ORIGINAL ARTICLE

Guiding therapeutic plasma exchange for antibody-mediated rejection treatment in lung transplant recipients – a retrospective study

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SUMMARY

Antibody-Mediated Rejection (AMR) due to donor-specific antibodies (DSA) is associated with poor outcomes after lung transplantation. Currently, there are no guidelines regarding the selection of treatment protocols. We studied how DSA characteristics including titers, C1q, and mean fluorescence intensity (MFI) values in undiluted and diluted sera may predict a response to therapeutic plasma exchange (TPE) and inform patient prognosis after treatment. Among 357 patients consecutively transplanted without detectable pre-existing DSAs between 01/01/16 and 12/31/18, 10 patients were treated with a standardized protocol of five TPE sessions with IVIG. Based on DSA characteristics after treatment, all patients were divided into three groups as responders, partial responders, and nonresponders. Kaplan–Meier Survival analyses showed a statistically significant difference in patient survival between those groups ($P = 0.0104$). Statistical analyses showed that MFI in pre-TPE 1:16 diluted sera was predictive of a response to standardized protocol ($R^2 = 0.9182$) and patient survival ($P = 0.0098$). Patients predicted to be nonresponders who underwent treatment with a more aggressive protocol of eight TPE sessions with IVIG and bortezomib showed improvements in treatment response ($P = 0.0074$) and patient survival ($P = 0.0253$). Dilutions may guide clinicians as to which patients would be expected to respond to a standards protocol or require more aggressive treatment.

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Key words
antibody titer, antibody-mediated rejection, donor specific antibody, lung transplantation, serum dilution, therapeutic plasma exchange

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Introduction

The development of donor-specific antibodies (DSA) after lung transplantation, a key feature of antibody-mediated rejection (AMR), occurs in approximately 30% of lung transplant recipients and is associated with poor post-transplant outcomes [1–3]. Therefore, AMR therapy is almost always initiated and may include some of the following agents: therapeutic plasma exchange (TPE), IVIG, rituximab, and/or bortezomib [5,7–9]. Several studies demonstrated that transplant recipients who cleared DSA as a result of AMR treatment showed improved survival [4–6], while the inability to clear DSA was associated with increased risk of CLAD and poor survival [7–9]. However, currently there are no guidelines for optimal AMR treatment after lung transplantation due to the absence of clinical trials and a lack of approved medications specific for treating AMR in lung transplant recipients.

Therapeutic plasma exchange is commonly used as a first-line AMR treatment due to its capability to quickly decrease circulating DSA and few adverse effects [4,9,10]. Previous studies established that a single 1–1.5 volume TPE procedure could remove up to 66–77% of IgG from plasma in a single session [11]. In the presence of steady-state antibody production by long-lived plasma cells (LLPC; e.g., pretransplant HLA antibody levels), five TPE procedures scheduled over ten days are supposed to reduce total IgG level to 10% of original levels [10,12]. However, DSA production post-transplant may be mediated by various mechanisms, including activation of naïve B-cells and/or memory B-cell followed by differentiation into plasma cells [13], formation of antibody-immune complexes with stimulating antigens leading to production of higher antibody titers [14], and antibody secretion by LLPC [15]. In other words, TPE efficacy may be difficult to predict in the context of AMR compared to pretransplant desensitization [16–19]. Despite AMR being a potentially reversible cause of graft dysfunction, the results of treatment often can be haphazard due to the absence of biomarkers to guide treatment regimens [16].

Many fields of medicine have steered toward the concept of precision and personalized medicine, in which predictive biomarkers are used to achieve the goal of determining the optimal intervention for an individual patient using objective data-driven criteria. The Single Antigen Bead (SAB) assays allow detection and characterization of DSA against human leukocyte antigens (HLA) [20]. Over the past decade, it became increasingly clear that the binary approach based on the presence or absence of DSA cannot inform effective AMR treatment. Instead, additional dimensions, such as antibody strength, subtype, and capability to activate complement should be guiding therapeutic interventions [20]. For example, in the context of pre and peri-operative transplant desensitization, DSA titers and 1:16 serum dilutions may serve as predictive biomarkers for TPE treatment [21,22]. In addition, C1q-binding can serve as a "response biomarker" when evaluating a response to AMR treatment in renal transplant recipients [23,24]. We recently showed that 1:16 dilution can serve as a biomarker to specifically predict response to a single TPE for a particular antibody using SAB assay [22]. Here, we hypothesized that some of these characteristics may predict DSA response to a standardized protocol of five TPE sessions with IVIG. To test this hypothesis, we studied how the differences in antibody titers, C1q-binding, and MFI values in undiluted and 1:16 diluted sera before and after treatment can predict response to treatment and patient survival.

Materials and methods

Study cohort

The charts for adult lung recipients consecutively transplanted at Temple University Hospital between 01/01/16 and 12/31/18 were reviewed to identify patients transplanted without *pre-existing* DSAs (*p*DSA) who developed *early* DSA or *de novo* DSA (*e*DSA or *dn*DSA) post-transplant. All DSA-positive patients without clinical AMR were excluded from the study as well as patients who had contraindication to TPE treatment. The DSA characteristics, including titers, C1q binding, and mean fluorescence intensity (MFI) values in undiluted and diluted EDTA-treated sera were collected for patients treated with five TPE sessions supplemented with low dose IVIG followed by high dose IVIG. Patient survival was defined as the time from day 0 of treatment to patient's death, and all patients alive were censored at the time of last follow-up. In the case of death with a functioning graft, we censored graft survival at the time of death. In addition, response to treatment and graft survival was evaluated in patients treated with a more aggressive protocol consisting of eight TPE sessions with IVIG and bortezomib. Some patients who developed AMR were treated with this aggressive protocol if the predominant DSA MFI did not decrease at 1:16 dilution. The study design is shown in Fig. S1. The study was approved through the Temple University Institutional Review Board (IRB).

Detection and characterization of HLA DSA

Human leukocyte antigens typing and DSA assignment: Two field high-resolution (2F-HR) HLA typing for donor and recipients was performed using HoloType HLA kits (Omixon, Inc., Budapest, Hungary) and sequenced on an Illumina MiSeq (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations. Next-generation sequencing (NGS) data were analyzed with Twin (Omixon) to define DSA at the 2F-HR HLA level as described [25].

Donor-specific antibodies testing: HLA class I and class II antibody testing was performed on 5 mm Ethylenediaminetetraacetic acid (EDTA) – treated sera using LABScreen Single Antigen beads (One Lambda; ThermoFisher) on a Luminex® platform (Luminex Corporation, Austin, TX, USA) as previously described [22]. All antibody specificities were confirmed by LSPRA (phenotype bead) and FlowPRA Screen assays (One Lambda; ThermoFisher, West Hills, CA, USA) as described [20,23]. A cutoff of 1500 MFI was used to identify positive DSA specificities. DSA specificities between 900 MFI and 1500 MFI were considered as weak positive if the background-corrected ratio was ≥4. Antibody titers were determined by performing serial 4-fold dilutions using PBS and EDTA-treated sera (undiluted, 1:4, 1:16, 1:64, etc.) until all antibodies fell below a threshold of 900 mean fluorescence intensity (MFI) units. Heat-inactivated sera were tested with C1qScreen (One Lambda; Thermo Fisher) for identification of complement-binding antibodies according to the manufacturer's recommendations.

Standard immunosuppression

Alemtuzumab or Basiliximab were used as induction therapy. The choice of an induction agent was driven by factors such as age, CMV status, and history of cancer and was not affected by a patient's immunologic risk at our institution. Standard immunotherapy consisted of tacrolimus, mycophenolate mofetil, and prednisone.

AMR treatment

Standard TPE treatment consisted of five procedures of one-plasma-volume exchange with 5% albumin replacement scheduled every other day followed by 100 mg/kg sucrose-free IVIG and 1000 mg/kg after the last TPE (Fig. S2a). All TPE procedures were performed using Spectra Optia (TerumoBCT, Lakewood, CO, USA).

Aggressive multi-modality TPE treatment consisted of four double TPE procedures (total eight TPE) of one-plasma-volume exchange with 5% albumin replacement scheduled every other day followed by 100 mg/kg IVIG and 1000 mg/kg after the last TPE (Fig. 2b). Four doses of bortezomib were given as 1–1.3 mg/m^2 every 72 h starting after the first double session of TPE.

Statistical analyses

Statistical analyses including log-linear regression, paired *T*-test, and Kaplan–Meier survival were performed using IBM® SPSS® Statistics (v26; IBM Corp., Armonk, NY, USA) and GRAPHPADPRISM software (v8.4; GraphPad Software Inc., San Diego, CA, USA). All $P < 0.05$ were considered to indicate statistical significance. Cohort demographics were summarized with descriptive statistics. One-way ANOVA was used when comparing three groups. Survival curves were plotted using the Kaplan–Meier method and groups were compared by log-rank testing.

Results

Study cohort

Prior reports of AMR treatments in lung transplant recipients used heterogeneous regimens [16–19]. In order to determine which DSA characteristics may predict an effective response to TPE as a primary therapy, we identified lung transplant recipients treated under the same protocol. Out of 379 consecutively transplanted patients between 01/01/16 and 12/31/18, 357 patients were transplanted without detectable pre-existing DSAs, 60 (16.8%) of whom developed DSA post-transplant. Out of those 60 patients, 43 (71.7%) patients did not have signs of allograft dysfunction due to AMR. Of the 17 (28.3%) patients with at least probable AMR, 7 patients had contraindications to plasma exchange and were excluded from this analysis, while 10 patients were treated with five sessions of TPE followed by high dose IVIG (Figs S1 and S2). There was no statistical difference in DSA production or AMR between patients receiving Alemtuzumab or Basiliximab as an induction agent. Table S1 shows clinical characteristics for patients who were treated using TPE/IVIG.

Patient survival after AMR treatment correlates with post-treatment DSA levels

Donor-specific antibodies levels were tested before and after TPE treatment using the following parameters:

MFI in EDTA-treated undiluted and 1:16 diluted sera, titers, and C1q binding (Fig. 1). Class I DSA had lower mean MFI and titers compared to class II DSA, which is in agreement with previously published reports [26–29]. Individual DSA demonstrated various responses to treatment (Fig. S3); however, there was no significant difference in mean MFI, titers, or C1q-binding after treatment (Fig. 1a–c) suggesting that single-modality TPE treatment was not always effective. Also, there was no significant difference in titers and MFI or response to treatment between early and late DSAs (Fig. 1d,e).

A recent study showed that an antibody with a titer of >1:512 cannot be effectively removed by 5 or more TPE sessions during the course of pretransplant desensitization [21]. Therefore, we divided patients into groups with predominant DSA titer ≥1:1024 (1:1024, 4096, 16384) and <1:1024 (1:64, 256). There was no significant difference in the patients' survival based on titer levels prior to treatment ($P = 0.4450$) suggesting that DSA with lower titers did not have a better response to treatment compared to high titer DSAs (Fig. 2a). Similarly, there was no significant difference in patient survival based on pretreatment MFI levels ($P = .1115$) (Fig. 2b). On the contrary, there was a significant difference in survival between patients with high and low post-treatment titer ($P = 0.0127$) and MFI levels ($P = 0.0127$; Fig. 2c,d), suggesting that effective AMR treatment improves patient survival. There was no statistically significant difference in C1q binding ($P = 0.1899$; Fig. 2e).

Recognizing the limitations of such a small cohort, our data support previous findings that inability to clear DSA is associated with poor survival after treatment. They also suggest that DSA with lower titers/MFI do not respond to treatment better than DSA with high titer/MFI, which highlights the importance of developing a better understanding of additional DSA characteristics aside from mere quantification.

Degree of response to TPE therapy correlates with patient survival after treatment

Next, we studied how a degree of titer and MFI reduction correlates with survival to define a satisfactory response to treatment.

In terms of titer reduction, five TPE sessions can effectively reduce titers by four logs during pretransplant desensitization [e.g., titer of 1:256 (2^8) could be decreased to 1:16 (2^4)] [21]. The average log reduction was 1.8 in our cohort, with maximal log reduction of 4

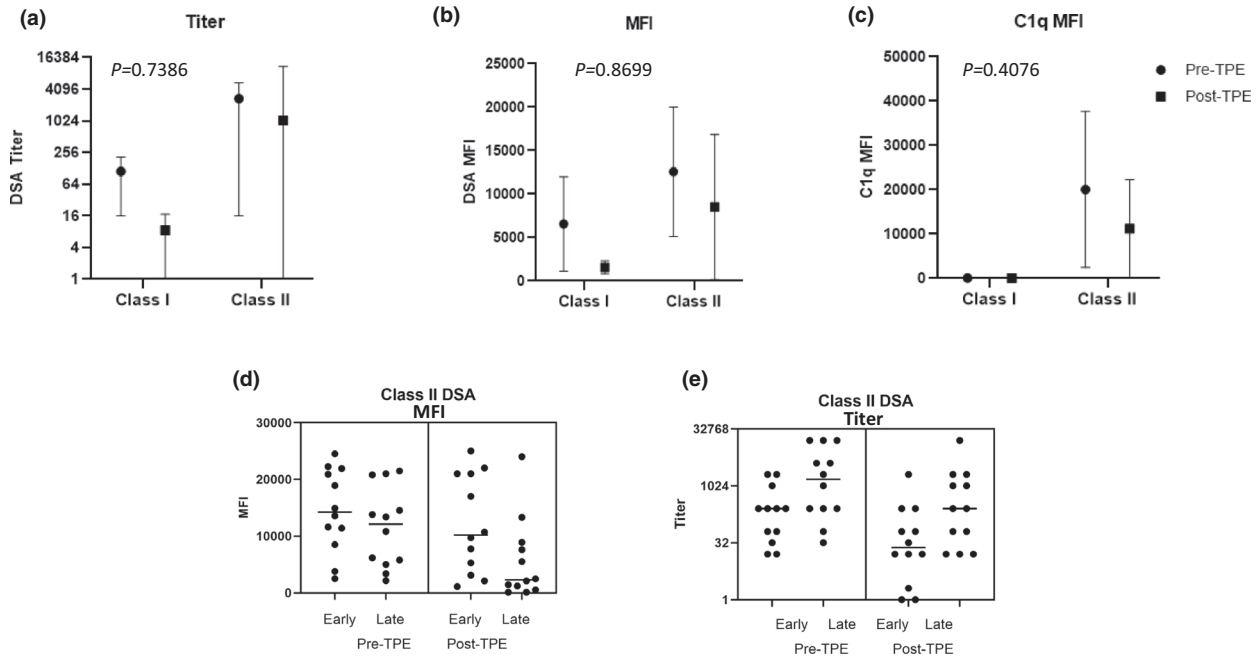

Figure 1 Donor-specific antibodies (DSA) levels before and after TPE treatment. (a) Mean DSA titers measured in EDTA-treated serum; (b) mean MFI levels in EDTA-treated serum (DSA was considered positive, if MFI were >1500); (c) mean C1q MFI levels (DSA was considered C1q positive, if MFI were >500). (d,e) Five patients have developed early DSA (within 4-month post-transplant) and five patients developed late DSA (between 8- and 20-month post-transplant). There was no statistically significant difference in MFI (d) or titers (e) between early and late DSAs before or after treatment. Only Class II DSA were compared, since class I DSA were detected only in patients who had early DSAs.

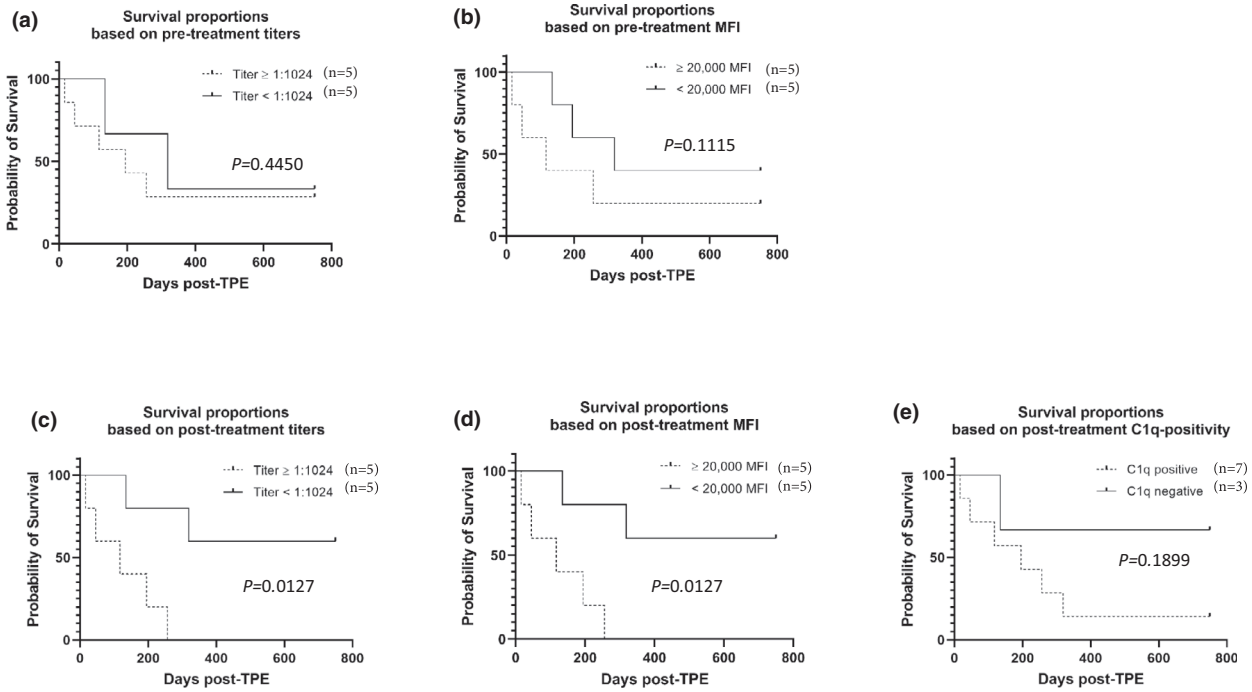

Figure 2 Kaplan–Meier survival analyses based on the predominant donor-specific antibodies (DSA) levels. (a,b) Overall survival (OS) does not differ in patients with higher (≥1:1024) and lower (<1:1024) DSA titers (a), higher (≥20,000 MFI) and lower (<20,000 MFI) DSAs (b) prior to treatment. However, OS does differ in patients with higher and lower DSAs titers (c) and MFI (d) after treatment. There was no statistically significant difference in OS between C1q-positive and negative DSA after treatment (e). All predominant DSA were C1q positive prior to treatment.

and the minimal reduction of 0. We divided all patients into three groups: responders (titers were reduced by the log of 4), partial responders (titer log reduction of 2–3), and nonresponders (titer log reduction of 0–1). In terms of MFI reduction, five TPE sessions are expected to decrease IgG levels by 70–90% from pretreatment levels [22]. We expressed post-treatment MFI as a percentage of pretreatment levels and divided patients into three groups as follows: responders (MFI levels decreased by >70%), partial responders (MFI decreased by 30–70%), and nonresponders (MFI decreased by <30%). Kaplan–Meier Survival analyses showed a statistically significant difference between responders vs. partial responders and vs. nonresponders using both MFI and titer levels (Fig. 3a,b).

To account for the contribution of DSA rebound after treatment on patient survival, we analyzed DSA levels up to one-year post-treatment (Fig. S4). Patients, who responded to standard TPE treatment remained DSA negative, while 3 out of 4 patients, who partially responded to treatment demonstrated some level of rebound between 3- and 12-month post-treatment. The "nonresponder" patients did not show decrease in DSA levels at any time after treatment.

Our results suggest that a degree of response to treatment correlates with patient survival after treatment. Therefore, it is important to identify potential "nonresponder" patients prior to initiating the standard treatment protocol.

Use of 1:16 dilution can guide treatment protocols

Previously, we showed that 1:16 serum dilution accurately predicts a response to a single TPE using 1.5 volume of plasma [22]. Here we hypothesized that dilution studies of pretreatment serum may predict DSA levels after treatment. We found strong positive correlation between MFI values in 1:16 diluted pre-TPE sera and post-TPE sera (Fig. 4a). Our data show that if there is no MFI decrease at 1:16 dilution, there would be no decrease in MFI levels after TPE treatment. Furthermore, MFI reduction in 1:16 diluted serum was prognostic of patient survival after treatment (Fig. 4b). DSA titers, MFI in undiluted serum, or MFI in 1:64 diluted serum were not predictive of response to treatment or patient survival (data not shown).

Based on these findings, we have modified our institutional AMR treatment protocol. Patients who did not

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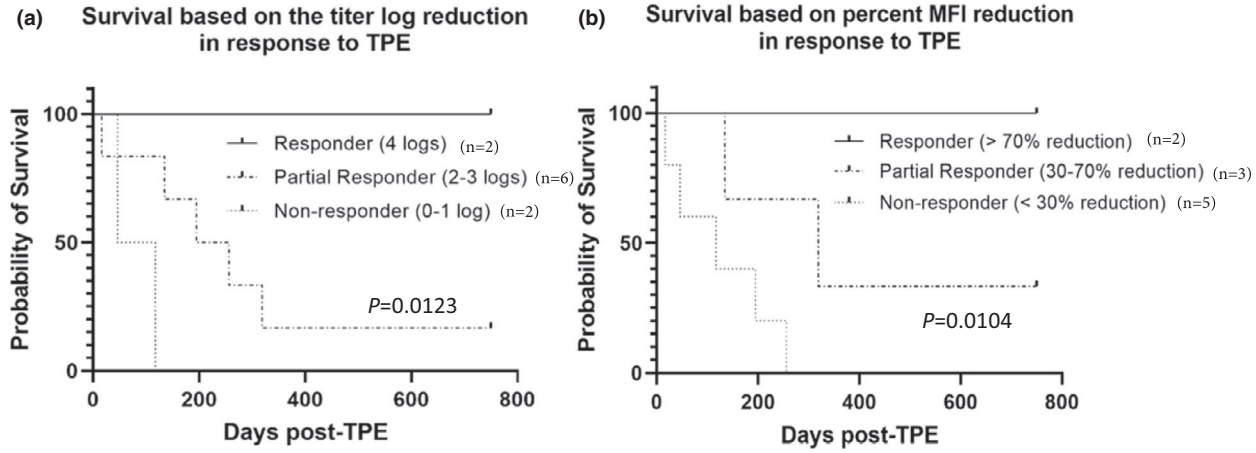

Figure 3 Responses to TPE correlate with OS after treatment. (a) Based on log reduction in titers, patients are divided to three groups as responders (log reduction of 4), partial responders (log reduction of 2–3) and nonresponders (log reduction of 0–1). Kaplan–Meier survival analyses show OS as a function of log titer reduction. (b) Based on percent of MFI reduction after treatment compared to pretreatment levels, patients are divided to three groups as responders (MFI reduction after treatment by >70%), partial responders (MFI reduction after treatment by 30–70%) and nonresponders (MFI reduction by <30%). Kaplan–Meier survival analyses show OS as a function of percent of MFI reduction. Log-rank tests show that OS significantly differs in patients classified as responders, nonresponders, or partial responders based on percent of MFI decrease and on log titer reduction. The significance was set at a *P*-value of 0.05.

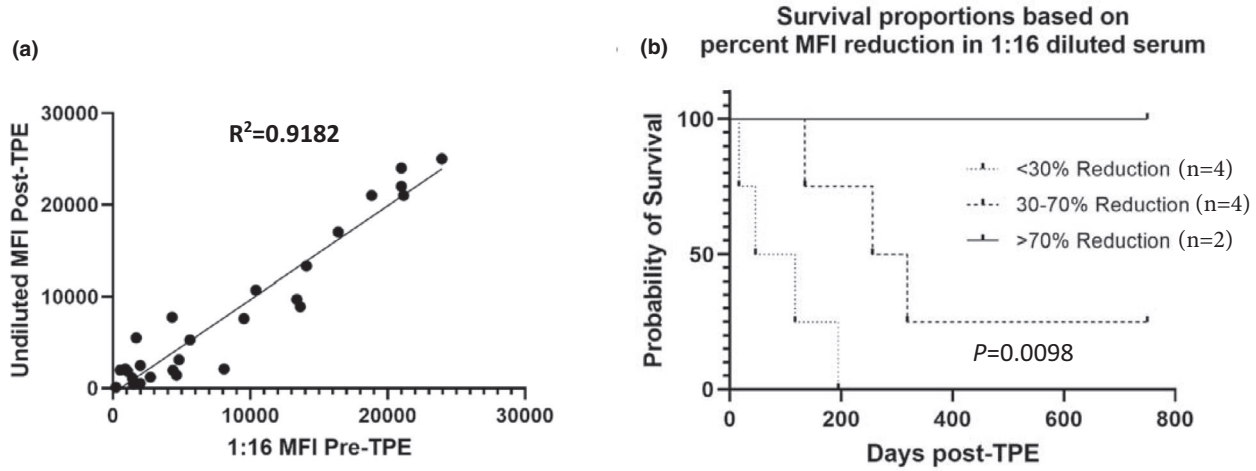

Figure 4 1:16 dilution is predictive of response to standard treatment. (a) MFI values in pretreatment 1:16 diluted EDTA-treated sera show strong positive correlation with MFI values in undiluted sera after 5th TPE. (b) Percent of MFI decrease in pretreatment 1:16 diluted sera compared to undiluted sera was predictive of patient's survival after treatment. Based on percent of MFI decrease in 1:16 diluted serum, all patients were divided into three groups (with <30% decrease, 30–70% decrease, and >70% decrease). Patients whose DSAs did not decreased at 1:16 had worst OS compared to patients whose DSAs showed at least partial decrease at 1:16 dilution.

show >30% decrease in MFI at 1:16 were immediately stratified into the "more aggressive" arm of AMR treatment protocol, which consisted of four sessions of double TPEs (total eight sessions) with bortezomib after every session. Compared to retrospective data for patients who were predicted to be nonresponders based on a lack of decrease in MFI at 1:16 dilution and were treated with our standard protocol, three patents who would be predicted nonresponders by the same criteria were treated with the aggressive protocol and demonstrated a response to treatment with improved survival (Fig. 5a,b).

Based on our limited data, using 1:16 dilution may help identify patients more likely to respond to a standard protocol vs those who require a more aggressive treatment. This approach could help save valuable time during on going graft damage in the context of AMR and achieve better outcomes for lung transplant recipients.

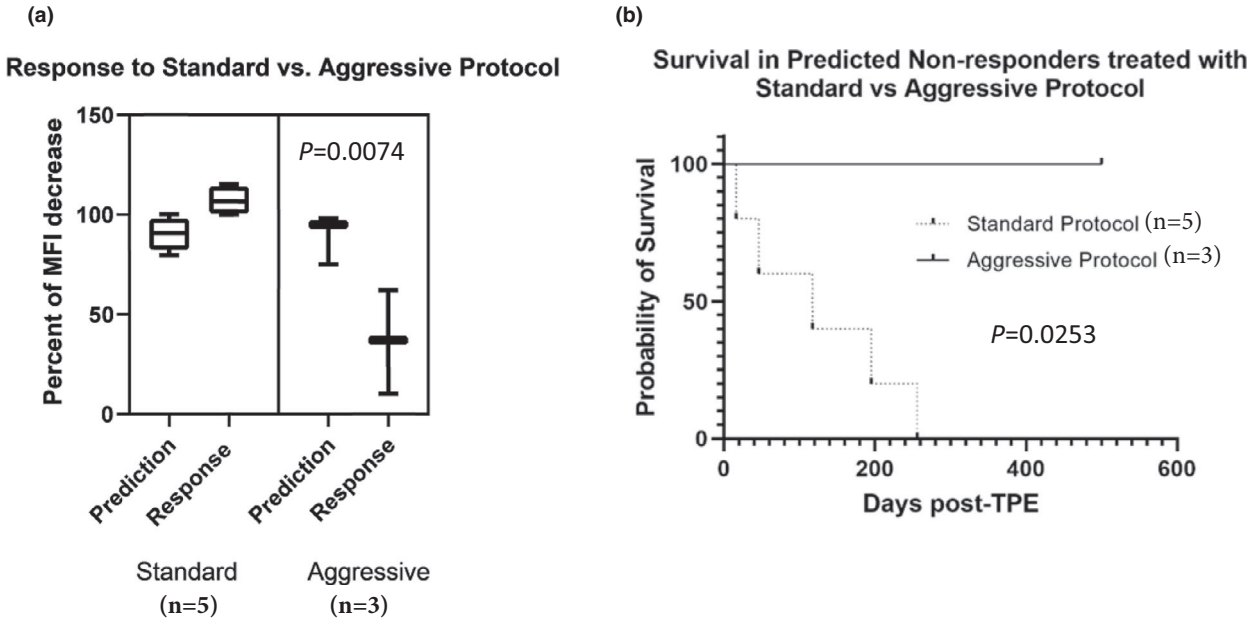

Figure 5 Patients predicted to be Nonresponders based on 1:16 dilution could benefit from more aggressive multi-modality treatment protocol. (a) "Prediction" indicates an expected decrease in MFI values after standard TPE protocol based on 1:16 dilutions. All patients are predicted to be nonresponders. "Response" shows actual percent of MFI decrease in response to the standard or the aggressive protocols. DSA treated with standard TPE protocol did not decrease, while DSA treated with more aggressive multi-modality protocol, consisting of four double session of TPE (total 8 TPE) with bortezomib, DSA levels significantly decreased. (b) Decreases in DSA levels in response to aggressive protocol were accompanied by improvement in patients' survival.

Discussion

While significant progress has been made toward improved outcomes in lung transplant recipients, AMR remains challenging to treat and, thus, negatively impacts patient's survival. During the last decade, it has been established that DSA reactive with mismatched HLA antigens are essential component of AMR. Although there are no FDA approved AMR treatments, several therapeutic strategies such as TPE, proteasome inhibitor bortezomib, high dose intravenous immunoglobulin, and rituximab show promising results in early observational studies. However, when it comes to daily practice, clinicians and immunologists often feel powerless because there are no guidelines as to how to determine which treatment may successfully decrease DSA production and reverse AMR. Commonly, a standard TPE protocol is initiated as first-line therapy. However, treatment outcomes are often disappointing and reinforce a need for tools that can guide therapeutic strategies to improve outcomes.

In this study, we attempted to answer the following questions. How response to treatment, in terms of degree of decrease in DSA levels, may inform patient survival after treatment? Can we predict the degree of DSA decrease prior to initiation of treatment? Finally, would this information help to guide treatment selection? In order to answer these questions, we identified patients who developed at least probable AMR due to *de novo* DSA and were treated under the same protocol. Decrease in DSA levels was characterized by titer reduction (in logs) and MFI reduction (% from pretreatment MFI values). In order to use MFI values, we had to address the limitations of SAB assays, including prozone-like effect mediated by complement interference, antibodies against denatured antigens, inter- and intra- laboratory variations, and the inconsistent correlation between the mean fluorescence intensity (MFI) and the clinical significance of an antibody [20,22,30–34].

An examination of treatment outcomes showed survival benefit in patients who responded to therapy completely or at least partially. The reduction in either titer or MFI was informative about patient survival after treatment (Fig. 3), but neither could predict a degree of response to treatment (data not shown). Instead, we found that MFI reduction in 1:16 diluted pretreatment serum was predictive of both, the response to standard TPE and the patient's survival after treatment (Fig. 4). We explain this as follows: a single TPE decreases IgG levels in the same manner as a 1:16 dilution [30]. If there is no decrease in MFI at 1:16 dilution, it means

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that MFI values are above the linear range in SAB assay (saturation zone), and no response to the first TPE will be detected. Such DSAs may respond to five sessions of TPE by decreasing their titers; however, they remain so strong that patients treated with standard protocol do not demonstrate any survival benefit after treatment. Pinelli *et al.* [21] showed that there is no benefit in increasing the number of TPE session beyond five sessions. This agrees with classical studies showing that the largest volume of IgG is removed from intravascular space during the 1st TPE, while remaining sessions help to reduce extravascular IgG levels via re-equilibration [11,12]. On another hand, if DSA is reduced by ~75% at 1:16 dilution and after the first TPE, it is likely to achieve a 90% decrease after five TPE sessions [11,12]. Overall, we found that the degree of MFI decrease in 1:16 diluted serum was predictive of a response to TPE in 90% (9 of 10) patients (Fig. S5). In one case, the 1:16 dilution predicted a complete response (MFI decreased by 76% at 1:16), while TPE treatment achieved only ~50% response. We speculated that this may have happened due to continuing rising DSA levels when DSA production was accelerating after initiation of treatment. Indeed, when we have measured DSA levels before and after each TPE session, we found a continuing increase in DSA levels prior to the 2nd session of TPE (data not shown). The DSAs started to decrease only after 4th TPE, and this was the reason for a partial response to treatment. Although our observations require further validation in a bigger cohort, our finding suggests that in order to successfully treat DSA with the standard TPE protocol, two conditions need to be met: (i) the pretreatment DSA levels should be reduced by 1:16 dilutions by >70% (or at least by 30–70% to achieve a partial response), and (ii) the DSA levels should also be measured prior to 2nd TPE to identify DSA with rising levels. These two steps can help to identify patients who would not benefit from the standard TPE treatment regimen and, therefore, need to be considered for more aggressive regiment treatment from the beginning. It has been shown that bortezomib can induce rapid apoptosis of long-lived plasma cells and halt IgG production within 8 h of treatment [35,36]. Our data show that early patient stratification into more aggressive treatment arm offers a way to effectively reduce DSA levels and improve survival in patients who were predicted to be nonresponders and would not be expected to benefit from the standard protocol (Fig. 5).

In summary, AMR is a major cause of graft failure in lung transplant recipients. Deciding which patients to treat, selecting the effective AMR therapy, and evaluating a response to AMR treatment are major challenges in lung transplantation. The findings from this study are significant in identifying a potential predictive biomarker to guide AMR and improve patient survival after treatment. This biomarker can be easily evaluated by all centers that use Single Antigen Bead assay by including 1:16 diluted serum for DSA evaluation. The primary limitation of our study is the small number of patients. However, our study underlines the need for prospective multicenter trials and translational mechanistic studies to validate these findings and develop individualized treatment strategies.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Study design.

Figure S2. AMR treatment protocols.

Figure S3. DSA titers and MFI levels before and after TPE treatment.

Figure S4. DSA levels after treatment.

Figure S5. Predicted and observed response to standard TPE.

Table S1. Patient's characteristics.

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