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Comparative analysis of how immune sensitization is defined prior to lung transplantation

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ABSTRACT

Background: Immune sensitization prior to lung transplantation may be associated with worse survival. Using solid phase assays to define sensitization, we assessed the relationship between PRA status, donor specific anti-HLA antibodies (DSA) pre-transplant, cytotoxic cross match results and the clinical outcomes following lung transplantation.

Methods: Luminex assays determined the presence of antibodies to class I and class II MHC molecules prior to lung transplantation. At the time of transplant, the PRA status, the presence of DSA and prospective cytotoxic cross match result were analysed in 195 patients undergoing lung transplantation between June 2008 and June 2012. Clinical outcomes analysed included acute cellular and antibody-mediated rejection, chronic lung allograft dysfunction (CLAD) and mortality.

Results: At the time of transplant, 45% of patients had a positive PRA and 29% had DSA. On univariate analysis, the presence of pre-transplant class I or II anti-HLA donor-specific antibodies was not associated with the development of chronic lung allograft dysfunction (CLAD) despite significant associations with PRA status and B-cell crossmatch.

Conclusion: Defining sensitization using solid phase assays provide additional details regarding donor-specific sensitization but did not provide additional prognostic information to that provided by historically available cell-based cross-match assays.

1. Introduction

Antibody-mediated rejection (AMR), particular when presenting immediately post lung transplant in its hyperacute form, is a well recognized entity [1], however its contribution to chronic lung allograft dysfunction (CLAD) following lung transplantation is less clear [2]. This contrasts to what is seen in other solid organ transplants where there is a wealth of evidence suggesting that AMR is a significant contributor to graft dysfunction, and indeed may be the leading cause of late grafts loss in kidney transplants [3].

While the presence of antibodies to human leukocyte antigens (HLA) prior to transplantation has been linked to poor post-transplant outcomes following non-pulmonary solid organ transplantation [4,5], the evidence for the same association in lung transplantation is less robust [6,7]. A two-center study of 656 lung transplant recipients (LTR) showed that patients with a pre-transplant panel reactive antibody (PRA) status of greater than 25% had reduced survival compared to patients with a lower PRA [8]. However a recent analysis of the United Network for Organ Sharing (UNOS) database gave conflicting results. When all patients were analysed (1987–2005) a PRA greater than 25% was associated with worse survival, however this effect was not seen when the analysis was restricted to the most recent cohort of patients undergoing transplantation between 1998 and 2005 [9].

Using PRA to define sensitization and the presence of antibodies to HLA antigens, does have some important limitations, namely that the antibody screen does not test donor cells directly nor defines antibody specificity. The PRA being a complement dependent cytotoxicity assay is further limited in as much that it cannot discriminate between HLA and non-HLA antibodies, can be confounded by autoantibodies and does not distinguish IgM antibodies from IgG antibodies. Increasingly newer solid phase assays

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are being used to define the degree of sensitization in patients awaiting solid organ transplantation.

Solid phase immunoassays, such as those based upon the Luminex (Luminex Corporation, TX, USA) platform, provide high sensitivity, specificity and throughput for the detection of class I and II HLA antibodies in general, and donor-specific antibodies (DSA), specifically. Additionally, the solid phase antibody tests provide quantification of detected antibody with Luminex results expressed as mean fluorescent intensity (MFI). A recent study by Kim et al. of 126 lung transplant recipients, demonstrated that the presence of anti-HLA DSA (MFI >3000) was associated with AMR [10].

In this single-center study cohort of lung transplant recipients, we assessed whether patients identified as being sensitized pre-transplant had poorer medium-term (within 12 months of transplant) and longer-term outcomes (up to 48 months post transplant) compared to non-sensitized transplant recipients. Similar to recent studies in renal transplantation [11], we performed a detailed analysis of the class, specificity and quantification of anti-HLA DSA, and how they correlate to other measures of immune sensitization, as well as their associations with post-transplant clinical outcomes.

2. Materials and methods

2.1. Lung transplant cohort

Patients undergoing lung transplantation at The Alfred Hospital between June 2008 and June 2012 and available for local follow up were included in a study investigating the relationship between pre-transplant sensitization, as defined by multiple techniques, and clinical outcomes following lung transplantation. Most patients received a standard triple immunosuppressant regimen consisting of prednisolone, azathioprine and tacrolimus. Induction therapy with the IL-2 receptor blocker, basiliximab, was given as a calcineurin-sparing agent to 63 patients who were identified pre-transplant as being at higher risk of developing renal dysfunction. The degree of pre-transplant sensitization did not influence the choice of induction or maintenance immunosuppression. Prior to transplantation, desensitization protocols were not used in sensitized patients. All patients at risk of CMV reactivation (either donor- or recipient- positive CMV serostatus) received prophylaxis with 2 weeks of intravenous ganciclovir followed by oral valganciclovir for a minimum of 5 months. Surveillance bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsies was performed at 2 weeks and 1, 2, 3, 6 and 12 months, or if clinically indicated. Censor date for the study was June 2013. All patients provided written consent and the study was approved by the Alfred Hospital ethics committee.

2.2. Immunologic evaluation

Pre-operative immunologic evaluation was routinely performed on all potential LTR. HLA typing (HLA-A, -B, -C, -DR, -DP and -DQ) was performed by standard complement-dependent microcytotoxicity assay and sequence-based typing (Victorian Transplantation and Immunogenetics Service, Victoria, Australia). The panel reactive antibody (PRA) status was assessed at listing with a complement-dependent cytotoxicity assay against a T-lymphocyte panel of 30 donors. The frequency of PRA was determined by tail analysis [12] and values greater than 5% considered positive. The presence of class I and class II HLA were defined pre-transplant for each potential transplant recipient using a Luminex screening assay, and if positive the highly sensitive Luminex single-antigen bead (SAB) assay was used to further delineate the DSA. Whilst on the waiting list, screening was performed every 6 months and positive patients had the SAB assay performed every 6 months. Quantification of HLA-DSA was given as mean fluorescent intensity (MFI), with the positive threshold set at >500. The local tissue typing laboratory grades anti-HLA DSA according to the MFI: weak = 500–2000; moderate = 2000–8000; and strong = >8000. A prospective donor–recipient T- and B-cell cross-match was performed by the classical complement-dependent cytotoxicity method in all cases, and positive results were confirmed following treatment with dithiothreitol (DTT). The decision to proceed with transplantation for any given donor–recipient pairing was largely made on the basis of a negative prospective T-cell complement-dependent cytotoxicity cross-match result. The degree of pre-transplant sensitization, as defined either by (i) positive PRA, (ii) the presence pre-transplant of class I or class II HLA, (iii) the presence of class I or class II HLA-DSA or (iv) the cytotoxic cross match result was determined in each transplanted patients and correlated to post-transplant clinical outcomes. In patients who had repeat SAB testing whilst awaiting transplant, the result with the highest MFI was included in the analysis.

2.3. Clinical outcomes

Clinical variables analysed included acute cellular and antibody-mediated rejection, chronic lung allograft dysfunction (CLAD) i.e. bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS), and mortality. Acute cellular rejection was diagnosed on histopathological changes identified on transbronchial biopsy specimens, according to the International Society of Heart Lung Transplantation pathological scoring system [13]. In the absence of an internationally agreed definition for pulmonary AMR, we applied a previously published definition of AMR as "the presence of DSA with MFI of greater than 5000 was applied as being clinically significant and a trigger for treatment in the presence of otherwise unexplained allograft dysfunction" [14]. BOS was diagnosed on physiologic criteria of a sustained and irreversible reduction in forced expiratory volume (FEV_1) compared with the best FEV_1 achieved post lung transplantation in the absence of any other identifiable cause [15]. RAS is defined by restrictive physiology and the presence of ground glass and interstitial fibrosis on imaging [16]. Financial costs associated with the Luminex platform prohibited routine post-transplant surveillance screening of DSA in all LTR, however post-transplant evaluation of DSA was performed in selected patients in the setting of unexplained clinical deterioration.

2.4. Statistical analysis

Descriptive statistics were used to summarise baseline and clinical characteristics of study subjects. Numerical data were compared using Student's *t*-test or Mann–Whitney *U*-test as appropriate. Categorical data were compared using the chi-square test for equal proportions or Fisher's exact test wherever appropriate. The association with graft loss [as defined by a composite of (i) death with a failing graft, (ii) re-transplantation and (iii) CLAD] was assessed in a univariate analysis including the following variables; recipient gender, age, pre-transplant disease, transplant type, donor–recipient HLA match and immune sensitization (positive B-cell crossmatch, presence of class I and/or class II HLA, presence of class I and/or class II DSA, panel reactive antibody status). Logistic regression analysis was used to assess factors associated with 12-month composite event end point whereas Cox proportional hazards regression was used to assess factors associated with time to composite event end point. Receiver operating characteristic (ROC) curve analysis was performed to assess