Viewpoint

Histocompatibility testing for haematopoietic stem cell transplantation: At the fronteer between clinical services and genetic research

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During the last three decades the success of organ and haematopoietic stem cell transplantation (HSCT) has been greatly facilitated by our increasing knowledge on the diversity of major histocompatibility complex antigens, the so-called human leukocyte antigens (HLA) in man. Barriers to transplantation reside in polymorphic cell surface antigens that differ between recipients and donors: major histocompatibility antigens, minor histocompatibility antigens (such as sex disparity), and ABO blood groups (crucial only for organ transplantation). In HSCT, knowledge of some key genetic characteristics of patients and potential donors is thus an obligatory step to optimise HSC donor selection. Incompatibilities for HLA antigens are recognised by the immune system and are associated with an increased risk of post-transplant complications and with a lower survival probability. High compatibility standards have significantly improved HSCT outcome and allowed an increasing number of patients with haematological malignancies or other disorders of the immune system to have access to this therapy. Genetic testing for histocompatibility is a rapidly moving field in terms of technical expertise. Advances in transplant protocols and stem cell-based new therapeutic approaches, such as in regenerative medicine, call for a re-evaluation of the ethical framework of genetic testing in HSCT at the crossroad of clinical service and research.

Role of HLA compatibility in haematopoietic stem cell transplantation (HSCT)

As opposed to organ transplantation where HLA matching is more permissive and detection of pre-existing anti-HLA antibodies the most crucial step for organ allocation, the HLA compatibility criteria are much more stringent in HSCT. For any given patient the best donor is a brother or sister that has inherited the very same HLA antigens, a so-called genotypically identical sibling, meaning that all HLA genes of the patient and the donor code for exactly the same HLA antigens. However an increasing number of patients do not have a compatible sibling donor (25% chance of compatibility) because of the lower number of offspring in most industrialised countries and because of the older age of the patients. For those patients without a compatible related donor a suitable unrelated donor may be identified in the international registry listing more than 19 million HLA-typed volunteer donors mostly of European descent. Alternatively HSC from cord blood units stored in public banks can be used. Actually over 50% of all HSCTs are performed with stem cells from unrelated donors or cord blood units. Because of the extreme diversity of the HLA system, still some patients, particularly those from non-European ancestry, will not have a single potential compatible donor in the international registry. To increase equity in access of patients to HSCT it has been advocated that donor recruitment campaigns may focus on such minorities. The peculiarities of the donation of HSC at the ethical and juridicial level in comparison to organ donation have been discussed previously [1]. When no HLA compatible donor is available, one option is the conception of a child as a potential HSC donor. Compared to natural conception, selection of embryos based on pre-implantation HLA testing offers a clear advantage. Ethical issues linked to HLA typing for pre-implantation genetic diagnosis (PGD) have been discussed elsewhere [2]. As a realistic alternative in the future, HLA testing of embryos will allow the deriving of compatible embryonic stem cells for cell therapy without the need for a child conception [3].

HLA antigens as disease susceptibility markers

Since the seminal observation in 1973 that the HLA-B27 antigen was strongly associated with susceptibility to ankylosing spondylarthritis, numerous reports have identified specific HLA alleles as susceptibility markers for several autoimmune diseases, such as coeliac disease, narcolepsy, rheumatoid arthritis, to name just a few. HLA alleles are also associated with a higher risk of severe allergic reactions to drugs, such as abacavir used in the anti-HIV tritherapies or carbamazepine, used to treat epilepsy. The identification of such HLA alleles in a given individual analysed for transplantation purposes raises the issue of explaining this result to the concerned individual that would express his/her intention to know about his/her HLA type. For example, a HSC donor that has been typed as HLA-B27-positive upon registration has a hundred-fold higher risk of developing ankylosing spondylarthritis com-

pared to a HLA-B27-negative individuals and has a 50% probability of transmitting this risk to his/her offspring. However in the general population 95–99% of HLA-B27-positive adults will never develop the disease.

According to the US National Bioethics Advisory Commission (NBAC) genetic results should be disclosed to genetic research participants only under strict criteria, among which the results must have "significant implications for the subject health" [4]. Although NBAC criteria would not apply for a mere disease susceptibility factor, such as HLA, that has no implication for the health of the donors at least in the short term. However the issue might need to be re-evaluated whenever larger scale genetic studies as performed in HSCT research projects could reveal complex genotypes associated with disease with a much higher probability coefficient.

Stem cell donors registries

The development of many national stem cell donor registries as well as cord blood unit registries, connected to the international database BMDW *(Bone Marrow Donor Worldwide)*, provides an increasing chance for any patient to find a suitable donor. As of June 2012 19'150'000 volunteer donors were registered in 67 registries from 49 countries, and 530'000 cord blood units were stored in 45 cord blood banks from 30 countries. In order to protect donors while giving patients the best chance of finding a donor for HSC transplantation, the WMDA *(World Marrow Donor Association)* has worked out standards that were first published in 2004 [5], updated in 2006 to comply with the regulations of the EU, then updated to take into account the cord blood banking activities *(http://www.worldmarrow.org)*. One of these standards indicates that donor records, including HLA genotype information, must be stored to ensure not only confidentiality, but also traceability. Anonymity and confidentiality during the search and donation process is required by the WMDA standards unless a given registry has a written policy stating the conditions under which patients and donors might be informed of each other's identity. Surprisingly 35% of the registries allow direct recipient-donor meetings [6]. However because of the increasing use of less intensive conditioning regimens for patients, more than one stem cell donation might be requested from any given donor. Under these conditions the policy of possible non-anonymity should probably be revised.

Stem cell donors as research subjects

Data on HSC donors, including HLA types, ABO blood groups, age, CMV status, are collected and used for an evaluation of the transplant results (i.e. efficacy of treatment), as requested by the national legislation in several countries. These data are also used in national or international research studies which have led to improvements in the therapeutic approaches. Technical advances in HLA typing and in analysing immune-related genetic polymorphisms have allowed a number of retrospective studies on patients and donors DNA available in the biobanks after approval by institutional review boards. Such studies have also contributed valuable information on the relevant parameters that might impact on the success of the transplantation. Laboratories providing histocompatibility typing are thus at the crossroads between clinical services and research in immunogenetics. With the rapid development of next generation sequencing tissue characteristics will become a small part of the total genetic information that will be provided. How should genetic information (so-called incidental findings) not related to the primary goal, i.e. tissue compatibility, be handled? Such genetic results could be viewed as clinically relevant, with for example a known mutation that confers a high risk of colon cancer [7]. The contribution of the >3 million sequence variants in each human genome in any disease is unknown. Based on genetic data for monozygotic twin pairs it has been estimated by mathematical modelling that the benefit of whole genome sequencing in the general population is small, as calculated for 24 different diseases including coronary heart disease, cancers, stroke [8]. However >90% of tested individuals might be alerted to a clinically significant predisposition to at least one disease [8].

Recently published recommendations for donor participation in research studies request that the research protocol should be approved in the country of origin of both patient and donor [9]. However this will be problematic for studies involving hundreds if not thousands or donors originating from many different countries. Concerns about these recommendations have been raised and it has been proposed that donors should be informed upfront 'of the implicit research nature of unrelated HSCT' [10]. Registries should thus make all efforts to include donors in their advisory boards. Biobanks such as those of HSC donor registries represent extremely valuable tools for medical research. For example HLA allele frequencies distribution among different populations or even within different geographical areas should be useful as reference data for epidemiological studies.

Genetic studies are now disclosing variants of non-HLA genes as relevant markers for clinical outcome of HSCT. Tests performed as part of research protocols may not be readily discriminated from those performed for the clinical management of patients [11]. It is indeed not easy to draw a scientifically sound fronteer between HLA genotyping for assessing the recipient-donor compatibility status and testing for other genes in the major histocompatibility complex or on other

chromosomes that do impact on the recognition of incompatibilities by the immune system, and on transplant outcome. Such genes may be crucial for the success of transplantation, for example by increasing the risk of graft-versus-host disease or rejection, by increasing the susceptibility to infections, or by limiting the response to pharmacological drugs. In many centres data on non-HLA genetic polymorphisms have been generated in retrospective studies with approval of institutional review boards, but without informed consent of the donors. New consent forms should take into account the technological advances from single gene analysis to multiple single nucleotide polymorphisms (SNPs) and whole genome sequencing and thus anticipate on the huge potential of these analytical tools. Also guidelines are needed on disclosure of non-HLA genetic information obtained by whole genome sequencing of the donors in the framework of research projects in HSCT and in stem cell-based regenerative medicine.

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