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Evolving experience of treating antibody-mediated rejection following lung transplantation

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ABSTRACT

Background: The importance of antibody-mediated rejection (AMR) following lung transplantation remains contentious. In particular, the diagnostic criteria suggested to define AMR, namely the presence of donor-specific antibodies (DSA), C4d immunoreactivity, histological features and allograft dysfunction are not always readily applicable or confirmatory in lung transplantation.
Methods: In a retrospective single-center study of 255 lung transplant recipients (LTR), we identified 9 patients in whom a clinical diagnosis of AMR was made within 12 months of transplant, and define the immunological, histological, clinical features, as well as the therapeutic response of this cohort.
Results: Nine LTR with AMR underwent combination therapy with high-dose intravenous corticosteroid, intravenous immunoglobulin, plasmapheresis and rituximab. Following therapy, while the total number of the original DSA dropped by 17%, and the median value of the mean fluorescence intensity (mfi) of the originally observed DSA decreased from 5292 (IQR 1319–12,754) to 2409 (IQR 920–6825) ($p < 0.001$), clinical outcomes were variable with a number of patients progressing to either chronic lung allograft dysfunction or death within 12 month.
Conclusion: AMR in lung transplantation remains both a diagnostic and therapeutic challenge, but when clinically suspected is associated with a variable response to therapy and poor long-term outcomes.

1. Introduction

Antibody mediated rejection (AMR) is well defined in renal transplantation [1,2], however, the diagnosis of AMR in lung transplant recipients (LTR) remains a clinical challenge. The four diagnostic tenets upon which AMR was first proposed in renal transplantation [1] – i) presence of anti-human leukocyte antigen (HLA) donor-specific antibodies (DSA), ii) positive C4d immunohistochemistry, iii) characteristic histological changes and iv) allograft dysfunction – are less robust when applied to lung transplantation. C4d staining, a marker for complement activation, has been shown to be poorly reproducible in lung tissue, and the specific histological abnormalities of AMR in the lung have yet to be defined [3]. Typically, the diagnosis of AMR following lung transplantation requires a multidisciplinary approach and is based upon the presence of clinical allograft dysfunction, circulating DSA and pathologic findings that are not suggesting an alternative diagnosis [3].

Treatment for AMR in LTR may include pulsed steroids, intravenous immunoglobulin (IVIg), plasmapheresis and adjunctive drugs such as rituximab and bortezomib; therapies that are largely proposed based on evidence in renal transplantation [4,5]. In the absence of randomized trial data, there is limited evidence for the efficacy of any of these treatments for AMR in lung transplantation. The literature is also limited in how each center defines AMR and the different thresholds for initiating

Abbreviations: AMR, antibody mediated rejection; BAL, bronchoalveolar lavage; BOS, bronchiolitis obliterans syndrome; CDC, complement-dependent cytotoxicity; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; CT, computed tomography; DAB, diaminobenzidine; DSA, donor-specific antibodies; DTT, dithiothreitol; H & E, hematoxylin-eosin; HLA, human leukocyte antigen; IVIg, intravenous immunoglobulin; LTR, lung transplant recipients; mfi, mean fluorescence intensity; PFT, pulmonary function testing; PP, plasmapheresis; RAS, restrictive allograft syndrome.

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therapy [6–10]. Additional important considerations include the significant financial costs related to both tests to diagnose AMR [11,12] and the resulting therapy [13,14].

2. Objective

In this single center study, we reviewed our evolving experience related to the diagnosis and treatment of AMR in LTR from 2009 to 2012. Specifically, we examined the effect of combination therapy including corticosteroids, plasmapheresis, rituximab, and intravenous immunoglobulin, on i) lowering levels of anti-HLA DSA and steroids, and ii) reversing clinical allograft dysfunction in patients diagnosed with AMR within 12 months of lung transplantation.

3. Materials and methods

3.1. Lung transplant cohort

We performed a retrospective review of all patients who underwent lung transplantation between January 2009 and January 2013 to identify patients who received therapy for suspected AMR. A prospective donor–recipient T- and B-cell cross-match was performed by the classical complement-dependent cytotoxicity (CDC) 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 made on the basis of a negative prospective T-cell CDC cross-match result. Typing of donor and recipient HLA was performed by serology (Table 1). The presence of class I and class II HLA was defined as pre-transplant for each potential transplant recipient using a Luminex screening assay (Bio-Strategy, Australia). At the time of transplant the highly sensitive Luminex single-antigen assay was used to detect the presence of DSA [10]. Quantification of HLA–DSA was given as mean fluorescence intensity (mfi), with the positive threshold set at >500.

Most patients received standard triple immunosuppressant regimen consisting of prednisolone (0.3 mg/kg in first 3 months reducing to 0.1 mg/kg beyond the first year), azathioprine (1.5 mg/kg) and tacrolimus (trough level 10–12 ng/ml in first 6 months, 8–10 ng/ml between 6 and 12 months, and 4–8 ng/ml thereafter). Immunosuppression was tailored depending on rejection history, infection, bone marrow suppression and renal failure. Induction therapy with the IL-2 receptor blocker, basiliximab, was given as a calcineurin-sparing agent to patients who were identified pre-transplant as being at higher risk of developing renal dysfunction. All patients at risk of CMV reactivation (either donor- or recipient-positive CMV serostatus) received valganciclovir antiviral prophylaxis for 5 months. The study was approved by the Alfred Hospital ethics committee.

Table 1
Serological typing of lung transplant recipient and associated donor.

Case	Donor HLA typing	Recipient HLA typing
1	A32, —; B18, 61; DR4, 17; DR52, 53; DQ2, 7	A31, 68; B8, 71; DR3, 13; DQ2, 6
2	A1, 2; B8, 57; Cw6, 7; DR3, 13; DR52, —; DQ2, 7	A2, 28; B44, 70; Bw4, 6; Cw5, 8; DR4, —
3	A2, 68; B18, 49; Bw4, 6; DR11, 14; DR52, —; DQ1, 7	A1, 24; B7, B39; Bw6, —; Cw7, —; DR4, 15; DQ6, 8
4	A1, —; B8, 57; Bw4, 6; Cw7, —; DR3, 7; DQ2, 9	A1, 32′ B7, 51; Cw1, —; DR15, —; DQ6, —
5	A2, 3; B35, 44; Bw4, 6; Cw4, 5; DR1, 3; DR52, —; DQ1, 2	A2, 11; B13, 40: DR1, 15
6	A1, 3; B8, 35; Cw4, 7; DR1, 4; DQ1, 3	A2, 11: B35, 44: DR1, —
7	A3, —; B15, 47: Cw3, 6; DR4, 13; DR52, 53: DQ1, 3	A1, 29; B65, 57; DR7, 13
8	A2, 68; B13, 62; DR1, 7: DR53, —; DQ1,2	A11, 24; B27, 40: DR10, 15
9	A1, —; B8, —; DR3, 15; DR51, 52; DQ2, DQ1	A1, 2; B62, 44; DR1, 15

3.2. Pulmonary function tests

Pulmonary function testing (PFT) was completed serially on each patient throughout followup. At minimum, monthly spirometry including FEV_1 and FVC was completed in the first two years post-transplantation with testing one to three monthly thereafter. Chronic lung allograft dysfunction (CLAD) was defined as a sustained loss of FEV_1 from baseline of greater than or equal to 20%. Decline was only deemed to be irreversible when two separate measures three weeks apart met the threshold [15]. The first date of decline in PFTs that met the criteria was recorded as the onset date. Individuals meeting criteria for CLAD were classified into two groups according to the pattern of loss based on the ratio of FEV_1 to FVC (FER) [16]. The spirometric phenotype was determined by the pattern of loss recorded at the date of onset with the *obstructive* (BOS) phenotype defined by an FER < 70% and the *non-obstructive* (RAS) by an FER > 70% [17].

3.3. Histological analysis

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. Transbronchial biopsies were assessed histologically for features of acute cellular and antibody-mediated rejection, as per ISHLT guidelines [18]. Paraffin-embedded, formalin-fixed transbronchial biopsy tissue samples were stained with hematoxylin–eosin (H&E) and Masson's trichrome. The presence of the complement product C4d was assessed by immunohistochemistry as previously described [19]. Four micron thick transbronchial biopsy sections on Fisher/Superfrost Plus slides were placed in a 60 °C oven, deparaffinized and rehydrated. After blocking the slides for non-specific staining with serum-free protein, C4d antibodies (Dako) were diluted 1:500 and incubated for 30 min. Staining was visualized with diaminobenzidine (DAB) chromagen (Dako) and counterstained in Richard Allen hematoxylin. C4d staining was considered positive if the staining was granular, and/or if it showed a continuous linear pattern with capillary and/or endothelial cell localization. Results were interpreted in the context of appropriate negative and positive C4d control tissue stains.

3.4. AMR diagnosis

Patients with declining lung function were further investigated with bronchoscopy and chest computed tomography (CT) scan, largely for determination of acute cellular rejection [18] or infection. C4d staining on transbronchial biopsies and measurement of anti-HLA DSA was requested by the clinician if AMR was suspected. The presence of class I and class II anti-HLA DSA were defined pre-transplant for each potential transplant recipient using a Luminex screening assay, and if positive the highly sensitive Luminex single-antigen assay was used to further delineate the DSA. Quantification of HLA–DSA was given as mean fluorescent intensity (MFI), with the positive threshold set at >500. The ability of DSA to fix complement was not routinely available and was not assessed as part of this study. Surveillance assessment of post-transplant DSA was not routinely performed in clinically stable asymptomatic patients. Initial characterization of patients' AMR status was performed using a modified classification based on recommendations from the 2004 National Conference on AMR in solid organ transplantation: i) presence of anti-human leukocyte antigen (HLA) donor-specific antibodies (DSA), ii) positive C4d immunohistochemistry, iii) characteristic histological changes and iv) allograft dysfunction [1]. Given concerns regarding the reproducibility of C4d staining in lung tissue, our center did not mandate positive