HLA typing in lung transplantation: does high resolution fit all?

Silvia Deaglio[1,2], Antonio Amoroso[1,2], Mauro Rinaldi[2,3], Massimo Boffini[2,3]

[1]Department of Medical Sciences, University of Turin, Turin, Italy; [2]AOU Città della Salute e della Scienza University Hospital, Turin, Italy; [3]Department of Surgical Sciences, University of Turin, Turin, Italy

Correspondence to: Prof. Massimo Boffini. Associate Professor, Heart and Lung Transplant Center, Cardiac Surgery Division, Surgical Sciences Department, University of Torino, Corso Bramante 88/90, 10126 Turin, Italy. Email: massimo.boffini@unito.it.

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The paper by Zhang and colleagues published in this issue of *Annals of Translational Medicine* reports on the value of using high definition of HLA typing in the setting of lung transplantation (1).

The results obtained by analyzing a cohort of 59 lung transplant recipients indicate that patients with a high degree of HLA mismatch, as determined using both genetic sequencing and the matchmaker software, are at higher risk of perioperative primary graft dysfunction (PGD) and acute rejection (AR). In addition, the authors suggest that systematic monitoring of donor specific antibodies (DSA) in the post-transplant setting may be helpful in identifying patients at high risk for AR. These conclusions suggest that improved HLA typing methods, as well as immunologic monitoring of transplanted patients may be helpful in early identification of rejection episodes.

HLA alleles were originally identified using sera from highly immunized individuals, typically patients that had received multiple blood transfusions or multiparous women, leading to the identification of groups of alleles defined by a panel of antibodies. Historically, HLA Class I was the first identified, with specificities defined through a series of consensus conferences known as International Histocompatibility Workshops in the late sixties and seventies (2). Typing by serology was then widely used worldwide also thanks to the implementation of consumer-friendly assays. While this kind of approach is simple, quick and low-cost, it offers a low-resolution picture of the HLA region, without providing allele identification and—in some instances—not resolving typing. HLA gene identification started only in the eighties with the progressive cloning of Class I and Class II genes. Since the beginning of the nineties, molecular tools for genetic typing of the HLA region became available. These tools exploit the polymerase chain reaction (PCR) and are based on the design of primers (SSP) or of oligonucleotides (SSO) that are specific for a given group of alleles, or—in some instances—for a specific allele. PCR-amplified sequences can then be run on a gel, as is the case for SSP, posing limitations on its use. In fact, SSP was originally set up to type for *DRB1* alleles, which were unsatisfactorily recognized by antibodies. Because gel resolution of the PCR products requires extensive manipulation and is hardly scalable, RT-PCR approaches were implemented, which are now mostly used in the setting of cadaveric donor typing, considering the rapidity and versatility of the assay, which is however quite costly and thus not yet universally employed. Alternative molecular methods use probes that can be immobilized on a membrane or a bead and mixed with the denatured DNA under analysis, which has been previously biotinylated. All these systems generally offer a low/intermediate resolution typing, even though for SSO typing newer kits allow to resolve most allelic combinations. The gold-standard for allelic typing for all loci is direct sequencing of the polymorphic exons of the HLA Class I (exons 2, 3 and 4) and Class II (exon 2) either by Sanger or by next generation sequencing. However, the two latter approaches are technically complex and require longer time compared to the other molecular methods. In addition, typing by NGS cannot be performed for a single patient, but is generally

performed for multiple samples in a row, restricting its employment by small/medium centers. Clinical applications of NGS typing has been developed in Hemopoietic Stem cell Transplantation. Typing costs vary widely, with SST being the least expensive and RT-PCR/NGS being the most expensive techniques.

Today, a donor and a recipient should be typed for HLA *A*, *B* and *C* genes (Class I) and *DRB1*, *DRB3*, *DRB4*, *DRB5*, *DQA1*, *DQB1*, *DPA1* and *DPB1* genes. Class II typing is complicated by the fact that the molecular products are dimers and hence alpha and beta subunits should be identified. HLA-DR is a special situation, as DRA is mostly monomorphic, but there are paralogues of DRB genes that are expressed in tight association with given *DRB1* alleles. Typing of both prospective recipients and donors should be performed using molecular biology approaches, which vary according to the context, the timing and relative costs.

The importance of HLA typing for solid organs is twofold. On the one side, it can be used to choose the most compatible individual out of a pool of possible recipients. On the other side, it is essential to identify donors that are *a priori* incompatible for pre-immunized patients. In organ transplantation, the first scenario is important essentially for kidneys, while it is of limited significance in the case of lung, heart and liver transplants, which are life-saving procedures. The second scenario is critical for all patients: in this context, allelic typing of the donor may be important, particularly if the prospective recipient has allele-specific antibodies (3).

The current guidelines of the Eurotransplant Network indicate that "every recipient and every organ donor must be typed for HLA-*A*, -*B*, -*C*, -*DR*, and -*DQ*. Serological and DNA typing for HLA-*A* and -*B* is accepted. For HLA-*DR*, HLA-*C* and HLA-*DQ*" (www.eurotransplant.org). The guidelines of the European Federation of Immunogenetics for typing of patients in the lung transplant waiting list indicate that typing should be performed for HLA-*A*, -*B* and -*DR*, if the patients are immunized (www.efi-web.org), implying that for non-immunized patients HLA typing is not strictly required, although performed by most Institutions.

According to the results of this work and of others it would seem appropriate to perform HLA typing for all HLA Class II loci, and preferably at a high resolution. While expensive and possibly technically challenging for some centers, this approach would allow a systematic evaluation of donor-recipient compatibility both in retrospective and prospective settings, enabling a clearer assessment of the role of HLA mismatch.

The related question that the work by Zhang and colleagues raises is how we should use improved HLA matching possibilities in the context of lung transplants. In the past 15 years, knowledge on HLA was improved by adding to genetic sequencing of the genes, data obtained from 3D structures of the HLA molecules, of the anti-HLA antibodies and of the T cell receptors binding to host HLA molecules complexed with peptides derived from HLA molecules of the donor. The resolution of crystals containing anti-HLA antibodies bound to HLA alleles was the starting point for the identification of the contact regions that are critical to the antigen-antibody interaction (termed epitope for the antigen and paratope for the antibody) and to the definition of the so-called functional epitope or eplet, i.e., the minimal set of amino acids defining the binding, usually between 3 and 5 (4). It is therefore now possible to predict donor-recipient mismatch on the basis of the epitope matching, defining an eplet mismatch load (5). In a recent publication it was shown that this parameter is likely a more accurate predictor of DSA production and subsequent rejection in lung transplant recipients (6). In a second work, eplet mismatch for *DR* and *DQ* loci correlated against incidence of chronic lung allograft graft dysfunction (CLAD) (7). Interestingly, eplet mismatch was positively associated to restrictive allograft syndrome, but not to bronchiolitis obliterans.

In addition to epitope matching, resolution of the T cell receptor structures, allowed the identification of the donor HLA-derived peptides that can indirectly activate an immune response in the host, when presented in association to the host HLA molecules (8). These discoveries constitute the basis for a new way of typing based on prediction of immunogenicity more than on simple sequence. This algorithm, termed PIRCHE for Predicted Indirectly Recognizable HLA Epitopes, determines compatibility between two individuals by defining the number of donor-derived HLA peptides that could be presented by recipient HLA Class II molecules to $CD4^+$ T cells, thereby starting an immune response that could lead to T and B cell activation (9).

Accordingly, two donors with a similar degree of incompatibility on the basis of genetic analysis, may be very different in terms of eliciting a response in the recipient. There are now softwares that are freely available to the scientific community that can be of help in identifying the number of immunogenic eplets in the donor/recipient pair (HLA Matchmaker) and softwares that can predict the immunogenicity of donor HLA-derived peptides in a given

recipient (PIRCHE).

While initial results using these softwares, such as the one presented in the work by Zhang and colleagues are encouraging, a note of caution should be raised concerning large-scale applicability of these procedures. In fact, even in the context of the work by Zhang and colleagues, high resolution typing for the donor—recipient pair was available only for about a third of all transplanted patients, underlining inherent technical difficulties and high costs. Accordingly, a prospective use is not so straightforward, at least in small to medium scale programs. Patients in the lung transplant waiting list may be in critical conditions and there may not be much room for choosing "the" most compatible donor, but simply "a" compatible donor. In addition, super selecting for HLA may pose significant limitations in terms of transplant accessibility in patients that have rare HLA alleles or that present a high degree of homozygosity.

A last consideration concerns the possibility of fine-tuning immune suppression for lung transplant recipients that are found to be high risk for developing CLAD or AR: if these findings will be independently validated and confirmed in larger cohorts, it will be possible to tailor immunosuppression according to the degree of both genetic mismatch and donor/recipient immunogenicity. In this respect, the field needs clinical trials with immunomodulatory drugs, building on the experience obtained with rituximab. The availability of tailored therapeutic options will confirm the need to know as much as possible about the immunologic situation of the recipient, including HLA typing. Future work will tell whether this scenario can become real.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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