histocompatibility and immunogenetics laboratories to develop a comprehensive program for HLA antibody detection and identification pre and post-transplantation in order to prevent kidney allograft losses.

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APPLYING VIRTUAL CROSSMATCH APPROACH IN PORTUGUESE KIDNEY TRANSPLANTS

Bruno A. Lima[1], Miguel Mendes[2], Helena Alves[3]

[1]Faculdade de Clinicias da Universdade do Porto, Porto, Portugal, [2]Ordem dos Biulogos, Porto, Portugal, [3]Instituto Nacional de Saude, Porto, Portugal
Correspondence: balima78@gmail.com

Presence of donor specific antibodies anti-human leukocyte antigen (HLA) is generally a contra-indication for transplantation and nowadays the identification of these antibodies are part of most pre-transplantation evaluations. In Portugal, the implemented protocol for the registration and maintenance of the active list for kidney transplant includes a complement-dependent cytotoxity (CDC) panel-reactive antibody (PRA) screening method, and Luminex technology for detecting and characterizing HLA alloantibodies. Under the current Portuguese kidney allocation system from deceased donors, implemented in August 2007, deceased donor kidneys are primarily allocated via ABO identical and time on dialysis with extra points to hyperimmunized patients, namely PRA CDC > 50%. Additional risk for the candidate or transplant organ can be represented by a proposed calculated PRA (cPRA) based upon unacceptable HLA antigens detected by Luminex to which the patient has been sensitized. These unacceptable HLA antigens used to generate cPRA represents a 'virtual' crossmatch (XM). Sensitized patients are less likely to be matched with a suitable donor organ. Even after clearing the hurdle of procuring a living donor, it is still possible that this is not sufficient due to the likelihood of having a XM-positive. In these cases and in the presence of incompatible blood type between recipients and their intended living donors, kidney paired donation (KPD) can provide an answer by facilitating exchanges between willing donors' kidneys. A national Portuguese KPD program, when realized, may prevent the current loss of a significant number of suitable living donors and reduce waiting list time for a deceased donor. An upgrade of a suggested point system in a Portuguese KPD program will be the use of cPRA instead of the values of PRA CDC. In Portugal, the virtual XM approach simply represents the optimization of an existing technique.

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THE EFFECT OF PRONASE ON HLA AND CD20 ANTIGENS

Ryan D. Stevens, Christopher Darke

Welsh Blood Service, Pontyclun, UK
Correspondence: ryan.stevens@wales.nhs.uk

As a prelude to establishing flow cytometry-based crossmatching for patients treated with Rituximab, we evaluated the effect of different concentrations of pronase on the expression of human leukocyte antigen (HLA)-class I (CI), class II (CII) and CD20. In all, 15 fresh peripheral blood lymphocyte (PBL) and 5 fresh splenic lymphocyte preparations were assessed. Each cell pellet was mixed and incubated for 30 min at 37°C with 1 ml of pronase at concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml in PBS and PBS only as a control. Antigen expression at each pronase concentration was evaluated using fluorescent antibodies, viz. anti-HLA-ABC/FITC (IM1838U), anti-HLA-DR, DQ, DP/RD1 (6604366) and anti-CD20/PC5 (A07773) (Beckman-Coulter, UK). For both cell types: average CD20 median channel fluorescence (MCF) with 0.5 mg/ml pronase dropped to ~10% of control values and was negligible with increasing concentrations of pronase thereafter; average CI MCF steadily decreased with increasing pronase concentration with an average CI MCF of 56% for PBLs and 41% for spleen cells with 2.0 mg/ml pronase. Average CII MCF, measured on PBL 'B cells', initially decreased to 79% at 0.5 mg/ml pronase, but then rose steadily to 97% MCF at 2.0 mg/ml pronase. Of the 15 PBL samples, with increasing pronase concentration, 7 had an increase in CII MCF, 6 a decrease and 2 showed an initial decrease followed by an increase in MCF. For spleen derived 'B cells', average CII MCF increased 30%–40% above control MCF at all concentrations of pronase. PBL cell counts decreased with increasing concentrations of pronase – down to 57% of the control count at 2.0 mg/ml. The total spleen cell count was seemingly unaffected but splenic B-cells decreased with increasing concentrations of pronase similar to PBLs. Pronase treatment is clearly suitable for removing lymphocyte CD20. However, HLA antigen expression and cell viability are also significantly affected with increasing concentrations. It is clearly vital that pronase concentrations and other technical parameters are well validated for crossmatching.

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DEVELOPMENT OF HARDY–WEINBERG IN THE POPULATION OF PATIENTS AWAITING A RENAL TRANSPLANT

Marian D. Witvliet, Geert W. Haasnoot, Frans HJ. Claas, Ilias IN. Doxiadis

ETRL, Leiden, the Netherlands
Correspondence: i.i.n.doxiadis@lumc.nl

Patients suffering from end stage renal disease can be offered a better quality of life by a transplant. Eurotransplant, the first international organ exchange organization, offers to up to 20% of the patient a fully human leukocyte antigen (HLA)-A,-B,-DR compatible graft. For transplantation of highly sensitized patients the acceptable mismatch program has shown to be a successful tool. Patients included in the Acceptable Mismatch (AM) program must have more 85% panel reactive antibodies and recently we could demonstrate that this selection leads to distortion of the Hardy–Weinberg equilibrium (HWE). Here we report on the HWE development in patients awaiting a first graft ($N = 356$),