DIAGNOSTIC SERVICES FOR THE BAMBINO GESU’ CHILDREN’S RESEARCH HOSPITAL IRCCS - DSB.AD007.041

According to the agreement (Prot. N. 0002974) between the CNR-IFT and the Children’s Research Hospital Bambino Gesù IRCCS, highly specialized diagnostic services were performed to assess the immunological status of transplant candidates and to monitor post-transplant humoral alloreactivity.

The IFT researchers worked at the Tissue Typing and Transplant Immunology Laboratory of the Lazio Regional Transplant Center (CRTL) providing academic and clinical leadership in diagnostic services, translational research studies, CRTL technologists training and quality system management of EFI & ASHI Accreditations (mandatory requirements for histocompatibility laboratories).

The human leukocyte antigen complex (HLA) contains the most polymorphic genes in the human genome. Genetic variation of HLA genes plays an important role in transplant medicine and immunology, because mismatches of HLA alleles can potentially evoke both cellular and antibody-mediated rejection (AMR). Although the control of cellular rejection has improved by immunosuppressant therapy, there is no doubt that AMR is detrimental for the graft clinical outcome. Preformed HLA antibodies in the patients' serum (developed after pregnancy, blood transfusion, or a previous transplant) represent a risk factor for transplant. "De novo" HLA antibody production in transplanted patients increases the risk of acute and chronic rejections.

The research and the clinical activity were synergetic in expanding the knowledge on histocompatibility and the humoral immune response to organ transplant, as well as in developing innovative technologies: the main goal is to guarantee the best care conditions for the patients.

The following histocompatibility tests were used to ensure the best immunological donor-recipient compatibility:

- low/high resolution PCR-based HLA typing using sequence-specific primers (SSP), sequence-specific oligonucleotide probes (SSOP) and the new Real Time (RT) PCR-SSP;
- anti-HLA antibody screening and characterization using Solid Phase assays, i.e. flow cytometric beads and Luminex beads coated with purified or recombinant HLA antigens;
- detection of antibodies directed towards donor lymphocyte-specific antigens using complement-Dependent Cytotoxicity Crossmatch (CDC-XM) and flow cytometry crossmatch (FC-XM).

Our Laboratory is the first in Italy to validate the new RT PCR-SSP technique and to introduce it into clinical laboratory practice to perform the HLA typing of deceased donors. This method is based on an innovative chemical process which allows a typing extended to all HLA Class I & II loci, with a workflow consistent with the urgency of organ donation. The introduction of the RT PCR-SSP has allowed the execution of a more precise pre-transplant "virtual crossmatch". This is the process of assessing the results of solid phase HLA antibody identification assays to predict, or correlate to, the results of a physical crossmatch, i.e. to predict the risk of transplant. Now, the RT PCR-SSP has become the technique of choice for an extended HLA typing of deceased donors in most histocompatibility laboratories, which improves the selection of the most suitable transplant candidates.

It is important to stress that our Laboratory is the first and among the few in Italy to perform a new Enzyme-Linked Immunosorbent Assay (ELISA) test to allow a quantitative determination (U/ml) of Angiotensin II type 1 receptor (AT1R)-antibody. This evaluation is crucial because the simultaneous production of anti-AT1R and donor-specific anti-HLA antibodies has an negative synergistic effect that may result in an accelerated loss of the transplanted organ function. The joint use of Solid Phase assays and ELISA test represents a non-invasive tool for identifying patients who need specific therapies to prolong graft survival.
In conclusion, the results obtained were particularly useful in pre-transplant because they have produced a significant improvement of the organ allocation policy, providing a reliable measure of clinical risk assessment. In post-transplant the above results have supported the clinician in the early diagnosis of AMR and in personalized management of transplant patients.

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