



# A virtual crossmatch-based strategy for perioperative desensitisation in lung transplant recipients with pre-formed donor-specific antibodies: 3-year outcome

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**A perioperative desensitisation protocol in lung transplant recipients with high pre-formed DSAs was associated with satisfactory outcome. Cleared pre-formed DSAs after desensitisation was identified as an independent predictor of graft survival.** <https://bit.ly/3mN6OkY>

**Cite this article as:** Parquin F, Zuber B, Vallée A, et al. A virtual crossmatch-based strategy for perioperative desensitisation in lung transplant recipients with pre-formed donor-specific antibodies: 3-year outcome. *Eur Respir J* 2021; 58: 2004090 [DOI: 10.1183/13993003.04090-2020].

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This article has supplementary material available from [erj.ersjournals.com](http://erj.ersjournals.com)

Received: 17 Nov 2020  
Accepted: 8 April 2021

## Abstract

**Background** Pre-formed donor-specific antibodies (DSAs) are associated with worse outcome after lung transplantation (LTx) and might limit access to LTx. A virtual crossmatch-based strategy for perioperative desensitisation protocol has been used for immunised LTx candidates since 2012 at Foch Hospital (Suresnes, France). We compared the outcome of desensitised LTx candidates with high DSA mean fluorescence intensity and those with low or no pre-formed DSAs, not desensitised.

**Methods** For all consecutive LTx recipients (January 2012 to March 2018), freedom from chronic lung allograft dysfunction (CLAD) and graft survival were assessed using Kaplan–Meier analysis and Cox multivariate analysis.

**Results** We compared outcomes for desensitised patients with high pre-formed DSAs (n=39) and those with no (n=216) or low pre-formed DSAs (n=66). The desensitisation protocol decreased the level of immunodominant DSA (class I/II) at 1, 3 and 6 months post-LTx ( $p<0.001$ ,  $p<0.01$  and  $p<0.001$ , respectively). Freedom from CLAD and graft survival at 3 years was similar in the desensitised group as a whole and other groups. Nevertheless, incidence of CLAD was higher with persistent high-level DSAs than cleared high-level ( $p=0.044$ ) or no DSAs ( $p=0.014$ ). Conversely, graft survival was better with cleared high DSAs than persistent high-level, low-level and no pre-formed DSAs ( $p=0.019$ ,  $p=0.025$  and  $p=0.044$ , respectively). On multivariate analysis, graft survival was associated with cleared high DSAs (hazard ratio 0.12, 95% CI 0.02–0.85 versus no DSAs;  $p=0.035$ ) and CLAD with persistent DSAs (3.04, 1.02–9.17 versus no pre-formed DSAs;  $p=0.048$ ).

**Conclusion** The desensitisation protocol in LTx recipients with high pre-formed DSAs was associated with satisfactory outcome, with cleared high pre-formed DSAs after desensitisation identified as an independent predictor of graft survival.

## Introduction

Lung transplantation (LTx) is now considered as a viable option for patients with end-stage respiratory insufficiency, with adequate selection criteria [1]. One limiting factor to list candidates may be related to

pre-LTx human leukocyte antigen (HLA) immunisation, which is assessed by Luminex technique with HLA single-antigen flow bead (SAFB) assay in most centres [2]. The proportion of candidates with Luminex-detected anti-HLA antibodies has increased to almost half of all candidates [3, 4] and represents a recurrent issue because pre-formed donor-specific antibodies (DSAs) have been associated with worse outcomes. Hence, allosensitisation in LTx candidates has been associated with increased risk of death while on a waiting list [5], increased risk of acute cellular rejection, chronic lung allograft dysfunction (CLAD) and worse survival [3, 6–9]. Lastly, pre-formed DSAs may trigger immediate post-operative hyperacute antibody-mediated rejection (AMR) [10, 11]. An LTx candidate with pre-formed DSAs at mean fluorescence intensity (MFI) >3000 may have positive real cytotoxicity crossmatch (CXM), which remains a contraindication to LTx. Hence, a “positive” virtual CXM considering pre-formed DSAs with MFI >3000 is still considered a barrier to LTx in many centres.

Since 2012, to overcome this hurdle for immunised LTx candidates, our centre has adopted a policy of systematic perioperative desensitisation for all sensitised LTx candidates with pre-formed DSAs at MFI >1000 on day 0 in an attempt to increase the pool of potential donors. Pre-formed DSAs at MFI >5000 were considered not authorised for the desensitisation protocol owing to the reported risk of positive real CXM [12]. The perioperative desensitisation protocol for patients with high pre-formed DSAs includes plasma exchange, rituximab and intravenous immunoglobulins (Ig), combined with high-dose mycophenolate mofetil.

Here, we report our experience with perioperative desensitisation in candidates with pre-formed DSAs by using this pre-emptive virtual CXM-based strategy. We compared the outcome of desensitised LTx candidates with high-level pre-formed DSAs and those without or with low-level pre-formed DSAs who did not undergo desensitisation. The incidence of CLAD, graft survival, pre-formed DSA MFI outcome, primary graft dysfunction (PGD) and acute rejection (including cellular acute rejection [13] and AMR [2]) were compared between desensitised LTx candidates and unsensitised patients.

## Materials and methods

### Patients

We reviewed data for all consecutive patients who underwent LTx between January 2012 and March 2018 at Foch Hospital (Suresnes, France) with at least one available anti-HLA antibody detection performed by SAFB Luminex assay (One Lambda, Canoga Park, CA, USA) within 6 months before LTx. Among 312 LTx patients, 239 (74%) were sensitised with pre-formed anti-HLA antibodies at MFI >500 on the date of listing, and among them, n=105 (33%) had pre-formed DSAs after allocation of a lung graft. For these 105 candidates with pre-formed DSAs, a pre-defined policy of graft allocation and perioperative desensitisation protocol was applied according to the MFI level both on historical serum at the date of listing and on day-0 serum at the date of LTx. Accordingly, these sensitised candidates were classified as having high- or low-level pre-formed DSAs according to MFI in day-0 serum (see Perioperative desensitisation protocol), and their post-LTx outcome was compared to that of unsensitised candidates. The flow of patients is in figure 1.

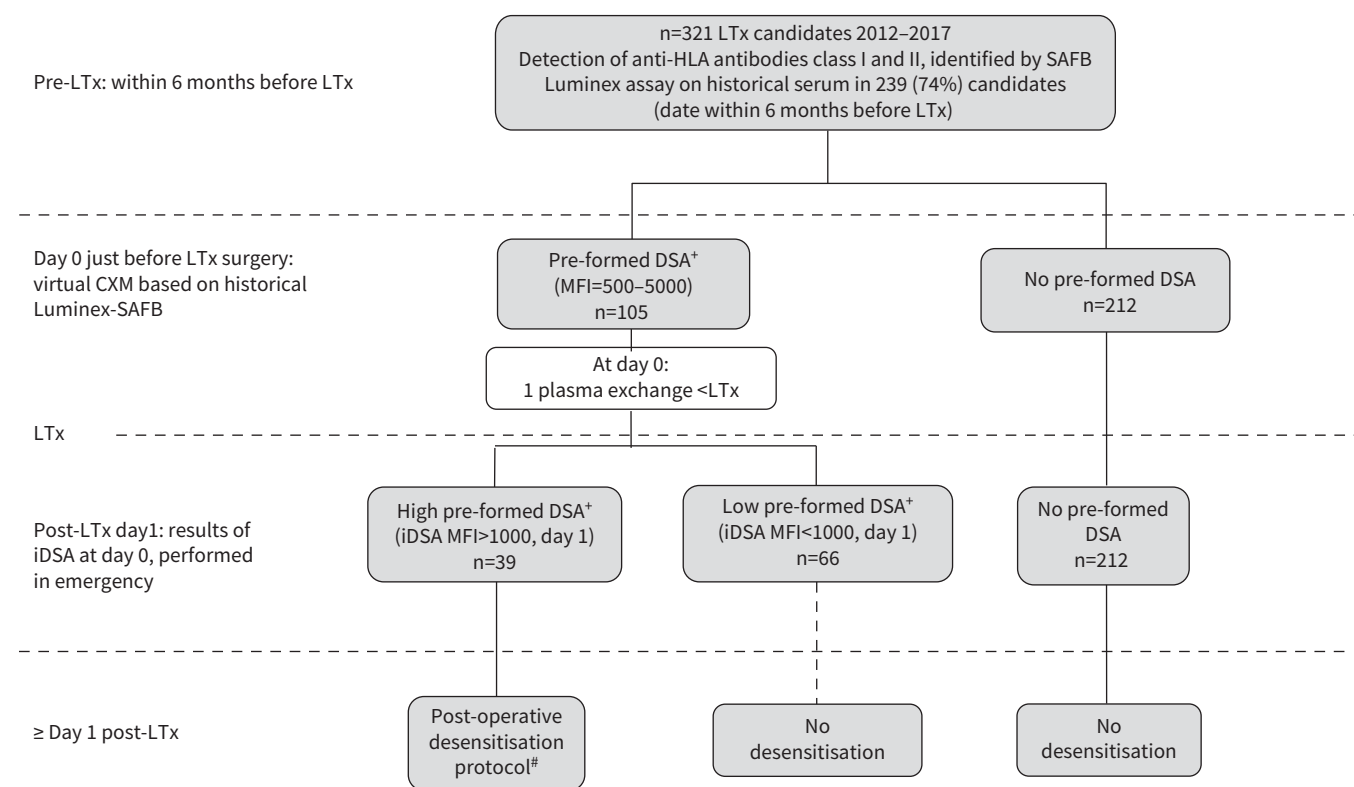
Our study complies with the Declaration of Helsinki, and the institutional review board approved the protocol. Informed consent was obtained at the time of LTx candidate evaluation.

### Immunology

Since 2009, Luminex assay has been routinely used in the laboratory of Saint-Louis Hospital (Paris, France) for screening anti-HLA antibodies against HLA class I and II antigens (LABScreen Autoantibody; One Lambda), with identification by SAFB Luminex assay, as reported previously [14] and detailed in the supplementary material. All beads with a normalised MFI >500 were considered positive, and the DSA nature of the detected antibody was assigned when at least one bead bearing the donor antigen was above the positivity threshold [14]. The HLA typing and crossmatching for this cohort have been reported previously [14] and are detailed in the supplementary material.

### Perioperative desensitisation protocol

Allocation of grafts was decided according to results of virtual CXM, performed with the historical pre-formed DSAs detected on the date of listing. “DSA” in the protocol description and results refers to pre-formed DSAs, because *de novo* DSAs were not considered in the description of this cohort. Allocation of a proposed graft to an immunised candidate with DSAs was allowed in case of corresponding DSAs with MFI ranging from 500 to 5000, but MFI >5000 was considered as not permitted. All immunised LTx candidates with DSAs (n=105) had one pre-operative plasma exchange session just before LTx surgery. The indication for desensitisation protocol was then readjusted on day 1 post-LTx according to MFI results



**FIGURE 1** Flow chart of patients. LTx: lung transplantation; HLA: human leukocyte antigen; SAFB: single-antigen flow bead; CXM: cytotoxicity crossmatch; DSA: donor-specific anti-HLA antibody; iDSA: immunodominant DSA; MFI: mean fluorescence intensity. #: post-operative: five plasma exchange sessions; day 1 post-LTx: 375 mg·m<sup>-2</sup> rituximab; day 3 post-LTx: 2 g·kg<sup>-1</sup> intravenous immunoglobulins; 3 g·day<sup>-1</sup> mycophenolate mofetil in maintenance immunosuppression.

for day-0 immunodominant DSAs. The immunodominant DSA was defined as the DSA with the highest MFI for a given patient. All patients with immunodominant DSAs that remained at MFI >1000 on day 0 (high-DSA group; figure 1) underwent a perioperative desensitisation protocol including 1) five plasma-exchange sessions, 2) rituximab, 3) *i.v.* Ig infusion on day 3 post-plasma exchange and 4) mycophenolate mofetil (3 g·day<sup>-1</sup>) (supplementary material). For other immunised patients with immunodominant DSA MFI <1000 at day 0, the desensitisation protocol was not performed on day 1, but they had already received a pre-LTx plasmapheresis session (low pre-formed DSA group; figure 1). A cleared DSA was arbitrarily defined as a DSA with MFI <1000. This cut-off was chosen because it corresponded to the threshold of MFI used to start our protocol of desensitisation. Retrospective true CXM was performed within the 24 h of LTx by complement-dependent cytotoxicity (CDC and anti-human globulin-CDC) on donor T- and B-lymphocytes in historical and current sera.

#### Immunosuppressive protocol

The immunosuppression protocol used at Foch Hospital has been described previously [14] and is detailed in the supplementary material.

#### Monitoring protocol

The monitoring protocols for surveillance and diagnosis of PGD [15], and acute rejection episodes, pulmonary infections and CLAD have been described previously [14, 16] and are detailed in the supplementary material.

#### Statistical analyses

Continuous data are presented as mean±SD or 95% confidence intervals or median (interquartile range (IQR)) and were compared by Kruskal–Wallis test or ANOVA. Categorical data are presented as n (%) and were compared by Chi-squared or Fisher's exact test. Comparisons between MFI levels of pre-formed DSA before and after desensitisation involved Mann–Whitney paired tests. Freedom from CLAD and graft

survival rates (time to death or re-transplantation) were estimated by the Kaplan–Meier estimator and were compared by log-rank test.

Cox univariate regression was used to evaluate the association between clinical and biological factors and outcomes. Cox multivariable models were built to assess the association between the immunological perioperative status (desensitised high DSA group, no DSA group or low DSA group) and CLAD onset and graft failure with adjustment for potential confounding factors. Factors associated with CLAD onset and graft failure on univariate analyses (at a significance of  $p < 0.2$ ) were selected for multivariate analyses. For all analyses,  $p < 0.05$  was considered statistically significant. Statistical analyses involved using SAS (version 9.4; SAS Institute, Cary, NC, USA).

## Results

### Characteristics of patients

During the study period, 321 patients underwent transplantation, including 39 patients with high DSAs who underwent desensitisation protocol, 66 patients with low DSAs and  $n=216$  patients with no DSAs (figure 1). The recipient, donor and transplant characteristics are presented in table 1. All but one patient in the no-DSA group underwent bilateral LTx. Mean $\pm$ SD time on the waiting list was  $39\pm 57$  days in the high-DSA group. Recipients with high DSAs were older, more frequently female and had a higher incidence of PGD grade 3 as compared with patients with no DSAs ( $p=0.031$ ,  $p=0.023$  and  $p=0.001$ , respectively; table 1). Other characteristics were similar between the three groups. Mean calculated panel reactive antibody (cPRA) at the date of listing in the high-DSA patients was 86% (95% CI 80–92%) taking into account the anti-HLA antibody MFI value  $>500$ , and was lowered to 28% (95% CI 17–39%) excluding all anti-HLA antibody MFI values 500–5000 which were authorised with the desensitisation protocol. No patient with high DSAs died while on the waiting list. Mean $\pm$ SD time on the waiting list was

TABLE 1 Baseline characteristics of the study population according to perioperative desensitisation therapy

	All patients	Densensitised high pre-formed DSAs MFI $>1000$	Not desensitised low pre-formed DSAs MFI $<1000$	Not desensitised no pre-formed DSAs	p-value <sup>#</sup>
<b>Patients</b>	321	39	66	216	
<b>Age years</b>	41 $\pm$ 13	46 $\pm$ 13	41 $\pm$ 13	39 $\pm$ 14	0.056, 0.031,* 0.029 <sup>¶</sup>
<b>Female</b>	167 (52)	27 (69)	105 (48)	35 (53)	0.059, 0.023,* 0.150 <sup>¶</sup>
<b>Diagnosis</b>					0.42
COPD	73 (22)	12 (30)	12 (18)	49 (22)	
Cystic fibrosis	161 (50)	18 (46)	32 (49)	111 (51)	
Fibrosis	40 (12)	7 (18)	7 (10)	26 (12)	
Other	33 (10)	2 (5)	10 (15)	21 (9)	
Re-transplant	12 (3)	0 (0)	4 (6)	8 (3)	
<b>Donor age years</b>	47 $\pm$ 15	49 $\pm$ 14	48 $\pm$ 15	46 $\pm$ 15	0.51
<b>Donor <math>P_{aO_2}</math> mmHg</b>	364 $\pm$ 87	357 $\pm$ 92	376 $\pm$ 79	361 $\pm$ 89	0.42
<b>Donor smoking</b>	130 (40)	18 (46)	26 (39)	86 (39)	0.74
<b>Induction therapy</b>					0.92
No induction	62 (12)	6 (15)	14 (21)	42 (19)	
ATG	87 (27)	12 (30)	18 (28)	57 (26)	
Anti-IL2	168 (53)	21 (53)	32 (50)	115 (53)	
<b>CMV mismatch (donor+/recipient<sup>-</sup>)</b>	53 (16)	4 (10)	11 (16)	38 (17)	0.52
<b>Perioperative ECMO</b>	142 (44)	18 (46)	30 (45)	94 (43)	0.93
<b>PGD grade 3 versus 0/1/2</b>	33 (15)	12 (30)	9 (19)	12 (9)	0.003, 0.001*
<b>Ischaemic time min</b>	450 $\pm$ 150	465 $\pm$ 156	453 $\pm$ 136	447 $\pm$ 153	0.77
<b>HLA A, B MM denominator 4</b>	3.06 $\pm$ 0.84	2.83 $\pm$ 1	3.06 $\pm$ 0.81	3.10 $\pm$ 0.80	0.22
<b>HLA DR, DQ MM denominator 4</b>	2.59 $\pm$ 1.08	2.72 $\pm$ 1.14	2.66 $\pm$ 1.13	2.55 $\pm$ 1.06	0.57

Data are presented as n, mean $\pm$ SD or n (%), unless otherwise stated. Chi-squared tests were used for the comparison of categorical variables, and the unpaired t-test for the comparison of continuous variables. DSA: donor-specific antibody; MFI: mean fluorescence intensity;  $P_{aO_2}$ : arterial oxygen tension; ATG: anti-thymocyte globulin; IL: interleukin; CMV: cytomegalovirus; ECMO: extracorporeal membrane oxygenation; PGD: primary lung graft dysfunction; HLA: human leukocyte antigen; MM: mismatch. <sup>#</sup>: comparison between the three groups. \*:  $p < 0.05$  for the comparison between high pre-formed DSA group and no pre-formed DSA group; <sup>¶</sup>:  $p < 0.05$  for the comparison between high pre-formed DSA group and low pre-formed DSA group.

39±57 days with high DSAs. Mean time from LTx to CLAD onset and from LTx to graft failure onset was 2.68 years (95% CI 2.60–2.76 years) and 2.57 years (95% CI 2.47–2.67 years), respectively, and did not differ between the three groups ( $p=0.135$  and  $p=0.417$ , respectively; table 1).

#### ***DSA outcome after perioperative desensitisation in patients with high pre-formed DSAs***

Nine patients had one DSA specificity, eight had two DSA specificities and 22 had three or more DSAs identified. 16 immunodominant DSAs (iDSA) had class I specificity, and 23 had class II specificity. Categories of MFI values of DSAs on day 0 for iDSAs (class I or II) and cumulative MFI values for DSAs in a given sample are shown in figure 2. Three patients with iDSA MFI 960–1000 were desensitised and included in the high-DNA group. Overall, 34% of patients had iDSA MFI >3000 and 10% had cumulative DSA MFI >5000 on day 0. The median MFI for iDSAs and cumulative DSAs on day 0 was 2207 (IQR 1382–3651) and 3546 (2594–6729), respectively. We observed a significant decrease in the amount of DSAs with desensitisation protocol, as assessed by median MFI for iDSA (class I or II) and cumulative DSAs between day 0 and month 6 (both  $p<0.001$  versus day 0; figure 3). The DSA level was still decreased at 6 months versus day 0 in both the cleared DSA group (mean difference  $-2081\pm940$ ,  $p<0.001$ ) and persistent DSA group (mean difference  $-2535\pm4139$ ,  $p=0.018$ ) (supplementary figure S1). Overall, 24 patients showed DSA clearance at 6 months (MFI <1000) ( $n=12$  class I and  $n=12$  class II), whereas 15 had persistent DSA. At 6 months post-LTx, 12 (75%) out of 16 patients with DSA class I showed cleared DSAs versus 12 (52%) out of 23 patients with DSA class II ( $p=0.19$ ). The amount of decrease in MFI at 6 months was greater with DSA class I than class II ( $-84.6\%$  versus  $-50.9\%$ ,  $p=0.047$ ).

In the high-DNA group, four patients had a retrospective positive CDC-lymphocytotoxicity crossmatch (four out of 36, 11% of CXMs performed) due to IgM T- and B-positivity ( $n=3$ ), and to IgG B-positivity ( $n=1$ ).

#### ***Freedom from CLAD***

Freedom from CLAD at 1 and 3 years post-LTx was similar in the high-DNA group and no-DNA groups (1 year median 92.1%, IQR 83.1–1.01% versus 97.4%, 95.1–99.7%; 3 years 86.8%, 75.6–98.1% versus 88.5%, 83.9–93.1%; log-rank,  $p=0.10$  and  $p=0.70$ , respectively; figure 4a), or as compared with the low-DNA group (1 year median 92.6%, IQR 85.4–99.8%; 3 years 88.5%, 83.9–93.1%; log-rank,  $p=0.933$  and  $p=0.257$ , respectively; figure 4a). Furthermore, when high-DNA patients were classified into those with cleared iDSAs at 6 months and those with persistent iDSAs at 6 months, the incidence of CLAD was higher with persistent high DSA than no DSAs (log-rank,  $p=0.014$ ) and cleared high DSAs (log-rank,  $p=0.044$ ) (figure 4b). Freedom from CLAD was similar for patients with DSA class I and class II (log-rank,  $p=0.54$ ; supplementary figure S2).

#### ***Graft survival***

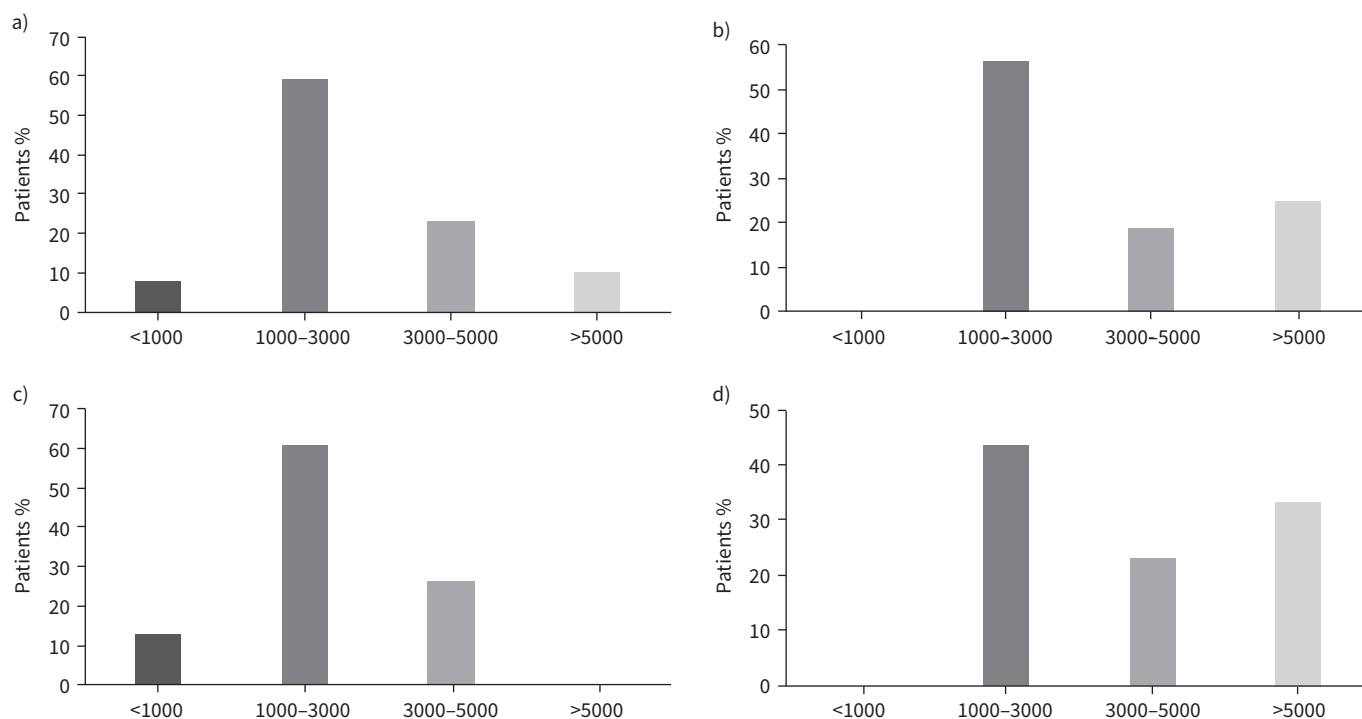
At 1 and 3 years post-LTx, graft survival was similar with high DSAs and no DSAs (1 year median 97.4%, IQR 92.2–1.03% versus 89.4%, 85.2–93.5%; 3 years 87.2%, 76.2–98.2% versus 79.2%, 73.7–84.6%; log-rank,  $p=0.449$  and  $p=0.284$ ; figure 5a). Graft survival was higher with high than low DSAs at 1 year (median 81.8%, IQR 72.2–91.4%, log-rank,  $p=0.02$ ), with no significant difference at 3 years: median 77.3%, IQR 66.8–87.7% (log-rank,  $p=0.155$ ; figure 5a). Furthermore, when high-DNA patients were classified into cleared versus persistent iDSA patients (figure 5b), graft survival was higher with cleared high DSA versus all other groups, including persistent high DSAs ( $p=0.019$ ), low DSAs ( $p=0.025$ ) and no DSAs ( $p=0.044$ ) (figure 5b). We observed a trend, although not significant, towards a higher graft survival in patients with DSA class I versus those with class II (log-rank,  $p=0.0509$ ; supplementary figure S3).

#### ***Determinants of CLAD onset and graft failure***

On univariate analysis, persistent high DSAs was associated with risk of CLAD onset, whereas fibrosis and other diagnoses, perioperative extra-corporeal membrane oxygenation (ECMO) and PGD grade 3 were associated with the risk of graft failure, regardless of DSA group (table 2). On multivariate analyses, both persistent high DSAs and low DSAs, male recipient and cytomegalovirus mismatch were associated with increased risk of CLAD onset. Furthermore, risk of graft failure was reduced with cleared high DSAs as compared with no DSAs, but was increased with PGD grade 3 and perioperative ECMO (table 2).

#### ***Primary graft failure***

Incidence of PGD grade 3 was higher with high DSAs than no or low DSAs (30% versus 9% and 19%, respectively;  $p=0.003$ ; table 1). On univariate analysis, fibrosis disease, lack of *ex vivo* procedure, perioperative ECMO and detection of high DSAs were associated with PGD grade 3 onset (supplementary table S1). On multivariate analysis, only high DSAs and perioperative ECMO remained associated with



**FIGURE 2** Distribution of patients with high levels of pre-formed donor-specific antibodies (pf-DSAs) at day 0 by amount of pre-formed DSAs into different ranges of mean fluorescence intensity (MFI) of the immunodominant DSA (iDSA). **a)** Class I and II: day 0; **b)** class I: day 0; **c)** class II: day 0; **d)** MFI cumulative class I and II: day 0.

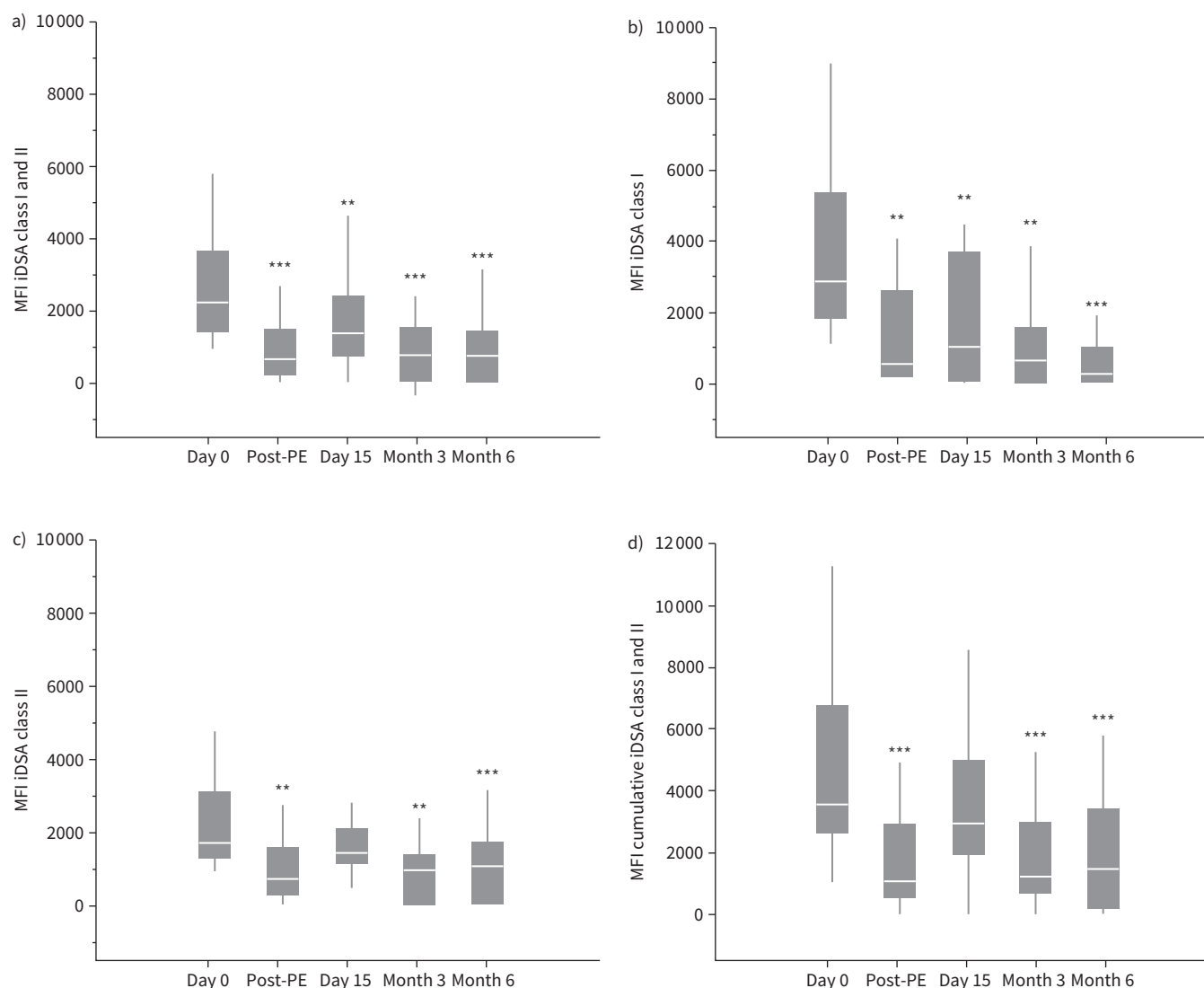
PGD grade 3 (hazard ratio (HR) 3.20, 95% CI 2.51–10.42,  $p=0.001$  and 3.05, 1.17–7.98,  $p=0.023$ ; supplementary table S1).

#### Acute rejection (cellular acute rejection and AMR)

Acute rejection score was  $0.73 \pm 0.94$ ,  $0.98 \pm 1.26$  and  $0.81 \pm 1.00$  with high, no and low DSAs, respectively, with no significant difference between the three groups ( $p=0.38$ ). Probable AMR within 1 year post-LTx occurred in six (15%) high DSA-patients, 15 (8%) patients without DSAs and five (7%) low DSA-patients, with no significant differences between the three groups ( $p=0.30$ ). Among high-DSA patients, probable AMR occurred in six out of 19 patients with persistent DSAs *versus* none out of 20 with cleared DSAs patients ( $p=0.008$ ). Mean  $\pm$  SD time after LTx to AMR with high DSAs was  $6.7 \pm 4.6$  months. In this group, CLAD developed in seven out of 38 patients who survived  $\geq 3$  months (bronchiolitis obliterans syndrome (BOS)  $n=6$ , restrictive allograft syndrome  $n=1$ ). Probable AMR diagnosed in the six patients with persistent DSAs after perioperative desensitisation was treated with an additional administration of rituximab ( $375 \text{ mg} \cdot \text{m}^{-2}$ ) and monthly *i.v.* Ig ( $2 \text{ g} \cdot \text{kg}^{-1}$ ) in the six cases [16]. In three out of these six patients who had AMR within the first 6 months, this additional desensitisation treatment was ineffective to clear their pre-formed DSAs at 6 months.

#### Discussion

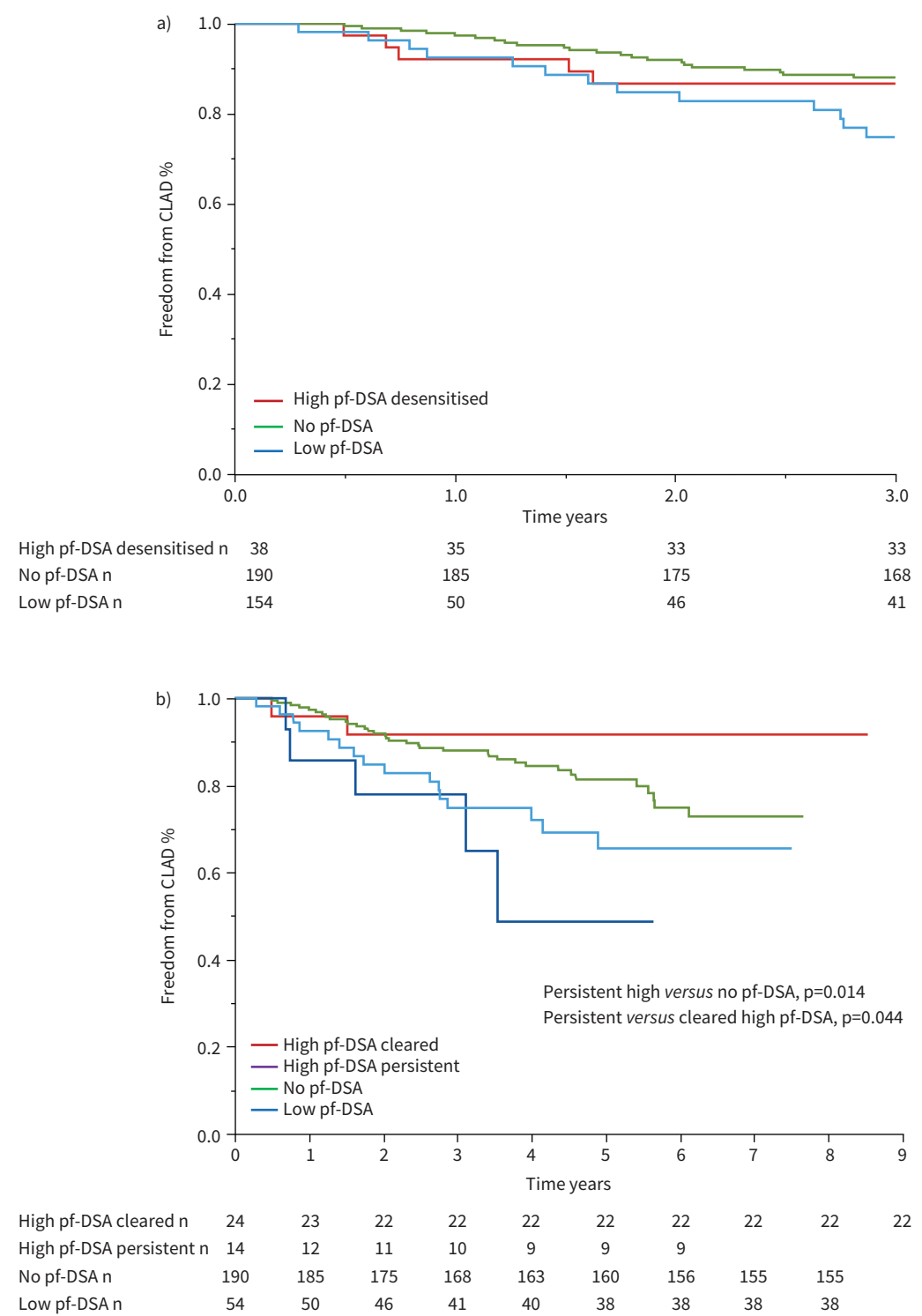
We analysed the impact of our desensitisation protocol on the outcome of LTx candidates transplanted with high DSAs. High-level DSA was cleared in approximately half of the cases, with significantly decreased MFI at 6 months post-desensitisation. Desensitised candidates showed similar outcomes (*i.e.* freedom from CLAD and graft survival) as compared with other patients. Furthermore, incidence of CLAD was increased in persistent high-DSA patients, as compared with no-DSA patients or patients with cleared high DSAs, which strongly argues for a deleterious role of these DSAs in our series. Conversely, and intriguingly, desensitised candidates with cleared DSAs at 6 months had even better survival than all other patients, including those with no DSAs. Multivariate analysis confirmed that cleared DSAs after desensitisation was an independent predictor of graft survival, and conversely, that persistent DSAs after desensitisation was a predictor of CLAD.



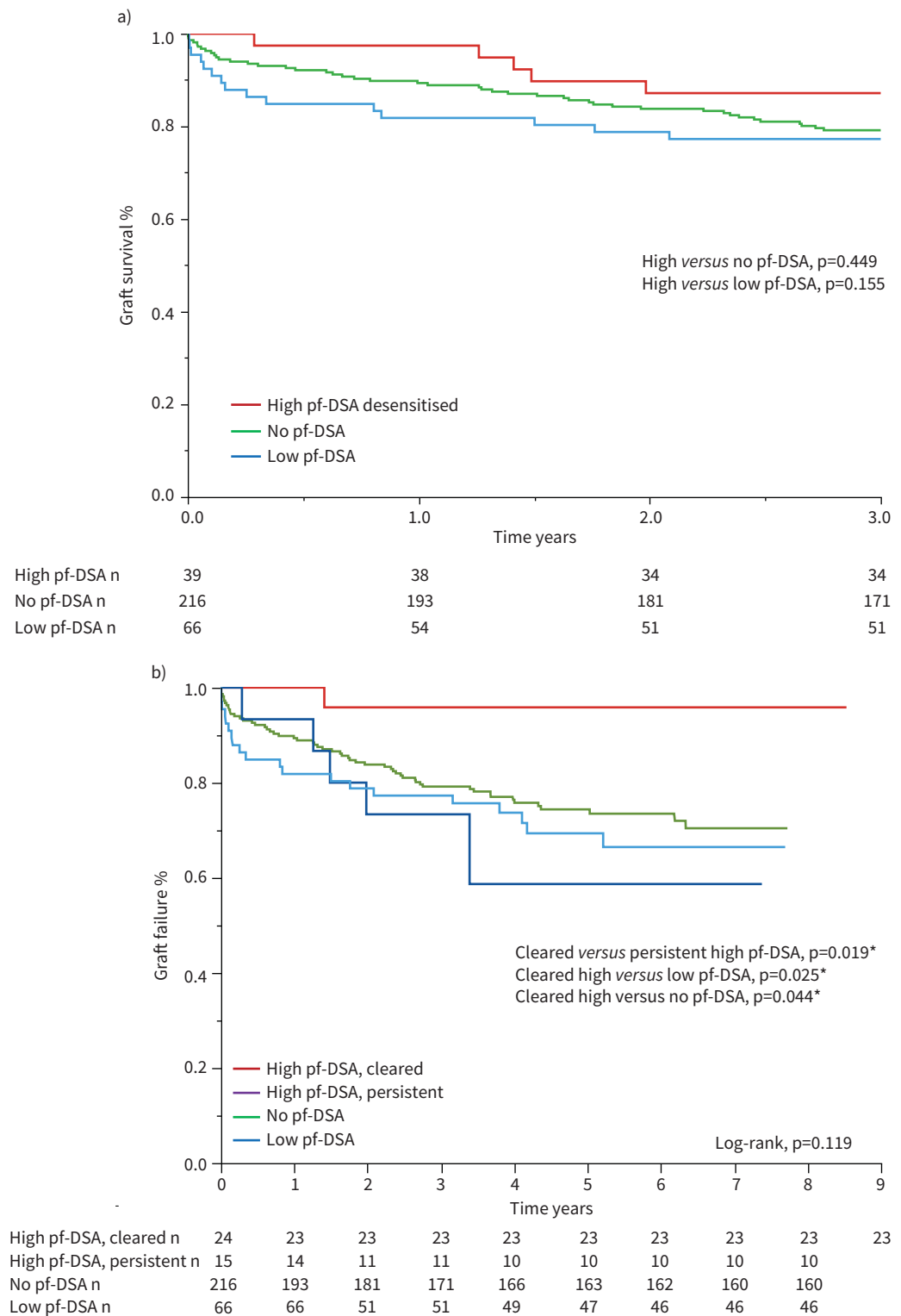
**FIGURE 3** Evolution of the amount of pre-formed donor-specific antibodies (DSA) mean fluorescence intensity (MFI, as assessed by median MFI), according to post-lung transplant (LTx) time points from day 0 to month 6. **a)** Evolution of immunodominant pre-formed DSA (iDSA, class I or II) from day 0 to post-plasma exchange (PE), day 15, month 3 and month 6. For iDSA, median (interquartile range (IQR)) MFI value was 651.5 (1291) at post-PE (*versus* 2207 (2269) at day 0,  $p < 0.001$ ), 760.5 (1533) at month 3 (*versus* day 0,  $p < 0.001$ ) and 736 (1462) at month 6 (*versus* day 0,  $p < 0.001$ ). **b)** Evolution of pre-formed iDSA class I from day 0 to post-PE, day 15, month 3 and month 6. **c)** Evolution of pre-formed iDSA class II from day 0 to post-PE, day 15, month 3 and month 6. **d)** Evolution of cumulative pre-formed DSA from day 0 to post-PE, day 15, month 3 and month 6. For cumulative DSA, median (IQR) MFI value was 1051 (2370) at post-PE (*versus* 3546 (4235) at day 0,  $p < 0.001$ ), 1215 (2274) at M3 (*versus* day 0,  $p < 0.001$ ) and 1441 (3273) at month 6 (*versus* day 0,  $p < 0.001$ ). \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

Our strategy, using a threshold of MFI >5000 as a cut-off for unacceptable donor HLA antigens, allowed for increased access to LTx for immunised candidates, with no significant increase in their immunological risk for CLAD or graft failure as compared with unsensitised patients, although a subgroup with persistent pre-formed DSAs showed increased incidence of probable AMR *versus* those with cleared DSAs.

Allosensitisation in LTx candidates has been associated with longer waiting times, and increased risk of death on the waiting list [5]. Furthermore, DSAs have been found to be associated with increased incidence of CLAD [3, 6–8] and reduced graft survival [3, 6–8]. In addition, allosensitisation might expose LTx candidates to life-threatening hyperacute rejection [17, 18]. To overcome these issues, perioperative desensitisation protocols have been investigated in some LTx centres. A first experience of perioperative deimmunisation with *i.v.* Ig and immunoadsorption in 12 sensitised candidates showed encouraging results with a trend toward greater freedom from BOS and reduced number of acute rejection episodes, as



**FIGURE 4** a) Comparison of freedom from chronic lung allograft dysfunction (CLAD) between patients with high-level pre-formed donor-specific antibodies (pf-DSA), low-pf-DSA patients and no-pf-DSA patients. Freedom from CLAD at 3 years post-lung transplant (LTx) was similar in the high-pf-DSA group compared to the no-pf-DSA group (median 86.8%, interquartile range 75.6–98.1% versus 88.5%, 83.9–93.1%; log-rank test,  $p=0.70$ ), or as compared with low-pf-DSA patients (88.5%, 83.9–93.1%; log-rank,  $p=0.257$ ). b) Comparison of freedom from CLAD by pf-DSA status and by persistence or clearance of pf-DSA after perioperative desensitisation at 3 years post-LTx. The incidence of CLAD was higher with persistent high pf-DSAs than no pf-DSAs.



**FIGURE 5 a)** Comparison of graft survival between patients with high-level pre-formed donor-specific antibodies (high pf-DSA), low pf-DSA patients and no pf-DSA patients. Graft survival at 3 years post-lung transplant (LTx) was similar in the high-pf-DSA group as compared to the no-pf-DSA group (87.2%, 76.2–98.2% versus 79.2%, 73.7–84.6%; log-rank,  $p=0.284$ ), and as compared to the low-pf-DSA group (87.2%, 76.2–98.2% versus 77.3%, 66.8–87.7%; log-rank,  $p=0.155$ ). **b)** Comparison of graft survival by pf-DSA status and by persistence or clearance of pf-DSA after perioperative desensitisation at 3 years post-LTx. Graft survival was higher with cleared high pf-DSA versus all other groups, including persistent high pf-DSA ( $p=0.019$ ), low pf-DSA ( $p=0.025$ ) and no pf-DSA ( $p=0.044$ ).

**TABLE 2** Clinical and immunological factors associated with chronic lung allograft dysfunction (CLAD) onset and graft survival after lung transplantation (LTx) (univariate and multivariate analysis)

	CLAD onset	p-value	Graft failure	p-value
<b>Variable univariate analysis</b>				
Recipient age (by 5-unit increase)	0.99 (0.97–1.01)	0.479	1.01 (0.99–1.03)	0.198
Recipient sex	1.47 (0.87–2.48)	0.147	1.13 (0.73–1.75)	0.571
Male <i>versus</i> female				
Initial disease		0.375		0.071
Cystic fibrosis	1.00		1.00	
Emphysema/COPD	1.38 (0.69–2.61)	0.354	1.66 (0.94–2.86)	0.081
Fibrosis	1.25 (0.54–2.94)	0.489	2.09 (1.08–3.85)	0.030
Other	1.05 (0.35–2.49)	0.927	2.08 (1.03–3.95)	0.042
Re-transplantation	2.57 (0.87–6.14)	0.082	0.85 (0.14–2.81)	0.820
Donor age	1.01 (0.99–1.03)	0.106	1.00 (0.99–1.02)	0.503
Donor sex (male <i>versus</i> female)	0.99 (0.59–1.68)	0.974	1.08 (0.70–1.68)	0.740
Donor smoking status (yes <i>versus</i> no)	1.16 (0.68–1.95)	0.570	1.31 (0.84–2.02)	0.228
CMV mismatch (yes <i>versus</i> no)	1.68 (0.90–2.95)	0.098	0.97 (0.53–1.68)	0.930
<i>Ex vivo</i> procedure (yes <i>versus</i> no)	0.81 (0.35–1.62)	0.572	0.82 (0.41–1.49)	0.543
Ischaemia	0.91 (0.75–1.08)	0.273	0.99 (0.84–1.13)	0.853
PGD grade 3 (yes <i>versus</i> no)	1.99 (0.80–4.22)	0.127	3.62 (1.99–6.27)	<0.001
Perioperative ECMO or cardiopulmonary bypass (yes <i>versus</i> no)	0.94 (0.55–1.58)	0.810	1.96 (1.27–3.06)	0.003
Induction therapy		0.670		0.219
No induction	1.00		1.00	
Thymoglobulin	0.85 (0.41–1.77)	0.656	0.64 (0.35–1.17)	0.146
IL-2 antagonist	0.75 (0.40–1.47)	0.383	0.63 (0.38–1.09)	0.098
No of HLA-A, B, DR, DQ mismatches (per 1-MM increment)	0.95 (0.79–1.13)	0.53	0.99 (0.85–1.16)	0.915
pf-DSA status and desensitisation group		0.037		0.048
No pf-DSA	1.00		1.00	
Low pf-DSA not desensitised	1.71 (0.95–3.09)	0.075	1.24 (0.74–2.06)	0.416
High pf-DSA persistent (desensitised)	2.91 (1.13–7.47)	0.038	1.52 (0.61–3.81)	0.367
High pf-DSA cleared (desensitised)	0.51 (0.12–2.12)	0.356	0.17 (0.02–0.98)	0.047
<b>Variable multivariate analysis</b>				
PGD grade 3 (yes <i>versus</i> no)	1.69 (0.67–4.20)	0.259	3.01 (1.57–5.80)	<0.001
Perioperative ECMO (yes <i>versus</i> no)			2.48 (1.34–4.57)	0.004
CMV mismatch (yes <i>versus</i> no)	1.97 (1.00–3.83)	0.049		
Recipient sex	1.88 (1.01–3.52)	0.048		
Male <i>versus</i> female				
Desensitisation of pf-DSA status group				
No pf-DSA	1.00		1.00	
Low pf-DSA	2.01 (1.01–4.00)	0.046	1.24 (0.67–2.28)	0.560
High pf-DSA persistent <sup>#</sup>	3.04 (1.02–9.17)	0.048	0.91 (0.34–2.42)	0.843
High pf-DSA cleared <sup>¶</sup>	0.67 (0.15–2.91)	0.590	0.12 (0.02–0.85)	0.035

Data are presented as hazard ratio (95% CI), unless otherwise stated. CMV: cytomegalovirus; PGD: primary graft dysfunction; ECMO: extracorporeal membrane oxygenation; IL: interleukin; HLA: human leukocyte antigen; MM: mismatch; pf-DSA: pre-formed donor-specific antibody. <sup>#</sup>: patients with high pf-DSA who had persistence of pf-DSA at 6 months post-LTx after desensitisation protocol; <sup>¶</sup>: patients with high pf-DSA who cleared their pf-DSA at 6 months post-LTx after desensitisation protocol.

compared with those not receiving desensitisation therapy and unsensitised patients [19]. The Toronto group reported their experience with a perioperative treatment including plasma exchange, rituximab and *i.v.* Ig in 53 patients with pre-formed DSAs (MFI >1200). They did not define a superior limit of pre-formed DSA MFI as a contraindication for LTx. Post-operative outcome was very satisfactory, with similar incidence of CLAD and graft failure in desensitised patients and unsensitised patients [20]. Satisfactory outcome was even observed in 26 patients with persistent cDSA MFI >20 000, both class I or II, at 6 months [20], although this immunological status was associated with poorer outcome in another analysis of the impact of pre-formed DSAs on CLAD [11]. Our strategy of desensitisation differed from this previous protocol in the MFI cut-off for unauthorised pre-formed DSAs, limited to 5000 in our centre. This cut-off was chosen first to avoid the risk of a true-positive CXM rapidly increased beyond this threshold [17, 21, 22]. In addition, it was planned in an attempt to lower the probability of persistent high-level DSA in the post-operative period, associated with subsequent CLAD [4, 11], particularly with anti-DQ DSAs [23, 24].

An intriguing finding was the higher graft survival observed in the desensitised group with cleared high pre-formed DSAs as compared with all other groups, including patients without any pre-formed DSAs. This result was unanticipated, and only hypotheses can be drawn, such as a possible supplemental benefit of desensitisation directed against non-identified DSAs with cleared DSAs, including non-HLA DSAs, undetected by SAFB which is directed to HLA antibodies. Indeed, a deleterious role of non-HLA DSAs has been found associated with AMR [25]. This better outcome of desensitised patients with cleared pre-formed DSAs also argues indirectly for an immunological risk with these pre-formed DSAs if persistent. This was also suggested by the exclusive occurrence of AMR episodes in the subgroup of desensitised patients with persistent DSAs (six out of 19 patients), and not in those with cleared DSAs (none out of 20 patients). Among three of these six patients, additional desensitisation with rituximab and *i.v.* Ig was administered within 6 months post-LTx, but was ineffective to clear their pre-formed DSAs at 6 months. These findings were not found in a previous report of desensitisation, possibly related to the lack of superior cut-off of unacceptable DSAs and persistence of pre-formed DSAs class I and II in most patients [20]. An unanticipated finding was also the increased risk of CLAD in patients with low DSAs as compared with unsensitised LTx candidates, because the impact of DSA with MFI 500–1000 on clinical outcome is infrequently recognised as deleterious [10, 11]. Hence, a deleterious role of low DSA level cannot be formally excluded, as was found in previous preliminary studies [3, 4], related to possible significant immune responses after implantation of the graft. Because of these uncertainties when initiating this protocol of desensitisation, the threshold of MFI >1000 was arbitrarily chosen because of the risk–benefit balance with the complexity and constraints of performing plasmapheresis sessions in the post-operative period.

Clearance of DSAs did not significantly differ between patients with class I or II anti-HLA DSAs, but we observed a tendency towards a higher clearance of DSAs for DSA class I *versus* class II. Also, the amount of decreased MFI at 6 months was greater with class I than class II DSAs, as was reported in desensitised LTx and kidney transplant recipients [19, 26]. Furthermore, we observed a tendency to an association between anti-DR/DQ DSA class 2 and their persistence, as observed in other studies [14, 24]. In another study that used a perioperative desensitisation protocol, persistence of pre-formed DSA class I and class II appeared similar at 6 months post-LTx [20].

The incidence of AMR was higher in the desensitised group than with no or low-level DSAs, but not significantly so, and similar to the reported incidence of AMR in LTx [16]. Only four patients had CXM due to IgM, both T- and B-positive or IgG B-positive retrospectively observed in the desensitised group. CXM due to IgM is usually considered as nonsignificant and associated with rather satisfactory graft outcome in renal transplantation [27]. Additionally, IgG B-positivity remains controversial, but some kidney transplant patients may show increased risk of rejection [28]. Hence, this low number of patients with possible immunological risk reflected by a positive CXM due to IgG B-positivity may suggest an adapted threshold of MFI >5000 for DSAs with unacceptable antigens in our protocol.

The incidence of PGD grade 3 was increased with high-level DSAs and multivariate analysis also found an independent association between high DSAs and PGD grade 3, as previously observed [29]. PGD is considered multifactorial and could include the inflammatory response associated with anti-HLA antibodies, as already suspected [6]. Nevertheless, clinically, this increased risk of PGD in the high-DSA group was not associated with an increased risk of CLAD, whereas severe PGD is a well-known risk of CLAD [30, 31], as was also found in this series. This finding also argues for a reasonable risk-taking with our desensitisation protocol.

The similar clinical outcomes we observed in desensitised LTx recipients may be due to the decrease in anti-HLA antibody titres, as observed in kidney transplantation or LTx [19, 32]. They may also be due to the induction of an immunologically quiescent state, potentially induced by *i.v.* Ig *via* different mechanisms including an anti-idiotypic effect, inhibition of B-cell function or downregulated antibody production [33], although these mechanisms still remain uncertain.

Our study has important limitations. A crucial point is the real benefit of such a strategy in patients with DSAs, because no control group of patients with high pre-formed DSA who did not undergo desensitisation was available. Nevertheless, several findings argue for a benefit of our targeted desensitisation protocol: 1) graft survival was similar in desensitised and no-DSA patients, whereas pre-formed DSAs have been found a strong independent predictor of death in LTx recipients without any desensitisation (HR 3.398,  $p=0.0010$ ) [11]; 2) clearance of DSAs in our desensitised patients was an independent predictor of graft survival on multivariate analysis, and much of the high-level DSAs was significantly decreased or cleared after LTx; and 3) cases of probable AMR with desensitisation ( $n=6$ , 15%) exclusively occurred with persistent DSAs.

The rationale for including all DSA (class I and II) to the protocol is an important issue, which is debatable. Indeed, DSA class II was almost associated with poor survival in case of persistence [14]. In a previous preliminary study, we observed that patients with pre-formed DSA class II had higher incidence of BOS as compared to those with pre-formed DSA class I [3]. Nevertheless, the total number of patients with persistence of identical pre- and post-LTx DSA class I (n=3) and II (n=7) was rather small in this study [3], and other studies have shown since then that both pre-formed class I and II could have a negative impact on post-LTx outcome [34]. It should be also mentioned that patients with pre-formed DSA class I had a lower survival than all other patients in the cohort of SMITH *et al.* [34] without a desensitisation protocol, which argues strongly for a deleterious impact of these pre-formed DSAs of class I, along with those of class II. Another important aspect is the risk of hyperacute rejection linked to pre-formed DSAs, which has been reported frequently as associated with a fatal outcome, sometimes due to pre-formed DSA class I [18, 35]. Nevertheless, we have to acknowledge that we were not able to demonstrate the deleterious effect on graft survival of pre-formed DSA class I *per se*, due first to the design of this study without a control arm, and also because most of these class I DSAs were cleared after desensitisation (75% of patients with DSA class I cleared at 6 months). This last result does not preclude their deleterious impact in case of persistence due to the lack of desensitisation protocol, as observed in a previous study [34]. In high-DSA patients, graft survival was higher in patients with DSA class I *versus* those with class II, with a near-significant difference. Hence, taken as a whole, our data suggest that pre-formed DSA class II seemed more difficult to clear with our perioperative desensitisation protocol, and that their persistence was more frequently associated with a poorer graft survival. Lastly, it should be mentioned that the close cooperation with our immunology laboratory allowed for the availability of day 0 result of SAFB assay within 24 h, which may not be applicable in all LTx centres.

Another point of uncertainty is the DSA MFI threshold of unacceptable antigens, which remains debated. The threshold of 5000 for unacceptable antigens, even with perioperative desensitisation, was chosen according to the risk of a positive real CXM [12] and/or hyperacute rejection after solid-organ transplantation [17, 21, 22]. Additionally, the lower limit of 1000 for the DSA MFI for starting a desensitisation was chosen according to recommendations [21, 22, 36] and previous studies which showed worse outcome with MFI as low as 500 [3, 11].

These results were observed in a single-centre nonrandomised study, so drawing firm conclusions about our protocol is difficult. Therefore, our results should be considered as primarily hypothesis-generating with a need for external validation in future prospective studies.

In summary, perioperative desensitisation protocols are important for decreasing cPRA and increasing access to LTx in immunised candidates with pre-formed anti-HLA antibodies. Because DSAs are frequently associated with poor outcome, the criteria for defining unacceptable antigens are a balance between immunological risk factors and the likelihood of finding a compatible organ. Our series strongly suggested a deleterious role of pre-formed DSAs by the increased incidence of CLAD and poor graft survival in sensitised patients with persistent DSAs after desensitisation, with a DSA MFI threshold of unacceptable antigens fixed at MFI 5000. Overall, we observed a benefit of this desensitisation protocol according to a risk stratification of LTx candidates with DSAs based on virtual CXM, with an acceptable median time of LTx after listing without death while on a waiting list, and similar overall risk of CLAD and graft failure in desensitised and non-desensitised patients.

Conflict of interest: None declared.

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