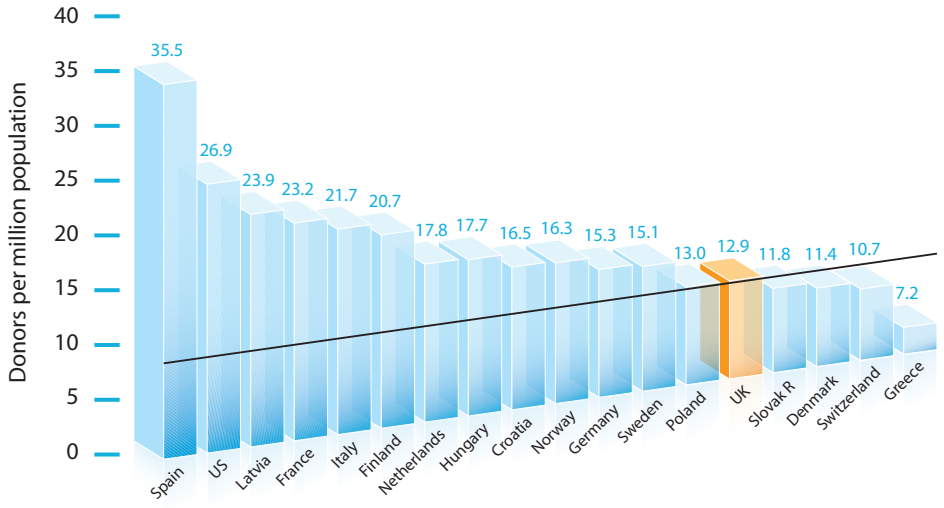
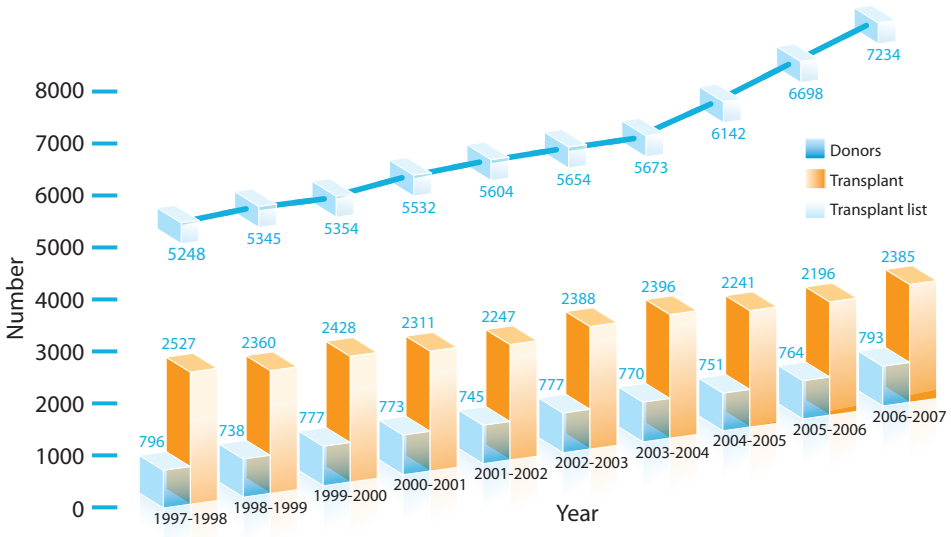


Figure 2
Number of deceased donors and transplants in the UK, 1 April 1997 – 31 March 2007, and patients on the active transplant lists at 31 March 2007



Within this the position for patients from a Black and Minority Ethnic (BME) background is considerably worse. People of Asian or African Caribbean origin are three to four times more likely than white people to need a kidney transplant, yet wait two to three times as long for a suitable organ. It is clear, therefore, that this sad situation needs to be urgently addressed.

In 2006 the then Minister of Health, Rosie Winterton, set up an Organ Donation Taskforce to identify what were the barriers to increasing organ donation and to consider what, within current legal and operational frameworks, could be done to overcome them.

Taskforce members bring with them a wide range of expertise, including transplantation and critical care medicine, donor co-ordination, health management, media, the perspective of patients and donor families, ethics and social research. Meetings were attended by representatives of the three Devolved Administrations as solutions need to work in a UK wide context.

The barriers to donation were quickly identified. The organ donation pathway has evolved over the past 40 years and is now unnecessarily complex. It calls on the expertise of several teams urgently and simultaneously, often from hospitals many miles apart. It relies on surgeons and anaesthetists, donor transplant co-ordinators (DTCs) and theatre staff working very long unsocial hours, often in unfamiliar surroundings and often in addition to their routine clinical commitments, in order to retrieve organs in optimum condition. And in the context of a financial system based on payment by results, the fact that there is no income to support donation activity doesn't encourage trusts to provide additional access to expensive critical care and theatre facilities. Properly supporting the families of dying patients to consider organ donation is a highly skilled role, and yet training for staff other than DTCs is limited, and in that context it is perhaps not surprising that, despite 90% of the UK population supporting organ donation in principle, 40% of relatives refuse to give consent when asked, rising to 70% for families of patients from a BME background. There are also legal and ethical concerns felt principally in critical care medicine where the law relating to the transition from patient to donor is not entirely clear.

The Taskforce was keen to learn from countries that have been more successful in this field in recent years. Fifteen years ago Spain had a donation rate very similar to that in the UK, but has achieved a rapid and sustained rise, with similar techniques successfully rolled out to Italy and several South American countries (Figure 3).

The USA has also substantially increased organ donation and many of the approaches in these countries are directly transferable to the UK. Both Raphael Matesanz from Spain and Frank Delmonico from the USA attended an early Taskforce meeting to reflect on their success and how the UK could apply similar principles. The Taskforce feel confident that, if its recommendations are adopted, families will feel better supported, the donation rate will substantially increase, patients will have access to the transplants they urgently need, staff will feel better equipped to provide a high quality service and the NHS, over the medium to long term, will use its resources much more effectively.

The Taskforce recommendations are with Ministers and their response is expected early in 2008.

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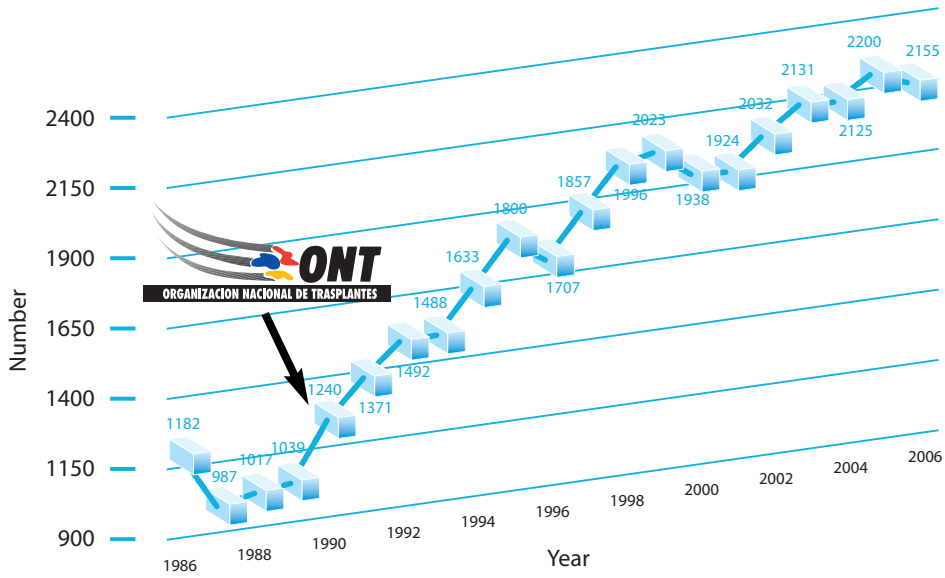
Figure 3
Increase in Spanish renal transplant rates 1989 – 2006



The Blood Transfusion Effect in Transplantation

Firstly, a brief historical note

The first cohort of patients to receive kidney allografts in the early 1960s were mostly survivors of many years on dialysis, and since erythropoietin was not available at that time, frequent multiple transfusions were usual in patients with renal failure as this was the only way to reverse anaemia which could sink as low as 2 gms/litre Hb in anephric patients. The first large scale trials of kidney transplantation with the introduction of azathioprine and steroids in the 1960s were remarkably successful, much to the surprise of many immunologists who found these agents much less effective in their experimental models. As renal transplantation was introduced in an increasing number of centres, the backlog of long-standing dialysis patients was cleared and thereafter transfusion could be avoided by simply proceeding rapidly to transplantation instead of dialysis. Because blood transfusion was known to lead to anti-HLA antibody production the advice of immunologists was to avoid blood transfusion wherever possible and this approach was taken in all centres.

In the early 1970's, over the course of five years or so, many clinicians were dismayed to find that the clinical results of renal allo-transplantation showed no improvement in graft survival, indeed it seemed to be worsening with more aggressive rejection treated by massive doses of steroid being a common response, and the complications produced were appalling. Immunologists may have felt vindicated in predicting poor outcome from their experimental models at that time. Fortunately since the start of transplantation the meticulous recording of clinical and laboratory data in large databases had been undertaken (which was in itself a pioneering step), and this allowed analysis of numerous factors that could be associated with this deterioration in graft outcome. HLA matching proved very important and blood transfusions were identified as a significant factor (Opelz & Terasaki, 1978), but to everyone's surprise the effect of transfusion was beneficial rather than deleterious.

There followed a long and fiercely contested debate as to whether the "blood transfusion effect" represented a true immunological mechanism or was rather just an artefact of selection of less reactive patients brought about by antibody-mediated sensitization. From the mid-1970's onwards experimental models of transplantation of various organs became established in animal models, and the phenomenon of blood transfusion, and subsequently infusion of a variety of other cell sources, was shown to markedly prolong the survival of vascularised organ allografts. Looking back it is interesting to speculate how the practice of kidney transplantation would have fared had there not been the fortuitous blood transfusion effect. Since the late 80's the mechanisms and characteristics of the blood transfusion effect have been meticulously dissected in experimental models, the most extensive studies being in the mouse.

Established Features of the Blood Transfusion Effect

Rodent models

In mouse models the effect of donor specific blood transfusion has been examined extensively by the Oxford transplantation group under the leadership originally of Peter Morris and Kathryn Wood and more recently Kathryn Wood alone. Using a mouse model, blood transfusion has been examined for its graft prolonging effect and the following principles established:

1. The effect is mediated by the content of leucocytes, not red cells and is a cell-based suppression mechanism.
2. Subsets of leucocytes or indeed the bone marrow can induce suppression phenomena indistinguishable from the blood transfusion effect.
3. The donor cells must express MHC antigens for the suppression effect to be produced, even a single MHC molecule transgenically expressed on a non-lymphoid cell such as a syngeneic fibroblast can suffice.
4. A time delay of at least 2-4 weeks is necessary between transfusion and organ allografting.
5. The effect of a single transfusion can be enhanced by the addition of some form of immunosuppression, especially anti-CD4 antibodies, but the suppression produced remains donor specific. However some immunosuppressive agents, for example calcineurin inhibitors at high dose, can prevent the suppression effect from developing.
6. The use of repeated transfusion can increase the power of the effect obviating the need for immunosuppression and spread the effect to included "third party" strains (i.e. become non-donor specific).
7. The mechanism of this suppression has been shown to be mediated by regulatory CD4 T cells is graft dependant and it has recently been shown to work via the indirect allo-recognition pathway.
8. The process occurs in the periphery and is independent of the thymus.
9. In vitro and in vivo studies suggest roles for CTLA-4, IL2, IL4, IL1 and TGF beta but probably secondary to a primary contact-based mechanism.

Human studies

It is wise to remember that experience shows direct extrapolation from mouse to man has often been broadly correct in principle, but incorrect in terms of detail, across many biological fields. Differences in the detailed functioning of phenomena such as the blood transfusion effect would be unsurprising in view of the known differences in the immune systems. As mentioned above numerous clinical studies in the 1970s established that a beneficial effect of transfusion existed as a repeatable

finding, not simply due to responder negative selection. For several years deliberate blood transfusion prior to renal transplantation became normal practice in most large transplantation centres, the typical regimen being three random transfusions at monthly intervals. Approximately 10% of patients developed antibodies to HLA with high panel reactivity, although this could be lowered by concomitant use of immunosuppressive drugs such as azathioprine. Donor specific transfusion under immunosuppression was also used by some centres for live donation of kidneys, usually for 1 or 0 haplotype matched pairs. Today blood transfusion is not used routinely in this way in transplantation. What brought about the change?

The widespread disquiet for using blood products as a result of the AIDS virus crisis was certainly a factor, but the introduction of cyclosporine in the early '80s was probably the major cause. The use of cyclosporine meant that graft survival rates as high as those achieved previously could be attained using cyclosporine (and later tacrolimus) without the apparent need for transfusion, and with the difficulties of blood transfusion following the AIDS crisis it was quietly dropped from the protocol of most centres. However, this change in practice was never supported by clinical trials, indeed a randomized prospective trial coordinated by Opelz from Heidelberg showed significant benefit from transfusion in a cyclosporine-based immunosuppression protocol (Opelz *et al*, 1997). Benefit of blood transfusion has been also shown using a tacrolimus-based regimen (Higgins *et al*, 2004). By the time these studies were published the results of cadaveric and live donor kidney transplantation had improved further, approaching 90 to 95% 1 year graft function in many centres, and it was hard to see how this could be bettered by re-introducing blood transfusion. Furthermore there are now considerable logistical difficulties in obtaining whole blood for transfusion since leukocyte depletion has become the norm for red cell replacement. However, it may still be worth undertaking in patient groups known to have high immune reactivity, for example pediatric renal transplantation (Niaudet *et al*, 2000).

Conclusions

The beneficial blood transfusion effect in transplantation is a proven phenomenon, which was arguably in part responsible for the early success of renal transplantation. For some years it was used clinically to good effect, but fell out of favour, partly due to the advent of AIDS and partly due to improved graft survival with the introduction of cyclosporine/tacrolimus. Nevertheless, recent carefully performed studies have shown that a beneficial effect is still demonstrable in the cyclosporine tacrolimus era, and it may be worth reconsidering in high rejector groups such as children. Intensive research into the mechanism of the effect continues and is justified as it seems to be based on the same mechanisms that may induce tolerance for organ transplantation (Niaudet *et al*, 2000).

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New Developments in Tissue Engineering

Conventional procedures aim to replace a damaged tissue or organ with a graft which does not grow but tissue engineering aims to regenerate and repair the tissue either by stimulating some of the remaining tissue to divide and re-grow or by adding a matrix into which cells can grow. To this end, tissue engineering is often described as Regenerative Medicine and usually involves some combination of matrix, cells and stimulating agents such as growth factors.

Tissue engineering is a vast research field and Regenerative Medicine hit the headlines in 2006 when it was reported by a research group in the United States that bladders had been grown in the laboratory and then transplanted into seven young patients where they had been working well for an average of four years. A portion of each patient's bladder, smaller than a postage stamp, was biopsied, cells were extracted and grown in cell culture such that 1 million cells divided to reach 1.5 billion before being added to a matrix and then transplanted into the patient (A. Atala *et al*, 2006).

NHSBT Tissue Services have worked with colleagues in the University of Leeds to develop a patented method of producing natural matrices from human donors where all donor cells are removed but the properties of the matrix have not been affected. We are now looking at how best to grow and add cells to these matrices. How to source, grow, add and differentiate cells on matrices is one of the major problems facing tissue engineering. Adult tissues primarily contain fully differentiated cells but also a small proportion of stem cells capable of self-renewal and differentiation but only into a small range of tissues. These adult stem cells have been found in bone marrow, heart, brain, adipose tissue, muscle, skin, eyes, kidneys, lungs, liver, gastrointestinal tract, pancreas, breast, ovaries and testis. However, getting to the cells and getting the cells to increase in number without differentiating and then differentiating when required creates a number of problems leaving researchers to look at other sources such as embryonic stem cells (ES) which are thought capable of unlimited cell division and possibly of differentiating into each of the 220 different cells types found in the adult human.

Although controversial, ES are investigated partially because of the need for large cell numbers in tissue engineering. If adult cells could extensively divide or other cells could act as ES, then the need for ES would diminish. One of the reasons cells stop dividing is that during each cell division, part of the chromosome, called a telomere, is lost - when a telomere is too short, the cell stops dividing. Addition of the enzyme telomerase induces longer telomeres to form and thereby allows many more cell divisions. This phenomenon is being extensively studied for tissue engineering purposes but one problem is that extended telomeres have also been found in some cancer cells and the possibility that increasing telomere length may increase the risk of cancer is being investigated.

Exciting new discoveries which allow normal adult cells to be re-programmed into "induced Pleuripotent Stem cells" (iPS) with ES-like properties and then be differentiated into e.g. blood, to replace sickle-cell erythrocytes, have been reported in the last quarter of 2007. One group of scientists at the University of Wisconsin took a small biopsy of normal human (or mouse) skin, harvested the cells and then transfected four genes, known as transcription factors, into the cells using retroviruses. The extra genes incorporated into the host chromosomes and when activated made the skin cells ES-like with an extensive cell proliferation potential and the ability to differentiate into at least eight different cell types. This ability of skin cells to re-programme, divide and differentiate has been used by another group in Cambridge, Massachusetts, to produce blood cells and "cure" a mouse of sickle cell disease. Skin cells from a mouse model of sickle cell disease were harvested and re-programmed into iPS by inserting the four transcription factors. The scientists then replaced the defective blood gene with a normal gene, induced the iPS to undergo red blood cell differentiation and then injected the cells into the animal. When the mouse blood was tested, there was no evidence of the sickle cells or the disease. The group leader Professor Rudolf Jaenisch stated "This demonstrates that iPS cells have the same potential for therapy as embryonic stem cells, without the ethical and practical issues raised in creating embryonic stem cells." (Whitehead, 2007).

While iPS cells offer tremendous promise for regenerative medicine, scientists caution that major challenges must be overcome before medical applications can be considered. First among these is to find a better delivery system, since retroviruses bring other changes to the genome that are far too random to let loose in humans.

From making and transplanting whole bladders to curing blood cell diseases, the future looks good for tissue engineering.

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Atala, A., Bauer, S. B., Soker, S. *et al.* (2006) Tissue-engineered autologous bladders for patients need cystoplasty. *Lancet*; 367; 1241-1246

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Cryopreservation of Cartilage for Transplantation

There is increasing interest in the possibility of treating diseased or damaged areas of synovial joint surfaces by grafts of healthy donor (i.e.allogeneic) cartilage. In the future such grafts could be obtained from cadaver tissue donors or they might be manufactured by tissue engineering methods. In either case the Tissue Services section of the NHSBT will be involved. To be effective the graft must contain living cells and, since it is avascular, cartilage is immunologically privileged - but of course that is of no advantage if the chondrocytes are already dead. Cartilage with living chondrocytes can be preserved for 10 – 14 days at temperatures above 0°C, but clearly much longer periods would be needed to ensure that there was an adequate supply of microbiologically tested tissue available for use. Isolated chondrocytes can be cryopreserved by standard methods, similar to those used, for example, for haemopoietic stem cells, but until now there has been no method that will preserve a high proportion of living chondrocytes *in situ* in surgical grafts, from the time of procurement or manufacture to clinical use. Many published papers indicate that survival of living chondrocytes *in situ* is inadequate at best and is also very variable (Pegg *et al*, 2006a). Long-term preservation methods that achieve survival of chondrocytes *in situ* are required to build up operational stocks of grafts for use and to enable living grafts of a practical size to be provided at the right time for patient and surgeon.

The first step in identifying the cause of the discrepancy between the cryobiological behaviour of isolated chondrocytes and cartilage tissue was to establish that the cryoprotectants we had chosen to use, dimethyl sulphoxide (DMSO) and propylene glycol, do actually penetrate into the tissue rapidly. Using knee joint cartilage from sheep as a model system we found that they do. Moreover, chondrocytes were shown to tolerate 10 or 20% DMSO and were not unusually susceptible to the osmotic stresses that can occur during cryopreservation. An experiment in which the effects of freezing with 10% DMSO to minus 50°C were separated from the effects of the concomitant rise in solute concentration showed that injury was associated with the formation of ice (Pegg *et al* 2006b). This was surprising: freezing injury can certainly occur if ice is allowed to form within cells but at the low cooling rates used ice would be expected to crystallize exclusively outside the cells where, as experience with isolated chondrocytes showed, it has no direct damaging effect on the cells. Freeze substitution microscopy of cryopreserved cartilage then showed that large ice crystals were formed within the chondron – this is defined as the cartilage cells (chondrocytes) plus the layer of acellular matrix that closely surrounds them – and some (at least) was within the chondrocytes, even when the cooling rate was optimal for isolated chondrocytes. We therefore proposed that the growth of ice within the chondron (rather than the surrounding acellular matrix) is responsible for the very poor survival of chondrocytes *in situ* when conventional methods of cartilage cryopreservation are used.

This finding established the need to avoid the crystallization of ice – in other words, a process called vitrification. There is one paper in the literature describing a vitrification method (Song *et al*, 2004). We confirmed the effectiveness of this method but found it to be impractical for clinical use because it required rapid cooling and ultra-rapid warming to avoid the crystallization of ice (Pegg *et al*, 2006c). However, we were able to develop a method in which the concentration of cryoprotectant was increased progressively during cooling and decreased during warming, so that the crystallization of ice was completely avoided and neither rapid cooling nor rapid warming was required. Because the cryoprotectant in the tissue was added stepwise so that its concentration followed the freezing point depression curve or 'liquidus line', we called the method the 'liquidus tracking' method (Pegg *et al*, 2006c). Using the ability of cartilage to incorporate sulphate into newly synthesized glycosaminoglycans (GAGs) as a viability test we were able to freeze cartilage so that it had 70% of the function of fresh control cartilage. In this method the rates of cooling and warming can be very low, which is essential for any method that is to be used in Tissue Banks to process the bulky grafts that are required by orthopaedic surgeons.

Our most recent experiments have shown that continuous stirring throughout the process resulted in a significant increase in the rate of ^{35}S sulphate incorporation into GAGs, now reaching 87% of the corresponding fresh control values. We have confirmed that the method is also effective for human knee joint cartilage and osteochondral dowels. The most important mechanical property (instantaneous compressive modulus) was unaffected by the process (Pegg *et al*, 2007).

We have also developed a closed circuit, continuous flow method in which both temperature and DMSO concentration are computer-controlled. With funding from the Department of Trade and Industry we are now collaborating with Planer plc to produce the necessary equipment for clinical use.

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Regulation of Tissue and Organ Donation: The Impact of the Human Tissue Act

The remit of the Human Tissue Authority (HTA) is broad and complex. Under two pieces of legislation, we regulate six diverse sectors, although it is our work in regulating living-donor organ transplants that seems to create the most public interest. Last July, two couples who had never met swapped kidneys to save a loved one. Two months later, a nurse, Barbara Ryder, made an altruistic non-directed kidney donation to a stranger. The HTA has allowed more flexibility in who can donate to whom by allowing these new forms of organ donation. This article summarises our work in regulating solid organ and bone marrow transplants.

Legislation

Human Tissue Act – donation of solid organs and bone marrow for transplantation

Consent is the cornerstone of the Human Tissue Act 2004 (HTAct). Storage and use of body parts, organs and tissue from the living or the deceased for specified health-related purposes and public display are covered by the HTAct. It also covers the removal of tissue from the deceased. The HT (Scotland) Act is based on authorisation rather than consent, but these are both expressions of the same principle.

Our role since September 2006, has been to approve all donations of solid organs from living donors, and any donations of bone marrow or PBSC from children, or from adults who do not have the ability to make an informed decision. We have trained and accredited 150 Independent Assessors (IAs) and 60 Accredited Assessors (AAs) to work on our behalf, and that of the donor, to assess applications for organ and bone marrow transplants, respectively. IAs and AAs recommend whether or not the HTA gives approval for the donation to go ahead from an ethical standpoint. The donor and recipient must be thoroughly assessed to make sure that informed consent has been given, that the risks of the procedure and its implications have been explained and understood and that there is no coercion or financial inducement. The process for straightforward organ transplant approvals takes around two days.

We recently achieved a major milestone in December 2007 – approving our 1,000th living-donor organ transplant.

As part of our statutory remit, we have issued Codes of Practice covering areas including consent, the donation of organs, tissue and cells for transplantation, the removal, storage and disposal of human organs and tissue, and the donation of allogeneic bone marrow and peripheral blood stem cells for transplantation.

EU Tissue and Cells Directive

In relation to licensing, our regulatory remit is expanded by the EU Tissue and Cells Directive (EUTCD). The EUTCD creates a common framework that ensures high standards in the procurement, testing, processing, storage, distribution and import/export of tissues and cells across the EU community. The Directive is primarily concerned with assuring the safety and quality of tissues and cells – including bone marrow and peripheral blood stem cells (PBSCs) – used for human application. The Directive came into force from 7 April 2006 throughout the EU and was transposed into UK law via the Human Tissue (Quality and Safety for Human Application) Regulations on 5 July 2007. The HTA is one of the two competent authorities checking the implementation of this Directive in the UK. (The Human Fertilisation and Embryology Authority is the competent authority for gametes and embryos.)

More than 200 tissue establishments applied for a human application licence by 7 April 2006. An HTA survey of the sector showed that 75 per cent of those who responded agreed that the EUTCD had a positive impact on the safety of tissue and cells used for transplantation.

In conclusion…

We believe we have created a system for donation that is transparent and which patients, families and professionals trust. Regulation of human tissue raises standards and plays a part in improving public health and confidence. We have been commended for our inclusive, risk-based approach, and we continue to ensure consent and patient safety remain at the heart of all the work we do.

We hope that in future, up to 50 couples a year will benefit from paired donation, and up to 10 altruistic kidney transplants could be approved. We work hand-in-hand with the transplant community to ensure consent and safety is paramount when people like Barbara Ryder want to "give something back" to society.

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CPD QUESTIONS FOR *BLOOD MATTERS* 24

1. HLA antibodies

a) Have no role in the rejection of solid organ transplants.
b) Can be implicated in TRALI.
c) Have no role in platelet refractoriness.
d) Are useful as a marker of disease.

2. Luminex Technology

a) Uses oligonucleotide probes for HLA antibody detection.
b) Does not require any further supplementary technique for HSC transplantation.
c) Has increased sensitivity, but may result in the detection of low level antibodies which may not be clinically relevant.
d) Has enabled tranplants to take place even in the presence of donor specific antibodies, due to the techniques lack of sensitivity.

3. Genome – Wide Association Studies (GWAS)

a) Coronary Artery Disease has been shown to have a strong association with a particular SNP on Chromosone 9 $_{p}$21.
b) GWAS has involved the study of 17,000 patients with Rheumatoid Arthritis.
c) GWAS has involved the study of 17,000 'shared-controls'.
d) Only one SNP showed association with Coronary Artery Disease.

4. The Application of Molecular Genetics to Fetal Blood Grouping

a) All 33 genes encoding the 262 antigens of the 29 Red Cell Blood Group system have been cloned and sequenced.
b) Amniocentesis and chorionic villius sampling are risk free procedures.
c) Any fetal DNA present in maternal plasma has originated directly from the fetus.
d) Fetal DNA represents between 3% and 6% of total cell-face DNA in maternal plasma.

5. The Application of Molecular Genetics to Fetal Blood Grouping

a) The fetal D phenotype can be predicted reliably from fetal DNA in the plasma of D-Negative pregnant women from the beginning of the second trimester.
b) All tests in D-Negative pregnant women are performed with adequate internal controls.
c) Only D testing on fetal DNA in maternal plasma is available.
d) Routine fetal D typing for all D-Negative pregnant women is available.

6. Managing the Risk of Transmission of vCJD and Blood and Tissues

a) Variant CJD is confined to the UK.
b) Best estimate at present is that there is a maximum likelihood estimate of 30,000 infected people.
c) Best estimate at present is that there is likely to be a prevalence of sub-clinical vCJD in the UK donor population in the order of 1/4,000 to 1/20,000.
d) Best estimate at present is a possibility of sub-clinical infection of vCJD of 1.93. Direct quote with correct answer of 0.93 changed to 1.93

7. Managing the Risk of Transmission of vCJD and Blood & Tissues

a) A maximum of only three years elapses between exposure and development of clinical vCJD
b) Variant CJD can be transmitted up to three years before the development of clinical vCJD.
c) Universal leucodepletion has a significant impact on plasma infectivity.
d) Universal leucodepletion has a significant impact on overall transmission ability.

8. Regulation of Tissue and Organ donation: The impact of the Human Tissue Act

a) The Human Tissue Act only regulates donation of solid organs.
b) The NHSBT is a competent Authority under the EU Tissue and Cells Directive.
c) Less than 100 tissue establishments applied for a Human Application Licence 7th April 2000.
d) Consent is the cornerstone of the Human Tissue Act.

9. Granulocyte Therapy

a) United Kingdom Blood Services regularly administer G-CSF and steroids to Granulocyte Donors prior to Apheresis.
b) HLA alloimmunisation is a risk, especially with "buffy coat" granulocyte transfusion.
c) There is a large body of published clinical evidence to demonstrate the benefits of Granulocyte Therapy.
d) Optimised Granulocyte Component is readily available.

10. The Blood Transfusion effect in Transplantation

a) In the 1970's blood transfusion was shown to be a beneficial factor in renal transplantation.
b) Re-introduction of blood transfusion prior to transplantation could significantly improve cadaveric for liver donor kidney transplantation.
c) Cyclosporine – based immunosuppression protocols did not benefit additionally from the "blood transfusion effect".
d) Even in patient groups known to have high immune reactivity, transfusion is currently rarely used prior to kidney transplantation.

Diary Dates Spring 2008

10 - 14 August

XXII International Congress of the Transplantation Society

Sydney, Australia.

Further information:

www.transplantation2008.org

10 September

Managing Massive Transfusion: Clinical and Blood Bank Perspectives

AABB Audioconference

Contact: AABB Education Department

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Fax: +1.301.215.6895

Email: education@aabb.org

Website: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audioconferences.htm

11 - 13 September

BBTS Annual Scientific Meeting

Llandudno, North Wales

Website: www.bbts.org.uk

17 September

What's New in Platelet Products?

AABB Audioconference

Contact: AABB Education Department

Tel: +1.301.215.6842

Fax: +1.301.215.6895

Email: education@aabb.org

Web site: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audioconferences.htm

17 - 19 September

Transfusion Practice & Transfusion Alternatives

Reval Latvia Hotel & Conference Centre

Website:

www.transfusionandalternatives.com

Information available from the organisers:

Eurocongress Conference Management,

Tel.: +31 20 679 3411

Fax: +31 20 673 7306

E-mail: itc.riga@eurocongres.com

22 - 26 September

40th Annual Course - Advances in Haematology

Hammersmith Conference Centre

For more information please contact Imperial on hcc@imperial.nhs.uk

Register online:

www.hhnt.org/hcc/conferences/advancesinhaematology2008/index.htm

24 September

Models for Effective Quarantine and Segregation of Cellular Therapy Products

AABB Audioconference

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Email: education@aabb.org

Web site: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audioconferences.htm

15 - 18 October

Platelets 2008 International Symposium

Woods Hole, Massachusetts, U.S.A.

For more information please contact, via website, on rsimak@platelets2008.org

View the programme and register online:

www.platelets2008.org

16 October

British Society of Blood and Marrow Transplantation Education Day (including Autumn Open Meeting)

RIBA, London

Further information: www.bsbmt.org

22 October

Intravenous Immunoglobulin (IVIG): Intended Use and Administration

AABB Audioconference

Contact: AABB Education Department

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Email: education@aabb.org

Web site: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audioconferences.htm

23-24 October

HHS Advisory Committee on Blood Safety and Availability Meeting

Washington, DC

Web site: www.hhs.gov/ophs/bloodsafety/index.html

24 October

Visualising Cellular Function in vivo

BioPark, Hertfordshire, United Kingdom

For more information please contact enquiries on: enquiries@euroscicon.com

You can view the programme and register online: www.euroscicon.com

4 - 6 November

BGS Durham

A Symposium on Clinical and Laboratory Aspects of Transfusion Science

St Mary's College, Durham

Further information:

www.bgsdurham.org/index.html

14 November

Recent Advances in Flow Cytometric Techniques and Instrumentation

London

For more information please contact enquiries on: enquiries@euroscicon.com

You can view the programme and register online: www.euroscicon.com

17 November

13th Meeting of the BSH Obstetric Haematology Group

St Thomas' Hospital, London

For more information please contact Julie Woolley on: julie.d.woolley@uhl-tr.nhs.uk

19 November

Platelet Refractoriness: Causes and Treatments

AABB Audioconference

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Email: education@aabb.org

Web site: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audioconferences.htm

6 - 9 December

American Society of Hematology

San Francisco, USA

You can view the programme and register online: www.hematology.org/meetings/2008/index.cfm

10 December

Differential Diagnosis of Suspected Pulmonary Transfusion Reactions

AABB Audioconference

Contact: AABB Education Department

Tel: +1.301.215.6842

Fax: +1.301.215.6895

Email: education@aabb.org

Web site: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audioconferences.htm

11-12 December

FDA/CBER Blood Products Advisory Committee Meeting

Bethesda, MD

Web site: www.fda.gov/CBER/advisory/bp/bpmain.htm

A full diary of events and training courses can be viewed on the following websites:

www.transfusionguidelines.org.uk

ww.blood.co.uk/hospitals

Readership Survey

We hope that you have enjoyed reading ***Blood Matters*** in its new format. Our aim has been to make ***Blood Matters*** clearer and easier to read, whilst keeping its professional appearance. We do value your feedback so please would you take two minutes to complete the enclosed survey and return it to us either by post at the address given or by completing it online at https://secure.blood.co.uk/bloodmatters/survey.asp

We value your response and in recognition of this we will select at random, one respondent who will receive a £30 book token.

Blood safety tracking pilot

NHS Connected for Health (NHS CFH) and the National Patient Safety Agency (NPSA) are working together with Mayday Healthcare NHS Trust on a blood tracking pilot scheme. This system uses modern technology to enable blood to be tracked from blood sampling to transfusion, helping to ensure that the correct blood is administered to all patients.

More information on the pilot is available at:
www.connectingforhealth.nhs.uk/systemsandservices/bloodpilot

TEAR HERE

MOISTEN HERE

BLOOD MATTERS READERSHIP SURVEY

We are seeking your views about the new look *Blood Matters*. Feedback will help ensure that we tailor the style and content of future editions to meet the needs of our readers.

We value your response and in recognition of this we will select at random, one respondent who will receive a £30 book token.

In order to be eligible for the book token prize, please complete the questions below.

1. How often do you read your copy of ***Blood Matters***?

Always ☐ Often ☐ Sometimes ☐ Rarely ☐ Never ☐

2. Please rate your level of satisfaction with the overall content of ***Blood Matters*** in terms of the following: (on a scale of 1 to 10, where 1 = very dissatisfied and 10 = very satisfied)

Breadth of subject matter	1	2	3	4	5	6	7	8	9	10
Information content	1	2	3	4	5	6	7	8	9	10
Level of detail	1	2	3	4	5	6	7	8	9	10
Technical level	1	2	3	4	5	6	7	8	9	10
Relevance to you	1	2	3	4	5	6	7	8	9	10

3. Do you have a preference for themed issues, focusing on particular subject matters, or do you prefer a mixture of topics?

Prefer themed issues ☐ Prefer mixed content ☐
No preference ☐

4. Would you value regular feature articles in each issue, e.g. update from SHOT, national blood use data, update from NHSBT Better Blood Transfusion Team?

Yes ☐ No ☐ No preference ☐

5. Suggestions for new content:

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6. How do you rate the length of ***Blood Matters***?

Too long ☐ About right ☐ Too short ☐

7 (a). The design of the current issue has been changed from previous issues. What is your overall impression of the new design and layout?

Excellent ☐ Good ☐ Satisfactory ☐ Poor ☐

7 (b). What do you think about the new cover design, style and colours used?

Excellent ☐ Good ☐ Satisfactory ☐ Poor ☐

7 (c). What do you think about the contents, style and colours used?

Excellent ☐ Good ☐ Satisfactory ☐ Poor ☐

7 (d). Do you feel that the changed format has improved ***Blood Matters***?

Yes ☐ No ☐ Don't know ☐

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Next Edition

The next edition of ***Blood Matters*** will contain a series of articles designed to bring you up-to-date on the way that NHS Blood & Transplant aims to provide blood components and diagnostic, stem cell and tissue services over the next decade.
It will also contain state of the art articles on:

- Labelling proposals for tissues and cell therapy products
- Iron and blood donation
- The supply and use of intravenous immunoglobulin in 2008
- Scientific and technical training
- Collaboration on Inspection in Europe: The EUSTITE Project
- IT development in hospitals – blood tracking

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