

# Challenges inherent to the diagnosis of antibody-mediated rejection in lung transplantation

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## Abstract

A bilateral sequential lung transplant was performed on a young female with cystic fibrosis-related bronchiectasis. She had negative prospective T- and B-cell crossmatch, and no known donor-specific antibodies. Post-transplantation, she developed bilateral pulmonary infiltrates of uncertain etiology, compounded by persistent tachycardia and questionable medication adherence. Despite aggressive intervention for suspected cellular rejection with high-dose intravenous corticosteroid, immunoglobulin, and anti-thymocyte globulin, her condition deteriorated to ultimately require ventilatory support. The eventual discovery of eplet donor-recipient mismatches on related DQB1 alleles raised the diagnosis of antibody-mediated rejection. Before plasmapheresis could be instituted, the patient rapidly succumbed to respiratory failure. Postmortem examination confirmed features of atypical allograft rejection, without evidence of classic acute cellular rejection. This is an unconventional case of antibody-mediated lung allograft rejection – an entity that is currently a difficult diagnostic and therapeutic challenge. Prevention of donor-specific antibodies by correct donor-recipient matching, and optimizing adherence post-transplantation are most important.

## Introduction

Antibody-mediated rejection (AMR) is relatively new in lung transplantation [1]. Despite the well-described Banff classification [2] of circulating donor-specific antibodies (DSA), C4d deposition, allograft dysfunction, and histopathology, we continue to encounter cases that confound us.

## Case Report

A 19-year-old woman underwent bilateral sequential lung transplant for end-stage cystic fibrosis. She had extensive bronchiectasis, pancreatic insufficiency, insulin-requiring diabetes mellitus, osteoporosis, and gastroesophageal reflux disease. Persistent sinus tachycardia and a single 23-beat monomorphic ventricular tachycardia were noted pre-transplant.

She underwent transplant following negative T- and B-cell crossmatch, non-detectable DSA on solid phase bead assay and serologically matched for cytomegalovirus and Epstein–Barr virus.

Three weeks later, she was discharged on immunosuppressants, antimicrobials, and insulin. Her home therapies included gastrostomy feeds, glucose monitoring, chest physiotherapy, and an exercise routine.

In the following three months, she has had difficulty managing her burgeoning medical regime as reflected by hyperglycemia and subtherapeutic tacrolimus level, 1.1 µg/L (target range 10–12).

Consequently, she was admitted with dyspnea thrice in short succession. Computer tomography of the chest revealed bibasal interstitial infiltrates with severe “pulmonary edema” pattern (Fig. 1), but post-transplant lymphoproliferative disorder (PTLD), acute cellular rejection, and atypical infection were also considered, investigated, and



**Figure 1.** Three representative transverse high-resolution computed tomography of the thorax with bibasilar infiltrates.

dismissed. Notably, her left ventricular ejection fraction was stable at 44% with no arrhythmias detected. She was diuresed and fluid-restricted. Alongside bilevel ventilatory support, broad-spectrum antibiotics were added. Bronchoscopies isolated no organisms, a reactive but benign cytopathological analysis, and the biopsies featured moderate acute nonspecific pneumonitis (graded ISHLT [International Society of Heart & Lung Transplantation] A0 and C4d negative). Noting the atypical picture and recent lapse in the tacrolimus level, she was thrice pulsed empirically with intravenous methylprednisolone; each provided transient respite. The suspicions of persistent acute allograft rejection remained high and rabbit anti-thymocyte globulin was administered, again with a brief response.

On reconsideration of the possibility of AMR, it was noted that the solid phase assay did not include the donor's DQB1\*05:03 allele bead. Careful compatibility evaluation revealed shared mismatched eplets between the donor's \*05:03 and recipient's \*05:01/\*05:02 alleles suggesting that these high non-DSA mean fluorescent intensity (MFI) could well be eliciting AMR (Table 1). With this new information and a tenuous clinical situation, plasmapheresis was planned.

Unfortunately, the patient's condition deteriorated rapidly. Despite aggressive resuscitation, it proved fatal 190 days post-lung transplant.

A limited autopsy demonstrated the proliferative phase of extensive diffuse alveolar damage (Fig. 2), with no evidence of acute cellular rejection or specific AMR, but features consistent with obliterative bronchiolitis and chronic rejection. The heart was macroscopically normal.

## Discussion

This case highlights the challenging, complex diagnostic, and therapeutic dilemmas faced by many lung transplant clinicians. The diagnosis of lung AMR was especially difficult given the atypical presentation confounded by persistent tachycardia and suspected heart failure, as well as the

**Table 1.** The recipients anti-HLA antibody profile.

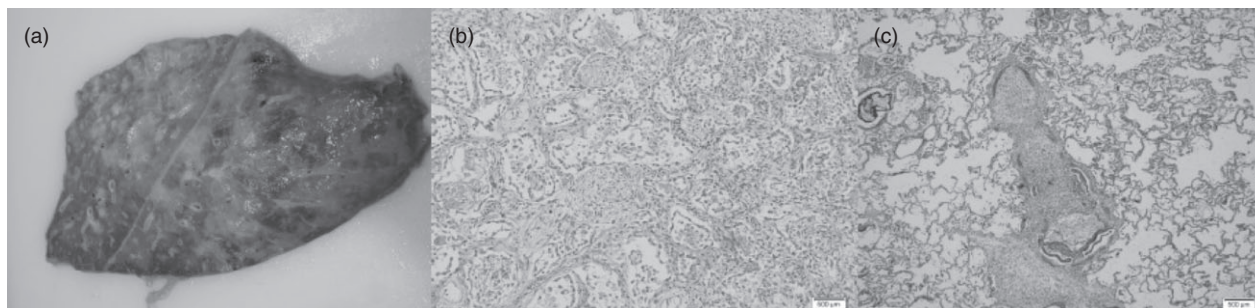
Date	Type of anti-HLA antibody	Specificity	Mean fluorescence intensity
June 2013	Class 1	A25	814
		B57	893
		Cw9	1304
	Class 2*	DQB1*05:01	8794
		DQB1*05:02	3051
		DR7	2613
July 2013	Class 1	None	–
	Class 2*	DQB1*05:01	5757
		DQB1*05:02	1847
		DR7	1164

\*The donor HLA typing included the DQB1\*05:03 antigen, which is not included in the standard OLI Luminex Class II Single Antigen Bead set. It does however share 10 mismatched eplets with DQB1\*05:01 and 9 with DQB1\*05:02, which are routinely tested beads.

potential for atypical/viral infection or PTLD in a young transplant recipient. Ultimately, based on history, radiological, histological, and solid phase bead assay interpretation, and after excluding other causes of allograft dysfunction, AMR was identified as the perpetrator.

Despite being well described in the transplant literature, the Banff classification [2] of renal AMR may not be directly applicable to the lung allograft [1].

This particular case did not show pre-transplant DSA, but with hindsight, we believe the non-donor-specific DQB1\*05:01 and \*05:02 antibodies (up to 8000 MFI) post-transplant were driving antibody-mediated graft damage. The standard Luminex™ (Luminex Corp, Austin, TX, USA) testing of the 100 commonest beads does not routinely include the detection of the donor's DQB1\*05:03. Despite belonging to the same class, they are not actually donor specific but are structurally related; consequently possess mismatched incompatibility as evaluated with HLA Matchmaker™ (Pittsburgh, PA, USA). The testing for extra alleles



**Figure 2.** (A) A macroscopic coronal section through left lung – the lungs were heavy and contained patchy peri-bronchial pale, firm regions involving all lobes. (B) H&E photomicrograph of lung ( $\times 10$  power) – shows hyaline membranes and areas of organizing pneumonia consistent with proliferative phase of diffuse alveolar damage. (C) Masson trichrome stain of lung ( $\times 5$  power) – shows almost complete luminal occlusion of bronchiole by hyaline fibrosis consistent with obliterative bronchiolitis, suggesting chronic rejection.

requires specific ordering-in and cost. This limitation to its clinical application demonstrated to us that antibody testing does have exceptions, thus an increased level of vigilant in DSA surveillance with closer collaboration with specialist HLA laboratory should be and has been prioritized since.

C4d deposition, a surrogate marker of complement activity, is neither reliable nor specific in the lung allograft. Its use is impeded by the very nature of classical pathway of the complement system that may be activated by surface proteins of gram-positive bacteria and C-reactive protein. This is especially troublesome in an organ that is prone to infection and inflammation.

Various measures are employed to characterize lung allograft dysfunction clinically, including symptoms, signs, spirometry, and radiology. Unfortunately, none are specific, with comparable appearances depicted in all forms of acute and chronic lung rejection, infection, and even cardiac dysfunction.

The histopathological list of lung AMR is so extensive that the latest 2012 Consensus from the ISHLT Pathology Council describes it as “nonspecific patterns of injury, that can be seen also in disorders such as severe acute cellular rejection, infection, reperfusion injury, and drug reactions” [3].

Adherence affects allograft rejection outcomes, survival, and healthcare costs. Indeed, non-adherence is perhaps the most important contributor to AMR beyond the first three months post-transplant. This is increasingly recognized among adolescents – affecting up to 80% of transplant recipients [4]. A variety of psychosocial traits including anxiety and depression, disease frustration, inadequate regimen knowledge, poor social supports, and substance misuse strongly affect adherence. The transition from pediatric to adult transplant service care, and the evolution from parental supervision to self-sufficiency are potential times of risk. The strategies for promoting adherence in adolescent transplant should thus be tailored individually

with collaboration between the adolescent, family and health care providers.

In pediatric transplant cohort, the standard deviation of tacrolimus has been implicated to predict rejection and hospitalization rates. Indeed, in one study, each single unit rise in standard deviation conferred a 23% increased risk of acute rejection [5]. Additionally, concomitant medications, intercurrent illnesses, postsurgical gut denervation, diabetic autonomic neuropathy, and pancreatic enzyme deficiency had all contributed to variability in this case.

In conclusion, lung AMR remains a difficult diagnosis with wide differential diagnoses. Currently, AMR is suspected on clinical basis, noting the presence of graft dysfunction (without other cause), circulating DSA and nonspecific pathological changes. The prevention of the development of AMR by correct donor-recipient matching and optimizing adherence post-transplant is most important to circumnavigate this conundrum. Further characterization of lung AMR must be a priority.

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### Disclosure Statements

No conflict of interest declared.

Appropriate written informed consent was obtained for publication of this case report and accompanying images.

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