IgM-Enriched Human Intravenous Immunoglobulin-Based Treatment of Patients With Early Donor Specific Anti-HLA Antibodies After Lung Transplantation

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Background. At our institution, until April 2013, patients who showed early donor specific anti-HLA antibodies (DSA) after lung transplantation were preemptively treated with therapeutic plasma exchange (tPE) and a single dose of Rituximab. In April 2013, we moved to a therapy based on IgM-enriched human immunoglobulins (IVIG), repeated every 4 weeks, and a single dose of Rituximab. Methods. This observational study was designed to evaluate the short-term patient and graft survival in patients who underwent IVIG-based DSA treatment (group A, n = 57) versus contemporary patients transplanted between April 2013 and January 2015 without DSA (group C, n = 180), as well as to evaluate DSA clearance in IVIG-treated patients versus historic patients who had undergone tPE-based treatment (group B, n = 56). Patient records were retrospectively reviewed. Follow-up ended on April 1, 2015. Results. At 6 months and 1 year of follow-up, group A had a survival similar to group C (P = 0.81) but better than group B (P = 0.008). Group A showed statistically nonsignificant trends toward improved freedom from pulsed-steroid therapy and biopsy-confirmed rejection over groups B and C. The DSA clearance was better in group A than group B at treatment end (92% vs 64%; P = 0.002) and last DSA control (90% vs 75%; P = 0.04). Conclusions. Patients with new early DSA but without graft dysfunction that are treated with IVIG and Rituximab have similarly good early survival as contemporary lung transplant recipients without early DSA. The IVIG yielded increased DSA clearance compared with historic tPE-based treatment, yet spontaneous clearance of new DSA also remains common.
invasive, because it does not require placement of a dialysis catheter and use of an extracorporeal blood pump. Moreover, it can be performed more easily in an outpatient setting and repeated at follow-up.

We designed a retrospective observational study to evaluate the IVIG-based treatment of early DSA in 2 ways. First, we compared patient and graft survival between DSA patients who were treated with IVIG and contemporary patients who were transplanted between April 2013 and January 2015 and did not develop early DSA. Second, we compared outcomes and efficacy in clearing early DSA between the IVIG-based and historic, tPE-based protocols.

MATERIALS AND METHODS

Patients
A retrospective observational study was performed in a single university center to evaluate the efficacy and safety of an IVIG-based treatment protocol of early DSA.

Three patient groups were defined. Patients who underwent LTX between April 2013 and January 2015, developed early DSA and underwent IVIG-based treatment, formed group A. Patients who showed de novo DSA or a positive complement-dependent cytotoxicity (CDC) crossmatch were included in group A. Instead, patients, who were transplanted during the same period, developed early DSA but were not treated, were excluded.

The DSA clearance in group A patients was compared with the DSA clearance in historic patients transplanted between January 2009 and June 2013 who developed early DSA or showed a positive CDC crossmatch but were treated with tPE. These patients formed group B.

Outcomes of group A patients were compared with the outcomes of the remaining contemporary patients who were transplanted between April 2013 and January 2015 but did not develop early DSA. These patients formed group C and served as the control group.

All patients received a single dose of anticytomegalovirus (CMV)-enriched human immunoglobulins immediately upon arrival at the intensive care unit (ICU), without any induction therapy. Posttransplant immunosuppressive therapy was based on a triple therapy (calcineurin inhibitor, mycophenolate mofetil, and prednisolone). Before January 2013, most patients received cyclosporine as first-line calcineurin inhibitor. However, all patients with assumed higher immunological risk, such as retransplants, patients’ posthuman stem-cell transplant as well as children, were discharged on tacrolimus. Since January 2013, tacrolimus was used first line in all patients after LTX at our institution, but patients with assumed low immunological risk were later switched to cyclosporine in the outpatient setting 3 months after LTX. At our institution, patients usually received mycophenolate mofetil as cell cycle inhibitor after transplantation.

Patient records and outpatient visits were retrospectively reviewed. Follow-up was 100% completed and ended on April 1, 2015.

The hospital ethical review board, being a retrospective study, waived the need for patient consent to the study.

Variables
The CMV risk profiles, the need for postoperative or secondary extracorporeal membrane oxygenation support, bronchiolitis obliterans syndrome (BOS) and primary graft dysfunction (PGD), and the variables pulsed-steroid therapy, biopsy-confirmed acute rejection and infection requiring hospitalization and treatment, have been defined elsewhere.6,19,21-24

At our institution, patients do not undergo transbronchial biopsy during initial hospitalization after LTX. Thereafter, protocol surveillance transbronchial biopsies are performed at 1, 3, and 6 months and 1 year after transplantation and upon indication.

In this study, incidence of antibody-mediated rejection was not reported. Because of unspecified pathologic diagnostic criteria for antibody-mediated rejection, lung pathologists at our institution never made the diagnosis.

Early DSA were defined as DSA, which were detected during initial hospitalization after LTX, before hospital discharge. The DSA clearance was defined as the absence of DSA in 2 consecutive Luminex-based solid phase multiplex bead array (SPA) (LIFECODES; Immucore Transplant Diagnostics, Inc., Stamfort, CT) controls, to avoid false-negative results, because it has been demonstrated that, in kidney transplantation, DSA may be bound to the graft while at the same time not being present in serum.26 The DSA recurrence was defined as a renewed positivity of previously cleared DSA at Luminex-based SPA control.

DSA Detection and Controls

The DSA detection protocol did not change during the study period. All groups A, B, and C patients were screened for anti-HLA antibodies at the time of listing to LTX, and, during hospitalization for LTX, immediately before LTX, on day 14 and before hospital discharge or upon indication using CDC (Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany) and Luminex-based SPA assays. Both B- and T cell crossmatches were performed using pretransplant serum. A low threshold of 1000 mean fluorescence intensity (MFI) was used to detect early DSA. If the lower cutoff MFI was set at higher values, for example, at 2000 or 5000 MFI, many patients, which anyway were at risk of developing humoral rejection, would have been missed. Therefore, to pick up these cases early, once sensitization had taken place, but humoral rejection was not undergoing yet, the MFI threshold value was set low at 1000 MFI. The highest MFI value against donor antigens was considered if more than 1 HLA coating bead with the same HLA specificity was detected. However, MFI values are only a semiquantitative measure of antibodies levels.27 At our institution, DSA against HLA DP were not measured.

At follow-up, in group A patients, Luminex-based SPA DSA controls were performed, according to the treatment protocol, at the beginning of each treatment session, after treatment end, and every 6 months. In group B patients, Luminex-based SPA DSA controls had been performed usually once per year. Instead, in group C patients, Luminex-based SPA DSA controls were not regularly controlled, but only upon indication, for example, in case of graft dysfunction.

IVIG-Based DSA Treatment Protocol

In April 2013, an IVIG-based treatment protocol was established and agreed by all the clinicians at our institution (Figure 1). This protocol was used to treat lung-transplanted patients who developed early DSA or showed a positive CDC crossmatch, instead of the tPE-based protocol, which had...
been used so far. Therapeutic plasma exchange was still used in combination with IVIG in patients with a positive cross-match or assumed acute rejection.

Immediately after evidence of early DSA, its confirmation through a control sample and evaluation of patient current clinical conditions, DSA treatment was initiated. The DSA treatment was usually performed preemptively, independent of graft dysfunction. In case of absence of lung dysfunction, treatment was performed only with IVIG and Rituximab. The first IVIG dose consisted of 2 g/kg of IgM-enriched human immunoglobulins. However, if graft dysfunction was suspected or in presence of a positive crossmatch, 3 or 5 sessions of tPE or, more recently, 2 sessions of immunoabsorption preceded the first IVIG dose, to accelerate DSA clearance. Then, a single dose of dose of Rituximab (375 mg/m²) was given. Originally, a second dose of Rituximab was considered in the protocol. However, because all peripheral blood fluorescence-activated cell-sorting assays for CD19+ and CD20+ lymphocytes were negative after the first dose, the second dose was never administered. If DSA control was still positive, treatment with an IVIG dose of 0.5 g/kg was performed every 4 weeks. Treatment was ended and considered successful only if 2 consecutive controls were negative for DSA.

The IVIG was given through a peripheral venous catheter. Premedication with an antihistaminic drug (usually 150 mg ranitidine and 10 mg clemastine fumarate) and 1 g paracetamol was given to avoid allergic reactions before Rituximab, and before IVIG, only if the patient had already shown allergic reactions against IVIG. No prophylactic antibiotic therapy was given during IVIG therapy.

The DSA treatment protocol based only on tPE and Rituximab, which was used between January 2009 and April 2013, has been recently described elsewhere.\textsuperscript{18}

**Statistics**

Data collection and analysis were performed retrospectively using SPSS 22.0 (IBM, NY). Endpoints were DSA clearance at treatment end and last Luminex-based SPA control, mortality, and freedoms from pulsed-steroid therapy, biopsy-confirmed acute rejection, and infection requiring hospitalization. Because of the short follow-up period for groups A and C in comparison to B patients, only incidence of BOS for each group was reported, without quantifying statistically significant differences among groups.

Categorical and continuous variables were summarized as percentages and median ± interquartile range, respectively. The independent samples nonparametric Kruskal-Wallis 1-way ANOVA or Mann-Whitney tests and the $\chi^2$ test or the Fisher exact tests were used for group comparisons of continuous and categorical variables, respectively.

Survival estimates along with freedom from pulsed-steroid therapy, biopsy-confirmed acute rejection, BOS, and infection requiring hospitalization were calculated by the product-limit method of Kaplan-Meier. Differences among groups, except for BOS, were quantified using the log-rank test. Survival and freedom curves were truncated at 2 years follow-up for group B patients.

Some patient characteristics may have conferred a degree of heterogeneity to each patient group, which could have biased outcomes. Therefore, outcomes and DSA clearance

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**FIGURE 1.** The process map reports the actual DSA treatment protocol at our institution.
were evaluated in groups A and B patients, after stratification according to presence of preformed versus de novo DSA; in group A patients, after stratification according to the presence of graft dysfunction or a positive crossmatch; in group B patients, after stratification according to cyclosporine use at discharge; and in group C patients, after stratification according to presence of preoperative donor nonspecific HLA antibodies.

Two-tailed $P$ values of 0.05 or less were considered significant.

RESULTS

Patient Groups

Between April 1, 2013, and January 31, 2015, among 243 patients who underwent LTX at our institution, 63 (26%) patients developed early DSA or showed a positive crossmatch. Among these patients, 57 (90%) underwent IVIG-based treatment and formed group A.

Six (10%) patients were not treated, because the DSA report came when patients were already discharged to the rehabilitation clinic and were not recalled for treatment. These patients were transplanted from October 2013 to February 2014, at the beginning of our experience with the IVIG-based treatment. More recently, because our experience with IVIG treatment has grown, patients where positive DSA evidence came after hospital discharge, were recalled and underwent treatment as well. Among these 6 patients, none showed DSA at the last LumineRx-based SPA control, and all were alive at end follow-up. However, these patients were too few to form a control group and therefore excluded from the study.

The remaining 180 (74%) contemporary patients without early DSA formed group C.

Fifty-six historic DSA patients who had undergone tPE-based treatment formed group B. Among these patients, 54 (96%) were transplanted between January 2009 and April 2013. The remaining 2 (4%) patients were transplanted between April and June 2013, during a transition phase from the tPE to IVIG treatment protocols.

Pre-, Intra- and Postoperative Patient Characteristics

Preoperative patient characteristics were mostly similar among groups (Tables 1 and 2), without any significant difference in the prevalence of preoperative anti-HLA antibodies, which have been demonstrated to be a risk factor for mortality and graft dysfunction elsewhere, but not at our institution. Groups A, B, and C showed a different pattern of cumulative HLA mismatches.

Postoperatively, group B showed a higher prevalence of complications and in-hospital mortality, as well as longer ICU and hospital stay times (Table 3). This difference was not due to the presence of graft dysfunction, because the evidence came after hospital discharge, were recalled and underwent treatment as well. Among these 6 patients, none showed DSA at the last LumineRx-based SPA control, and all were alive at end follow-up. However, these patients were too few to form a control group and therefore excluded from the study.

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Fifty-six historic DSA patients who had undergone tPE-based treatment formed group B. Among these patients, 54 (96%) were transplanted between January 2009 and April 2013. The remaining 2 (4%) patients were transplanted between April and June 2013, during a transition phase from the tPE to IVIG treatment protocols.

Preoperative data

| Variable | Group A (n = 57) | Group B (n = 56) | Group C (n = 180) | $P$ |
|----------|-----------------|-----------------|-----------------|-----|
| Female sex | 31 (54) | 36 (64) | 93 (52) | 0.25 |
| Age, y | 50 (35-57) | 49 (34-56) | 50 (35-57) | 0.69 |
| Blood group | | | | |
| A | 31 (54) | 28 (50) | 78 (43) | 0.30 |
| B | 4 (7) | 9 (16) | 15 (8) | 0.13 |
| AB | 1 (2) | 1 (2) | 12 (7) | 0.16 |
| 0 | 21 (37) | 18 (32) | 75 (42) | 0.41 |
| CMV risk | | | | |
| Low | 10 (18) | 10 (18) | 52 (29) | 0.09 |
| Intermediate | 25 (45) | 22 (39) | 71 (40) | 0.80 |
| High | 21 (37) | 24 (43) | 55 (31) | 0.26 |
| Transplant indication | | | | |
| COPD | 15 (26) | 11 (20) | 49 (27) | 0.52 |
| Pulmonary fibrosis | 21 (37) | 19 (34) | 57 (32) | 0.76 |
| Cystic fibrosis | 7 (12) | 10 (18) | 46 (26) | 0.08 |
| Pulmonary hypertension | 5 (9) | 7 (12) | 1 (0.5) | <0.001 |
| Retransplant | 7 (12) | 5 (9) | 10 (6) | 0.22 |
| Other | 2 (3) | 4 (7) | 17 (9) | 0.36 |
| Preoperative invasive mechanical ventilation | 0 (0) | 4 (7) | 5 (3) | 0.08 |
| Preoperative ICU | 7 (12) | 10 (18) | 19 (11) | 0.35 |
| Preoperative ECMO | | | | |
| Venovenous | 3 (5) | 4 (7) | 9 (5) | 0.82 |
| Venoarterial | 4 (7) | 4 (7) | 1 (0.5) | 0.007 |
| LAS score | 39 (34-47) | 38 (34-50) | 37 (34-44) | 0.32 |
| Donor | | | | |
| Age, y | 53 (39-63) | 45 (35-53) | 49 (35-59) | 0.27 |
| Ventilation, d | 3 (2-6) | 4 (2-7) | 4 (2-8) | 0.25 |
| $pO_2$ (100%, mm Hg) | 409 (306-455) | 389 (273-435) | 365 (305-446) | 0.40 |

* Lung allocating scores (LAS) available from January 2012.
Values are expressed as median (IQR) or N (%).
COPD indicates chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; IQR, interquartile range.
prevalence of postoperative need for pulsed-steroid therapy for presumed rejection was similar among groups. Moreover, prevalence of PGD was higher in groups A and B than group C, but similar among groups A and B (P = 0.12 and 0.16 for PGD scores 2-3 at 24 and 48 hours, respectively).

**DSA and Therapy**

The DSA characteristics and treatment are reported in Table 4. Fifty-one (90%) group A versus 48 (86%) group B patients developed de novo DSA (P = 0.36) after transplantation. More group A than group B patients developed DSA against recipient HLA DQ antigen (P = 0.004). No patient had undergone desensitization therapy with IVIG or tPE before transplantation.

The first dose of IVIG was preceded by tPE or immunosorption in 10 group A patients, due to positive cross-match (n = 5) or assumed acute rejection (n = 5), according to the treatment protocol reported in Figure 1. Among patients with assumed acute rejection, 2 patients showed worsening of lung function tests and increase of infection parameters without evidence of active bacterial, fungal, or viral infection. The remaining 3 patients, during their initial ICU stay after transplantation, developed respiratory insufficiency with hypoxemia requiring reintubation of mechanical ventilation, without evidence of infection. Two patients were successfully weaned from ventilation. The remaining patient died of sepsis 3 weeks after treatment.

A single dose of Rituximab was given at the end of the first cycle of IVIG and tPE in 52 (91%) group A and 51 (91%) group B patients, respectively. Five group A patients did not receive Rituximab due to recent infection (n = 4) or abdominal surgery (n = 1). Instead, 5 group B patients did not receive Rituximab due to evidence of carcinoma in the explanted lungs (n = 1) or concomitant steroid treatment for acute rejection (n = 4).

At follow-up, 8 (15%) group A patients did not require any further admission for IVIG treatment, because these patients did not show DSA at discharge any more (n = 7) or showed evidence of malignancy (n = 1).

Complications associated with IVIG therapy are reported in Table 4.

**DSA Clearance**

At treatment end, group A showed a better DSA clearance than group B (92% vs 64%; P = 0.002). Among the 4 group A patients who still showed DSA, 1 patient had evidence of malignancy soon after transplantation, and did not undergo further IVIG treatment. Another patient had undergone retransplantation. The remaining 2 patients showed persistent DSA against HLA-DQ antigen after 5 and 7 admissions for IVIG treatment.

At the last Luminex-based SPA control, performed after a median of 13 (6-26) months, DSA clearance was better in group A than group B (90% vs 75%; P = 0.04). Among group A patients, only one showed DSA recurrence 11 months after treatment end. The patient had developed chronic rejection due to unsatisfactory adherence to immunosuppressive therapy.

At treatment end and at last Luminex-based SPA control, DSA clearance showed no difference in groups A and B, after intragroup stratification according to the presence of preformed versus de novo DSA (Table S1, SDC, http://links.lww.com/TP/B222); in group A, after stratification according to presence of graft dysfunction or positive cross-match (Table S2, SDC, http://links.lww.com/TP/B222); in group B, after stratification according to therapy with cyclosporine at discharge (Table S3, SDC, http://links.lww.com/TP/B222); and among group A versus excluded patients (Table S4, SDC, http://links.lww.com/TP/B222). Moreover, among groups A and B patients, DSA clearance at treatment end and at last control showed a similar trend before and after stratification according to the presence of preformed versus de novo DSA (Table S1, SDC, http://links.lww.com/TP/B222), with better clearance in group A patients.

**Survival, Acute Rejection, and Infection**

Median follow-up amounted to 12 (7-18) months for group A, 37 (25-43) months for group B, and 12 (7-18) months for group C, and is reported in Figures 2 and 3, Table 5, and Tables S1-S5 (SDC, http://links.lww.com/TP/B222).
At 6 months and 1 year follow-up, group A showed better overall survival than group B and similar to group C (Table S1, SDC, http://links.lww.com/TP/B222). Sixteen patients died in-hospital (Table 3). Survival conditioned to hospital discharge was similar among groups (Table 5; Table S1, SDC, http://links.lww.com/TP/B222).

At 6 months and 1 year follow-up, there was no statistically significant difference among groups regarding freedom from pulsed-steroid therapy, biopsy-confirmed rejection and infection requiring hospitalization, although group A showed a trend toward better freedom from pulsed-steroid therapy and biopsy-confirmed rejection than the other 2 groups (Table 5; Table S1, SDC, http://links.lww.com/TP/B222).

Endpoints showed no difference in groups A and B after intragroup stratification according to presence of preformed versus de novo DSA (Table S1, SDC, http://links.lww.com/TP/B222); in group A, according to presence of graft dysfunction or positive crossmatch (Table S2, http://links.lww.com/TP/B222); in group B, after stratification according to therapy with cyclosporine at discharge (Table S3, SDC, http://links.lww.com/TP/B222); in group C, after stratification according to preoperative evidence of DSA (Table S5, SDC, http://links.lww.com/TP/B222); and among group A and the 6 excluded patients (Table S4, SDC, http://links.lww.com/TP/B222). Moreover, outcomes among groups A and B patients were similar before and after stratification according to presence of preformed versus de novo DSA (Tables S1, SDC, http://links.lww.com/TP/B222 and Table 5).

**DISCUSSION**

This study presents the short-term results of an IVIG-based treatment with Rituximab in patients with early humoral sensitization after LTX.

The IVIG therapy yielded optimal DSA clearance (92%), which persisted at last DSA control (90%) and was better than that in tPE-treated patients. Prevalence of IVIG-related side effects was low. Patients with DSA, which confer a

### TABLE 3.
Intraoperative and postoperative data

| Variable                              | Group A (n = 57) | Group B (n = 56) | Group C (n = 180) | P     |
|---------------------------------------|-----------------|-----------------|------------------|-------|
| **Intraoperative**                    |                 |                 |                  |       |
| Single lung                           | 1 (2)           | 1 (2)           | 2 (1)            | 0.89  |
| Double lung                           | 56 (98)         | 55 (98)         | 178 (99)         | 0.89  |
| ECMO                                  |                 |                 |                  |       |
| Venoarterial                          | 15 (26)         | 15 (29)         | 32 (18)          | 0.14  |
| Venovenous                            | 2 (3)           | 4 (8)           | 8 (4)            | 0.54  |
| Postoperative-continued ECMO          | 6 (11)          | 8 (14)          | 10 (6)           | 0.89  |
| **Ischemic time, min**                |                 |                 |                  |       |
| Right                                 | 408 (339-511)   | 431 (352-527)   | 408 (319-497)    | 0.20  |
| Left                                  | 542 (450-617)   | 551 (484-636)   | 501 (412-604)    | 0.83  |
| **Postoperative**                     |                 |                 |                  |       |
| Rethoracotomy for bleeding            | 2 (3)           | 7 (12)          | 12 (7)           | 0.16  |
| Temporary dialysis post transplantation| 2 (3)           | 11 (20)         | 11 (6)           | 0.002 |
| PGD grade 2 or grade 3                |                 |                 |                  |       |
| 24 h                                  | 12 (21)         | 19 (34)         | 17 (9)           | <0.01 |
| 48 h                                  | 12 (21)         | 18 (33)         | 18 (10)          | <0.01 |
| Atrial fibrillation                    | 7 (12)          | 13 (24)         | 12 (7)           | 0.002 |
| Postoperative pulsed steroid therapy   | 19 (33)         | 24 (44)         | 49 (27)          | 0.05  |
| Superficial secondary wound infection  | 5 (9)           | 12 (21)         | 9 (9)            | 0.01  |
| Secondary ECMO                         | 2 (3)           | 4 (7)           | 3 (2)            | 0.11  |
| Tracheostomy                          | 6 (10)          | 19 (34)         | 13 (7)           | <0.001|
| Ventilation time, d<sup>1</sup>       | 1 (1-1)         | 2 (1-9)         | 1 (1-1)          | <0.001|
| ICU stay, d<sup>1</sup>               | 2 (1-5)         | 4 (2-17)        | 2 (1-3)          | <0.001|
| Hospital stay, d<sup>1</sup>          | 25 (22-34)      | 30 (23-54)      | 23 (21-30)       | <0.001|
| In-hospital mortality                  | 2 (3)           | 9 (16)          | 5 (3)            | 0.001 |
| Sepsis after a prolonged hospital stay| 2 (3)           | 5 (9)           | 2 (1)            |       |
| Failure of transplanted lungs          | 0               | 1 (2)           | 1 (0.5)          |       |
| Malignancy                            | 0               | 1 (2)           | 0                |       |
| Pulmonary bleeding                     | 0               | 1 (2)           | 0                |       |
| Cardiac arrest                         | 0               | 1 (2)           | 0                |       |
| Mechanical ileus                       | 0               | 0               | 1 (0.5)          |       |
| Bacterial encephalitis                 | 0               | 0               | 1 (0.5)          |       |
| Imnosuppressive therapy<sup>a</sup>   |                 |                 |                  |       |
| Cyclosporin                            | 0 (0)           | 16 (34)         | 0 (0)            | <0.001|
| Tacrolimus                             | 55 (100)        | 31 (66)         | 175 (100)        | <0.001|

<sup>a</sup> Patients who died in-hospital were censored.

Values are expressed as median (IQR) or N (%).
higher risk for mortality and acute and chronic rejection,\(^1^9\) showed survival, freedom infection and forced expiratory volume in 1 second similar to transplanted patients without DSA, after IVIG therapy. Although IVIG patients showed better survival than tPE patients, results were confounded by the higher prevalence of postoperative complications in tPE than IVIG patients and different immunotherapy at discharge. In this last case, however, DSA clearance and outcomes were not significantly different in tPE patients after stratification according to cyclosporine use at discharge (Table S3, SDC, http://links.lww.com/TP/B222). Moreover, it remains controversial whether the immunosuppressive therapy has an impact on DSA clearance\(^28^3^0\) and survival.\(^3^1\)

In solid-organ transplantation, immunoglobulins have been used successfully for clearing DSA or desensitizing patients with DSA before transplantation, either alone or in combination with other treatment modalities, such as tPE and immunoabsorption.\(^1^0^1^1^1^5^1^7^1^9\) Hachem et al\(^1^7\) reported the first case series on preemptive DSA treatment with IVIG and Rituximab in LTX.

In comparison to the study of Hachem et al, however, we included patients with preformed DSA or positive CDC

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**TABLE 4.** Anti-HLA DSA: immunology and treatment.

| Variable | Group A (n = 57) | Group B (n = 56) | \(P\) |
|----------|-----------------|-----------------|------|
| Postoperative DSA HLA A | 5 (9) | 4 (7) | 1.0 |
| MFI\(^a\) | 17603 (5424-22259) | 2997 (1563-6522) | 0.11 |
| HLA B | 8 (14) | 11 (20) | 0.46 |
| MFI\(^a\) | 2988 (1485-12360) | 6679 (3886-12897) | 0.18 |
| HLA C | 0 (0) | 0 (0) | 0.004 |
| HLA DQ | 50 (88) | 37 (66) | 0.58 |
| MFI\(^a\) | 4441 (2784-10464) | 3233 (2302-9002) | 0.54 |
| HLA DR | 6 (10) | 8 (14) | 0.68 |
| MFI\(^a\) | 12124 (5520-15485) | 8077 (4968-16058) | 0.36 |
| De novo DSA | 51 (90) | 48 (86) | 0.11 |
| Positive crossmatch | 5 (9) | 9 (16) | 0.24 |
| Time to DSA development, d | 11 (10-18) | 12 (6-22) | 0.88 |
| DSA therapy at initial hospitalization | | | |
| IVIG-based therapy | 57 (100) | | |
| IVIG | 48 (84) | | |
| IVIG + tPE | 7 (12) | 4 | |
| 3 sessions tPE | 4 | | |
| 5 sessions tPE | 3 | | |
| IVIG + immunoabsorption | 3 (5) | | |
| tPE-based therapy | | 56 (100) | |
| 3 sessions tPE | | 47 | |
| 5 sessions tPE | | 10 | |
| Rituximab | 52 (91) | 51 (91) | 1.0 |
| DSA therapy at follow-up | | | |
| IVIG\(^b\) | 47 (85) | | |
| No. treatments | 3 (2-5) | 34 (64) | 0.02 |
| Treatment time, mo\(^c\) | 5 (3-6) | 35 (75) | 0.04 |
| Patients still on treatment at end FU | 4 (7) | 0 | 0.34 |
| DSA clearance | | | |
| At completion of DSA treatment\(^d\) | 46 (62) | 34 (64) | 0.06 |
| At last DSA control\(^e\) | 45 (90) | 35 (75) | 0.17 |
| DSA at last control HLA A | 1 (2) | 0 | |
| HLA B | 0 | 3 (6) | 0.34 |
| HLA DQ | 5 (10) | 9 (19) | 0.06 |
| HLA DR | 0 | 0 | |
| IVIG side effects\(^f\) | | | |
| Headache, nausea and vomit | 4 (2) | | |
| Hemolytic anemia | 1 (0.5) | | |
| Allergic reaction | 1 (0.5) | | |

\(a\) MFI values at first positive control are reported.

\(b\) patients who died in-hospital were censored.

\(c\) Patients still on treatment at end follow-up were censored.

\(d\) \(n = 50\) for Group A and \(n = 53\) for Group B.

\(e\) \(n = 50\) for Group A and \(n = 47\) for Group B.

\(f\) Overall IVIG treatments, \(n = 234\).

Values are expressed as median (IQR) or N (%).
crossmatch. This intragroup heterogeneity might have influenced the DSA clearance and outcomes in IVIG and tPE patients. In fact, in kidney transplantation, patients, who before transplantation were sensitized against donor HLA antigens, were at significant high risk of humoral rejection and graft loss after transplantations.\textsuperscript{32,33} Moreover, mechanisms and strength of antibody production in patients with preformed versus de novo DSA are different.\textsuperscript{2,8} In our study, outcomes and DSA clearance did not differ in patients with preformed DSA versus patients with de novo DSA in each group (Table S1, SDC, http://links.lww.com/TP/B222). In addition, outcomes and DSA clearance among IVIG and tPE patients showed similar results before and after stratification according to presence of preformed versus de novo DSA (Table S1, SDC, http://links.lww.com/TP/B222 and Table 5).

The improved DSA clearance in IVIG versus tPE patients may be due to the IVIG pleiotropic and long-lasting immunomodulatory effects: IVIG can downregulate B-cell activation and antibody production and provoke apoptosis of mature B cells; they can induce anti-inflammatory cytokines and contain blocking antiidiotypic antibodies to anti-HLA antibodies; and they can block complement-mediated injury through inhibition of C3 activation.\textsuperscript{11-16,34} Meanwhile, tPE, as well as immunoabsorption, only remove DSA from plasma. Therefore, we deem that the combination of the immunomodulatory effects of IVIG with the apoptotic effects of Rituximab on pre-B and mature B cells\textsuperscript{35,36} yielded the optimal DSA clearance obtained in our study. We still use tPE but only in combination with IVIG at our center.

**FIGURE 2.** Overall survival. Group A patients showed better survival than group B patients and similar to group C patients. Patients at risk are reported above the x-axis. Survival curve for group B patients is truncated at 2 years of follow-up.

**FIGURE 3.** Freedom from pulse steroid therapy (A), biopsy-confirmed rejection (B), BOS (C), and from infection requiring hospitalization (D) are reported. Patient at risk are reported above the x-axis. The curves for group B patients have been truncated at 2 years of follow-up.
Other types of immunoglobulins given for specific indications may also clear DSA, such as CMV immunoglobulins and antithymocyte globulins. The IVIG preparation mostly applied in kidney transplantation for treatment of DSA is not enriched for any specific components. At our institution, we use IgM enriched IVIG due to their largest inhibition of lymphocytoxicity and their immunomodulatory and antibacterial properties, because they have been used mainly in patients with sepsis so far. Thus, it is possible that IVIG did not only clear DSA in our patients but also protected them from infection. The IVIG patients, although potentially more immunosuppressed through repeated IVIG therapy and Rituximab, showed freedom from infection requiring hospitalization similar to patients without DSA.

We have recently shown in patients mainly transplanted before implementation of the IVIG protocol that development of early DSA was a risk factor for mortality. In the present study, although follow-up was short, survival of IVIG patients was similar to survival of patients without DSA. Moreover, interestingly, IVIG patients showed improved freedom from pulsed-steroid therapy and biopsy-confirmed rejection than patients without DSA, although not statistically significant, pointing at a potential immunoprotective effect of IVIG. Longer follow-up and more studies are required to evaluate this effect on graft and patient survival.

| Variable | Group A (n = 55) | Group B (n = 47) | Group C (n = 175) | P       |
|----------|-----------------|-----------------|------------------|---------|
| Survival (%) |                 |                 |                  |         |
| 6 mo     | 96 ± 3          | 84 ± 5          | 95 ± 2           |         |
| 1 y      | 94 ± 3          | 79 ± 5          | 94 ± 2           |         |
| P value group A vs group B: 0.008; P value group A vs group C: 0.81 |
| Survival after hospital discharge (%) |                 |                 |                  |         |
| 6 mo     | 98 ± 2          | 94 ± 4          | 99 ± 1           |         |
| 1 y      | 98 ± 2          | 94 ± 4          | 97 ± 2           |         |
| P value group A vs group B: 0.13; P value group A vs group C: 0.55 |
| Death causes |                |                 |                  |         |
| BOS      | 0 (0)           | 6 (13)          | 0 (0)            |         |
| Infection| 0 (0)           | 1 (2)           | 4 (2)            |         |
| Malignancy| 1 (2)          | 1 (2)           | 2 (1)            |         |
| Other\(a\) | 0 (0)          | 5 (11)          | 0 (0)            |         |
| Freedom from pulsed-steroid therapy (%) |                 |                 |                  |         |
| 6 mo     | 78 ± 6          | 60 ± 7          | 68 ± 4           |         |
| 1 y      | 65 ± 7          | 47 ± 7          | 61 ± 4           |         |
| P value group A vs group B: 0.12; P value group A vs group C: 0.38 |
| Freedom from biopsy-confirmed rejection (%) |                 |                 |                  |         |
| 6 mo     | 85 ± 5          | 82 ± 6          | 68 ± 4           |         |
| 1 y      | 78 ± 7          | 70 ± 7          | 64 ± 4           |         |
| P value group A vs group B: 0.97; P value group A vs group C: 0.15 |
| ISHLT grade |              |                 |                  |         |
| A1       | 11 (23)         | 14 (31)         | 44 (29)          | 0.68    |
| A2       | 1 (2)           | 1 (2)           | 13 (9)           | 0.133   |
| Freedom from BOS (%) |               |                 |                  |         |
| 6 mo     | 100             | 96 ± 4          | 99 ± 1           |         |
| 1 y      | 100             | 87 ± 5          | 96 ± 2           |         |
| Freedom from infection (%) |               |                 |                  |         |
| 6 mo     | 86 ± 5          | 81 ± 6          | 88 ± 3           |         |
| 1 y      | 80 ± 6          | 64 ± 7          | 81 ± 3           |         |
| P value group A vs group B: 0.15; P value group A vs group C: 0.84 |
| Immunosuppressive therapy at last control\(b\) |             |                 |                  |         |
| Cyclosporin | 1 (2)         | 9 (19)          | 32 (18)          | 0.009   |
| Tacrolimus| 54 (98)         | 38 (81)         | 142 (81)         | 0.007   |
| Everolimus| 2 (4)           | 2 (4)           | 5 (3)            | 0.87    |
| Mycophenolate mofetil | 54 (98)     | 47 (100)        | 174 (99)         | 0.52    |
| FEV\(_1\) (% predicted) |             |                 |                  |         |
| 1 y      | 84 (64-101)     | 71 (59-88)      | 84 (73-106)      | 0.01    |
| Last control (12, 6-18 mo) |         | 86 (58-100)    | 66 (55-85)       | 83 (63-104) | 0.001 |

\(a\) Sudden at home, pulmonary embolism, acute myocardial infarction, acute type A dissection.
\(b\) Patients who died in-hospital were censored.

Values are expressed as mean ± SD, median (IQR) or N (%).
FEV\(_1\), forced expiratory volume in 1 second; ISHLT, International Society for Heart and Lung Transplantation.
treatment implies costs and impairs quality of life of patients who have to repeat treatment every 4 weeks. Regarding the former problem, careful control of patients during IVIG administration is paramount. Regarding the latter, a combination of tPE or immunoabsorption with IVIG in all patients with early DSA may accelerate their clearance. Therapy costs and side effects may raise some concerns about the necessity of DSA treatment,20 because some DSA patients cleared DSA without any treatment. However, there is enough available scientific evidence showing that DSA are risk factors for survival and acute and chronic rejection, not only in LTX, but also in other solid organ transplantation.1,9,14 A randomized trial will be helpful in clearing the real impact of DSA and their treatment on patient and graft survival in LTX.

STUDY LIMITATIONS

The retrospective nature of the analysis introduced inherent limitations. To obviate the lack of randomization and account for in-group heterogeneity, outcomes were evaluated after stratification according to presence of positive cross-match or graft dysfunction in group A, preformed versus de novo DSA in and among groups A and B, preoperative donor nonspecific HLA antibodies in group C and baseline immunotherapy in group B.

Only patients who developed early DSA during initial hospitalization after LTX were allocated to IVIG treatment. The remaining patients (group C), who were discharged from the hospital without having developed early DSA, could have developed DSA thereafter. However, the prevalence of DSA at follow-up could not be estimated in these patients, because they did not routinely undergo DSA controls at our institution.

CONCLUSIONS

The present treatment protocol with IgM-enriched IVIG and Rituximab showed better long-lasting DSA clearance than our historic tPE-based treatment. However, spontaneous DSA clearance remains frequent. Although the better survival in IVIG patients in comparison with tPE patients was influenced by confounders related to the retrospective nature of this study, patients with early new DSA but without graft dysfunction treated with IVIG have midterm survival and lung function equivalent to patients not developing early DSA at all.

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